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OXIDATIVE CLEARANCE IN AGE REALATED MACULAR DEGENERATION: EFFECTS OF NATURAL ANTIOXIDANTS SUPPLIMENTATION

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ABSTRACT
Malonaldehyde and dehydroascorbic acid were found to be highly increased in Age Related Macular Degeneration, a retinal disorder, suggesting intense oxidative stress in this condition. Superoxide dismutase was also found to be increased probably as a part of natural defensive mechanism against oxygen damage. Reduced level of gluthione peroxidise in this condition seems to be associated with lipid peroxidation. The abnormal glucose utilisation and insulin response to glucose load found in the study cases has been attributed to pancreatic dysfunction and/or receptor-mediated mechanism which could be the toxic manifestation of dehydroascorbic acid. High triglyceride, very low density lipoprotein cholesterol and a high triglyceride to high density lipoprotein cholesterol ratio suggest the relevant risk of clinical vascular disorders. Ascorbic acid, Vitamin E and mixed carotenoids supplementation separately to these patients could reverse the altered biochemical parameters more or less in the similar fashion. The mode of action of these antioxidants probably cantered around the free radical metabolism.

KEYWORDS: Age Related Macular Degeneration, Oxidative Stress, Antioxidants, Plasma Lipids, Dehydroascorbic Acid.

INTRODUCTION
Age Related Macular Degeneration (ARMD) is a degeneration of retina and this retinal pigment epithelium in the macular region occurring late in life; it is the leading cause of new cases of legal blindness and hardly any treatment is available for most of the patients. The events in ARMD have been associated with the generation of free radicals.[1]

Enzymes, Vitamin C and Vitamin E, β Carotene and mixed carotenoids are capable of converting free radicals to stable compounds before they can inflict major damages on the cell membranes. Superoxide dismutase (SOD) converts superoxide radicals to oxygen and hydrogen peroxide. Gluthione peroxidise (GP_X) continues this process of detoxifying the free radicals by converting the hydrogen peroxide to water. GP_X is also capable of converting the lipid peroxyl radicals formed in cell membranes to non radical lipids thereby preventing the cellular damage caused by these free radicals. When the balance between the free radical generation and quenching by antioxidant agents is disrupted the development of ARMD occurs. The vulnerability of retina and retinal pigment epithelium in ARMD has been attributed to low antioxidant protection of foveal crest because of low Vitamin E and caritenoids concentration at the foveal center.[2]

Oxidative stress has been found to be greatly increased in diabetes of prolonged exposure to hyperglycemia. The advanced glycated end products produced by the redox reaction have been implicated in the pathology of diabetic complications e.g. retinopathy and nephropathy.[3] Thus it was of interest to study some biochemical features in ARMD e.g. oxidative enzymes, insulin and glucose response to a glucose load and plasma lipids along with oxidative stress biomarkers malonaldehyde and dehydroascorbic acid. The presence of oxidised low density lipoprotein in vivo has been established and antibody titres against this product correlate well with the progression of atherosclerosis.[4] As such it was necessary to study the role of dietary supplementation of antioxidants e.g. ascorbic acid, Vitamin E and mixed carotenoids on the biochemical parameters to explore the biochemical events in ARMD.

