CLINICAL STUDY

Effect of vitamin E supplementation on microalbuminuria, lipid peroxidation and blood prostaglandins in diabetic patients

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Abstract

Background: **Oxidative stress is an important pathogenic factor in the development of diabetic vascular complications.**
Aims: **To study the effect of vitamin E supplementation on microalbuminuria, plasma levels of malondialdehyde (MLD) and metabolites of prostaglandins TXA_2 (TXB_2) and PGI_2 (6-keto-$PGF_1\alpha$) and to evaluate the relation between plasma MLD and thromboxane B2 (TXB_2) in diabetic patients.**
Patients and methods: **Diabetic microalbuminuric patients were supplemented with vitamin E 1200 IU daily (EVIT, Rodisma, Germany) and measurements of microalbuminuria, MLD, TXB_2 and 6-keto-$PGF_1\alpha$ were repeated after 4 months of treatment.**
Results: **Vitamin E supplementation lowered microalbuminuria (93.8±45.6 vs 67.95±28.4 µg/min, $p<0.05$), MLD (0.55±0.26 vs 0.32±0.16 µmol/l, $p<0.001$) and also TXB_2 level (115.14±22.7 vs 15.32±14.7 ng/l, $p<0.001$) in diabetic microalbuminuric patients. The changes of 6-keto-$PGF_1\alpha$ after treatment were not significant.**
Conclusions: **Our results did not show any significant relationship between levels of MLD and TXB_2. Vitamin E supplementation significantly lowered microalbuminuria, MLD and TXB_2.** ***(Tab. 2, Fig. 2, Ref. 35.)***
Key words: diabetes mellitus, vitamin E, malondialdehyde, prostaglandins

Diabetes mellitus alters cellular production of eicosanoids in a number of tissues (1) and these agents have been implicated in the pathogenesis of vascular complications in diabetic patients. Thromboxane A_2 (TXA_2) is a biologically active derivate of arachidonic acid and has potent vasoconstrictive and platelet-activation functions. Some studies have found elevated blood levels of TXA_2 in diabetic patients in comparison with age-matched healthy control subjects (2) and increased TXA_2/prostacyclin I_2 (PGI_2) ratio in patients with diabetes (3). The results of these studies suggest that TXA_2/PGI_2 ratio reflects the pathological conditions of diabetes, especially the changes of vasculature and the monitoring and improvement of this ratio could be useful for the prevention of diabetic vascular complications.

The mechanism of the effects of diabetes in increasing the levels of vasoconstrictors, such as TXA_2, while decreasing levels of vasodilatators, such as PGI_2, has still not been fully elucidated. Our view is that these effects might be mediated by increased levels of free radicals. Many studies have documented, that hyperglycemia can cause increased oxidative stress, accumulation of lipid peroxidation products, such as malondialdehyde in the blood of diabetic patients (4, 5, 6). Elevated oxidative stress subsequently increases phospholipase A_2 activity and stimulates the release of arachidonic acid, which induces further generation of TXA_2.

Vitamin E is a potent antioxidant. In clinical studies vitamin E supplementation was effective in preventing diabetic vascular complications, such as retinopathy and nephropathy (7). Vitamin E treatment in experimental studies decreased oxidative stress and also corrected the lowering of the PGI_2/TXA_2 ratio found in diabetic rats (8).

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Tab. 1. Group characteristics of patients.

	Group A n=19	Group B n=10	Group C n=10
Age (y)	55.2±7.6	54.4±6.1	53.6±9.4
Diabetes duration (y)	13.4±9.3	14.2±10.6	0
Treatment of DM (D/PAD/I)	19/12/12	10/6/6	-
HbA_1c (%)	8.4±1.7	8.2±1.0	5.7±0.4[a,b]
Albuminuria (µg/min)	93.9±45.6	14.8±7.8[c]	12.4±3.4[a]
Total cholesterol (mmol/l)	5.16±1.04	5.27±1.02	5.24±0.9
Triglycerides (mmol/l)	1.55±0.9	1.59±1.0	1.49±0.88

All results are expressed as mean±SD. Group A = diabetics with microalbuminuria, group B = diabetics with normoalbuminuria, group C = healthy subjects, D = diet, PAD = oral antidiabetic drugs, I = insulin
[a]p<0.001 group C vs group A
[b]p<0.001 group C vs group B
[c]p<0.001 group B vs group A

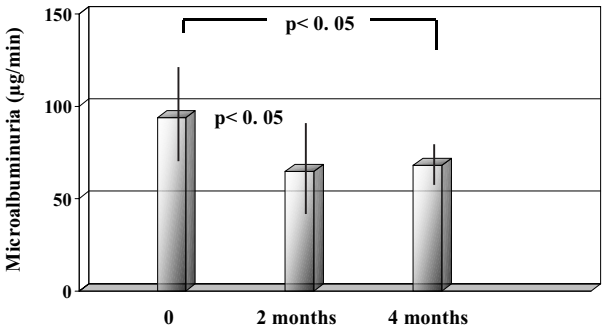

Fig. 1. Changes of microalbuminuria after vitamin E supplementation in group A. Results are expressed as mean±SD.

