Turkish Journal of Endocrinology and Metabolism, (1999) 4 : 153-162 **ORIGINAL ARTICLE**

Oxidative Stress and Anti-Oxidant Therapy in Type-2 Diabetes Mellitus

Nur Kebapçı* **Belgin Efe*** **Fahrettin Akyüz**** **Emine Sunal**** **Canan Demirüstü*****

* *Osmangazi University, Internal Medicine Department, Endocrinology and Metabolism Subdivision, Eskişehir, Türkiye*

* *Osmangazi University, Department of Biochemistry, Eskişehir, Türkiye*

** *Osmangazi University, Department of Biostatistics, Eskişehir, Türkiye*

Alpha-tocopherol (A-T) ,ascorbic acid (AA),malondialdehide (MDA) concentrations, superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities were measured before and after a 60 day period to define the basal oxidative status in type 2 diabetic patients and to assess the effects of A-T and AA supplementation as an anti-oxidative therapy. The results were compared to those of 10 healthy volunteers. Type 2 diabetic patients were followed up for 60 days in four main groups. Glubornuride or insuline was given to all the patients. Group 1: No additional vitamin was given. Group 2: AA (1 g/day) was given. Group 3: A-T (600 mg/day) was given. Group 4: Both AA (1 g/day) and A-T (600 mg/day) were given. At the end of 60 days normoglycaemic (HbA_{1c} < 8%) and hyperglycaemic (HbA_{1c} > 8%) groups of patients were formed. Initial A-T and AA concentrations of patients were statistically lower, MDA concentration, MDA/SOD and MDA/GPX ratios were statistically higher than in the control group. SOD and GPX activities were similar to the control group. At the end of 60 days, normoglycaemic patients had significant increments in A-T and AA levels without any vitamin supplementation. Supplementation of A-T in combination in AA besides normoglycaemia restoration resulted in the highest significant increments in A-T and AA and the highest significant reduction in MDA levels. In conclusion: anti-oxidative therapy with A-T and AA may be beneficial in addition to glycaemia regulation.

Abbreviations: A-T, alpha tocopherol; AA, ascorbic acid; MDA, malon-dialdehide;

SOD, superoxide dismutase; GPX, glutathione peroxidase; DM, diabetes mellitus.

Key words: type 2 diabetes mellitus , alpha-tocopherol, ascorbic acid, superoxide dismutase, glutathione peroxidase, malondialdehide.

Introduction

It is generally accepted that chronic hyperglycaemia is responsible for most of the long-term complications of diabetes mellitus. Accelerated non-enzymatic glycation of proteins may be the underlying mechanism by which hyperglycaemia causes complications. Chronic hyperglycaemia also may change the redox potentials. Glucose and glycated proteins can reduce molecular oxygen under physiological conditions, catalysed by transition metals,

Correspondence address:

Nur Kebapçı
Osmangazi Üniversitesi Tıp Fakültesi,
İç Hastalıkları Anabilim Dalı
Endokrinoloji ve Metabolizma Bilim Dalı
26048, Eskişehir, Türkiye
Tel : 9 0 222 2392979
Fax : 9 0 222 2393774

yielding alphaketoaldehydes and oxidizing intermediates. Evidence suggests that free radicals such as superoxide anions and hydrogen peroxide are slowly produced by glucose autoxidation. These are substantial causes of the structural damage which results when protein is exposed to glucose in vitro (1). Over-production of precursors to reactive oxygen radicals and / or decreased efficiency of inhibitor and scavenger systems may result in oxidative stress in diabetes mellitus. Hyperglycaemia, accelerated non-enzymatic glycation of proteins and the alterations of redox potential may be included in the pathogenesis of chronic diabetic complications (2, 3).

The basic therapeutic approach is the regulation of blood glucose level and the reduction of the risks of long-term complications. Antioxidants such as A-T and AA have been demonstrated to reduce protein glycation both in vivo and in vitro. In addition they also act as scavengers of free radicals generated by non-enzymatic glycation (4). These findings may lead to new therapeutic approaches for the prevention of complications by limiting the damage caused by non-enzymatic glycation and oxidative stress.

The aim of the present study was to investigate A-T, AA, MDA levels, SOD and GPX activities to define the basal oxidative status in type 2 DM patients and to assess the effects of supplementation with A-T or AA separately or in combination in the existence of normoglycaemia restoration or hyperglycaemia.

