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CLINICAL STUDY

Comparative study of serum/plasma glycation and lipid peroxidation of young patients with type 1 diabetes mellitus in relation to glycemic compensation and the occurrence of diabetic complications

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Abstract: *Aim of the study:* We tried to investigate whether the AGEs in serum and lipoperoxides (LPO) monitoring were suitable for an early prediction of diabetic complications (DC) development in diabetological practice. We wanted to find whether it is better to divide the file according to the presence of DC or in terms of glycemic compensation in this study.
Patients and methods: 79 diabetic patients with duration of disease for at least 5 years were divided in respect to DC presence/absence and also to long-time glycemic compensation. HbA1c was measured by LPLC in fair capillary blood, s-AGEs were estimated spectrofluorimetrically and LPO iodimetrically and spectrophotometrically in serum.
Results: HbA1c, s-AGEs and LPO were significantly higher in the group with DC (+DC) vs. controls and also in –DC vs. controls. HbA1c and s-AGEs were significantly higher in +DC vs. patients without DC (–DC). HbA1c, s-AGEs and LPO were significantly higher in patients with poor glycemic compensation (PGC) compared to controls and HbA1c and LPO in patients with good glycemic compensation (GGC) compared to controls. HbA1c and s-AGEs were significantly higher in PGC vs. GGC. In the group of GGC we have found interesting significant correlations of HbA1c with HDL ($r=0.451$, $p<0.05$) and with LDL ($r=–0.450$, $p<0.05$).
Conclusions: Our findings suggest that the monitoring of s-AGEs in poorly compensated diabetic patients and LPO in all may be very useful to recognize the risk of complications. The dividing of patient file in terms of long time glycemic compensation is more reliable for research of this issue *(Tab. 3, Fig. 6, Ref. 41).* Full Text in free PDF *www.bmj.sk.*
Key words: diabetic complications, glycemic control, HbA1c, s-AGEs, lipoperoxides.

Abbreviations: +DC – with diabetic complications, AGEs – advanced glycation end products, DC – diabetic complications, -DC – free of diabetic complications, DD – duration of diabetes, GGC – good glycemic compensation, HDL – high density cholesterol, LDL – low density cholesterol, LPLC – low pressure liquid chromatography, LPO – lipoperoxides, NGSP – National Glycohemoglobin Standardization Program, PGC – poor glycemic compensation, ROS – reactive oxygen species, s-AGEs – serum advanced glycation end products, T1DM – Type 1 diabetes mellitus, TAG – triacylglycerols, TC – total cholesterol, UAER – urinary albumin excretion rate.

Type 1 diabetes mellitus (T1DM) is characterized by absolute or nothing short of absolute endogenous insulin deficiency which results in hyperglycemia (1) that is considered to be a primary cause of diabetic complications (DC) and is associated with glycation, glycoxidation and oxidative stress (2, 3). Persistent hyperglycemia accelerates the formation of early and advanced glycation products. This causes long-lived proteins to become more heavily modified, in addition to rendering shorter-lived molecules as targets for advanced glycation (4). Accumulation of advanced glycation end products (AGEs) has several toxic effects and takes part in the development of diabetic complications – nephropathy, neuropathy, retinopathy and angiopathy (5). Also the reactive oxygen species (ROS) formed during the formation of AGEs cause a self-perpetuating cycle of ROS/AGE formation. The proposed sources of ROS in the Maillard

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reaction are many fold, including the autoxidation of glucose (Wolff pathway), Schiff bases (Namiki pathway) and Amadori adducts (Hodge pathway), as well as AGE proteins themselves (4). The overproduction of ROS leads to oxidative modification of biologically important compounds and damage of them. LDLs that are highly sensitive to oxidation may be modified by these reactions, or incorporated into immune complexes (6). Highly glycated or oxidized LDL particles escape detection by LDL receptors and trigger recognition by macrophage receptors and are internalized by macrophages by means of the scavenger receptors on the surfaces of these cells. The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells (7, 8).

The increased risk of DC development is particularly associated with poor glycemic control (9–11).

An effective prevention of vascular complications plays a very important role in diabetes treatment. The early detection of complications affords to use the preventive and therapeutic proceedings aimed at stopping or delaying the disease progression. Therefore the monitoring of suitable markers of glycation and glycoxidation focused on early detection of eventual complications risk and antiglycation and antioxidation treatment aimed at strategies to interrupt the formation or action of glycation, glycoxidation or oxidation products or their accumulation, may be very beneficial.

