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Original Article

Oxidative Status and Insulin Resistance in Diabetic Retinopathy: Effect of Natural Antioxidants

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Abstract

The homeostatic model assessment (HOMA) Index was found to be increased both in uncomplicated diabetes mellitus and in diabetic retinopathy indicating the degrees of insulin resistance. Malondialdehyde as a biomarker for oxidative stress has also been found in these groups with a decreased tolerance to glucose load. When these groups were further analyzed and compared according to their insulin status, it was observed that hyperinsulinemia in diabetic retinopathy enhanced the level of oxidative stress with a decrease in plasma ascorbic acid and an increase of plasma dehydroascorbic acid level, paralleling the levels of malondialdehyde in these patients. The role of natural antioxidants e.g. ascorbic acid, Vitamin E and mixed carotenoids in lessening insulin has been discussed. It seems that ascorbic acid is a better antioxidant in comparison to other antioxidants in normalization of insulin resistance and glucose utilization. A persistent long-standing insulin resistance has been suggested as one of the responsible factors for vascular complications in diabetic retinopathy. Dehydroascorbic acid seems to be a better sensitive biomarker for oxidative stress.

Keywords: *Diabetic Retinopathy, Antioxidants, Insulin Resistance, Oxidative Stress, Dehydroascorbic Acid*

Introduction

Diabetic patients are at greatly increased risk of developing atherosclerosis and microvascular disease, manifesting as retinopathy and nephropathy. Increased production of free radicals has been strongly implicated in the pathophysiology of both these conditions (Chawla *et al.* 2016, Cade, WT. 2008). Diabetes mellitus involves a wide range of consequences, including macro and microvascular problems, which lead to the development of severe coronary artery disease in diabetic individuals as opposed to non-diabetic patients (Kumar.*et.al* 2020). Oxidative stress is greatly increased in diabetes because of prolonged exposure to hyperglycemia. This advanced glycosylation and the products produced by these redox reactions have been implicated in the development of diabetic complications (Giacco *et.al*. 2010, Singh *et.al*.2014). Insulin resistance is often a cardinal feature of non-insulin dependent diabetes mellitus (NIDDM). Hyperinsulinemia and insulin resistance which precede by

many years the development of NIDDM have been implicated in the development of diabetic complications (Tangvarasittichai.*et.al.*2015). But no correlation has yet been observed between the extent of hyperglycemia, degree of hyperinsulinemia and the nature of complication in diabetes mellitus. Thus, it was our interest to find out these correlations, if any, in diabetic patients, with or without retinopathy, in relation to a normal control group. The role of some natural antioxidants, e.g. ascorbic acid, Vitamin E and mixed carotenoids were also explored on these relationships.

Materials and Methods

Thirty-five adult normal individual, aged between 55 to 70 years were selected as control for the study. They did not have immediate past illness and their biochemical and hematological parameters were within normal biological reference interval. The body mass index (BMI) were between 22 to 24 Kg/m^2. Sixty-seven patients of the same age group and BMI, suffering from diabetic retinopathy were selected for the study. All these patients were suffering from uncontrolled diabetes mellites of Type II category (NIDDM) for more than 15 years. Ophthalmoscopic examination in these patients with full mydriasis revealed microaneurysms exudates (hard and soft), retinal hemorrhage, venous obstruction and neovascularization. Forty-three cases of uncomplicated NIDDM of less than 15 years duration were also selected for the study to note the difference, if any, between the uncomplicated diabetes mellitus and diabetic retinopathy. They were also of the same age group and BMI as in other groups. To avoid the sex variation criterion, all the subjects selected were male. They were informed about the implication of the study and consent was obtained from them. They were asked not to smoke as to take any medicine and / or vitamins besides the maintenance dose of antihypertensive or any emergency drug, when required.

Thirty-five normal controls, forty-three uncomplicated diabetic patients and sixty-seven diabetic retinopathy patients were involved in the project. All the subjects were asked to fast overnight (10-12 hours) and fasting blood samples were collected in the morning and analyzed 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 analyzed for plasma glucose (FPG)(Ambade.*et.al.*1998), ascorbic acid (AA) and dehydroascorbic acid (DHAA)(Margolis,*et.al.*1990), insulin (FINS)(Besch,*et.al*.1987) and malondialdehyde (MDA)(Banjare,*et.al*.2017). Post prandial samples were analyzed for one-hour plasma glucose (1HPPG), two-hour plasma glucose (2HPPG), one-hour insulin (1HPPINS) and two-hour insulin (2HPPINS).