Subjects, Materials and Methods

Subjects: 80 patients with type 2 diabetic patients (50 women and 30 men; 56. 21 ± 0. 95 years of age) with a mean duration of diabetes of 7. 01 ± 0. 69 years and 10 healthy volunteers (7 women, 3 men; 53. 9 ± 2.54 years of age) as control subjects were included in the study. The clinical characteristics are compared to control subjects in Table 1. The patients with and without chronic complications are shown in Table 2.

Informed consent was obtained and the study design was in accordance with the Helsinki declaration. Patients with an inflammatory disease, pancreatic, liver, lung, Fe or Cu storage disease, recent cerebral or myocardial infarction and these with creatinin clearence below 50 ml/min were excluded. Alcoholics and smokers were excluded, too. The patients had not used vitamin supplements previously. They were given diabetic diets and insulin or an oral hypoglycaemic agent during the study (Glubornurid, Roche). According to the design of the study, A-T, 100 mg tablet (200 mg tid); AA, 1 g effervescant tablet were given.

The Control group included healthy, non-smoker volunteers who were consistent with the patients according to age, sex and body mass index.

Materials: Thiobarbituric acid, reduced and oxidized forms of glutathione, 2, 9, 6 tripidyl-S-triazine (TPTZ), nitrobluetetrazolium (NBT), and beta-Nicotinamide adenine dinucleotide phosphate (reduced form) were from Sigma Xylol was from Merck.

Spectrophotometry: UV-120 Shimadzu spectrophotometry was used.

Preparation of Samples: 8 ml of venous blood were drawn from 80 type 2 diabetic patients and 10 control subjects following a 12 hour fast, at 8 a. m. at the beginning (day 0) and at the end of the study

Table 1. Clinical features of NIDDM patients are statistically compared to control subjects with student t-test.

	Control n=10	Patients n=80	Result
Sex Female/Male	7/3	50/30	P>0. 05
Age (year)	53.9 ± 2.54	56.21 ± 0.95	P>0.05
BMI (kg/m^2)	26.49 ± 0.94	29.14 ± 0.48	P>0.05
Fasting blood glucose (mg/dl)	89.6 ± 1.83	166.73 ± 7.42	P<0.001 ***
HbA1C (%)	2.98 ± 0.08	8.14 ± 0.27	P<0.001 ***

Table 2. Diabetic patients with chronic complications are shown.

Retinopathy	Absent (n=28)		Background (n=42)	Proliferative (n=10)
Nephropathy	Ccr>120 ml/dk (n=16)		Ccr=80-120 ml/dk (n=49)	Ccr=50-80ml/dk (n=15)
	Normoproteinuria (n=63)		Microproteinuria (n=17)	
Neuropathy	Absent (n=24)	Peripheric (n=12)	Autonomic (n=9)	Peripheric+Autonomic (n=35)

(day 60). 2 ml of each sample was kept for serum glucose measurement, the rest of the blood was heparinized (10 units/ml blood). 1 ml was for HbA_1C and 6 ml was used for obtaining plasma and erytrocyte hemolysate.

Plasma was separated by centrifugation at 3500 rpm, for 5 min. at 5°C in order to measure AA, A-T and MDA levels. Then it was is again centrifugated to remove platelets and buffy coat. Erytrocytes were washed three times with isotonic buffered saline solution and frozen at -20° C until the study day. Fasting blood glucose was measured on automated analyzer Hitachi 747, by Boehringer Mannheim. Glycated HbA_{1C} was determined by microcolon test system according to Bisse (5) with Sigma diagnostic kits.

A-T : It was estimated spectrophotometrically as described by Hashim (6). The principle of this method depends on the measurement of the optic density of the blue color of the mixture in which A-T reduces ferric ions to ferro and then TPTZ forms a blue color with ferro ions. Absolute alcohol (99.9 %), xylol and distilled water were added to the plasma. Then it was centrifugated at 2000 rpm for 5 min. TPTZ and FeCl3 were added to the supernatant and the optic density was measured at 600 n. m., being compared to the A-T (1 mg %) as a standard. It is shown as mg/dl .