Useful predictors of DC and also new and effective methods of their detection are still searched. Because DC may occur as early as in childhood and adolescence, this issue is still current. It would be beneficial to find additional predictors that distinguish between patients who have a higher risk of developing complications from those who do not.

Approximately the third of AGEs exert the fluorescent properties. Also, we assume that measurement of AGEs in serum may help to reveal a risk of later diabetic complications aforetime the measurement of them in tissues. Therefore we focused on the determination of serum AGEs (s-AGEs) levels by fluorescence spectroscopy. Moreover we aimed to evaluate lipoperoxides (LPO) as a marker of oxidative stress. We tried to recognize whether the fluorescence AGEs and LPO (both in serum) were appropriate for the early prediction of the risk of diabetic complications diabetological practice.

Patients and methods

Study patients and design

The study group consisted of 79 patients with T1DM as defined by the National Diabetes Data Group (12) with duration of disease (DD) at least 5 years (mean age 15.2 ± 2.7 years) regularly attending 1st Department of Pediatrics, Children Diabetological Center of the Slovak Republic, University Hospital, Faculty of Medicine, Comenius University, Bratislava. The patients with T1DM were divided according to presence/absence of DC into the 2 groups: with diabetic complications (+DC) – 40 patients and without diabetic complications (-DC) – 39 those, regardless of glycemic compensation. Moreover, the same patients were divided to those of poor long-time glycemic compensation (mean HbA1c levels in the last 2 years > 8.5 %) (PGC), 59 subjects, and those of good glycemic compensation (GGC), 20 subjects, regardless of the presence/absence of DC. Blood samples for analysis of HbA1c were drawn approximately every 3 months. Using of the American Ophtalmologic Academy no changes (fundus diabetic retinopathy) were found by the ophthalmologist examining the eyes. Diabetic nephropathy was assessed by the urinary albumin excretion rate (UAER). Microalbuminuria was considered to be present when the UAER was > 20 μg/min in at least 2 samples collected overnight for 12 hours during six months. Diabetic neuropathy was excluded/confirmed using electromyography. 31 healthy children were used as controls. The procedures used in the study were approved by the ethics committee at our institution.

Sample analysis

Total cholesterol (TC), high density cholesterol (HDL), low (TAG) density cholesterol (LDL) and triacylglycerols (TAG) were estimated from recent serum enzymatically and colorimetrically using automatic analyzer Cobas Integra 400 (Roche, Switzerland).

Serum creatinine was estimated using an enzymatic method (Vitros 250, Johnson and Johnson Comp., USA).

UAER was determined by immunoturbidimetric assay in urine collected during 12 nocturnal hours using Cobas Integra 400 (Roche, Switzerland).The samples were collected at least 2-times.

Analyses of lipoproteins, creatinine and UAER were performed as part of routine examination of patients by Department of Clinical Biochemistry Laboratory, Children University Hospital, Bratislava, Slovakia.

HbA1c was determined by LPLC (DiaSTAT, Bio-RAD, USA) in fair capillary blood using the NGSP calibration.

The s-AGEs were determined by spectrofluorimetric assay at 418 nm by analyzer Perkin-Elmer LS-3 (USA) in serum samples, which were stored at –20 °C and were defrost only once. The samples were diluted in ratio 1:5 by deionized water. The chinine sulphate was used to calibrate the instrument and monitor its performance.

LPO were estimated by iodimetric assay and spectrophotometrically in serum samples which were stored at –20 °C and were defrost only once. The measurement was realized according to El-Saadani et al. (13). Total amount of lipid peroxides was monitored at 365 nm at analyzer Biochrom 4060 (Pharmacia Biotech, USA).

Statistical analysis

Baseline characteristics of the patients were compared using the Student's t-test. (Excel 2006), the results are expressed as mean ± SD. Variables not normally distributed (s-AGEs, LPO) were log transformed before performing statistical analyses or compared using the non-parametric Mann Whitney´s test (StatDirect). The results were expressed as median (1st quartile, 3rd quartile). The data distribution normality was tested using the Shapiro-Wilk´s test (StatDirect). The correlation between the

Table 1. Clinical and biochemical parameters in patients with diabetes and controls with respect to presence/absence of diabetic complications.