The changes in microalbuminuria in diabetic patients reflect not only changes in the severity of renal disease (9, 10), but microalbuminuria also independently predicts total and cardiovascular mortality in patients with both Type-1 and Type-2 diabetes mellitus (11), and is associated with several biochemical risk factors for atherosclerosis such as atherogenic lipid profiles and hyperinsulinemia (12, 13).

In this study, our aim was to evaluate the effect of vitamin E supplementation on microalbuminuria and the production of eicosanoids in diabetic patients and to reveal the possible mechanism of this effect.

Materials and methods

We studied 19 diabetic microalbuminuric patients – group A (aged 55.2±7.6 years, diabetes duration 13.4±9.3 years, HbA_1c 8.4±1.7 %, 4 patients with type 1, 15 patients with type 2 diabetes), 10 diabetic normoalbuminuric patients – group B (aged 54.4±6.1 years, diabetes duration 14.2±10.6 years, HbA_1c 8.2±1.0 %, 2 patients with type 1, 8 patients with type 2 diabetes) and 10 healthy controls-group C (aged 53.6±9.4 years). In group A the microalbuminuria (20–200 µg/min) was confirmed in two samples of 12 hour urine and the mean value was used for analysis. The baseline characteristics of patients are summarized in Table 1. There were no significant differences in age comparing groups A, B and C and in glycemic control between group A and B. Diabetic patients in group A and B were treated with diet, oral hypoglycemic agents or insulin. The patients were not treated with acidum aetylosalicylicum or nonsteroidal antiinflammatory drugs, the treatment with angiotensin-converting-enzyme inhibitors was not started 3 months before beginning or during the study. The study was approved by the local ethical committee and written informed consent was obtained from all patients in accordance with the protocol. Microalbuminuria, plasma total cholesterol, triglycerides, plasma malondialdehyde (MLD) level, the metabolite of serum thromboxane A_2 – thromboxane B_2 (TXB_2) and the metabolite of plasma PGI_2 – 6-keto-$PGF_1\alpha$ were measured in groups A, B and C.

Methodology of HbA_1c measurement

Determination of HbA_1c, one of glycated hemoglobins, is considered a useful index of long-term glycemic control. Venous blood anticoagulated by EDTA was used. HbA_1c and total Hb were determined from hemolysate prepared on board of the COBAS chemistry system from whole blood. HbA_1c was measured from the hemolysate by a latex enhanced turbidimetric immunoassay, total Hb was measured by colorimetric cyanide free alkaline hematin method. The final HbA_1c test result was determined from HbA_1c/Hb ratio, including a conversion formula to match a HPLC reference method (Roche Diagnostic GmbH, Mannheim, Germany).

Methodology of microalbuminuria measurement

A portion of carefully timed (12 hour collection period from 6:00 p.m. untill 6:00 a.m., patients had avoided any physical activity) well-mixed sample of urine was used. Samples were stored at ±2 to ±8 degrees of Celsius prior to testing. Analysis was made the same day after urine collection by immunoturbidimetric assay for urinary albumin (Microalbumin, Randox Laboratories Ltd., Crumlin, United Kingdom) on autoanalyser (COBAS Mira S, Roche Diagnostics GmbH, Mannheim, Germany).

Methodology of malondialdehyde measurement

Serum malondialdehyde was measured with high-performance liquid chromatography after reaction with thiobarbituric acid.

Methodology of serum TXB_2 measurement

Since TXA_2 is rapidly converted to TXB_2, a chemically stable but biologically inactive hydration product, thromboxane synthesis in biological tissues has been monitored by measuring TXB_2. TXB_2 is produced ex-vivo in blood after clotting. Serum TXB_2 thus produced can be monitored as an index of platelet cyclo-oxygenase activity.