AA: Plasma AA concentration was estimated colorimetrically as described by Tiettz (7). AA was oxidized by Cu (ll) to form dehydroascorbic acid (DHAA), which reacted with acidic 2, 4 dinitrophenylhydrazine to form a red bishydrozone. Its absorbance was measured at 520 n. m. It is shown as mg/dl.

MDA : Plasma MDA concentration was measured by a modified method of Okhawa and et al. (8). It depends on the optic density of the reaction of MDA with thiobarbituric acid. The plasma samples were boiled for 60 min. at 90° C in a mixture of sodium dodesil sulphate, acetic acid (20 %, PH:7. 5) and TBA (8 %). The absorbance of butanol pridine extraction of plasma at 520 n. m. was measured, then compared to 1. 1. 3. 3. tetraetoksiprophane as a standard. The results are shown as nmol/ml.

Preparation of Hemolysates : The hemoglobin concentration of each erytrocyte package was determined by Drabkin's method (9). 0. 5 ml of erytrocytes with 3. 5 ml distilled water, 1 ml of ethanol and 0. 6 ml of chloroform were mixed together in order to get chloroform and ethanol extraction. It was centrifugated at 3000 rpm for 10 min., then the supernatant was kept as hemolysates of erytrocytes.

Enzyme Assay :

SOD: Erytrocyte SOD activity was measured according to Beaucham and Fridovich's method which was modified by Winterborn, et al. (10). The principle of this method is the inhibiting rate of SOD on the reaction in which superoxide anion reduces NBT. Each patient's erytrocyte hemolysate was mixed with 0. 1 M EDTA, NaCN (1. 5 ml/dl), 1. 5 mM NBT and 0. 12 mM riboflavine and then phosphate tamponade (M/5, PH:7. 8) was added. The standard tube was without erytrocyte hemolysate. Each tube was shaken and then left for 15 min. in a uniformly lit box. The optic densities were measured at 560 n. m. and compared to the standard tube. The percentage of inhibition of reduction of NBT was calculated by the optic densities. The results are shown as U/grHb.

GPX : It was measured spectrophotometrically as described by Beutler (11). KPO4 (1M, PH:7), 0. 1 M GSH, 0. 2 M EDTA (neutral), GSSG reductase (10 units/ml), 0. 4M Na-Azide, and 2 Mm NADPH were mixed together with hemolysate and distilled water was added. The adsorbance of the reaction in which NADPH was produced during the reaction of GSSR to GSH was measured at 340 n. m. Then 10 mM H_2O_2 was added and the change in the adsorbance of the mixture was again measured. GPX activity was estimated by the change in the absorbance. It is shown as ıu/L.

Design of The Study : Venous blood was drawn from each patient to measure the basal levels of A-T, AA, MDA and SOD, GPX activities, fasting blood glucose and HbA_{1C} (Day 0). All the patients were using glubornurid or insulin. They were randomly divided into four main groups.

Group 1 : No additional vitamin supplement was given (n=20).

Table 3. The number of hyperglycaemic (H) and normoglycaemic (N) diabetic patients in the four main treatment groups are shown.

	Hyperglycæmic (H)		Normoglycaemic (N)
Group 1 H	n=11 (No additional vitamin)	Group 1N	n=9 (No additional vitamin)
Group 2 H	n=11 AA (1 g/day)	Group 2 N	n=9 AA (1 g/day)
Group 3 H	n=10 A-T (600 mg/day)	Group 3 N	n=10 A-T (600 mg/day)
Group 4 H	n=10 AA (1 g/day) and A-T (600 g/day)	Group 4 N	n=10 AA (1 g/day) and A-T (600mg/day)

Group 2 : AA (1 g/day) was given for 60 days (n=20).

Group 3 : A-T (600 mg/day) was given for 60 days (n=20).

Group 4 : AA (1 g/day) and A-T (600 mg/day) were given together for 60 days (n=20).

The patients were followed up over a two week period in the outpatients clinic in order to be sure that they were using the medication properly. At the end of 60 days venous blood was drawn from each patient to measure A-T, AA, MDA levels, SOD, GPX activities, fasting blood glucose and HbA_{1C}. During the study, if a patient's HbA_{1C} increased or remained above 8 % (HbA_{1C}>8%), the patient was accepted as hyperglycaemic (H). But if it decreased or remained below 8 % (HbA_{1C}>8%) then the patient was accepted as normoglycaemic (N).

