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CHANGES IN PLASMA LIPIDS AND ANTIOXIDANT ENZYMES IN INSULIN RESISTANT DIABETIC RETINOPATHY: EFFECTS OF NATURAL ANTIOXIDANTS SUPPLIMENTATION

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ABSTRACT
Plasma lipids and antioxidant enzymes were assayed in uncomplicated diabetes mellitus, type 2 and in diabetic retinopathy patients. According to their plasma insulin status these groups were further subdivided into normoinsulin groups and insulin resistant groups. Oxidative stress was present in all the groups as evident by increased malonaldehyde and decreased ascorbic acid/ dehydroascorbic acid ratio. Triglyceride, very low density lipoprotein cholesterol and triglyceride/ high density lipoprotein cholesterol molar ratio have been found to be highly increased along with superoxide dismutase in insulin resistant retinopathy patients. The insulin resistance was found to be maximum in this group which paralleled the increased level of dehydroascorbic acid. Oral supplementation of Ascorbic acid, Vitamin E and mixed carotenoids could normalize all the parameters in the different groups except in insulin resistant diabetic retinopathy group where the values remained partially elevated. The prognostic value of the elevated parameters and the role of insulin vis-à-vis oxidative stress have been discussed.

KEYWORDS: Insulin resistance, Dehydroascorbic acid, Antioxidant enzymes, Plasma lipids, Diabetic Retinopathy.

INTRODUCTION
Production of reactive oxygen species in the body and antioxidant defenses are approximately balanced in a healthy individual. A tilting of balance in favor of reactive oxidation species can create the situation of oxidative stress leading to major cellular damage. Premature infants who were associated with high oxygen concentration while placed in an incubator showed an abrupt increase in the incidence of eye damages. The oxygen toxicity in these infants was later attributed to formation of oxygen radicals which could be decreased by careful monitoring of oxygen as well as administration of α tocopherol.[1,2] Oxidative stress is greatly increased in diabetes mellitus because of prolonged exposure to hyperglycemia. The advanced glycosylation products produced by these redox reactions have been implicated in the development of diabetic complications.[3] Lipid peroxides and thiobarbituric acid reactivity as the markers of oxidative stress have been found to be increased in diabetes.[4] Superoxide dismutase (SOD) can remove superoxide by accelerating its rate of conversation to H_2O_2 by about forty folds at pH 7.4 while H_2O_2 can be removed predominantly by glutathione peroxidase (GP_X) and assisted by glutathione reductase (GR).[5]

On the other hand, free radicals have been implicated in the metabolism of lipids also. Besides formation of lipid peroxides, free radicals react with low density lipoprotein (LDL) producing oxidized LDL which was which was found to be associated with microvascular complications.[6,7]

The natural antioxidants e.g. ascorbic acid, Vitamin E and mixed carotenoids have been found to quench the free radicals; but their effectiveness and mode of action differ widely. Thus it was of interest to note the role of oxidative enzymes and plasma lipids in diabetes and in one of its complications, diabetic retinopathy. As insulin resistance is associated with diabetes mellitus very often[8], both the experimental groups were divided into insulin-sensitive and insulin-resistant groups for the study. Each of the three natural antioxidants was

supplemented with the food of the subjects of study to explore their role in the mechanism of oxidative stress.

MATERIALS AND METHODS

Thirty five normal adult individual, forty three diabetic patients of Type 2 category (NIDDM) of less than fifteen years duration without any complication and sixty seven diabetic retinopathy patients of more than fifteen years duration were selected for the project after a detailed clinical and biochemical check up. On ophthalmological examinations the retinopathy patients revealed microaneurism exudates (hard and soft), retinal hemorrhage, venous obstruction and neovascularisation. All the subjects were male aged between 55 to 70 with a body mass index (BMI) between 22 to 24. The implication of the project was explained to them and consent was obtained from them. They were asked to take only their maintenance drugs for seven days prior to the experiment. A 12 hours fasting blood samples were collected from all the subjects in the morning and analyzed immediately for different biochemical parameters e.g. plasma ascorbic acid (AA)[9] and dehydroascorbic acid (DHAA)[9], immunoreactive insulin (INS)[10], malonaldehyde (MDA)[11], superoxide dismutase (SOD)[11], glutathione peroxidase (GP_X)[11], glutathione reductase (GR)[11], triglyceride (TG)[12], cholesterol (CH)[12], low density lipoprotein cholesterol (LDLC)[12], very low density lipoprotein cholesterol (VLDLC)[12] and high density lipoprotein cholesterol (HDLC).[12]