Both the uncomplicated diabetic group and diabetic retinopathy group were further subdivided into normo-insulin diabetic group, insulin resistant diabetic group, normo-insulin diabetic retinopathy group and insulin resistant diabetic retinopathy group according to their insulin status. As there was not much difference between diabetic groups and the normo-insulin diabetic retinopathy group; only the diabetic retinopathy groups were subjected oral vitamin supplementation. They were further subdivided into three sub groups. One subgroup patientwas asked to take supplementary ascorbic acid 500 mg twice daily for six weeks, another subgroup patientwas given supplementary Vitamin E 400 IU twice daily for six weeks; and the last subgroup patients were fed mixed carotenoids, containing 3 mg lutein and zeaxanthin, 2 mg β carotene and 100 mg carrot, twice daily for six weeks. After six weeks, their blood was withdrawn again and the same parameters, stated before were studied again to see the effect of these antioxidants on these parameters of the experimental groups.

The whole protocols as well as the dosage of vitamins were approved by the ethical committee of the institution.

Results

Both uncomplicated diabetic group and diabetic retinopathy group showed low plasma AA, high DHAA, high MDA and increased glucose values ($p \leq 0.001$) when compared with normal controls.

Fasting insulin (p≤ 0.02), one hour and two-hour PP insulin (p≤ 0.001) were also found to be impaired (Table 1).

Table 1: Comparative Study of Biochemical Values in Diabetes Mellitus and Diabetic Retinopathy in relation to Normal Controls

Parameters	Unit	Normal Control (n = 35)	Diabetic Mellitus (n = 43)	Diabetic Retinopathy (n = 67)
MDA	nmol/ ml	1.03 ± 0.2	2.6 ± 0.5	3.1 ± 0.5
AA	mg/ dl	1.01 ± 0.013	0.76 ± 0.09	0.68 ± 0.1
DHAA	mg/ dl	0.002 ± 0.022	0.26 ± 0.05	0.29 ± 0.06
FPG	mg/ dl	89 ± 10	170 ± 22	178 ± 60
1HPPG	mg/ dl	138 ± 18	300 ± 37	314 ± 47
2HPPG	mg/ dl	102 ± 13	257 ± 51	341 ± 84
F-INS	mIU/ L	19 ± 8	25 ± 8	47 ± 45
1HPPINS	mIU/ L	47 ± 20	76 ± 38	72 ± 43
2HPPINS	mIU/ L	30 ± 10	74 ± 50	72 ± 53

Values as Mean± Standard Deviation (SD)

When the uncomplicated diabetes group were divided according to their insulin status, the hyper insulin group showed only higher one hour and two-hour PP insulin when compared to normo-insulin group (Table 2).

Table 2: Comparative Study of Biochemical Values in Diabetes Mellitus and Diabetic Retinopathy with or without Insulin Resistance.

Parameters	Unit	Diabetic without Insulin Resistance (n = 23)	Diabetic Mellitus with Insulin Resistance (n = 20)	Diabetic Retinopathy without Insulin Resistance (n = 33)	Diabetic Retinopathy with Insulin Resistance (n = 34)
MDA	nmol/ ml	2.6 ± 0.5	2.6 ± 0.5	2.7 ± 0.2	3.4 ± 0.5
AA	mg/ dl	0.78 ± 0.09	0.75 ± 0.08	0.76 ± 0.06	0.60 ± 0.66
DHAA	mg/ dl	0.27 ± 0.04	0.24 ± 0.05	0.26 ± 0.04	0.33 ± 0.05
FPG	mg/ dl	170 ± 18	169 ± 26	155 ± 51	200 ± 60
1HPPG	mg/ dl	305 ± 41	294 ± 23	298 ± 47	330 ± 40
2HPPG	mg/ dl	271 ± 53	241 ± 45	320 ± 92	362 ± 70
F-INS	mIU/ L	24 ± 7	26 ± 10	15 ± 8	78 ± 45
1HPPINS	mIU/ L	53 ± 25	100 ± 29	39 ± 14	105 ± 35
2HPPINS	mIU/ L	32 ± 9	121 ± 32	28 ± 17	115 ± 39

Values as Mean± SD

Ehen the diabetic retinopathy group were divided according to their insulin status, the hyper insulin group showed higher DHAA, MDA, FPG, one-hour PPG, all the insulin values (p≤ 0.001) and two-hour PPG (p≤ 0.05) with a low value of AA when compared with normo-insulin group (Table 2).