Type 2 diabetic patients in the four main treatment groups mentioned above were classified as normoglycaemic (N) or hyperglycaemic (H) for example as G1N or G1H. Therefore the patients were presented in eight different groups as shown in Table 3.

Statistical Analyses : The results are given as mean values ± SEM. Differences between groups were analysed by student t-test. Paired t-test was used to compare the basal (Day 0) and the final (Day 60) values of each treatment group, included in systat 5.01 for Windows Copywrite 1990-1992 by Systat Inc. P<0. 05 was considered statistically significant.

Results

Fasting blood glucose and HbA_{1C} levels were significantly elevated in all diabetic patients compared with the control group (p<0. 001, p<0. 001). A-T and AA concentrations were significantly lower (p<0. 001, p<0. 001), MDA level was significantly higher (p<0. 001) in the patient group as compared to the control group. The ratios of MDA to SOD and MDA to GPX were higher in diabetic patients (p<0. 001, p<0. 01) (Table 4).

Table 4. Basal (Day 0) AA, A-T, MDA, SOD, GPX, MDA/SOD, MDA/GPX values of NIDDM patients are statistically compared to control subjects with student t test.

	Control n=10	Patients n=80	Result
AA (mg/dl)	1.31 ± 0.05	0. 76 ± 0.03	P<0.001***
A-T (mg/dl)	1.08 ± 0.03	0. 88 ± 0.01	P<0.001***
MDA (nmol/ml)	2.96 ± 0.13	5. 01 ± 0.16	P<0.001***
SOD (u/gHb)	3929.3 ± 165.75	3531.87 ± 69.38	P>0.05
GPX (u/g Hb)	19.83 ± 1.55	21. 51 ± 0.7	P>0.05
MDA/SOD	0.00076 ± 0.00005	0.00146 ± 0.00006	P<0.001***
MDA/GPX	0.16 ± 0.01	0.25 ± 0.01	P<0.01**

The change in A-T concentration was -7 % in (G1H) (p<0. 01), + 4.5 % in (G1N) (p<0.05), +2.25 % in (G3H) (P<0.05), +3.4 % in (G3N) (p<0. 001), +12.94 % in (G4H) (p<0. 001) and +15 % in (G4N) (p<0.001).

The change in AA concentration was +16 % in (G1N) (p<0.01), +27 % in (G2H), +30.9 % in (G2N) (p<0.01), +38 % in (G4H) (p<0.05) and +67 % in (G4N) (p<0.001).

The change in MDA concentration was -24. 5 % in (G2H) (p<0.01), -18.3 % in (G2N) (p<0. 05), -12.92 % in (G3N) (p<0.01), -21.2 % in (G4H) (p<0. 05) and -34.5 % in (G4N) (p<0.05) (Table 5).

Table 5. The final (Day 60) values of the A-T, AA and MDA are compared to the basal (Day 0) values with paired t-test. The values presented here are shown as mean± SEM.
Δ : The difference between the basal and the final values are shown in percentage.

	A-T(mg/dl)				AA (mg/dl)				MDA (nmol/ml)			
	Day 0	Day 60	Δ	Result	Day 0	Day 60	Δ	Result	Day 0	Day 60	Δ	Result
G1 H	0.88±0.01	0.82±0.01	-7	P<0.01**	0.75± 0.06	0.77±0.07	+2.6	P>0.05	4. 77± 0.39	4. 22± 0.32	-11. 54	P>0.05
G2 H	-	-	-	-	0.74± 0.06	0.94±0.05	+27	P<0.01**	5. 47±0.4	4. 13± 0.4	-24. 5	P<0.01**
G3 H	0.89±0.01	0.91±0.01	+2. 25	P<0.05*	-	-	-	-	5. 51± 0.4	5. 21±0.42	-5. 5	P>0.05
G4 H	0.85±0.01	0.96±0.02	+12.94	P<0.001***	0.84± 0.08	1. 16±0.09	+38	P<0.05*	5. 54±0.54	4. 37±0.14	-21. 2	P<0.05*
G1 N	0.89± 0.01	0.93±0.02	+4.5	P<0.05*	0.73±0.05	0.85±0.06	+6	P<0.01 **	4. 8±0.52	3. 92±0.37	-19. 4	P>0.05
G2 N	-	-	-	-	0.71±0.05	0.93±0.08	+30.9	P<0.01 **	4. 38±0.38	3. 58±0.18	-18. 3	P<0.05*
G3 N	0.89±0.01	0.92± 0.01	+3. 4	P<0.001***	-	-	-	-	4. 18±0.18	3. 64±0.16	-12. 92	P<0.01**
G4 N	0.88±0.02	1.02±0.04	+15	P<0.001***	0.79±0.09	1. 32±0.12	+67	P<0.001***	5. 20±0.56	3. 41±0.22	-34. 5	P<0.05*

