No Association between Serum Lipids Levels and Lipids Oxidizability in Type 2 Diabetes

Robab Sheikhpour[1*], Bemanali Jalali[2], Mohammad Afkhami-Ardekani[3]

1-Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2-Shahid Sadoughi University of Medical Sciences, Yazd. Iran
3-Yazd Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

***Correspondence:**
Robab Sheikhpour, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Tel: (+98) 351-6282884.
Email: sheikhpour_r@yahoo.com

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Abstract

Objective: Diabetes is the most common metabolic disease. One of the most common problems in diabetic patients is atherosclerotic cardiovascular disease which induced by hyperlipidemia. Also there has been currently great interest in the potential contribution of oxidative stress in development of diabetes complications. The present study was performed to associate between lipid oxidizability and serum lipid levels in diabetic patients.

Materials and Methods: In this study, 55 diabetic patients in Yazd Diabetes Research Center were chosen. Lipid oxidizability, HbA1c and serum lipid levels were analyzed in patients. Lipids were measured by enzymatic method. HbA1c was estimated by the ion exchange chromatography. The lipid oxidation procedure was performed by addition of CuCl2. The kinetics of conjugated dienes formation was monitored by spectrophotometer and parameters such as ODmax, Lag Time, Vmax and Tmax were analyzed.

Result: In present study, there was no association between lipid oxidizability and serum lipids levels. Also there was no association between lipid oxidizability parameters and HbA1c.

Conclusion: This study showed that high levels of blood lipids such as cholesterol and triglyceride do not have any effect on maximal amount of lipids peroxide products accumulation and maximal rate of oxidation during the lipid oxidation course.

Key Word: Lipid, Lipid oxidizability, Diabetes

Introduction

Diabetes mellitus is one of the most common problems caused by a combination of insulin resistance and impaired insulin secretion by pancreatic β cells (1,2). One of the most common problem in diabetic patients is atherosclerotic cardiovascular disease which is induced by hyperlipidemia (3,4). Lipid abnormalities such as hypertriglyceridemia and fatty acid distribution changes could participate in the development of vascular lesions in diabetes (5). Currently, there has been great interest in the potential contribution of oxidative stress to the development of diabetes complications

(6,7). Oxidative stress is a condition characterized by a disturbance in the prooxidant-antioxidant balance in favour of the former, which leads to a potential harm to the cell. Reactive oxygen species (ROS) can damage proteins, lipids, nucleic acids and other cellular components under oxidative stress conditions (8). In addition, the activation of stress-sensitive signaling pathways that regulate gene expression can also result in cellular damage (9).

Several mechanisms seem to be involved in the pathogenesis of the oxidative stress, which include glucose autoxidation, protein glycation, formation of advanced glycation end products, and the polyol pathway which is induced by hyperglycemia. Many studies have shown an association between serum lipids and lipids oxidizability in such diseases as diabetes, hypertention, atherosclerosis and so on, but in our country, it has not been considered much. The purpose of this study was to evaluate the association between serum lipids levels and lipids oxidizability in type 2 diabetic patients (10).

Materials and Methods

Sample collection: In this study, 55 diabetic patients aged 40 to 60 years in Yazd Diabetes Research Center in 2009 were chosen.

Inclusion criteria: Type 2 diabetes mellitus for more than 5 years (diagnosed according to American Diabetes Association criteria) with glycated hemoglobin (HbAlc) more than 7%, triglyceride (TG) less than 400 mg/dl and total cholesterol (TC) less than 250 mg/dl.

Exclusion criteria: Cigarette smoking, current treatment with anti-oxidant drugs or other medications except for oral hypoglycemic agents, diabetic complications, using vitamins or minerals supplements in the last two months, renal failure, ischemic heart disease, congestive heart failure, uncontrolled hypertension, history of stroke and chronic liver disease, pregnancy and lactation. Moreover, patients who had to change dosage or types of medications were excluded.

Blood collection: Blood samples were obtained from patients. Serum was separated from the clots after complete coagulation (l h in room temperature) by low speed centrifugation (15 min at 2000 g), and stored in -70°C refrigerator.

Lipid profiles and HbA1c assay: Cholesterol, triglyceride and high-density lipoprotein (HDL) were measured by enzymatic method and Pars Azmoon kit. Low-density lipoprotein-C (LDL-C) was analyzed by Friedewald method (11). HbA1c test is assay in which lysed whole blood samples. HbA1c was estimated by the ion exchange resin kit method.

