The effects of glycemic control on malondialdehyde modified low-density-lipoprotein-immunglobulin G levels in type 2 diabetics

Alper Tunga Ozdemir[1*], Hale Aral[2], Fusun Erdenen[3], Rabia Bilge Ozgul Ozdemir[4], Omer Emecen[5], Guvenc Guvenen[6], Cuneyt Muderrisoglu[3]

Abstract

Objective: The objective of this study was to investigate the effects of glycemic control on the levels of malondialdehyde-low-density-lipoprotein-immunglobulin G (MDA-LDL-IgG) which is supposed to be positively correlated with myocardial infarction risk in subjects with type 2 diabetes mellitus (DM).

Material and Method: Glucose, triglyceride, total cholesterol, high-density-lipoprotein-cholesterol (HDL-C), low-density-lipoprotein-cholesterol(LDL-C), hemoglobin A1c (A1C) and MDA-LDL-IgG levels were evaluated in subjects with well-controlled DM (W-DM, <7% HbA1c, n=18), poorly-controlled DM (P-DM, >7% HbA1c, n=22) as well as in non-diabetics (Non-DM, n=15).

Results: There were no significant differences between P-DM and W-DM groups in terms of triglyceride, total cholesterol and LDL-C levels, however the test results were significantly low for the Non-DM group in comparison with other groups (respectively, p=0.002, p<0.001 and p=0.001). There was no significant difference between W-DM and Non-DM groups with regard to MDA-LDL-IgG levels, however they were significantly higher in P-DM group compared to W-DM and Non-DM (p=0.002). There was a positive correlation between A1C and MDA-LDL-IgG levels (r=0.463, p=p=0.001).

Conclusion: These findings suggest that the normalization of blood glucose levels in type 2 diabetics may persuade the reduced rate of formation of new antigenic epitopes on the LDL via non-enzymatic glycosylation. The regulation of diabetes may be improved by reducing antibody formation against the MDA-LDL although there is no effect on lipid levels. A1C may not only be a good indicator of blood glucose control but also a good predictor for diabetes-related macro vascular complications

Key words: Type 2 diabetes mellitus, A1C, glycemic control, Cholesterol, MDA, LDL

Introduction

Diabetes mellitus (DM) is a complex disease associated with obesity, autoimmunity and inflammatory disorders (1–3). Type-2 DM is the most common type of DM worldwide and its incidence is increasing in Western societies (4). Severe complications may occur as a result of all these factors related with DM. Most common complications can be classified as macro-vascular (myocardial infarction, stroke, and peripheral arterial disease) and microvascular (retinopathy, nephropathy, neuropathy) (5,6).

Lipid abnormalities are common in patients with Type 2-DM and are generally characterized by decreased serum levels of high density lipoprotein cholesterol (HDL-C), increased triglycerides (TG), total cholesterol (TC) and low density lipoprotein (LDL-C) particles (1). Atherosclerosis is an inflammatory disease which is strictly related with serum lipid abnormalities and diabetics are predisposed to atherosclerosis process in earlier stages of their lives (3).

Cellular uptake of cholesterol takes place via clathrin mediated low-density-lipoprotein (LDL) receptors and these receptors regulate themselves with feedback mechanisms (7,8). However, macrophage cells phagocyte the modified LDL molecules in the pathogenesis of atherosclerosis by endocytosis via scavenger receptors which are distinct from classical LDL receptors.

The accumulation of modified LDL in macrophages and other phagocytes leads to the formation of foam cells (9). Due to exposure to different molecular modifications, LDL is a critical molecule which plays an important role in development of atherosclerosis. Advanced glycosylation and oxidation of LDL are both well-known modifications for pathogenesis of atherosclerosis leading to formation of neo-antigenic epitopes on the LDL thus transforming it into a highly immunogenic molecule (10–12). Modified LDL induces the innate and adaptive immune cells, as a result, stimulating the formation of the foam cell and auto-antibodies against LDL(13,14).

MDA-LDL is an oxidatively modified LDL molecule and plays an important role in the progression of atherosclerosis. Macrophage cells transform into foam cells by the over-uptake of MDA-LDLs via scavenger receptors while in addition , humoral immune response develops against neoepitopes resulting from lipoprotein modification (9,11). The immune complexes of oxidized-LDL and IgG have been demonstrated to be more potent activators of macrophages in comparison with just oxLDL(26). MDA-LDL-IgG is an autoantibody against MDA-LDL and previous studies have shown that immune complexes of MDA-LDL and IgG are strong predictors for acute events such as myocardial infarction and stroke in patients with type 2-DM (23).