The uncomplicated diabetic group and the diabetic retinopathy group were further subdivided into (a) normoinsulin diabetic group (23 cases), (b) insulin resistant diabetic group (20 cases), (c) normoinsulin diabetic retinopathy group (33 cases) and (d) insulin resistant diabetic retinopathy group (34 cases). This was done according to their insulin status where a fasting level of insulin more than 20µIU/L is considered as hyperinsulinemic.[13] The degree of insulin resistance was determined by homeostatic model assessment index (HOMA).[12] As no differences in biochemical parameters were observed between the diabetic groups and normoinsulin diabetic retinopathy group, only diabetic retinopathy groups were selected for further study on the role of antioxidants. These groups were further divided into three subgroups. One group was asked to take ascorbic acid 500mg twice daily; another to take Vitamin E 400 IU twice daily and the third group to take mixed carotenoids containing 3mg lutein and zeaxanthine, 2mg β carotin and 100mg carrot extract twice daily. The project was continued for six weeks after which their blood samples were analyzed again as usual and the parameters were compared with the earlier observations, The dosage of the antioxidants employed in this project were in consonant with the other works.[14] The whole project was approved by ethical committee of the institution.

RESULTS

Diabetic groups showed higher TG and VLDLC ($p < 0.001$) with no change in SOD and GR levels were observed when compared with normal control. A decrease in GP_X and AA/ DHAA ratio and high MDA were evident in this group ($p < 0.001$). (Table – I).

In diabetic retinopathy patients TG, VLDLC and MDA ($p < 0.001$) with SOD ($p < 0.02$) were found to be increased with a decrease in AA/ DHAA ratio and GP_X ($p < 0.001$) when compared with normal control. (Table – I).

When diabetic group was compared as a whole with the diabetic retinopathy group, the retinopathy group showed increased TG and VLDLC ($p < 0.01$), MDA ($p < 0.02$) and SOD ($p < 0.001$) with a decreased AA/ DHAA ratio ($p < 0.001$) in relation to uncomplicated diabetic group. (Table – I).

When the uncomplicated diabetic group was divided according to their insulin status, the normoinsulin group did not differ much from hyperinsulin group except their insulin status. (Table – II).

When diabetic retinopathy patients were divided according to their insulin status, the hyperinsulic group showed higher levels of TG, CH, LDLC, VLDLC, MDA ($p < 0.001$) and HDLC ($p < 0.01$) and decreased AA/ DHAA ratio when compared with notmoinsulin group. No change was observed in GP_X and GR levels. (Table – II).

In normoinsulin diabetic retinopathy group, ascorbic acid supplementation decreased TG, VLDLC ($p < 0.01$) and MDA ($p < 0.001$) with an increase in AA/ DHAA ratio ($p < 0.001$) and in GP_X level ($p < 0.02$) showing no change in SOD activity. Vitamin E administration in this group decreased MDA, TG ($p < 0.001$) and VLDLC ($p < 0.01$). Increased AA/ DHAA ratio ($p < 0.05$) was also observed. Mixed carotenoids supplementation could decrease TG, VLDLC ($p < 0.001$) and MDA ($p < 0.001$) with an increase in GP_X ($p < 0.05$) and AA/ DHAA ratio ($p < 0.01$). (Table – III).

In insulin resistant diabetic retinopathy group, ascorbic acid supplementation showed no change on the plasma lipids levels. SOD was restored to normal level ($p < 0.01$) with an increase in GP_X and AA/ DHAA ratio ($p < 0.001$) and a decrease in MDA level ($p < 0.001$).

Vitamin E supplementation in this group could decrease MDA ($p < 0.001$) and SOD ($p < 0.01$) with an increase in AA/ DHAA ratio ($p < 0.001$). GP_X was restored to almost normal level ($p < 0.02$).

Mixed carotenoids supplementation in this group decreased MDA ($p < 0.001$) and SOD ($p < 0.01$). AA/ DHAA ratio and GP_X levels improved significantly ($p < 0.001$). (Table – IV).

TABLE – I: Biochemical Parameters in Normal Control, Uncomplicated Diabetes Mellitus and Diabetic Retinopathy.