As a whole, when the hyperinsulinemic diabetic group was compared with the hyperinsulinemic diabetic retinopathy group, higher values of DHAA (p≤ 0.01), MDA (p≤ 0.02) F-INS (p≤ 0.001) and 2H PPG (p≤ 0.001), and a decreased level of AA (p≤ 0.001) were noted in diabetic retinopathy group (Table 2).

In diabetic retinopathy, without insulin resistance, ascorbic acid supplementation showed decreased level of MDA and DHAA (p≤ 0.001), one-hour PPG and two-hour PPG (p≤ 0.05), and an increased level of AA (p≤ 0.001).

No change in insulin level was observed.

Vitamin E supplementation also decreased MDA (p≤ 0.001), and DHAA (p≤ 0.02) with an increase in plasma AA, while mixed carotenoids could decrease MDA (p≤ 0.001) and DHAA (p≤ 0.01) only (Table 3).

Table 3: Effect of Antioxidants on Biochemical Parameters in Diabetic Retinopathy without any Insulin Resistance.

Parameters	Unit	Before AA Supplementation (n = 11)	After AA Supplementation (n = 11)	Before Vitamin E Supplementation (n = 11)	After Vitamin E Supplementation (n = 11)	Before Carotenoids Supplementation (n = 11)	After Carotenoids Supplementation (n = 11)
MDA	nmol/ ml	2.7 ± 0.3	1.3 ± 0.3	2.8 ± 0.2	1.2 ± 0.2	2.6 ± 0.2	1.1 ± 0.1
AA	mg/ dl	0.77 ± 0.04	1.76 ± 0.06	0.75 ± 0.08	0.82 ± 0.07	0.75 ± 0.05	0.78 ± 0.04
DHAA	mg/ dl	0.27 ± 0.05	0.16 ± 0.04	0.25 ± 0.04	0.19 ± 0.06	0.26 ± 0.04	0.20 ± 0.03
FPG	mg/ dl	146 ± 56	127 ± 33	151 ± 45	145 ± 37	171 ± 47	158 ± 32
1HPPG	mg/dl	299 ± 34	259 ± 31	289 ± 49	264 ± 42	308 ± 56	287 ± 43
2HPPG	mg/dl	325 ± 73	265 ± 43	297 ± 91	274 ± 69	341 ± 105	309 ± 80
F-INS	mIU/ L	12 ± 5	13 ± 5	21 ± 8	21 ± 6	11 ± 5	12 ± 3
1HPPINS	mIU/ L	39 ± 10	42 ± 6	49 ± 16	45 ± 7	28 ± 9	29 ± 11
2HPPINS	mIU/ L	30 ± 17	27 ± 13	35 ± 18	38 ± 13	17 ± 9	18 ± 5

Values as Mean± SD

In diabetic retinopathy patients with insulin resistance, ascorbic acid supplementation increased AA level (p≤ 0.001) with decreased DHAA and MDA (p≤ 0.001). The one-hour PPG and two-hour PPG (p≤ 0.02), F-INS (p≤ 0.01), 1HPPINS (p≤ 0.01).

Vitamin E supplementation could decrease DHAA and MDA (p≤ 0.001), one-hour PPG (p≤ 0.01), two-hour PPG (p≤ 0.05), F-INS (p≤ 0.01), 1HPPINS (p≤ 0.02), 2HPPINS (p≤ 0.001), while AA was restored almost to normal level (p≤ 0.001).

Mixed carotenoid supplementation decreased MDA and DHAA (p≤ 0.001), 1HPPG (p≤ 0.001), 2HPPG (p≤ 0.05).

F-INS (p≤ 0.050, 1HPPINS (p≤ 0.01), and 2HPPINS (p≤ 0.001). Also, the AA level was restored almost to normal level (p≤ 0.001) (Table 4).

Table 4: Effect of Antioxidants on Biochemical Parameters on Diabetic Retinopathy with Insulin Resistance.