The formation of immune complexes of immunoglobulin G (IgG) and malondialdehyde modified LDL (MDA-LDL) increases the pro-inflammatory immune response and leads to progression of atheroma plaque (19,20,21). Clinical studies on type1-DM have shown that high levels of MDA-LDL and Ig complexes are associated with increased odds for developing diabetic nephropathy and progression of retinopathy (22). Furthermore, it is reported that increased levels of MDA-LDL immune complexes are strong predictors of myocardial infarction in patients with type2-DM (23).

Currently, studies usually focus on the effects of MDA-LDL in the pathogenesis of diabetic complications. Most of these studies suggest a strong correlation between MDA-LDL/Ig complexes and the diabetic complications. Based on these studies, we tried to investigate whether there is a relationship or not between diabetes regulation and serum levels of MDA-LDL-IgG in subjects with type 2-DM. In this study, we evaluated the levels of glucose, TG, TC, HDL-C, LDL-C and MDA-LDL-IgG in subjects with well-controlled DM (W-DM, <7% HbA1c), poorly-controlled DM (P-DM, >7% HbA1c) and non-diabetic (Non-DM) subjects.

Material and Methods

This study was performed at the Istanbul Research and Education Hospital, Medical Biochemistry Laboratory.

The serum samples and demographical data were obtained from our laboratory and laboratory information system (LIS). The groups were created according to ICD-10 (International Statistical Classification of Diseases and Related Health Problems) E11 codes. Serum samples were separated into groups as P-DM (n=22), W-DM (n=18) and Non-DM (n=15) according to inclusion criteria. MDA-LDL-IgG ELISA analyses were performed on waste serum samples.

A1C levels were evaluated using Synchron LX-20 (Beckman-Coulter) auto-analyzer in the complete blood count samples drawn into vacutainer 2 mL volume tubes containing 3.6 mg K2 EDTA. The serum glucose, TG, TC, HDL-C and LDL-C (direct method) levels were evaluated via Synchron LX-20 (Beckman-Coulter) auto-analyzer in the serum samples drawn into vacutainer 10mL volume SST™ tubes with Silica Clot Activator (Becton Dickison). Finally, serum MDA-LDL-IgG levels were evaluated via the ELISA method according to manufacturer instructions (LDN, Labor Diagnostika Nord GmbH & Co. KG).

Descriptive statistics for the studied variables (characteristics) were presented as median, mean, standard deviation, minimum and maximum values (Table 1). One-way ANOVA test was performed to compare all groups. The Bonferroni method was used for homogeneous variances and the Tamhane's T2 method was used for nonhomogeneous variances. The correlation analysis of parameters was performed using nonparametric Spearman's rho correlation method. Statistical significance level was considered as 5% and SPSS (ver: 20) statistical program was used for all statistical computations.

Results

All groups of glucose, TG, TC, HDL-C, LDL-C, HbA1c and MDA-LDL-IgG levels were summarized in Table-1. There were no significant differences between P-DM and W-DM groups for TG, TC and LDL-C levels, howeverthese tests were significantly low in Non-DM group in comparison with other groups (respectively, p=0.002, p<0.001 and p=0.001). No statistically significant difference was observed between groups in terms of HDL-C (p=0.081). There were significant differences between the three groups in terms of glucose (p<0.001) and A1C (p<0.001) levels.

There was no significant difference between W-DM and Non-DM groups in terms of MDA-LDL-IgG levels, however they were significantly higher for the P-DM group in comparison with W-DM and Non-DM (p=0.002). There was a strong positive correlation (Correlation Coefficient=0.463) between A1C and MDA-LDL-IgG levels (p=0.001). The comparison chart of all parameters is shown in Figure 1.

Table 1: Evaluation of all parameters and comparison between the groups of poorly controlled DM (P-DM, n=22), well controlled DM (W-DM, n=18), and non-diabetic subjects (Non-DM, n=15).

