Acta Biomed 2021; Vol. 92, N. 3: e2021100 DOI: 10.23750/abm.v92i3.9505

Original article

The Association between Glycemic Control with Oxidant Status Parameters in Type 2 Diabetic Patients

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Abstract. *Purpose:* Glycemic control is important in order to avoid LDLs increased susceptibility to oxidation in diabetic patients. This study assess the relationship between diabetes control with serum prooxidant-antioxidant balance (PAB), oxidized LDL cholesterol (oxLDLc), homocysteine and vitamin D levels in patients with type 2 diabetes. *Material and methods:* This was a cross-sectional study on three groups including 80 subjects as well (WGC) and poor (PGC) glycemic control and 40 healthy subjects. Presence of nephropathy and retinopathy were determined using IDF criteria. HbA_1c level was determined with columnar chromatography using BioSystems kit. Serum PAB, homocysteine, oxLDLc and vitamin D levels were measured by the standard tests. *Results:* There was a significant association between PAB with PGC (P< 0.001), diabetic retinopathy (P< 0.01) and nephropathy (P< 0.01) in type 2 diabetic patients. Moreover, the results showed that vitamin D serum levels was significantly lower in PGC patients (P< 0.01), and diabetic patients with retinopathy (P< 0.01). Multiple linear regression analysis revealed that the vitamin D deficiency can predict the HbA1c variations by 77.7% (β= - 0.775) in subjects with type 2 diabetes mellitus (P<0.001). *Conclusions:* There was a significant association between prooxidant-antioxidant balance and vitamin D serum levels with diabetic complications. (www.actabiomedica.it)

Keyword: Type 2 diabetes mellitus; Prooxidant-antioxidant balance; Homocysteine; Oxidized LDL; Vitamin D

Introduction

Diabetes mellitus can be considered as one of the serious and costly multifactorial diseases with micro and macrovascular complications (1). According to the International Diabetes Federation (IDF), 285 million people worldwide are currently living with diabetes and 90-95% have type 2 diabetes (2). This number is expected to reach over 300 million by the year 2025 (3).

It was shown increased risk of cardiovascular disease (CVD) can explain the mortality in type 2 diabetes. Lipid peroxidation is high in type 2 diabetic patients and could be due to hyperglycemia, hyperhomocysteinemia and probably decreased antioxidant enzymes activity (4). It has been shown that hyperglycaemia has an important role in LDL susceptibility to oxidation (5). Elevated levels of oxidized LDL cholesterol (oxLDLc), which consider as a biochemical risk marker for CVD, have found in diabetic patients (6). Disruption of the prooxidant-antioxidant balance (PAB) can cause oxidative stress (7) which is well-documented in groups of patients with type 2 diabetes (8). Many studies reported conflicting results regarding the LDL cholesterol oxidation (9,10) and homocysteine level (11,12) in patients with type 2 diabetes. Epidemiological evidence supports the link between hypovitaminosis D and increased risk of mortality due to diabetes (13). It has been suggested that vitamin D exerts its anti-oxidant features by activating the MEKs/ERKs/SirT-1 axis (14).

Diabetes microvascular complications including retinopathy and nephropathy were associated with higher levels of HbA1c (15). The major cause of type 2 diabetes mellitus complications has not yet been clarified. Understanding the role of various nutritional or other modifiable risk factors that may contribute to the pathogenesis of diabetes, is important in the effort to combat the rising tide of diabetes and CVD worldwide (16). This study was conducted to assess the relationship between diabetes control, serum PAB, oxidized LDL cholesterol, homocysteine and vitamin D levels and microvascular diabetic complications in patients with type 2 diabetes mellitus in Iran.

Material and Methods

Study population

This cross-sectional study was approved by Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran. One hundred and twenty volunteers were included (80 with and 40 without type 2 diabetes). Questionnaires containing past medical history, social and family history were obtained and written informed consent was obtained from all individuals.