MATERIALS AND METHODS

Thirty five (35) adult normal individual aged between 55 to 70 were selected as control for the study. They did not have any immediate past illness and their biochemical and haematological parameters were within normal range. Their body mass index was between 22 to 24. A group of thirty five (35) patients suffering from ARMD, exudative type, of the same age group and body mass index were selected for the study. They were diagnosed on the basis of Macular Photocoagulation Study (MPS) group criteria e.g. Best Corrected Visual Acquity (BCVA) of either eye 6/9 or worse, retinal pigment epithelium abnormalities, drusen etc, All the subjects were male; this was to avoid the sex variation criteria. They were informed about the implication of the study and consent was obtained from them. All the subjects were asked not to smoke and not to take any medicine and/ or vitamins besides the maintenance dose of antihypertensive drug where required/ during the study period. Some emergency ophthalmic drugs were allowed when needed. All the subjects were asked to fast overnight for 10 – 12 hours and fasting blood samples were collected in the morning and analysed for different biochemical parameters. The subjects were then asked to take a glucose drink containing 75 gm of glucose and blood samples were withdrawn exactly after one hour and again on two hours for analysis. Fasting samples were analysed for Glucose (FPG)[5], Cholesterol (CH)[6], Low Density Lipoprotein Cholesterol (LDLC)[6], Very Low Density Lipoprotein Cholesterol (VLDLC)[6], High Density Lipoprotein Cholesterol (HDLC)[6], Triglyceride (TG)[6], Insulin (FINS)[7], Superoxide dismutase (SOD)[8], Glutathione Peroxidase (GP_X)[8], Glutathine Reductase (GR)[8], Ascorbic acid (AA)[8], dehydroascorbic acid (DHAA)[8] and Malonaldehyde (MDA).[8] The postprandial samples were analysed for one hour Glucose (1HPPS), two hour Glucose (2HPPS), one hour Insulin (1HPPINS) and two hour Insulin (2HPPINS).

The ARMD patients involved in the project were divided into three groups. One group was asked to take supplementary ascorbic acid 500 gm twice daily; the second group was given Vitamin E 400 IU twice daily; and third group was supplemented with mixed carotenoids containing 3 mg Lutein and Zeaxanthine, 2 mg β carotene and 100 mg Carrot extract twice daily. The supplementation continued for six weeks and then again the blood samples were analysed for the parameters studied earlier to observe the effects of each individual antioxidant on these parameters. The doses of the antioxidants used in the experiment were in consonant with other studies.[8]

RESULTS

In ARMD cases, serum TG, HDLC, VLDLC, SOD activity, DHAA, MDA, FPG, 1HPPG, 2HPPG and 2HPPINS were all found to be increased ($p < 0.001$) while AA and GP_X were diminished ($p < 0.001$) when compared with normal controls (Table – I). Ascorbic acid supplementation in ARMD decreased MDA and DHAA ($p < 0.001$), TG, VLDLC and 2HPPINS ($p < 0.01$) and 2HPPG ($p < 0.02$). SOD level restored to normal level ($p < 0.01$). AA ($p < 0.001$) and GP_X level ($p < 0.01$) were found to be increased.

Vitamin E supplementation could decreases MDA, DHAA and SOD ($p < 0.001$), TG and VLDLC ($p < 0.02$). AA ($p < 0.05$) and GP_X ($p < 0.001$) level were observed to be normal.

Almost a similar pattern was observed in mixed carotenoids supplementation. MDA ($p < 0.001$), TG and VLDL ($p < 0.02$), SOD and DHAA ($p < 0.01$) and 2HPPINS ($p < 0.05$) were decreased with an increase in GP_X level ($p < 0.001$) (Table – II).

Table I: Biochemical Parameters in Normal Control and in Age Related Macular Degeneration Patients.

Parameters	Unit	Normal Control	Age Related Macular Degeneration (ARMD)
MDA	nmol/ml	1.03 ± 0.2	3.8 ± 0.4
SOD	U/gm Hb	962 ± 138	1082 ± 90
GP_X	U/ gm Hb	55 ± 14	39 ± 6
GR	U/ gm Hb	6.7 ± 1.5	6.4 ± 0.6
TG	mg/dl	108 ± 13	134 ± 40
CHOL	mg/dl	181 ± 15	191 ± 30
HDLC	mg/dl	46 ± 4	53 ±10
LDLC	mg/dl	114 ± 12	111 ± 27
VLDLC	mg/dl	21 ± 3	27 ± 8
AA	mg/dl	1.01 ± 0.01	0.61 ± 0.1
DHAA	mg/dl	0.002 ± 0.002	0.38 ± 0.05
FPG	mg/dl	89 ± 10	103 ± 17
1HPPG	mg/dl	138 ± 18	185 ± 31
2HPPG	mg/dl	102 ± 13	154 ± 22
FINS	µIU/L	19 ± 8	18 ± 10
1HPPINS	µIU/L	47 ± 20	47 ± 22
2HPPINS	µIU/L	30 ± 10	39 ± 15

Values are Mean ± SD

Table II: Effects of Antioxidants on the Biochemical Parameters of Age Related Macular Degeneration Patients.