Parameters	Group	Median	Mean	SD	Min	Max	p
Glucose (mg/dL)	P-DM	181,50 a	215,31	97,37	93	551	<0,001
	W-DM	135,50 b	155,27	37,36	100	233	
	Non-DM	93,00 c	94,26	6,56	79	110	
Triglyceride (mg/dL)	P-DM	185,00 a	198,63	80,40	110	465	0,002
	W-DM	209,90 a	221,66	135,25	60	595	
	Non-DM	75,00 b	100,33	57,39	45	210	
Total Cholesterol (mg/dL)	P-DM	247,00 a	236,54	37,81	156	313	<0,001
	W-DM	225,50 a	227,77	29,23	161	300	
	Non-DM	176,00 b	174,93	12,47	152	196	
HDL-C (mg/dL)	P-DM	54,00 a	55,09	12,25	37	89	0,081
	W-DM	48,00 a	48,61	7,26	38	67	
	Non-DM	55,00 a	54,53	6,71	41	68	
LDL-C (mg/dL)	P-DM	146,50 a	141,72	39,49	62	222	0,001
	W-DM	134,00 a	134,83	29,05	84	196	
	Non-DM	108,00 b	100,33	19,21	71	134	
A1C (%)	P-DM	9,80 a	10,26	1,73	8,6	14,8	<0,001
	W-DM	6,80 b	6,60	0,67	5,3	7,9	
	Non-DM	5,00 c	4,95	0,26	4,5	5,5	
MDA-LDL-IgG (U/L)	P-DM	358,35 a	504,27	260,67	267,30	1102,20	0,002
	W-DM	320,60 b	342,15	115,58	204,80	619,60	
	Non-DM	332,70 b	298,91	63,85	188,90	605,20	

It is a statistically significant difference between the groups with different letters; a, b and c (p<0.05) Min:Minumum; Max:Maximum; SD: standard deviation
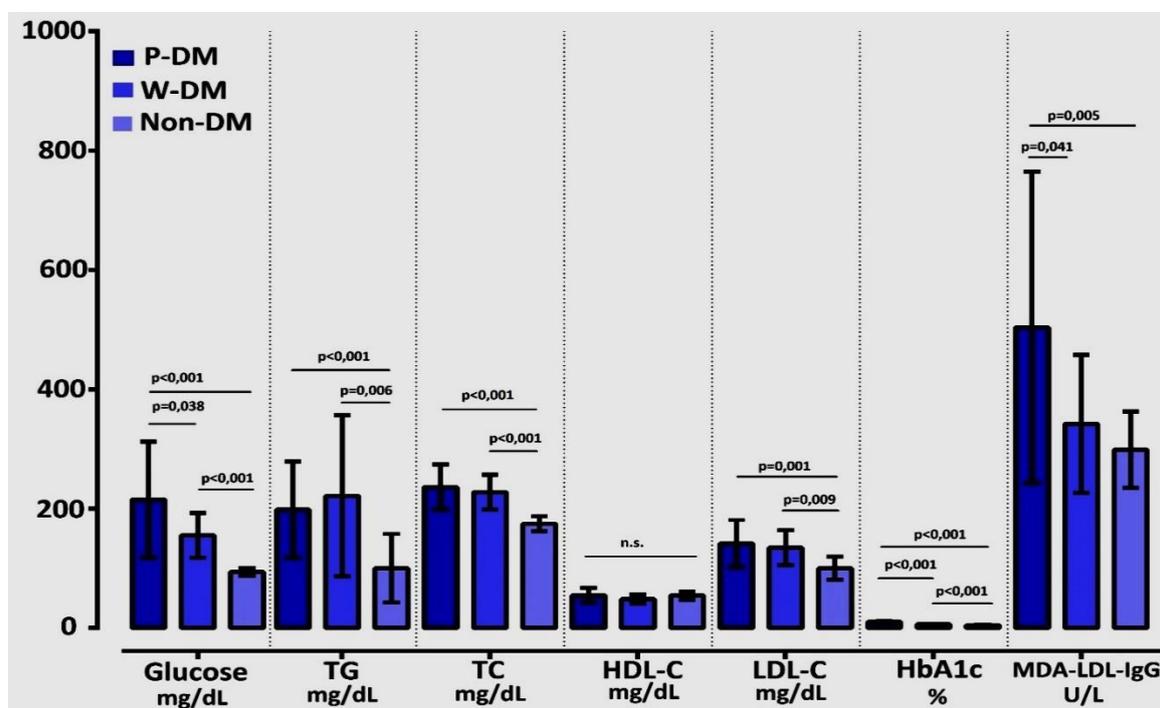

Figure 1: The comparison chart of the levels of all parameters. Data are presented as mean±SD (standard deviation). P-DM: poorly controlled diabetes mellitus, W-DM: well controlled diabetes mellitus, Non-DM: non-diabetic subjects, TG: Triglyceride, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, MDA-LDL-IgG: Malondialdehyde modified low density lipoprotein IgG

Discussion

In this study, we observed that the regulation of DM did not affect serum lipid levels. There was no significant difference between P-DM and W-DM subjects for TG, TC, HDL-C and LDL-C, but serum lipid levels of both groups were significantly higher in comparison with the Non-DM group. Subjects of the P-DM group have the highest level of MDA-LDL-IgG but there was no significant difference between the W-DM and Non-DM groups.