Tab. 2. Comparison of malondialdehyde. thromboxane B_2 and 6-keto-$PGF_1\alpha$ in diabetics with microalbuminuria (group A), diabetics with normoalbuminuria (group B) and healthy subjects (group C).

	Group A n=19	Group B n=10	Group C n=10
Malondialdehyde (μmol/l)	0.55±0.26	0.25±0.06[a]	0.22±0.02[a]
Thromboxane B_2 (ng/l)	115.4±22.7	22.33±25.71[a]	21.91±32.89[a]
6-keto-$PGF_1\alpha$ (pg/ml)	85.12±40.49	80.20±30.54	86.14±29.40
TxB_2/6-keto-$PGF_1\alpha$	1.47±0.98	0.36±0.28[b]	0.35±0.27[b]

All results are expressed as mean±SD. Group A = diabetics with microalbuminuria, group B = diabetics with normoalbuminuria, group C = healthy subjects, TxB_2 = thromboxane B_2.
[a]$p<0.001$ group B vs group A and group C vs group A
[b]$p<0.01$ group B vs group A and group C vs group A

Blood samples were collected in pre-chilled plastic tubes, centrifugated after clotting and stored at -30 degrees of Celsius prior to testing.

The TXB_2/2,2-dinor-TXB_2 (^{125}I) assay system (TXB_2/2,3-dinor-TXB_2 (^{125}I) RIA kit, IZOTOP, Institute of Isotopes Co.Ltd, Budapest, Hungary) enables quantitative determination of TXB_2 in serum, based on the competition between unlabelled TXB_2 and a fixed quantity of ^{125}I-labeled TXB_2 for a limited number of binding sites on TXB_2 specific antibody, was used for the determination of prostanoids in serum. Radioactivity was counted by gama-coulter JNG (Spišská Nová Ves, Slovakia). Levels of TXB_2 in the unknown samples were determined by interpolation from the standard curve.

Methodology of plasma 6-keto-$PGF_1\alpha$ measurement

PGI_2 is rapidly converted in aqueous medium into 6-keto-$PGF_1\alpha$, a chemically stable but biologically inactive hydration product. In view of the short half-life of the active metabolites, prostacyclin synthesis in biological tissues can only be monitored by measuring 6-keto-$PGF_1\alpha$, the primary breakdown product.

Blood samples were collected in pre-chilled plastic tubes containing anticoagulant (EDTA) and plasma was stored at -20 degrees Celsius.

For the extraction of 6-keto-$PGF_1\alpha$ from plasma, solid-phase extraction using Amprep TM (Amersham International, USA) minicolumns were applied according to the procedure described in the assay kit (see below).

The 6-keto-$PGF_1\alpha$/2,3-dinor-6-keto-$PGF_1\alpha$ (^{125}I) assay system (6-keto-$PGF_1\alpha$/2,3-dinor-6-keto-$PGF_1\alpha$ (^{125}I) RIA kit, IZOTOP, Institute of Isotopes Co. Ltd, Budapest, Hungary) enables the quantitative determination of 6-keto-$PGF_1\alpha$ in plasma, based on the competition between unlabelled 6-keto-$PGF_1\alpha$ and a fixed quantity of ^{125}I-labeled 6-keto-$PGF_1\alpha$ for a limited number of binding sites on 6-keto-$PGF_1\alpha$ specific antibody, was used for the determination of prostanoids in plasma. Radioactivity was counted by gama-coulter JNG (Spišská Nová Ves, Slovakia). Levels of 6-keto-$PGG_1\alpha$ in the unknown samples were determined by interpolation from the standard curve.

After biochemical analysis the patients from group A were supplemented with natural vitamin E 1200 IU daily (EVIT, Rodisma, Germany) for 4 months. Measurements of microalbuminuria, total cholesterol, triglycerides, HbA_1c, MLD, TXB2 and 6-keto-$PGF_1\alpha$ were repeated after 2 and 4 months of treatment.

Statistical analysis: Results were expressed as mean±SD. Our three groups of patients were compared using the Mann-Whitney U test. The Wilcoxon rank sum test was performed to assess changes of microalbuminuria, total cholesterol, triglycerides, HbA_1c, MLD, TXB_2 and 6-keto-$PGF_1\alpha$ after vitamin E supplementation in group A. Pearson correlation coefficient was performed to examine the correlation between the MLD and TXB_2 levels.