Parameters	Unit	Normal Control	Uncomplicated Diabetes Mellitus	Diabetic Retinopathy
MDA	nmol/ml	1.03 ± 0.2	2.6 ± 0.5	3.1 ± 0.5
SOD	U/g Hb	962 ± 138	924 ± 47	1035 ± 119
GP_X	U/g Hb	55 ± 14	41 ± 6	40 ± 7
GR	U/g Hb	6.7 ± 1.5	6.2 ± 0.5	6.4 ± 7
TG	mg/dl	108 ± 13	130 ± 37	168 ± 69
CH	mg/dl	181 ± 15	190 ± 24	189 ± 30
HDLC	mg/dl	46 ± 4	49 ± 5	48 ± 6
LDLC	mg/dl	114 ± 12	115 ± 22	110 ± 24
TG/HDLC Ratio		1.63	1.24	1.53
AA/DHAA Ratio		Above 50	2.9	2.3
HOMA INDEX		4.1	10.5	20.6

Values are Mean ± SD

TABLE – II: Comparative Study of Biochemical Values in Diabetes and Diabetic Retinopathy with or without Insulin Resistance.

Parameters	Unit	Diabetes without Insulin Resistance	Diabetes with Insulin Resistance	Retinopathy without Insulin Resistance	Retinopathy with Insulin Resistance
MDA	nmol/L	2.6 ± 0.5	2.6 ± 0.5	2.7 ± 0.2	3.4 ± 0.5
SOD	U/g Hb	929 ± 52	918 ± 40	963 ± 100	1105 ± 91
GP_X	U/g Hb	42 ± 6	41 ± 7	41 ± 7	38 ± 8
GR	U/g Hb	6.1 ± 0.6	6.3 ± 0.4	6.4 ± 0.7	6.4 ± 0.7
TG	mg/dl	129 ± 32	130 ± 41	139 ± 29	197 ± 83
CH	mg/dl	188 ± 23	192 ± 25	174 ± 24	204 ± 27
HDLC	mg/dl	50 ± 6	48 ± 4	46 ± 5	50 ± 6
LDLC	mg/dl	112 ± 23	118 ± 23	101 ± 20	120 ± 23
VLDLC	mg/dl	26 ± 6	26 ± 8	27 ± 6	34 ± 8
TG/HDLC Ratio		1.13	1.20	1.32	1.72
AA/DHAA Ratio		2.9	3.1	2.9	1.8
HOMA INDEX		10.0	10.8	6.0	38.5

Values are Mean ± SD

TABLE – III: Effects of Antioxidants on the Biochemical Parameters of Diabetic Retinopathy Patients without any Insulin Resistance.

Parameters	Unit	Before AA Supplementation	After AA Supplementation	Before Vitamin E Supplementation	After Vitamin E Supplementation	Before Carotenoids Supplementation	After Carotenoids Supplementation
MDA	nmol/L	2.7 ± 0.3	1.3 ± 0.3	2.8 ± 0.2	1.2 ± 0.1	2.6 ± 0.2	1.1 ± 0.1
SOD	U/g Hb	982 ± 112	933 ± 96	957 ± 112	942 ± 93	945 ± 58	947 ± 49
GP_X	U/g Hb	38 ± 7	45 ± 5	42 ± 7	46 ± 6	44 ± 4	48 ± 4
GR	U/g Hb	5.7 ± 0.6	5.8 ± 0.4	6.8 ± 0.4	6.3 ± 0.6	6.7 ± 0.5	6.5 ± 0.5
TG	mg/dl	133 ± 30	96 ± 20	143 ± 32	84 ± 23	141 ± 21	106 ± 11
CH	mg/dl	174 ± 28	170 ± 21	163 ± 22	160 ± 15	185 ± 13	179 ± 8
HDLC	mg/dl	46 ± 5	47 ± 5	43 ± 5	44 ± 5	48 ± 4	49 ± 3
LDLC	mg/dl	101 ± 25	104 ± 19	93 ± 17	99 ± 12	109 ± 13	109 ± 7
VLDLC	mg/dl	27 ± 6	19 ± 4	27 ± 6	17 ± 5	28 ± 4	21 ± 2
TG/HDLC Ratio		1.26	0.9	1.46	0.84	1.28	0.9
AA/DHAA Ratio		2.9	11.0	3.0	4.3	2.9	3.4
HOMA INDEX		4.3	4.1	7.8	7.5	10.5	4.7

Values are Mean ± SD

TABLE – IV: Effects of Antioxidants on the Biochemical Parameters of Diabetic Retinopathy Patients with Insulin Resistance.