Glycosylation is the reaction by which a carbohydrate is covalently attached to a target protein. Glycosylation is a mandatory process to produce functional proteins and is usually performed with the assistance of an enzyme (24). However, there is also another non-enzymatic version of glycosylation. The non-enzymatic glycosylation of proteins is an uncontrolled process and is responsible for the immunogenicity of protein (10). The oxidation of glycosylated proteins plays an important role in initiating lipid oxidation. Furthermore the resultant lipid peroxidation products, which have "neoself determinants" recognized by the immune cells (12,25).

Glycemic control significantly reduces the risk of microvascular complications in diabetic patients. It is considered that a 1% reduction in A1C decreases the risk of retinopathy, neuropathy and nephropathy by about 40% (15,16).

In contrast, there is a limited number of studies on the effect of glycemic control on macro vascular complications of DM, such as myocardial infarction, stroke (17,18).

Persistent high blood glucose is one of the major causes which leads to non-enzymatic glycosylation of proteins. A1C is the well-known form of glycosylated proteins and it is a laboratory test that shows how well your diabetes is being controlled (27). In this study subjects diagnosed with type 2-DM were divided into two groups as W-DM and P-DM according to A1C levels. Although there were significant differences between the levels of glucose and HbA1c, a significant difference was not found between the two groups in terms of serum lipid levels and especially LDL. However, we observed a significant difference between the MDA-LDL-IgG levels for P-DM and W-DM groups in which it is reported as a predictor for macro vascular complications of DM (11,12,14).

There are some limitations in this study. The appearance of diabetes-related complications is related to disease duration. High levels of MDA-LDL-IgG in subjects with P-DM may be associated with the duration of the disease. It is not easy to acquire reliable information about the age of diabetes, because the subjects do not know the beginning of the diabetic process, as a high serum glucose level, for themselves; they are followed later in the duration of diabetes. And even the known duration of diabetes mellitus for each subject was not recorded in the data system. Furthermore, serum samples were obtained from different individuals, therefore we were unable to evaluate the regulation of diabetes and whether there is a direct impact on the reduction of MDA-LDL-IgG levels or not. We also could not reach clinical data related with macro vascular complications.

Conclusion

We observed a significant difference between P-DM and W-DM groups in terms of their MDA-LDL-IgG levels. Furthermore, it was observed that the levels of MDA-LDL-IgG were similar in subjects with W-DM and Non-DM, and were significantly lower compared to P-DM subjects. These findings suggest that the normalization of blood glucose levels in type 2 diabetics may persuade the reduced rate of formation of new antigenic epitopes on the LDL via non-enzymatic glycosylation. Although there is no effect on lipid levels, the regulation of diabetes may be improved by reducing antibody formation against MDA-LDL.

In addition, there was a positive correlation between A1C and MDA-LDL-IgG levels in type 2 diabetics. A1C may not only be a good indicator of blood glucose control but is also a good predictor for diabetes-related macro vascular complications. Further prospective studies are needed to confirm these findings.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The authors are solely responsible for the content and writing of the paper.

References

1. Calti Gur C, Polat H, Muderrisoglu C, Altunoglu E, Yilmaz M. In Patients with Type-2 Diabetes, Diabetes Regulation, Hba1c, Duration of Diabetes, BMI, Dyslipidemia, and Microalbuminuria Compared with Macrovascular Complications. Istanbul Medical Journal. 2013 Dec 19;14(4):243–7.

2. Çetinkalp DŞ. Tip 1 Diyabet ve Otoimmunite. Turkiye Klinikleri J Endocrin-Special Topics. 2010;3(2):51–8.

3. Dursunoğlu D, Evrengül H, Kaftan A, Kiliç M, Sermez Y. Koroner Ateroskleroz ve Diyabet. Turkiye Klinikleri J Cardiol. 2004;17(1):55–60.

4. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2014 [Internet]. 2014. Available from: http://www.cdc.gov/diabetes/data/statistics/2014statisticsreport.html

5. Çeviker T, Sametoğlu F, Aksoy F, Özdemir AT, Aral H, Güvenen G. The Evaluation of TNF-α and CRP as Inflammatory Markers in Type 2 Diabetes-Related Complications. Istanbul Med J. 2008;9(4):58–60.

6. Gurcan Z, Polat H, Muderrisoglu C, Besler M, Ozgul RB. A Comparison of Insulin Resistance Between Hemodialysis and Peritoneal Dialysis Patients. Istanbul Medical Journal. 2011;12(2):65–8.

7. Poda M. Kalıtsal Dislipdemi Fenotipleri ve Genetik İlişkiler Üzerine (Derleme). Deneysel Tıp Araştırma Enstitüsü Dergisi. 2011;1(2):14–9.

8. Goldstein JL, Brown MS. History of Discovery: The LDL Receptor. Arterioscler Thromb Vasc Biol. 2009 Apr;29(4):431–8.

9. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem. 1983;52:223–61.

10. Parmaksız İ. Advanced Glycation End-Products in Complications of Diabetes Mellitus. MMJ. 2011;24(3):141–8.

11. Kurban S, Mehmetoğlu İ. Okside Düşük Dansiteli Lipoprotein Otoantikorları ve Klinik Önemi. Turkiye Klinikleri J Med Sci. 2005;25(1):73–84.

12. Binder CJ, Shaw PX, Chang M-K, Boullier A, Hartvigsen K, Hörkkö S, et al. Thematic review series: The Immune System and Atherogenesis. The role of natural antibodies in atherogenesis. J Lipid Res. 2005 Jul 1;46(7):1353–63.

13. Hansson GK, Libby P, Schönbeck U, Yan Z-Q. Innate and Adaptive Immunity in the Pathogenesis of Atherosclerosis. Circulation Research. 2002 Aug 23;91(4):281–91.

14. Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. Clinical Immunology. 2010 Ocak;134(1):33–46.

15. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998 Sep 12;352(9131):837–53.

16. Lathief S, Inzucchi SE. Approach to diabetes management in patients with CVD. Trends in Cardiovascular Medicine [Internet]. [cited 2015 Nov 13];0(0). Available from: http://www.tcmonline.org/article/S1050173815001504/abstract

17. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes. New England Journal of Medicine. 2008 Oct 9;359(15):1577–89.

18. Long-Term Effects of Intensive Glucose Lowering on Cardiovascular Outcomes. New England Journal of Medicine. 2011 Mar 3;364(9):818–28.

19. Palinski W, Rosenfeld ME, Ylä-Herttuala S, Gurtner GC, Socher SS, Butler SW, et al. Low density lipoprotein undergoes oxidative modification in vivo. Proc Natl Acad Sci U S A. 1989 Feb;86(4):1372–6.

20. Virella G, Lopes-Virella MF. Lipoprotein autoantibodies: measurement and significance. Clin Diagn Lab Immunol. 2003 Jul;10(4):499–505.

21. Li W, Febbraio M, Reddy SP, Yu D-Y, Yamamoto M, Silverstein RL. CD36 participates in a signaling pathway that regulates ROS formation in murine VSMCs. J Clin Invest. 2010 Nov;120(11):3996–4006.

22. Lopes-Virella MF, Baker NL, Hunt KJ, Lyons TJ, Jenkins AJ, Virella G, et al. High concentrations of AGE-LDL and oxidized LDL in circulating immune complexes are associated with progression of retinopathy in type 1 diabetes. Diabetes Care. 2012 Jun;35(6):1333–40.

23. Lopes-Virella MF, Hunt KJ, Baker NL, Moritz T, Virella G. The Levels of MDA - LDL in Circulating Immune Complexes predict Myocardial Infarction in the VADT study. Atherosclerosis. 2012 Oct;224(2):526–31.

24. Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, et al. Essentials of Glycobiology. 2009 [cited 2015 Nov 17]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK1908/

25. Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. Proc Natl Acad Sci U S A. 1993 Jul 15;90(14):6434–8.

26. Saad AF, Virella G, Chassereau C, Boackle RJ, Lopes-Virella MF. OxLDL immune complexes activate complement and induce cytokine production by MonoMac 6 cells and human macrophages. J Lipid Res. 2006 Sep;47(9):1975–83.

27. Erciyas F, Taneli F, Arslan B, Uslu Y. Glycemic control, oxidative stress, and lipid profile in children with type 1 diabetes mellitus. Arch Med Res. 2004 Apr;35(2):134–40.