Parameter	T1DM total	n	+DC	n	–DC	n	Controls	n
Age (yr.)	15.2±2.7	79	15.8±1.9	40	14.5±1.9	39	9.2±4.9	31
DD (yr.)	8.7 ± 3.0	79	9.8 ± 3.1	40	7.5±2.6	39	-	-
TC (mmol/l)	4.32±0.67	79	4.29±0.66	40	4.36±0.69	39	3.89±0.73[a,b,c]	**31**
HDL (mmol/l)	1.57±0.42	79	1.46±0.36	40	1.69±0.45[b]	39	1.28±0.32[a,b,c]	31
LDL (mmol/l)	2.66±0.77	79	2.70±0.80	40	2.62±0.75	39	2.42±0.64	31
TAG (mmol/l)	1.16±0.82	79	1.40±0.93	40	0.92±0.47[b]	35	1.29±0.57[c]	29
Creatinine (μmol/l)	59±12.5	78	60±9.9	40	58±14.5	38	42.2±12.3[a,b,c]	29
HbA1c (%)	9.51±1.90	79	10.60±1.73	40	8.40±1.37[b]	39	5.0±0.39[a,b,c]	21
s-AGEs (A.U.)	67.9(61.6, 76.4)	70	72.5(66.2, 76.6)	34	63.5(60.4, 74.1)[b]	36	58.2(52.0, 65.5)[a,b,c]	29
LPO (nmol/ml)	119(100.3, 156.3)	48	124(102.8, 151.5)	22	116.5(98.8, 159.8)	26	99(67, 106) [a,b,c]	11

[a] significant difference in comparison with all patients with T1DM
[b] significant difference in comparison with +DC
[c] significant difference in comparison with –DC
The results are presented as mean ± SD in data with normal distribution and as median(1st quartile, 3rd quartile) in data with abnormal distribution

measured parameters was examined using the Pearson´s correlation test (r, p) or by the Spearman's rank correlation test (R, p) (Statistixl 1.8, Excel 2006).

A p value < 0.05 was considered as statistically significant.

Results

The levels of glycation and oxidation parameters were significantly higher in the group of all patients with T1DM compared to controls (Tab. 1, Figs 1, 2, 3).

Diabetic patients with and without complications

Comparison of clinical and biochemical parameters

As shown in Table 1, there were significantly higher the levels of HbA1c, s-AGEs (Fig. 1) and LPO (Fig. 2) in +DC group vs. controls. HbA1c, s-AGEs (Fig. 1) and LPO (Fig. 2) were significantly higher also in –DC vs. controls. HbA1c and s-AGEs (Fig. 1) were significantly higher in +DC compared with –DC.

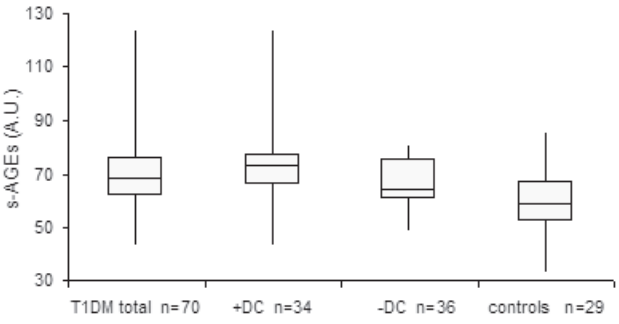

Fig. 1. Comparison of s-AGEs levels in diabetic patients and controls: s-AGEs levels were significantly higher in all patients with T1DM compared to controls (67.9 (61.6, 76.4) A.U. vs 58.2(52.0, 65.5) A.U., p<<0.001), in +DC compared to controls (72.5 (66.2, 76.6) A.U. vs 58.2(52.0, 65.5) A.U., p<<0.001), in –DC compared to controls (63.5 (60.4, 74.1) A.U. vs 58.2(52.0, 65.5) A.U., p<0.01) and also in +DC against –DC (72.5 (66.2, 76.6) A.U. vs 63.5(60.4, 74.1) A.U., p<0.05). The results in figure are presented as [min.-1st quartile-median-3rd quartile-max.]. +DC – diabetic patients with diabetic complications, –DC – diabetic patients free of diabetic complications.