Venous blood samples (8-10 ml of whole blood) were obtained after a 10-h overnight fasting. EDTA was used as anticoagulant. HbA_1c was determined in whole blood samples with columnar chromatography using BioSystems kit (Barcelona, Spain). Volunteers were divided into three age- and sex- matched groups including 40 subjects as well glycemic control: (HbA1c < 8%), 40 subjects as poor glycemic control, (HbA1c >10%) and 40 healthy subjects.

Biochemical measurements

The whole blood samples were centrifuged at 3000 rpm for 10 min, and the serum aliquots were separated and stored at -20°C for the subsequent measurement of PAB, homocysteine, oxidized LDL cholesterol and vitamin D levels.

The PAB assay is based on 3,3',5,5'- tetramethylbenzidine (TMB) and its cation (17), and measure the balance of oxidants and antioxidants simultaneously in one experiment. It uses two different kinds of reactions: one is an enzymatic reaction where the chromogen TMB is oxidized to a color cation by peroxides and the second is a chemical reaction where the TMB cation is reduced to a colorless compound by antioxidants. The photometric absorbance is then compared with the absorbances given by a series of standard solutions (17). The standard solutions were prepared by mixing varying proportions (0–100%) of 250 mM hydrogen peroxide with 3mM uric acid (in 10mM NaOH). A low PAB value means that antioxidants are present at greater concentration than oxidants, while a high PAB value means more oxidants are present than antioxidants (18).

Serum homocysteine level were measured using immunoturbidometry based on assays to co-substrate conversion product (Demeditec Diagnostic GmbH, Lise-Meitner-strabe 2, D-24145 Kiel Germany). Serum homocysteine level was determined with an intra-assay precision CV of 5.08%.

Oxidized LDL cholesterol levels were measured using a specific immunometric assay for human ox-LDLc using an enzyme-linked immunosorbent assay kit (Mercodia Oxidized LDL ELIZA; Mercodia AB,

Uppsala, Sweden) and results were multiplied by dilution factor (6561) for this factor. Intra-assay precision CV was 6.2% for measuring serum oxidized LDL cholesterol levels.

Serum 25- hydroxy vitamin D (25OHD) levels were measured using a competitive electroluminescence protein binding assay (Roche Diagnostics vitamin D total assay kit; Roche Diagnostics, Mannheim, Germany) on a Cobas e411 analyzer. Serum 25OHD was measured with an intra-assay precision CV of 3.07%. Vitamin D insufficiency and deficiency were defined as a serum 25OHD of 50-75 nmol/L and <50 nmol/L, respectively (19). Presence of nephropathy and retinopathy were determined using the IDF criteria (20).

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS version 11.5). The normality of distribution was assessed by the Kolmogorov Smirnov test. Categorical variables were compared using Chi-square test. Quantitative data were expressed as the mean ± SD for normally distributed variables or the median and IQR for not normally distributed variables. Analysis of normally distributed variables is included independent-samples T tests, One-Way ANOWA tests. Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance used for not normal distribute variables. In order to assess the independent effects of vitamin D, homocysteine, PAB, oxidized LDL cholesterol, smoking, sex and age on measures of HbA1c, multiple regression analyses was performed separately in each group. A P-value <0.05 is considered as level of statistical significance for all tests.

Results

Basic characteristics

There were three groups in this study including 40 subjects as well glycemic control, (HbA1c <8%, comprised 11 male and 29 female, aged 50.27±8.00 years), 40 subjects as poor glycemic control, (HbA1c >10%, included 15 male and 25 female, aged 48.5±11.00 years) and 40 healthy subjects comprised 11 male and 29 female (aged 46.02±7.71years). There was no significant difference in gender distribution between the groups (P= 0.535). Demographic and some clinical characteristics in each group of study are shown in Table 1. Data indicate significant differences in clinical factors such as cardiovascular disease, retinopathy and nephropathy between three groups. Poor control diabetic patients had a significantly higher CVD, diabetic retinopathy and nephropathy (P <0.001, for all), (Table 1).