Results

There was no significant difference between group A and B in glycemic control measured with HbA_1c levels (8.4±1.7 vs 8.2±1.0 %, p=NS), and duration of diabetes (13.4±9.3 vs 14.2±10.6 years, p=NS). The distribution of age between groups A, B and C was also not significant (55.2±7.6 vs 54.4±6.1 vs 53.6±9.4 years, p=NS). The level of MLD – a lipid peroxidation product, was elevated in group A compared with B group ($p<0.001$) and C group ($p<0.001$). The level of TXB_2 was increased in group A compared with group B ($p<0.001$) and group C ($p<0.001$). The ratio TXB_2/6-keto-$PGF_1\alpha$ was also higher in group A compared with group B ($p<0.01$) and group C ($p<0.01$) (Tab. 2). TXB_2 level had no significant correlation with MLD ($r=0.21$, $p=0.46$). Vitamin E supplementation lowered microalbuminuria (93.8±45.6 vs 67.95±28.4 μg/min, $p<0.05$) (Fig. 1), MLD (0.55±0.26 vs 0.32±0.16 μmol/l, $p<0.001$) and TXB_2 level (115.14±22.7 vs 15.32±14.7 ng/l, $p<0.001$) (Fig. 2) in diabetic microalbuminuric patients. The changes of 6-keto-$PGF_1\alpha$ were not significant (85.12±40.49 vs 80.16±40.25 pg/ml, p=NS). Treatment with vitamin E positively influenced the TXB_2/6-keto-$PGF_1\alpha$ ratio (1.47±0.98 vs 0.36±0.24, $p<0.01$).

There was no significant difference in the baseline levels of total cholesterol and triglycerides between our groups. However,

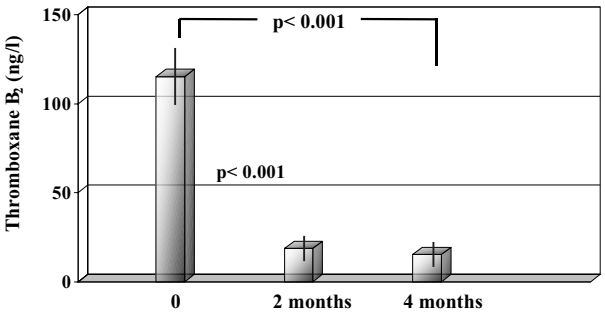

Fig. 2. Changes of thromboxane B_2 after vitamin E supplementation in diabetics with microalbuminuria. Results are expressed as mean±SD.

we found a tendency for total cholesterol level to increase after vitamin E supplementation compared with respective baseline values (5.16±1.04 vs 5.57±1.02 mmol/l, p=NS). Triglycerides did not differ compared with respective baseline values (1.55±0.9 vs 1.50±0.8 mmol/l, p=NS) after treatment. There were no significant changes in HbA_1c values after treatment (8.4±1.7 vs 8.39±1.39 %, p=NS). Systolic blood pressure control was not influenced after treatment (143.47±16.3 vs 143.26±15.3 mmHg, p=NS) and diastolic blood pressure increased (82.11±9.3 vs 87.84±8.6 mmHg, p<0.05).

Discussion

Vascular diseases in diabetes are characterized by impaired homeostasis of vasoactive substances – raised levels of vasoconstrictor factors (endothelin, thromboxane) and reduction of vasodilatating factors (prostacyclin, EDRF — Endothelin Derived Relaxing Factor). TXA_2 is produced by the sequential enzymatic metabolism of arachidonic acid through the cyclooxygenase or prostaglandin H (PGH) synthase pathway (14). Arachidonic acid is released from phospholipids in the cell membrane by the actions of lipases, including phospholipases A and C. Free arachidonic acid is sequentially converted to prostaglandin (PG)G_2 and then PGH_2 by the cyclooxygenase and peroxidase actions of cyclooxygenase (PGH synthase) enzymes 1 and 2 (15). PGH_2 is converted to active TXA_2 by the action of thromboxane synthase. TXA_2 has a number of biological effects; it stimulates vascular smooth muscle contraction and promotes platelet aggregation, resulting in potent hemodynamic and procoagulant effects that are thought to play a role in cardiovascular disease (16). Changes in eicosanoid metabolism, with increased production of TXA_2 have also been implicated in the pathogenesis of microvascular complications of diabetes, diabetic nephropathy (17, 18, 19) being an example. In an experimental study urinary excretion of TXB_2 by diabetic rats was higher than that of healthy controls and the results of this study suggest that altered renal production of TXA_2 and PGI_2 is involved in the pathogenesis of diabetic nephropathy in rats with type 2 diabetes (20). Also clinical studies have shown elevated blood levels of TXB_2 in diabetic patients (2) and increased TXA_2/PGI_2 ratio in patients with microvascular complications of diabetes (3). In agreement with these studies, we found elevated levels of serum TXB_2 in diabetic microalbuminuric patients compared with diabetic normoalbuminuric patients and healthy subjects in our study. The ratio TXB_2/PGI_2 was also increased in patients with microalbuminuria (group A) compared with the other two groups of patients (B and C group).