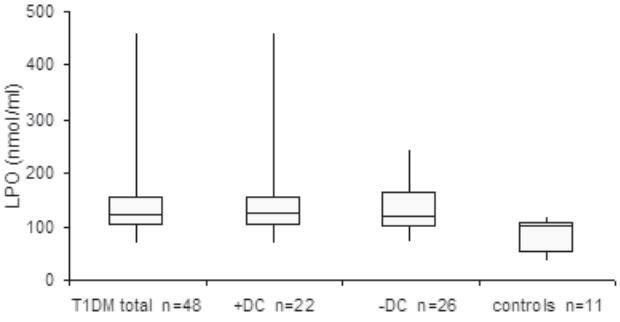

Fig. 2. Comparison of LPO levels in diabetic patients and controls: LPO levels were significantly higher in all patients with T1DM compared to controls 119 (100.3, 156.3) nmol/ml vs 99 (67, 106) nmol/ml, p<0.01), in +DC compared to controls ((124 (102.8, 151.5) nmol/ml vs 99 (67, 106) nmol/ml, p<<0.01), in –DC compared to controls (116.5 (98.8, 159.8) nmol/ml vs 99 (67, 106) nmol/ml, p<0.01. The difference between +DC and –DC was not significant. The results in figure are presented as [min.-1st quartile-median-3rd quartile-max.]. +DC – diabetic patients with diabetic complications, –DC – diabetic patients free of diabetic complications.

Correlations between measured parameters

As shown in Table 2, in the group of all patients with T1DM, significant correlations of creatinine in serum with DD and HDL with age was found, HbA1c correlated with DD, with TAG, s-AGEs correlated with TC, with TAG, and also with the mean of HbA1c during the last 2 years. The significant correlations of LPO with TAG and with s-AGEs were found (Tab. 2).

In the group of +DC patients we have found a significant correlation between creatinine and DD. HbA1c correlated with the lipid profile parameters: with TC, negatively with HDL, and with TAG. The significant correlations were found between s-AGEs and TAG and also between s-AGEs and the mean of HbA1c during the last 2 years.

Table 2. Correlations and relationships between parameters in patients with diabetes and controls.

Correlation	T1DM total	+DC	-DC	PGC	GGC	Controls
Age – HDL	NS	NS	r=-0.429[b]	NS	r=-0.488[a]	NS
Age – LDL	NS	NS	r=0.353[a]	NS	NS	NS
Age - TAG	NS	NS	r=0.369[a]	NS	r=0.532[a]	NS
DD - creatinine	r=0.447[c]	r=0.458[b]	r=0.471[b]	r=0.490[c]	r=0.621[b]	NS
HbA1c* - DD	r=0.294[b]	NS	NS	NS	NS	NS
HbA1c* - TC	NS	r=0.339[a]	NS	NS	NS	r=-0.469[a]
HbA1c* - HDL	NS	r=-0.312[a]	NS	r=-0.303[c]	r=0.451[a]	NS
HbA1c* - LDL	NS	r=0.325[a]	NS	NS	r=-0.450[a]	r=-0.460[a]
HbA1c* - TAG	r=0.435[c]	r=0.407[b]	NS	r=0.437[c]	NS	NS
s-AGEs – TC	r=0.236[a]	NS	NS	NS	NS	NS
s-AGEs - TAG	r=0.546[c]	r=0.541[c]	r=0.386[a]	r=0.537[c]	NS	NS
s-AGEs - HbA1c**	r=0.490[c]	r=0.490[b]	NS	r=0.486[c]	NS	NS
LPO – HDL	NS	NS	r=-0.444[a]	NS	NS	NS
LPO – TAG	r=0.531[c]	r=0.435[a]	r=0.751[c]	r=0.479[b]	R=0.660[e]	NS
LPO – HbA1c**	NS	NS	NS	NS	R=0.508[d]	NS
LPO – s-AGEs	r=0.354[a]	R=0.393[d]	NS	r=0.375[a]	NS	NS

* recent data, ** mean value during last 2 years, r – Pearson´s correlation coefficient, R– Spearman´s correlation coefficient, [a] $p<0.05$, [b] $p\leq0.01$, [c] $p\leq0.001$, [d] $\alpha=0.05$ by

LPO correlated with TAG, and there was a significant relationship between LPO and s-AGEs in this group (Tab. 2).

In the group of -DC patients we have found significant correlations of lipid profile parameters and creatinine with the age of patients (see Table 2): HDL, LDL and TAG; and creatinine in serum correlated significantly with DD. No parameter correlated with HbA1c in –DC group. As shown in Table 2, the correlation between s-AGEs and TAG was small, but significant, and LPO correlated significantly with HDL and also with TAG.

Diabetic patients with good and poor glycemic compensation

Comparison of clinical and biochemical parameters

As shown in Table 3, there were significantly higher parameters in PGC group compared to controls: HbA1c, s-AGEs (Fig. 3) and LPO (Fig. 4).

There were significantly higher levels of HbA1c and LPO (Fig. 4) in GGC group compared to controls, whereas the difference of the s-AGEs levels was not significant.

The levels of HbA1c were significantly higher in patients of PGC compared to those of GGC. Also the difference of s-AGEs between the groups was significant (Fig. 3). The levels of LPO are similar in patients mentioned above.