AA concentration of the control group is compared to the basal (Day 0) and final (Day 60) AA levels of each group of patients with student t-test. The basal and the final AA concentrations of G1H, G2H, G1N, G2N were significantly lower than those of thecontrol group. But in group G4H and G4N the basal differences were changed as follows: in G4H $p<0.001 \rightarrow NS$, in G4N $p<0.001 \rightarrow NS$.

A-T concentration of the control group is compared to the basal (Day 0) and final (Day 60) A-T levels of each group of patients with student t-test. The basal and the final A-T concentrations of G1H, G3H, G4H, G1N, G3N were significantly lower than those of the control group. Only the difference in basal A-T levels of G4N compared to the control group became insignificant on day 60. The statisticaly difference was changed as $p<0.001 \rightarrow NS$.

Although the final MDA concentrations of G1N, G2N, G3N were statistically decreased on day 60, they were still significantly higher than those of the control group. The statistically differences were changed as follows: in G1N $p<0.01 \rightarrow p<0.05$, in G2N $p<0.01 \rightarrow p<0.05$, G3N $p<0.001 \rightarrow p<0.01$. Only in G4N, the basal MDA level decreased to a level similar to the control group ($p<0.01 \rightarrow NS$).

The data in tables 5, 6, 7 are shown in figure 1: A, B, C.

The control group is illustrated in the first column of each figure. S1 shows the basal (Day 0) and S2 shows the final (Day 60) values of AA and A-T in A and B. Although it is not shown it is the same in C.

Table 6. AA concentration of the control group is compared to the basal (Day 0) and the final (Day 60) AA levels of each group of patients with student t-test. The values are shown as mean±SEM. NS: Non significant.

		AA (mg/dl)	
Control		1. 31 ± 0. 05	Result
G1H	Day 0 (n=11)	0.75 ± 0.06	P<0.001***
	Day60 (n=11)	0.77 ± 0.07	P<0.001***
G2H	Day 0 (n=11)	0.74 ± 0.06	P<0.001***
	Day60 (n=10)	0.94 ± 0.05	P<0.001***
G4H	Day 0 (n=9)	0.84 ± 0.08	P<0.001***
	Day 60 (n=9)	1.16 ± 0.09	NS
G1N	Day 0 (n=9)	0.73 ± 0.05	P<0.001***
	Day 60 (n=8)	0.85 ± 0.06	P<0.001***
G2N	Day 0 (n=9)	0.75 ± 0.05	P<0.001***
	Day 60 (n=9)	0.93 ± 0.05	P<0.001***
G4N	Day 0 (n=10)	0.79 ± 0.09	P<0.001***
	Day 60 (n=10)	1.32 ± 0.12	NS

Table 7. A-T concentration of the control group is compared to the basal (Day 0) and the final (Day 60) A-T levels of each group of patients with student t-test. The values are shown as mean±SEM.NS: Non significant.

	A-T(mg/dl)	
Control	1. 08 ± 0.03	Result
G1 H Day 0 (n=11)	0.88 ± 0.01	P<0.001***
Day60 (n=11)	0.82 ± 0.01	P<0.001***
G3 H Day 0 (n=10)	0.89 ± 0.01	P<0.001***
Day60 (n=9)	0.91 ± 0.01	P<0.01***
G4 H Day 0 (n=10)	0.85 ± 0.01	P<0.01***
Day 60 (n=9)	0.96 ± 0.02	P<0.01**
G1 N Day 0 (n=9)	0.89 ± 0.01	P<0.001***
Day 60 (n=8)	0.93 ± 0.02	P<0.01**
G3 N Day 0 (n=10)	0.89 ± 0.01	P<0.001***
Day 60 (n=9)	0.92 ± 0.01	P<0.001***
G4 N Day 0 (n=10)	0.88 ± 0.02	P<0.01***
Day 60(n=10)	1.02 ± 0.04	NS

Table 8. MDA concentration of the control group is compared to the basal (Day 0) andfinal (Day 60) MDA levels of each group of patients with student t-test. The values are shown as mean±SEM. NS: Non significant.