Parameter	Unit	Ascorbic Acid Supplementation		Vitamin E Supplementation		Mixed Carotenoids Supplementation	
		Before	After	Before	After	Before	After
MDA	nmol/L	3.5 ± 0.4	1.8 ± 0.3	3.8 ± 0.2	2.0 ± 0.3	4.0 ± 0.3	1.9 ± 0.2
SOD	U/gm Hb	1140 ± 92	1008 ± 85	1035 ± 49	907 ± 38	1062 ± 84	935 ± 65
GP_X	U/gm Hb	39 ± 4	52 ± 5	38 ± 7	53 ± 5	41 ± 6	53 ± 4
GR	U/gm Hb	6.7 ± 0.6	6.7 ± 0.4	6.4 ± 0.5	6.4 ± 0.4	6.2 ± 0.3	6.3 ± 0.2
TG	mg/dl	140 ± 39	104 ± 12	125 ± 37	93 ± 16	137 ± 42	98 ± 15
CHOL	mg/dl	207 ± 30	189 ± 14	174 ± 24	170 ± 13	186 ± 29	175 ± 17
HDLC	mg/dl	53 ± 11	51 ± 7	49 ± 9	49 ± 8	53 ± 7	52 ± 4
LDLC	mg/dl	124 ± 24	117± 13	104 ± 30	103 ± 17	105 ± 22	103 ± 13
VLDLC	mg/dl	28 ± 8	21 ± 2	25 ± 8	18 ± 3	28 ± 8	20 ± 3
AA	mg/dl	0.63 ± 0.1	1.69 ± 0.06	0.63 ± 0.09	0.71 ± 0.07	0.58 ± 0.04	0.61 ± 0.05
DHAA	mg/dl	0.39 ± 0.03	0.24 ± 0.05	0.39 ± 0.03	0.24 ± 0.05	0.35 ± 0.06	0.25 ± 0.07
FPG	mg/dl	94 ± 13	91 ± 9	112 ± 18	102 ± 10	103 ± 14	98 ± 8
1HPPG	mg/dl	169 ± 27	159 ± 18	199 ± 32	184 ± 19	188 ± 26	181 ± 13
2HPPG	mg/dl	151 ± 14	138 ± 9	160 ± 25	150 ± 20	150 ± 25	137 ± 16
FINS	µIU/L	15 ± 7	15 ± 4	21 ± 10	19 ± 6	20 ± 12	19 ± 6
1HPPINS	µIU/L	48 ± 25	50 ± 11	41 ± 19	43 ± 16	54 ± 20	54 ± 9
2HPIMS	µIU/L	41 ± 14	27 ± 4	34 ± 18	26 ± 9	42 ± 12	32 ± 7

Values are Mean ± SD

DISCUSSION

In ARMD, the oxidative stress was found to be very high as evidenced by high MDA and DHAA. The high SOD level was probably to cope up with the increased oxidative stress. SOD has been considered a part of natural defence mechanism against oxygen damage by acting as a scavenger for the toxic superoxide radical. Antioxidants by decreasing MDA and DHAA could lessen the load of oxidative bringing back the SOD activity to its normal level. The magnitude of an oxidative stress could be assessed by the level of DHAA since it is independently modulated and does not depend on the AA level. It is not unlikely that high DHAA could lead to pancreatic dysfunction and/ or receptor mediated altered insulin action leading to abnormal glucose utilisation. The toxic effect of DHAA could be due to the change in the redox system which may be due to or leading to oxidative stress.[9] Normally gluthione protects AA and no DHAA is formed until all the gluthione is converted to reduced gluthione. In spite of the presence of normal gluthione reductase in this experiment, gluthione concentration was probably low so that AA had to be converted to DHAA. Decreased gluthione content of pancreas has been observed in ascorbic acid deficiency and said to be related to decreased insulin content of pancreas.[9] A diminished tolerance of glucose challenge with a sluggish action of insulin in ARMD could be associated with oxidative stress as antioxidants supplementation in these cases could reverse the situation. A single fasting insulin sample greater than 16.5 µIU/ml has been shown to be inversely correlated with insulin sensitivity.[10] On the other hand hyper insulinemia has been defined as a serum insulin level of 60.4 µIU/ml or more at 120 minutes after 75 gm of glucose challenge.[11] Thus in spite of its sluggishness, the term insulin resistance could not tagged with the biochemical disorders in ARMD. But the impaired glucose utilisation is definitely associated with the biochemical mechanism in ARMD, which were ameliorated by AA and partially by mixed carotenoids with no role of Vitamin E in the process.