Parameters	Unit	Before AA Supplementation	After AA Supplementation	Before Vitamin E Supplementation	After Vitamin E Supplementation	Before Carotenoids Supplementation	After Carotenoids Supplementation
MDA	nmol/L	3.42 ± 0.5	1.24± 0.2	3.3 ± 0.5	1.23 ± 0.2	3.45 ± 0.2	1.17 ± 0.1
SOD	U/g Hb	1078 ± 86	961 ± 50	1136 ± 99	1002 ± 71	1100 ± 74	962 ± 94
GP_X	U/g Hb	44 ± 6	50 ± 4	39 ± 8	48 ± 5	34 ± 5	43 ± 3
GR	U/g Hb	6.9 ± 0.6	6.7 ± 0.6	6.0 ± 0.7	6.2 ± 0.4	6.4 ± 0.6	6.2 ± 0.5
TG	mg/dl	222 ± 102	182 ± 67	185 ± 78	154 ± 51	183 ± 49	152 ± 32
CH	mg/dl	204 ± 27	189 ± 14	210 ± 35	194 ± 19	199 ± 11	192 ± 10
HDLC	mg/dl	50 ± 5	50 ± 4	52 ± 8	51 ± 6	48 ± 3	49 ± 2
LDLC	mg/dl	118 ± 24	106 ± 13	126 ± 25	114 ± 15	117 ± 15	113 ± 13
VLDLC	mg/dl	36 ± 7	33 ± 7	32 ± 8	29 ± 7	34 ± 7	30 ± 7
TG/HDLC Ratio		1.93	1.59	1.56	1.3	1.78	1.35
AA/DHAA Ratio		1.8	7.5	1.9	3.3	1.8	3.0
HOMA INDEX		29.8	15.1	47.6	20.8	38.0	21.0

Values are Mean ± SD.

DISCUSSION

It is quite evident from the results obtained in the present study that the state of insulin levels plays a major role in the interactions of antioxidants and oxidative stress. The increased susceptibility of diabetic to vascular complications may, in part, be related to the increased rates of lipid peroxidation as evidence by increased MDA and reduced level of GP_X. GP_X is one of the enzymes responsible for the removal of H_2O_2 produced as part of cellular metabolism. The unaltered GR level confirmed that the supply of reducing equivalents was not hampered; but due to low GP_X, accumulated H_2O_2 was tackled by ascorbic acid which by converting a part into dehydroascorbic acid maintained the level of oxidized glutathione. The increased activity of SOD found in hyperinsulinic retinopathy group was probably to cope up with increased oxidative stress. SOD has been considered a part of the natural defense mechanism by acting as a scavenger for the toxic superoxide radicals. In uncomplicated diabetes the body adapted itself gradually to increasing oxidative stress and a homeostatic balance was maintained till it reached a saturation point. In insulin resistant diabetic retinopathy high levels of MDA and DHAA were countered by increased SOD activity. Antioxidants by decreasing the MDA and DHAA levels lessened the load of oxidative stress bringing back the SOD activity to normal level.

Though cholesterol level was unresponsive to AA and other antioxidants, TG and VLDLC were found to be diminished by all the antioxidants. AA has been found to enhance the lipoprotein lipase activity.[15] It is likely that this action could be mediated by the redox system. The unresponsive effect of plasma lipids in insulin resistant retinopathy indicates that some of the actions of antioxidants are countered by insulin resistance. It may have some impact on the LDL particle size and thus on the pathophysiological process in diabetic retinopathy leading to vascular disorders. The slight increase in HDLC when expressed in terms of percentage of total cholesterol was found to be insignificant. But high TG and HDLC ratio in the subjects indicated that insulin resistance in diabetic retinopathy may be involved in alteration of LDL size leading to vascular complications. Since a cut-off point of 1.33 has been accepted for most of the patients having small LDLC[16], this ratio gave us some indication in the progression of vascular pathology. The diminished level of TG after the administration of antioxidants could lead to altered size of LDLC particles improving the prognosis of vascular disorders. Its failure to decrease in insulin resistant retinopathy signifies that the mechanism of insulin resistance in some way may have some impact on the LDLC metabolism not unlikely through free radical involvement. The magnitude and duration of insulin resistance seems to be a deciding factor whether the antioxidants will take a longer time or with a higher does for their effectiveness.

A gradual decrease in AA/ DHAA ratio in the patients paralleling high MDA level signified increased oxidative stress which was maximum when the insulin resistance was very high as evidenced by the high HOMA index. The homeostatic balance was maintained as much as possible by AA leading to the formation of DHAA which is the reversibly oxidized form of AA. Thus a low AA/ DHAA ratio could be utilized to predict the prognostic outcome in the disorder of free radical metabolism. Though the antioxidants combat the oxidative stress almost in a similar manner, Vitamin E and mixed carotenoids could spare the action of AA as evidenced by higher AA/ DHAA ratio.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval
All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Institutional research committee.

Informed Consent
Informed consent was obtained from all the participants individually who were included in the study.

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