	MDA (nmol/ml)	
Control	2. 96 ± 0.13	Result
G1 H Day 0 (n=11)	4.77 ± 0.39	P<0.001***
Day60 (n=11)	4.22 ± 0.32	P<0.001***
G2 H Day 0 (n=10)	5.47 ± 0.40	P<0.001***
Day60 (n=10)	4.13 ± 0.23	P<0.001***
G3 H Day 0 (n=10)	5.51 ± 0.40	P<0.001***
Day 60 (n=10)	5.21 ± 0.42	P<0.001***
G4 H Day 0 (n=10)	5.54 ± 0.54	P<0.001***
Day60 (n=10)	4.37 ± 0.14	P<0.001***
G1 N Day 0 (n=9)	4.80 ± 052	P<0.01**
Day 60 (n=9)	3.92 ± 0.37	P<0.05*
G2 N Day 0 (n=9)	4.38 ± 0.38	P<0.01**
Day 60 (n=9)	3.58 ± 0.18	P<0.05*
G3 N Day 0 (n=9)	4.18 ± 0.18	P<0. 001***
Day 60 (n=9)	3.64 ± 0.16	P<0.01**
G4 N Day 0 (n=10)	5.20 ± 0.56	P<0.01**
Day 60 (n=10)	3.41 ± 0.22	NS

Figure 1(A): The basal AA levels of G4H and G4N increased to a level similar to the control group (G4H p<0.001→NS, G4N p<0.001→NS).

Figure 1(B): The basal AT levels of G4N increased to a level similar to the control group (G4N p<0.001→NS).

Figure 1(C): Only in G4N the basal MDA level decreased to a level similar to the control group (p<0.01→NS).

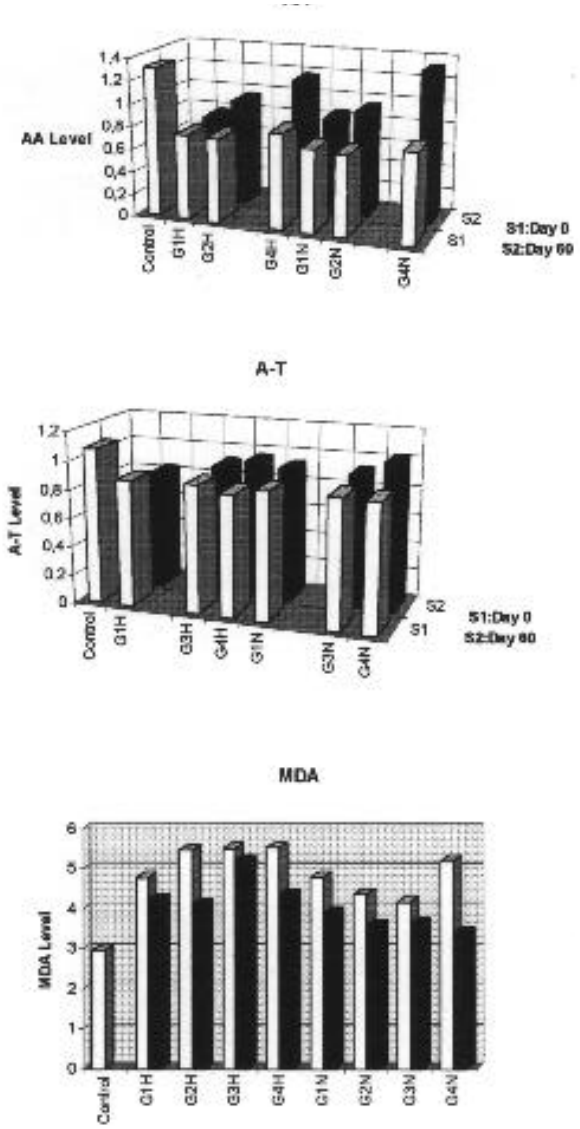

Figure 1. The basal (Day 0) and the final (Day 60) AA, A-T and MDA levels of each treatment group were compared to the control group by student t-test (respectively in A, B, C).