Reduced level of GP_X may indirectly lead to lipid peroxidation as evidenced as evidenced by high MDA since lipid hydroperoxides are destroyed by GP_X. The supplies of reduced equivalents were not hampered as GR was found to be normal. The accumulation of reduced glutathione could be relieved by AA which them self was converted to DHAA, altering AA/DHAA ratio. The interrelationship between plasma lipids, ascorbic acid status and insulin activity has been reported earlier. The report showed a diminished glucose tolerance and hypoinsulinism in scorbutic rhesus monkeys with high TG and VLDLC; these could be reversed both by the insulin as well as ascorbic acid suppliments.[12]

The increase in HDLC when expressed in terms of percentage of total cholesterol was found to be insignificant. But TG to HDLC ratio which is related to the processes involved in LDLC size pathophysiology is relevant with regard to the risk of clinical vascular diseases.[13] Thus the diminished levels of TG and VLDLC after antioxidants treatment in ARMD could lead to altered size of the LDLC particles improving the prognosis of the associated vascular disorders. Since all the antioxidants employed in the study had more or less the same biological effect, it is possible that their mode of action centred around the free radical metabolism.

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.

REFERENCES

1. Marshall J. The macula, aging and age related macular degeneration. In J Marshall Ed. The susceptible visual apparatus. Boca Raton F L, CRC Press, 1991; 76-92.
2. Crabtree D V, Adler A J, Snodderly D M. A local minimum Vitamin E in macaque retina is automatically coincident with a common locus of atrophy in human retina. Invest. Opthalmol. Vis. Sci., 1994; 35: 1392.
3. Hunt J V, Wolff S P, Oxidative glycation and free radical production: a casual mechanism of diabetic complications. Free Radic. Res. Commun, 1991; 12 & 13: 115-23.
4. Salonen J T, Yla-Herttuala S, Yamomoto R, et al, Autoantibody against oxidised LDL C and progression of carotid atherosclerosis. Lancet, 1992; 339: 883-87.
5. Goswami K, Mazumdar I, Sridhar K, Narayanan M, Correlation of HbA1C levels with average Estimated blood Glucose levels in improvement of Diabetes Management. Int. J Biotech. Biochem, 2017; 13(3): 205–10.
6. Goswami K, Bandyopadhyay A, Lipid Profile in Middle Class Bengali Population of Kolkata. Ind. J Clin. Biochem, 2003; 18(2): 127-30.
7. Jorgensen K R, Radioimmunoassay of Insulin in plasma and urine in normal subjects. Acta. Endocrinol, 1969; 60: 327-51.
8. Goswami K, Banerjee S K, Ali S, Bhattacharya B, Ascorbic acid therapy in lead exposed Jewellery workers of Bangladesh, 2001; 13(1): 11-6.
9. Banerjee S, Physiological role of dehydroascorbic acid. Ind. J Physiol. Pharmacol, 1977; 21(2): 85-93.
10. Meigs J B, Nathan D M, Wilson P W F, Cupplus L A, Singer D E., Metabolic risks factors worsen continuously across the spectrum of nondiabetic glucose tolerance. Annals. Int. Med., 1998; 128: 524-33.
11. Burchfiel C M, Curb J D, Arakaki R, Abott R D, Sharp D S, Rodriguez B L, Yano K. Cardiovascular risk factors and hyperinsulinemia in elderly men: The Honolulu Heart Program. Annals of Epidemiology, 1996; 6(6): 490-97.
12. Banerjee S, Bandyopathyay A, Plasma lipids in ascorbic acid deficient rhesus monkeys. Amer. J Physiol, 1965; 208: 329-33.
13. Adams A K, Wermuth E O, Mcbride P E, Antioxidant vitamins and the prevention of coronary heart disease. Amer. Family Physician, 1999; 60(3): 895-902.