The mechanism accounting for impaired eicosanoid metabolism in diabetes has still not been fully elucidated. Recent studies have documented that hyperglycemia can cause generation of oxygen radicals and accumulation of lipid peroxidation products, such as malondialdehyde, in the blood of diabetic patients (4, 5, 6). Oxidative stress was increased and antioxidant status decreased in diabetic patients in some clinical studies (21). A study of 40 Type-1 and 40 Type-2 diabetic patients showed that oxidative stress was more pronounced in Type-2 than Type-1 diabetics (22). Elevated oxidative stress is known to increase phospholipase A_2 activity and stimulates the release of arachidonic acid and further generation of TXA_2 (23, 24). Based on these data our hypothesis was, that the increased production of TXA_2 in diabetic patients might be a consequence of increased oxidative stress and therefore antioxidants, such as vitamin E might be beneficial in preventing vascular complications of diabetes.

Many studies demonstrated yet a reduction in oxidative stress and vascular complications after antioxidant supplementation in diabetic animals (25, 26, 27, 28). In an experimental study d-alpha-tocopherol treatment diminished albuminuria in diabetic rats (29) and improved the arachidonic acid cascade in the kidney of diabetic rats (30). Beneficial effect of vitamin E on microalbuminuria was confirmed also in diabetic patients (31). In other clinical studies vitamin E was beneficial in normalizing retinal and renal hemodynamic abnormalities without changing glycemic control, and it was most beneficial in those cases, where glycemic control was poorest and renal and retinal hemodynamic abnormalities were greatest (7).

We assessed malondialdehyde as a marker of lipid peroxidation in our study. In agreement with previous studies the level of MLD was significantly elevated in diabetic microalbuminuric patients compared with diabetic normoalbuminuric patients and healthy subjects. Diabetic microalbuminuric patients were treated with vitamin E 1200 IU daily for a period of 4 months. A decrease in levels of MLD was observed after 2 and 4 month of treatment in our patients. We also confirmed the effect of vitamin E on blood prostaglandins – the level of TXB_2 significantly decreased after treatment, however the level of 6-keto-$PGF_1\alpha$ was not significantly influenced. Therefore the ratio TXB_2/PGI_2 was positively influenced (decreased) with the treatment. Vitamin E supplementation also lowered microalbuminuria. It suggests beneficial effect of vitamin E treatment in patients with incipient diabetic nephropathy. To evaluate the relation between MLD and serum TXB_2 level, the Pearson correlation coefficient was performed. The correlation was not significant, and we did not establish a relation between MLD and

TXB_2, therefore we can not confirm our hypothesis, that increased production of TXA_2 might be a result of elevated oxidative stress.

The results of clinical studies on the effect of vitamin E on cholesterol, triglycerides or glycosylated hemoglobin have not been conclusive. In these studies vitamin E was shown to decrease (32) or have no effect (33) on blood glycosylated hemoglobin and to reduce (34) or have no effect (35) on triglycerides levels. In our patients the changes of glycosylated hemoglobin and triglycerides after treatment were not significant. In agreement with other studies we observed nonsignificant trend for total cholesterol to increase (7, 35).

Our study suggests, that there is an increase in lipid peroxidation and serum TXB_2 level in diabetic microalbuminuric patients. We confirmed changes in eicosanoid metabolism in diabetic patients, the TXB_2/PGI_2 ratio was elevated in diabetics with microalbuminuria in comparison with diabetic normoalbuminuric patients and healthy controls. These changes may contribute to vascular diseases, which are particularly prevalent in diabetic patients with microalbuminuria. Our results did not show any significant relationship between MLD and TXB_2 levels. We confirmed the beneficial effect of vitamin E supplementation in diabetic patients with microalbuminuria. Treatment with 1200 IU daily for a period of 4 months significantly lowered microalbuminuria and lipid peroxidation. Based on our results, vitamin E also positively influenced changes in eicosanoid metabolism with improvement of TXB_2/PGI_2 ratio, but the mechanism of this effect has not been fully elucidated.

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