Discussion

In this study, in type 2 diabetic patients, increased oxidative factors as a consequence of non-enzymatic glycation were shown by increased MDA concentrations, and weakened anti-oxidant defence system by reduced A-T, A. A and increased MDA/SOD, MDA/GPX ratios as compared to the control group. The benefits of anti-oxidant therapy were proposed as follows:

In hyperglycaemic patients: When A-T or AA was given alone for 60 days their final concentrations were significantly higher than the basal values. Combined AA and A-T supplementation caused an increment in AA concentration reaching a similar

level to that in the control group, so the initial difference between patients and controls became insignificant. Nevertheless the increment in A-T concentration was not enough to reach a similar A-T level to the in control group. If AA and A-T were given in combination, the increments in their concentrations were higher than when supplemented separately.

Supplementation of AA alone or in combination with A-T caused significant reductions in MDA levels in patients. But these reductions were not enough because MDA levels of the patients were still significantly higher than in the healhy control group.

In normoglycaemic patients: Without AA or A-T supplementation, glycaemia control besides a balanced diet, AA and A-T concentrations increased significantly after 60 days but MDA concentration did not change.

When AA or A-T was given alone, the increment of each vitamin concentration in percentage was higher than in the hyperglycaemic groups. Although they were increased, final A-T or AA were still lower than in the control group.

After supplementation of both vitamins in combination, the final A-T and AA concentrations reached a similar level to the control group.

MDA concentrations decreased in each normoglycaemic group after 60 days. Diet alone caused a weak, A-T caused a mild, AA caused a moderate, but combined AA and A-T vitamins caused the highest decrement in MDA levels. So glycaemia control together with combined AA and A-T supplementation may strengthen the anti-oxidant defence system by increasing AA and A-T concentrations to similar levels to the control group.

Oxidative stress may be defined as a measure of the steady state level of reactive oxygen or oxygen radicals in a biological system. Possible sources of oxidative stress and damage to proteins in diabetes mellitus include free radicals generated by autoxidative reactions of glucose and glucose adducts to proteins as a result of non-enzymatic glycation. Autoxidation of polyunsaturated free fatty acids in plasma and membrane proteins also contributes to this (12). Oxydative stress may be amplified by a continuing cycle of metabolic stress, tissue damage and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems, which further exacerbate the oxidative stress (13). In the present study, we have shown that type-2 diabetic patients had lower concentrations of AA and A-T which act in the anti-oxidant defence system and increased MDA concentrations as the product of lipid oxidation.

Glucose, glycated proteins and advanced glycation end products cause excessive production of superoxide anion. SOD is the first line enzyme which converts superoxide to hydrogen peroxide (14, 15) Hydrogen peroxide is converted to hydroxyl anion by metallo enzymes. Neither superoxide nor hydrogen peroxide are harmful like hydroxyl anion. Hydrogen peroxide is decomposed by GPX or by catalase. GPX and AT are inhibitors of peroxidation of polyunsaturated acids in biologic membranes. GPX inhibits the lipid peroxidation at the initial stages or if the reaction occurs, gluathione is used as an electron donor by GPX to stop the peroxidation reaction. AT is the most abundant lipid soluble antioxidant in the body (16). It is known as a chain breaker in lipid peroxidation chain reactions (17).

AA is the most abundant water soluble antioxidant. It has a sparing effect on A-T by reducing A-T radical to A-T.

Free radicals, due to their reactive nature, are short lived, so indirect methods of measurement are employed in clinical studies, including the demonstration of reduced plasma and intracellular antioxidant concentrations or the measurement of free radical damage to endogenous substrates such as lipids, often measured as lipid peroxides. Conjugated dienes, such as MDA reflect the amount of lipid peroxidation. Oxidative stress is evaluated by the combined measurement of several antioxidants (intracellular SOD, GPX, Catalase, AA or A-T) together with lipid peroxidation end products (MDA) (18, 19).