Lipid peroxidation assay: Copper-induced serum lipid peroxidation was estimated in a 60-fold diluted serum in 20 m Mphosphate buffer containing 720 µ M sodium citrate, pH 7.4. The lipid oxidation procedure was performed at 37°C and was initiated by addition of $CuCl_2$ to give a final concentration of 60 µM. The kinetics of conjugated dienes formation were monitored by spectrophotometer (Perkin-Elmer UV.VIS Double beam spectrophotometer 505S) by measuring absorbance in a 1-cm quartz cuvette at 245 nm, every 10 min for 300 min. For plotting the kinetic curves of the accumulation of lipid peroxide products (change of absorbance at 245 nm versus time in min) Microsoft Excel Software was used. A number of quantitative oxidation parameters including lag-time (the interval between the addition of $CuCl_2$ to the serum and the beginning of extensive oxidation), maximal rate of oxidation (V_{max}), maximal amount of lipid peroxide products accumulation (OD_{max}), and the time needed to gain maximal rate of oxidation (T_{max}) were evaluated (18). Before processing the samples, method of serum lipid oxidation was optimized and an inter-individual coefficient of variation (Cv) of 6% (for lag-time), 7.4% (for OD_{max}) and 7.5% (for V_{max}) was obtained. SPSS software (V.11.5) and Pearson's test were used for statistical analysis.

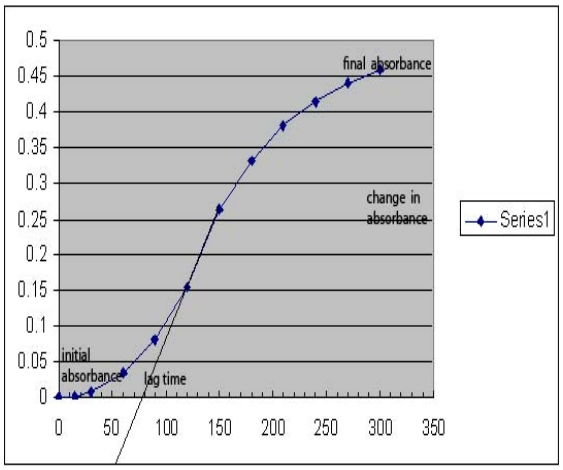

Figure 1: The curve of lipid oxidizabili 1

Results

In this study 55 patients were chosen. Serum lipids levels, oxidizability of lipids and HbA1c were evaluated according to the above criteria. Figure 1 shows the curve of lipid oxidizability. Table 1 shows mean of quantitative serum oxidation parameters and table 2 shows mean of quantitative serum lipids level in diabetic patients. Table 3 shows the association between lipid oxidizability and HbA1c.

Also, there was no association between lipids peroxidation and HbA1c. Table 4 shows the association between lipid oxidizability and serum lipids. There was no association between serum lipid oxidation and serum lipids levels.

Discussion

One of the major hypotheses proposed to explain the hyperglycemia-induced onset of diabetic complications is an increase in oxidative stress (12).

Oxidative stress is an important mediator of diabetic complications (12). Also, Lipid abnormalities such as hypertriglyceridemia and fatty acid distribution changes could participate in the development of vascular lesions in diabetes. A significant elevation in Malondialdehyde (MDA) level and decrease in glutathione and protein content was observed in both male and female diabetic patients in comparison to non-diabetic controls (13). One study has shown that there is an association between lipid peroxidation and serum lipid levels in hypertensive patients. But we could not find an association between lipid

Table 1- Mean of quantitative serum peroxidation parameters

Variable	Mean ±SD
OD max	0.325±0.073
T max	138.00±20.3
V max	2.088±0.532
Lag time	74.00±14.1

Table 2- Mean of quantitative HbA1c and lipids serum

Variable	Mean ±SD
Triglyceride	195.60±70.7
Cholesterol	184.90±34.478
HDL-C	30±7.4
LDL-C	119.1±33
HbA1c	8.99±1.79

Table 3- Association between lipid oxidizability parameters and HbA1c

Variable	HbA1c
T max	r=0.199 p=0.166
V max	r= -0.08 p=0.578
Lag time	r= -0.17 p=0.905