Biochemical factors

Level of biochemical factors in the three groups of the study are shown in Table 1. Poor control diabetic patients had a significantly lower vitamin D concentration and higher PAB, homocysteine and oxidized LDL cholesterol levels (P <0.001, for all), (Table 1). There was a significant difference in frequency of vitamin D deficiency between the study groups. The frequency of vitamin D deficiency was 100% and 95% in subjects with poor glycemic control and healthy subjects, respectively. The frequency of vitamin D insufficiency was 100% in well control glyceamic subjects (Table 1).

Prooxidants antioxidants levels

There was a significant higher PAB in poor glycemic control than well glycemic control (p <0.001). Also, positive retinopathy (p <0.01), and nephropathy (p <0.01) diabetic patients had higher serum PAB levels compared to without retinopathy and nephropathy subjects, (Table 2).

Homocysteine levels

Average homocysteine level in poor glycemic control patients was higher than other groups, (p <0.001), (Table 1). As shown in Table 2, poor glycemic control patients had a significantly higher serum homocysteine concentration than well glycemic control subjects, (p <0.01). Positive nephropathy patients had a higher serum homocysteine compared to without nephropathy diabetic subjects (p <0.05).

Table 1. Demographic and some clinical characteristics in each group of study

		Diabetic patients			
Variable		**Poor Control (N=40)**	**Well Control (N=40)**	**Control (N=40)**	**P-Value**
Age (yr)		48.5±11.00	50.27±8.00	46.02±7.71	0.111
Sex, %	**Male**	37.5	27.5	27.5	0.535
	Female	62.5	72.5	72.5	
HbA1C %		11.19 (10.45-12.80)	6.90 (6.42-7.57)	5.28 (4.91- 5.72)	<0.001*
Smoking %		32.5	12.5	2.5	<0.01
CVD %		57.5	17.5	0.0	<0.001
DR%		85	15	0.0	<0.001
DN %		27.5	5	0.0	<0.001
PAB (HK arbitrary unit)		90.69±33.21	57.12±36.10	52.32±8.86	<0.001
Homocysteine (µmol/l)		27.90±4.02	24.07±10.44	18.70±3.18	<0.001
Oxidized LDL cholesterol (U/L)		50.60±14.67	40.84±18.24	28.23±10.70	<0.001
Vitamin D (nmol/L)		19.57 (14.25-26.84)	58.00 (56.79-59.73)	20.28 (17.05-23.92)	<0.001*
Vitamin D status	**Vitamin D deficiency (%)**	40 (100%)	0	38(95%)	<0.001
	Vitamin D insufficiency (%)	0	40 (100%)	0	
	Normal Vitamin D (%)	0	0	2 (5%)	

CVD: Cardiovascular disease, DR: Diabetic retinopathy, DN: diabetic nephropathy, PAB: prooxidant-antioxidant balance; Oxidized LDL cholesterol: oxidized low density lipoprotein cholesterol. P-value determined by One-Way ANOVA test for continuous variable and Pearson Chi-Square for categorical variables.*P-value determined by Kruskal-Wallis test.

Oxidized LDL cholesterol levels

Average oxidized LDL cholesterol was higher in poor control subjects compared to well control group (p <0.05), (Table 2). As shown in Table 2, there was no difference in serum oxidized LDL cholesterol level between subjects with and without retinopathy, or nephropathy (p = 0.134 and p = 0.407, respectively).

Vitamin D

There was a significant difference in serum vitamin D levels between well glycemic control diabetic patients, poor glycemic control diabetic patients and control group (p <0.001), (Table 1). Serum vitamin D levels in well glycemic control patients were higher than poor glycemic control ones; this rate was 58.00 (56.79-59.73) and 19.57 (14.25-26.84) nmol/L, respectively (p <0.001). There was a significantly lower in serum vitamin D levels in positive retinopathy diabetic patients than no-retinopathy diabetic patients (p <0.001), (Table 2).