The ratios of MDA to SOD and to GPX respectively were suggested to indicate the ratio of oxidant factors to the anti-oxidative defence system (20). Both MDA and MDA/SOD, MDA/GPX

were higher in type-2 diabetic patients than in the control cases in this study.

HbA1c is a valuable index of hyperglycaemia and protein glycation of the previous 3-4 months (2). It is also shown to be a source of free radicals by reducing molecular oxygen (3, 21). Superoxide, hydrogen peroxide and lipid peroxides are precursors to more reactive species such as hydroxyl radicals. There is no evidence that once oxidative damage occurs, it may be reversed by chemical or enzymatic reduction. The kinetics of turnover of the protein molecule appears to be the critical factor limiting the accumulation of oxygen radical damage. Since collagen is a long lived protein molecule, products of radical reactions may accumulate with time and cause alterations in protein structure and function. These oxidation products may contribute to the development of chronic complications. Glycation of lysine and hydroxylysine residues of collagen, forms fructoselysine and fructosehydroxylysine and their oxidation products are shown as N-(carboxymethyl)lysine and N-(carboxymethyl) hydroxylysine. Because these products accumulate in collagen normally as a function of age and at an accelerated rate in diabetes, diabetes may be described as accelerated ageing (13). So the results of the clinical studies about oxidative stress in diabetes mellitus may be related to the differences of patients' age, body mass index, type of diabetes mellitus, duration of illness, the degree of glucose regulation and the existance of a concomittant chronic or inflammatory illness. Therefore it was important for us to form a homogeneous group of patients with the control group.

The reduced concentrations of A. A and A-T may be the evidence of a weakened anti-oxidant defence system in type-2 DM. Similar results have been reported in the literature (19, 20-26).

There are variable results about the SOD activity for instance McRury and et al., reported that diabetic SOD activity was not statistically different from that in control group as we have found (19, 23), but L. L. Tho et al., and some other investigators have found decreased SOD activity in diabetic patients (22, 27, 28). Total SOD concentration was found to be high while its activity decreases because of glycation (29).

The results about GPX activity are also variable so that L. L. Tho showed decreased GPX activity but Kaji et al., showed increased plasma GPX activity while erytrocyte GPX activity was not different from that of the control group. Fujiwara et al., reported reduced SOD activity and increased lipid peroxidation and no difference in GPX activity in diabetic patients (30). Decreased concentrations of A. A and A-T were reported, as we have shown (30-36).

The depletion of A. A in diabetic patients may be related to its role as a scavenger of free radicals. On the other hand chronic hyperglycaemia inhibits Na-K ATP-ase activity, so renal tubular A. A reabsorbtion is inhibited. In healthy human beings, erytrocytes and leucocytes take up dehydroascorbic acid (DHAA) to reduce to A. A intracellularly. Since glucose and DHAA are shown to be transported by the same transporter, chronic hyperglycaemia competetively inhibits DHAA transport and A. A recruitment (37). Therefore DHAA loss in the urine and so total body A. A loss may be the consequence of impaired cellular DHAA uptake (38). This inverse relationship between glycaemic control and intracellular A. A concentration requires restoration of glycaemic control and A. A replacement therapy. Glycaemia regulation, itself, may cause a significant increase in A. A concentration as we have shown in our study.

A-T is consumped in lipid peroxidation chain reactions as a chain breaker. In the lack of A. A, recruitment of A-T from the A-T radical is inhibited, so A-T may be reduced in diabetic patients. This mechanism may be the probable reason that A-T was not as effective as AA or combined AA and A-T in lowering MDA in this study. A-T also helps metabolic control of hyperglycaemia because its membrane stabilisation effect is shown to increase the sensivity of insulin receptors (39). Both A. A and A-T are shown to inhibit the early phase of non-enzymatic glycation (4, 40, 41) All these findings suggest that anti-oxidant therapy with A. A and A-T may be useful in complementing existing glycaemia control especially in those with long-term complications.

In conclusion, The benefits of anti-oxidant therapy were proposed because combined AA and A-T

supplementation together with glycaemic control resulted in the highest significant reduction in MDA levels and the highest significant increments in AA and A-T levels. If augmented free radical activity is one of the mechanisms leading to chronic complications, then antioxidant therapy may help to decrease the cytotoxic end products of lipid peroxidation.

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