Correlations between measured parameters

As shown in Table 2, in the group of patients with PGC there was a significant correlation between creatinine and DD, HbA1c correlated significantly with HDL and with TAG. Serum AGEs correlated significantly with TAG and with the mean of HbA1c in the last 2 years, no correlation was found between s-AGEs and actual levels of HbA1c. LPO correlated significantly only with TAG and slightly, but significantly with s-AGEs (Tab. 2).

In the group of GGC some lipid profile parameters correlated with age: HDL and TAG, and furthermore, creatinine correlated with DD (Tab. 2). We have found interesting significant correlations of HbA1c: positive with HDL (Fig. 5) and negative with LDL (Fig. 6). No significant correlations were found between s-AGEs and any lipid profile parameter.

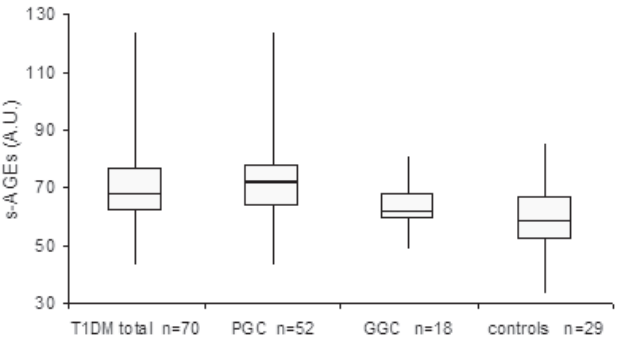

Fig. 3. Comparison of s-AGEs levels in diabetic patients and controls: s-AGEs levels were significantly higher in PGC compared to controls (71.9 (63.3, 77.2) A.U. vs 58.2 (52, 65.7) A.U., p<0.01), and in PGC against to GGC (71.9 (63.3, 77.2) vs 61.9 (59.6, 67.8) A.U., p<0.01), whereas the difference between GGC and controls was negligible. The results in figure are presented as [min.-1st quartile-median-3rd quartile-max.]. PGC – diabetic patients with poor glycemic compensation during the last 2 years, GGC– diabetic patients with good glycemic compensation during the last 2 years.

We have found a significant relationship of LPO with TAG, with the mean of HbA1c in the last 2 years (Tab. 2), whereas no one between LPO and the current HbA1c levels was found.

Discussion

Many studies deal with the impact of glycative and oxidative stress on the development of diabetic complications. We studied glycative and oxidative stress parameters in regard to dia-

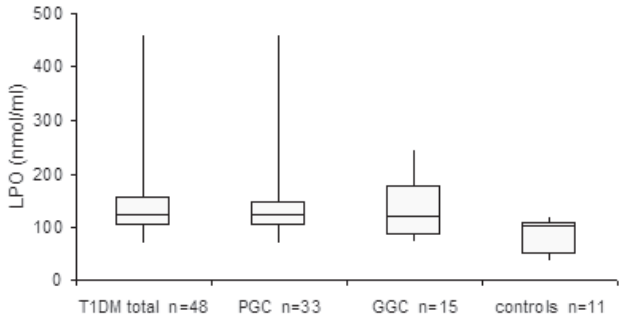

Fig. 4. Comparison of LPO levels in diabetic patients and controls: LPO levels were significantly higher in PGC compared to controls (121 (101, 138) nmol/ml vs 99 (67, 106) nmol/ml, p<0.01), in GGC compared to controls (117 (91.5, 172) vs 99 (67, 106) nmol/ml, p=0.01), whereas the difference between PGC and GGC was negligible. The results in figure are presented as [min.-1st quartile-median-3rd quartile-max.]. PGC – diabetic patients with poor glycemic compensation during the last 2 years, GGC– diabetic patients with good glycemic compensation during the last 2 years.

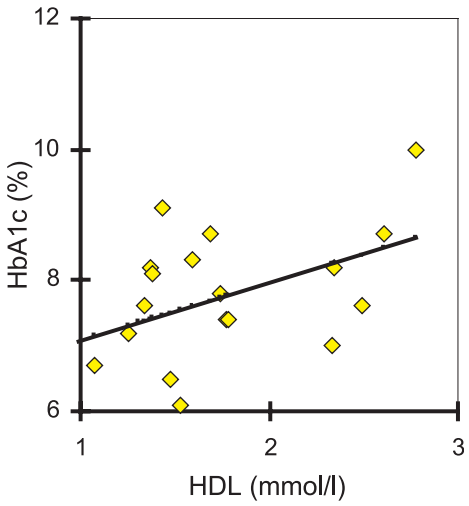

Fig. 5. Correlation of HbA1c and HDL in diabetic patients with good glycemic compensation during the last 2 years: HbA1c significantly positively correlates with HDL (r=0.451, p<0.05, n=20).

betic complications presence/absence and with respect to glycemic compensation.