Table 4- Association between lipid oxidizability parameters and serum lipids

Lipid oxidizability	Lipid			
	Cholesterol	**Triglyceride**	**HDL-C**	**LDL-C**
OD max	r=0.242,p=0.09	r=0.086,P=0.554	r=0.19,p=0.17	r=0.48,p=0.10
V max	r=0.197,p=0.17	r=-0.213,p=0.137	r=0.12,p=0.45	r=0.44,p=0.7
Lag time	r=0.016,p=0.913	r=-0.36,p=0.802	r=0.160,p=0.19	r=0.005,p=0.97

OD max: Maximal amount of lipids peroxide products accumulation during the lipid oxidation course
V max: Maximal rate of oxidation during the lipid oxidation course
Lag-time: The time needed (in min) for initiation of lipid oxidation products accumulation during the lipid oxidation course after addition of Cucl2
T max: Time needed (in min) to gain the maximal rate of lipid peroxide products accumulation during lipid oxidation

oxidizability and serum lipids levels in diabetic patients. Jalali showed that there is an association between OD max and cholesterol (r=0.5, p=0.001), triglyceride (r=0.3, p=0.003) and LDL (r=0.4, p=0.001) respectively and an association between Vmax and cholesterol (r=0.46 P=0.001), triglyceride (r=0.34 P=0.008) and LDL (r=0.45, P=0.001) respectively (14).

Another study showed that, there was an inverse relationship between insulin action and oxidative stress. Insulin resistance and increased oxidative stress have been observed in obese type 2 diabetic patients. The relationship between insulin action and oxidative stress was therefore suggested. This finding of an inverse relationship between plasma malondialdehyde concentration and glucose disposal rate during hyper-insulinemic clamp is in agreement with this suggestion. A decrease of oxidative stress could therefore improve insulin action in subjects with insulin resistance (15). But in this study, we did not observe any significant association between lipid peroxidation and HbA1c.

Another study showed that MDA significantly increases ($p<0.05$) in both uncomplicated and complicated DM compared to the control group, in agreement with the findings of Mahreen et al. and Ozdem et al. MDA showed a statistically significant positive correlation with HbA1c ($r=0.30$, $p<0.05$), in agreement with Turk et al. MDA also showed a statistically significant positive correlation with cholesterol, triglycerides, and LDL-C ($r=0.39$, $r=0.32$, $r=0.35$, respectively ; $p<0.05$) and negative correlation with HDL-C ($r=-0.31$, $p<0.05$) (16).

Another study showed that oxidative stress is greatly increased in patients suffering from diabetic neuropathy and is inversely related to glycemic control (17).

Simmi Kharb reported that glycated proteins might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may also be thought to play a role in increased lipid peroxidation in diabetes mellitus (18).

Hanachi reported patients with diabetes have higher MDA levels in their blood serum and that glycated hemoglobin and serum glucose level in these patients are related to each other. The interpretation of the data could be biased by such confounding factors, particularly because plasma lipid peroxidation seems to be linked to lipid concentrations and the degree of hyperglycemia. In conclusion, they found that oxidative susceptibility in vivo is increased in subjects with type 2 diabetes compared with non-diabetic subjects. The increased plasma lipid peroxidation and decreased plasma HDL that we observed in patients with type 2 diabetes mellitus may suggest that it may predispose the patient to the development of cardiovascular complications.

Supplementation with dietary free radical scavengers such as vitamins E and C has a potential role in boosting antioxidant-related defenses and is probably important in patients with diabetes. They propose that diabetic patients may have elevated requirement for antioxidants (19).

Another study showed that there is a relationship between the levels of cholesterol peroxidation products and HbA1c in erythrocytes of diabetic and healthy subjects. There was a significantly increased ratio of 7-oxocholesterol to cholesterol in diabetic erythrocytes compared to control erythrocytes. The ratio of 7-oxocholesterol to cholesterol was significantly correlated with the level of HbA1c (20).

Inouye reported that there were significantly increased ratios of conjugated linoleic acid (CLA) to linoleic acid (LA) in diabetic erythrocytes compared to control erythrocytes. The peak height ratio of CLA to LA was used as a biomarker of lipid peroxidation. These ratios of conjugated linoleic acid (CLA) to LA were also significantly correlated with HbA1c values (21).

Gillery reported that products of lipid peroxidation (MDA) bind to proteins and amplify glycoxidation-induced damages.

Glycoxydation intensity increases in diabetes mellitus, ageing, renal failure and other pathological states with oxidative stress (22).

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