Multiple regression analyses results

According to Table 3, efficient variables can explain the variation of HbA1c 73.8%, in diabetic patients (Adjuster R square = 0.738 ± 1.39, p = 0.0001). Multiple linear regression analysis showed that the serum vitamin D can predict the HbA1c variations by 77.7% (β = - 0.775) in type 2 diabetes (p <0.001), (Table 3).

PAB: prooxidant-antioxidant balance; Oxidized LDL cholesterol: oxidized low density lipoprotein cholesterol. **In healthy subjects:** Adjuster R square = -0.017 ± 0.758, p = 0.511, SE = Standard Error. **In diabetic subjects:** Adjuster R square = 0.738 ± 1.39, p = 0.0001, SE = Standard Error.

Discussion

In current study, some oxidant status parameters including serum pro-oxidant-antioxidant balance, oxidized LDL cholesterol, homocysteine and vitamin D levels determined in well and poor control diabetic

Table 2. PAB, homocysteine, oxidized LDL cholesterol and vitamin D association with diabetic complications

Variables\Groups	Well Control (N=40)	Poor Control (N=40)	P-Value	No-Retinopathy	Retinopathy	P-Value	No-Nephropathy	Nephropathy	P-Value
PAB (HK arbitrary unit)	57.12±36.10	90.69±33.21	< 0.001	62.75±38.78	85.77±34.68	< 0.01	69.40±37.05	98.98±33.28	< 0.01
Homocysteine (µmol/l)	24.07±10.44	27.90±4.02	< 0.01	24.53±10.47	27.44±4.34	0.112	25.37±8.79	28.63±2.88	< 0.05
Oxidized LDL cholesterol (U/l)	40.84±18.24	50.60±14.67	< 0.05	48.65±15.86	42.84±18.08	0.134	44.73±15.96	49.10±22.67	0.407
Vitamin D (nmol/l)	58.00 (56.79-59.73)	19.57 (14.25-26.84)	< 0.001[b]	26.39 (19.42-57.83)	22.85 (15.06-33.34)	< 0.001[b]	24.54 (18.36-57.22)	29.34 (17.39-54.38)	0.098[b]

PAB: prooxidant-antioxidant balance; Oxidized LDL cholesterol: oxidized low density lipoprotein cholesterol. P-value determined by Independent-samples T test. [b]P-value determined by Mann-Whitney U test.

patients and healthy subjects. Our results are largely confirmatory in a small number of patients. The most important finding of this study was the fact that the poor control diabetic patients had significantly higher serum PAB, homocysteine and oxidized LDL cholesterol levels compared to the well control diabetic patients. Additionally, the serum level of vitamin D was lower in poor control diabetic patients than the well control diabetic patients.

Another interesting finding was the high frequency of vitamin D deficiency in our subjects that has not been widely reported. The major reasons for low vitamin D levels have not been known in the Iranian population. Clothing due to cultural issues can decrease sun exposure in Iran (21). Daneshvar et al. have reported that the intake of vitamin D is lower than recommended dietary allowances in adults in Isfahan (22). Bonakdaran et al. have suggested that sedentary life style and sunscreen use are other reasons for high frequency of vitamin D deficiency in Iranian population. They have shown that the frequency of vitamin D deficiency was 80.7% in patients with metabolic syndrome in Iranian population (23).

In current study, there was a significant lower in serum vitamin D levels in positive retinopathy diabetic patients compared to no-retinopathy ones. Additionally, multiple linear regression analysis showed that the vitamin D deficiency can predict the HbA1c variations by 77.7%. A number of studies have reported that vitamin D supplementation has no significant effects on HbA1c level in type 2 diabetic patients (24, 25). Calvo-Romero et al. have found a small non-significant reduction in HbA1c in patients with type 2 diabetes mellitus after supplementation with vitamin D at least for 8 week (26). While, Ahmadieh et al. have reported hypovitaminosis D is an independent predictor of HbA1c, diabetic neuropathy and retinopathy in patients with type 2 diabetes (27). It seems that low vitamin D receptor signaling is the potential mechanism by which vitamin D deficiency can mediate risk of cardiovascular disease in type 2 diabetic patients (28). One study showed serum vitamin D level were lower in subjects with diabetic retinopathy than subjects without diabetic retinopathy (29). This result is in concordance with our finding.