Lipid profile

Lipid and lipoprotein disorders are frequently detected in patients with T1DM. Hyperglycemia is the major factor, inducing metabolic lipid and lipoprotein changes by increasing hepatic synthesis of triglycerides and promoting lipoprotein glycosylation and oxidation. The lipid profile in children with PGC is similar to that already described for adult patients. The main abnormalities found were following: increased levels of TAG, LDL, decreased values of HDL (14).

There were significantly higher levels of TC in the group of all diabetic patients compared to controls and also in patients both, +DC and –DC, compared to controls, in our study. The

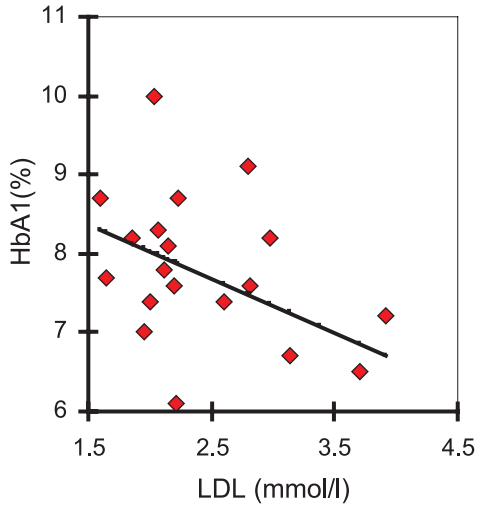

Fig. 6. Correlation of HbA1c and LDL in diabetic patients with good glycemic compensation during the last 2 years: HbA1c significantly negatively correlates with LDL (r=–0.450, p<0.05, n=20).

difference between +DC and –DC, between PGC and GGC and between GGC patients and controls was not significant. These results corresponds with the previous study (15).

HDL was lower in the group of patients in worse condition – "worseness groups" (i. e. in patients +DC or PGC) compared with those in better condition (i. e. in patients –DC or GGC). The LDL levels were higher in all diabetic groups compared to controls and also in +DC compared to –DC, but only the difference between PGC and controls was significant in our study. The levels of TAG in +DC patients were higher compared to controls in the present study. The TAG levels in all treated –DC patients and also in GGC were unexpectedly lower compared to controls, but these results correspond with previous studies (9, 16). Probably just because of long term good glycemic control, the levels of LDL in GGC were similar to controls. So we believe that the glycation of LDL is not increased in this group. Also the synthesis of TAG is not increased in diabetics in the present study, apart from +DC group. The differences between all patients with T1DM vs. controls and PGC or GGC groups vs. controls are noteworthy, but an explanation may be a strict dietary adherence, insulin treatment and/or exercise in diabetic patients on the one hand and consumption of fat-rich diet in controls on the other hand.

Parameters of glycative and oxidative stress

Glycation, glycoxidation and oxidation have both, physiological and pathophysiological significance (17) and are a minority feature of physiological metabolism (18). The exogenous route of AGEs and their precursors is especially the food that may be a major source of intracellular and serum AGEs (19). Circulating AGEs are detoxified by enzymes to AGE peptides that are excreted by the kidneys, the normal capacity of which may be easily exceeded, especially in the presence of renal disease, diabetes, or high AGE intake. When AGEs accumulate, a large por-

Table 3. Clinical and biochemical parameters in patients with diabetes and controls with respect to glycemic compensation.

Parameter	PGC	n	GGC	n	Controls	n
Age (yr.)	15.4±2.4	59	14.4±3.2	20	9.2±4.9	31
DD (yr.)	9.2 ± 3.0	59	7.1±2.7	20	-	-
TC (mmol/l)	4.35±0.65	59	4.36±0.74	20	3.89±0.73[a,b]	31
HDL (mmol/l)	1.52±0.37	59	1.71±0.51	20	1.28±0.32[a,b,c]	31
LDL (mmol/l)	2.73±0.64	59	2.47±0.65	20	2.42±0.64[b]	31
TAG (mmol/l)	1.26±0.92	59	0.88±0.30[b]	20	1.29±0.57[b]	29
Creatinine (µmol/l)	58±11.2	58	62±14.3	20	42.2±12.3[a,b,c]	29
UAER (µg/min)	48.3±135.5	54	9.6±5.5[b]	20	-	-
HbA1c (%)	10.12±1.74	59	7.71±0.99[b]	20	5.0±0.39[a,b,c]	21
s-AGEs (A. U.)	71.9(63.3, 77.2)	52	61.9(59.6, 67.8)[b]	18	58.2(52, 65.7)[a,b]	29
LPO (nmol/ml)	121(101, 138)	33	117(91.5, 172)	15	99(67, 106)[a,b,c]	11

[1] significant difference in comparison with all patients with T1DM, [2] significant difference in comparison with PGC, [3] significant difference in comparison with GGC
The results are presented as mean ± SD in data with normal distribution and as median(1st quartile, 3rd quartile) in data with abnormal distribution

tion of ingested AGEs is retained in tissues, contributing to increased OS and ultimately, to impaired organ function (20).

Apart from the HbA1c as an indicator of glycemic compensation and its relations with clinical and biochemical parameters, we have dealt with the s-AGEs in diabetic patients with DC and without them and also in patients with good and poor glycemic compensation. We have searched a relation of s-AGEs with glycemic compensation and also with oxidative stress parameters in all groups.

The glycated hemoglobin HbA1c reflects the integrated blood glucose concentration of the preceding 8–12 weeks, and is the most widely used index of chronic glycemia. Clinical trials have documented the relationship between glycated hemoglobin levels and the development of long-term complications of diabetes (21). In our study there were significantly higher HbA1c levels in all diabetic groups compared to controls and also in both "worseness groups" compared to those in "better condition". These results correspond with the previous studies (15, 22) that present higher HbA1c levels in both, +DC and –DC groups than in controls. Chiarelli et al (23) present significantly higher HbA1c levels in patients with persistent microalbuminuria or with retinopathy compared to –DC group and also to controls. Vander Jagt et al (24) describe significantly higher HbA1c levels in patients with neuropathy compared to –DC and controls. Expected, the correlation between HbA1c and HDL was negative in the PGC group. A slight but significant positive correlation between HbA1c and HDL and negative correlation between HbA1c and LDL in GGC are interesting and surprising. We have not found the similar results in another study. It is interesting that the similar discrepancies have not appeared when dividing our patients into groups with respect to DC presence/absence. We suggest that the organism of good glycemic compensation can keep the antioxidant status and the balance in lipoproteins. We think that the discrepancies of relations between HbA1c and lipoproteins ought to be studied in more GGC patients to confirm or invalidate the results given in this study. The fact that we have found discrepancies in the relations of HbA1c with lipoproteins just when dividing the group in terms of glycemic compensation, but not DC presence/absence, suggests an impact of glycemic compensation on the ability of well compensated organism to protect itself against glycation and ROS affect. A highly positive impact of good glycemic compensation on serum lipids in children and young adults presented the previous study (25). Several studies (26, 27) present significant correlations between HbA1c and lipoproteins in patients with diabetes. Petitti et al. (28) observed a trend of higher concentrations of TC, LDL, and TAG with higher HbA1c values in poorly compensated patients with T1DM and according to their study, glycemic compensation affects the concentrations of lipoproteins and intensive glucose control significantly decreased the concentrations of TC, LDL, and TAG in patients with T1DM.

The role of AGEs in DC development and predicting is still under investigation. The role of AGEs not only in tissues but also in serum in DC pathogenesis is accepted by more studies, but there exist various opinions of the s-AGEs as a predictor of the DC development. The authors (29, 30) have expressed the opinion that serum AGEs might predict the progression of early morphological kidney damage in patients with T1DM. Sampathkumar et al (31) recommend the measurement of AGEs for clinicians and researchers concerned in the management and prevention of diabetic vascular disease, whereas Busch et al (32) do not consider the s-AGEs to be a marker with a great predictive value. We chose s-AGEs measurement mainly because we supposed that elevated s-AGEs levels might contribute to the risk of AGEs accumulation, thus also to the risk of complication. The study (33) demonstrated that if the quality of patient control was good the concentrations of the AGEs were typically lower. When the complications are already present, the improvement of glycemic compensation alone may not be sufficient to prevent the continued progression of these pathologic processes, potentially due to the irreversibility of AGE formation as well as poor clearance mechanisms (33). This may be suggested also by our study: the s-AGEs levels in GGC and controls were similar, whereas the differences between PGC and controls and between PGC and GGC was significant. But we have found higher levels of s-AGEs not only in +DC compared to –DC, but also in –DC compared to controls, what did not appear in comparison of GGC vs. controls. In accordance with the studies (23, 29, 34), we have found significantly elevated levels of s-AGEs in the group of all patients with T1DM compared to controls. Chiarelli et al (23, 34) studied the s-AGEs in patients with nephropathies and retinopathies and without DC and according to their results, the severity of diabetic