Table 3: Effect of efficient variables on HbA1C using linear regression

Variables	Regression Coefficient (B) ± SE	Coefficient (β	P-value	95% C.I.for	
Healthy subjects					
PAB (HK arbitrary unit)	-0.020±0.016	-0.221	0.224	-0.054	0.013
Homocysteine (μmol/l)	-0.046±0.060	-0.184	0.448	-0.167	0.076
Oxidized LDL cholesterol (U/l)	0.014±0.013	0.203	0.285	-0.012	0.041
Vitamin D (nmol/l)	-0.002±0.007	-0.062	0.742	-0.016	0.011
Smoking %	0.013±0.050	0.058	0.797	-0.089	0.115
Sex %	0.492±0.429	0.295	0.260	-0.385	1.370
Age (yr)	-0.010±0.023	-0.094	0.657	-0.056	0.036
Diabetic subjects					
PAB (HK arbitrary unit)	0.012±0.005	0.175	0.01	0.003	0.022
Homocysteine (μmol/l)	-0.010±0.020	-0.031	0.620	-0.051	0.030
Oxidized LDL cholesterol (U/l)	0.002±0.010	0.014	0.821	-0.017	0.022
Vitamin D (nmol/l)	-0.105±0.010	-0.777	0.0001	-0.124	-0.086
Smoking %	0.517±0.399	0.081	0.199	-0.278	1.313
Sex %	0.074±0.356	0.013	0.836	-0.636	0.783
Age (yr)	0.023±0.017	0.082	0.184	-0.011	0.058

In current study, average PAB and oxidized LDL cholesterol levels were higher in poor control subjects compared to those well glycemic control diabetic patients. A high PAB value means more oxidants are present than antioxidants (18). Also, positive retinopathy and nephropathy diabetic patients had higher serum PAB levels compared to without retinopathy and nephropathy subjects. It seems that change in PAB levels is thought to be the cause of chronic diabetic complications, consistent with the literature. Kumawat et al. have reported that alteration in anti-oxidant status can help to predict the risk of diabetic retinopathy (30). Our results is consistent with a previous study shown that glycemic control is very important in order to avoid an increased susceptibility of LDLs to oxidation in diabetic patients (31). It is well documented that oxidized LDL cholesterol can activate the pathways associated with innate immunity and trigger pro-inflammatory events (32). Alkalin et al. showed that oxidized LDL cholesterol has been increased in diabetic patients and this may contribute to the increased atherogenesis in diabetes (33). Therefore, the outcome of our study shows the supportive role of well glycemic control decreasing the level of oxidant status parameters in type 2 diabetic patients.

Timar et al., have reported that high dose vitamin D supplementation (50000IU) once weekly during 9 weeks can increase the serum PAB levels in both groups of adolescents girls (aged 12-18) years with BMI <25 and ≥25 (kg/m^2). This result may be because of the high dose, or long-term of vitamin D in this study (34). Therefore, whether mega dose is better than low dose is controversial among scholars yet.

Study limitations

Present study was a cross-sectional analysis which had some limitations. Firstly, it lacks baseline data on seasonal changes, sun exposure and thus vitamin D level. Secondly, we don't have enough information about drug use, vitamin D injection and nutritional status of volunteers.

Conclusions

This study indicates that there was a significant association between glycemic control with prooxidant-antioxidant balance and vitamin D serum levels.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Received: 30 March 202
Accepted: 9 April 2020
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