angiopathy is related to the serum levels of AGEs (34). In children with poorly compensated diabetes, long-term (2 years) improvement of glycemic compensation resulted in significant reduction of s-AGEs levels in preschool and prepubertal children, as well as in pubertal individuals. The authors (23) suggested that the risk of microvascular complications may be present at an early age and the improvement of glycemic control may be associated with a significant decrease of s-AGEs. Similarly to our study, another study (2) have also found elevated values of s-AGEs in +DC vs. –DC, but unlike us, they have not found any significant difference between –DC and controls. Unlike us, no significant differences in the s-AGEs levels between +DC and –DC diabetic patients were found in the study (35). This discordance may be due to number of samples (the authors compared 13 patients +DC with 5 those –DC), our group of patient was bigger. Similarly to us, Buongiorno et al (36) have also found no difference between GGC and controls, but significantly higher s-AGEs in GGC compared to controls were found e. g. in the study (23). The results of the various authors are different. The s-AGEs did not correlate either with age or DD accordingly to the studies (9, 29, 35). We have found significant correlations of the s-AGEs with TAG according to authors (35, 37). A strong association between serum AGEs and serum triglycerides and cholesterol might be explained by a possible loss of optimal regulation of lipid metabolism (9). It could suggest a link between triglycerides and formation of AGEs. Maybe, also a fat-rich food might affect these relationships. Furthermore, in our study the AGEs correlated with FAM and also with the mean HbA1c during the last 2 years, but not with actual HbA1c in the worseness groups. We have not found this kind of correlations either in –DC group, or GGC one. These findings are unexpected and surprising, but in accordance with the previous studies (38, 39). Additional, these findings do not mean that s-AGEs have no relationship to glycemic compensation. Maybe, in well compensated patients, the oxidative processes independent on glycemia or glycemic compensation participate in forming of the AGEs more then glycation. We also believe that well compensated patients with T1DM may have sufficient protective mechanisms against the oxidative processes or their impact.

In healthy organism, free radicals act under strong control at exactly designated site of their action and antioxidant activity counterbalances free radicals production or eliminates the deleterious effects of their action by means of antioxidant systems. But in some diseases such as diabetes, the balance between ROS and antioxidant activity is shifted toward free radicals, causing oxidative stress. Free radical mediated oxidative stress is mainly involved in the pathogenesis of diabetic complications. Proteins and lipids are among the prime targets for oxidative stress. In the present study, we have evaluated the oxidative stress by estimating the lipid peroxidation. We have focused on LPO in serum. In the present study the levels of LPO were significantly higher in all diabetic groups against controls, but the differences between „better“ and „worseness“ groups were not significant. Our results are in accordance with the previous studies (22, 24, 40). Unlike us, Hsu et al (41) have found the LPO levels significantly higher in well compensated diabetic patients against to poorly compensated ones. The author suggested that supportive therapy aimed at oxidative stress may help to prevent clinical complications in children with T1DM. According to the previous study (24) it is not clear whether serum markers of oxidative stress are produced in the circulation or produced in specific cells or tissue and then released into the plasma, much like liver enzymes that increase in the circulation in response to hepatocyte injury. That authors support an idea that the mentioned markers may have been produced in tissues with subsequent release into the plasma. In our study, LPO have correlated significantly with TAG in all diabetic groups. We have found also a significant relationship LPO with the mean HbA1c in the previous 2 years in GGC, but not with actual data. The correlation of LPO with s-AGEs may suggest a linkage of glycative and oxidative stress with subsequent damage of biogenic molecules with the pathogenesis of complications in diabetes. The measurement of LPO may consistently provide an index of oxidative stress.

Conclusions

Our results suggested an association of glycation and oxidation processes with long-term glycemic compensation. Glycemic compensation, rigorous insulin treatment and healthy life style are important factors contributing to the ability of organism to protect itself against glycative and oxidative damage.

Our findings suggested that the monitoring of s-AGEs in poorly compensated patients with T1DM by specific fluorescence and one of LPO in all diabetic patients may be very useful in recognition of risk of complications. Moreover, the fluorimetric assay is advantageous because this method is simple, time saving and not expensive.

It is interesting to study glycation or oxidation parameters and their predictive value in respect to presence/absence of DC, but we assume it is more useful to study them in respect to glycemic compensation for the purpose mentioned above.

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