Parameters	Unit	Before AA Supplementation (n = 12)	After AA Supplementation (n = 12)	Before Vitamin E Supplementation (n = 11)	After Vitamin E Supplementation (n = 11)	Before Carotenoids Supplementation (n = 11)	After Carotenoids Supplementation (n = 11)
MDA	nmol/ ml	3.42 ± 0.5	1.24 ± 0.14	3.3 ± 0.5	1.23 ± 0.2	3.45 ± 0.2	1.17 ± 0.1
AA	mg/ dl	0.06 ± 0.07	1.73 ± 0.06	0.6 ± 0.07	0.7 ± 0.08	0.6 ± 0.05	0.76 ± 0.07
DHAA	mg/ dl	0.33 ± 0.04	0.23 ± 0.05	0.32 ± 0.05	0.23 ± 0.03	0.34 ± 0.04	0.25 ± 0.05
FPG	mg/ dl	201 ± 67	170 ± 47	191 ± 58	165 ± 52	211 ± 50	175 ± 28
1HPPG	mg/dl	332 ± 45	285 ± 37	319 ± 39	252 ± 58	347 ± 28	270 ± 41
2HPPG	mg/dl	349 ± 71	275 ± 52	256 ± 70	293 ± 50	386 ± 60	337 ± 41
F-INS	mIU/ L	60 ± 38	36 ± 17	101 ± 50	51 ± 14	73 ± 30	49 ± 22
1HPPINS	mIU/ L	102 ± 32	75 ± 11	122 ± 41	85 ± 12	89 ± 19	66 ± 12
2HPPINS	mIU/ L	104 ± 37	60 ± 12	135 ± 44	71 ± 10	105 ± 25	67 ± 15

Values as Mean± SD

Discussion

Hyperinsulinemia is defined in a report as fasting insulin ≥ 95th percentile (20 µIU/ ml) among the 504 subjects, who were non-obese and free of clinical diabetes and glucose intolerance(Burchfiel,*et.al*.1998). Fasting levels of insulin in the control group of this study was below this level, but was at a higher level, when compared to another study (Gozashti,*et.al*.2015). A single fasting insulin sample has been shown to be inversely correlated with insulin sensitivity or whole-body glucose uptake as measured by using the euglycemichyper insulinemic clamp method (Gutch, *et.al.2015*). On the other hand, hyperinsulinemia was defined as a serum level of insulin level ≥ 60.4 µIU/ ml at 120 minutes after 75 gm of glucose challenge, in a study, where more emphasis has been given to post prandial insulin than the fasting insulin.

In this present study, both the criteria were fulfilled. It has been proposed that peripheral insulin concentration reflects post hepatic insulin delivery rather than the actual secretory rates of insulin [11]. Thus, hyperinsulinemia could be due to increased rate of secretion, diminished excretion by liver, a decreased rate of degradation elsewhere or a failure of internalization at receptor site. The high insulin level was found to be related to the prevalence of insulin resistance, as this was particularly important in the Asian sub-group presenting with myocardial infarction and unstable angina (Stubbs,*et.al*. 1999). The homeostatic model assessment (HOMA) Index has been used to estimate the degree of insulin resistance. Using this Index, it has been reported that while mean HOMA Index in normal control was 2.09, in Type II diabetes mellitus, it was 6.3(Gutch,*et.al.*2015, Niemczyk,*et.al* 2013). In the present study, the mean HOMA Index for normal control was 4.17, while in Type II diabetes mellitus, it was 10.49, and in diabetic retinopathy, it was found to be 20.64. Thus, it was obvious that a higher degree of insulin resistance prevalent in our population could lead to greater vascular complications leading to diabetic retinopathy, coronary artery disease, nephropathy, etc.

In complicated diabetic patient's higher insulin level in hyper insulin group was the only significant finding, when compared to the normo-insulin group. The oxidative stress was present as evidenced by high MDA and DHAA level in both the groups. The increased oxidative stresses as well as intolerance to glucose level with higher insulin levels were evident in diabetic retinopathy. Thus, persistent insulin resistance of a long-standing nature could lead to altered parameters as seen in diabetic retinopathy leading to its further complications.

DHAA is unstable and is constantly being produced. Its disappearance indicates either reduction back to ascorbic acid or catabolism through di keto gluonic acid to oxalic acid (Knight,*et.al*.2016). Since DHAA is independently modulated and does not depend on AA level, its serum concentration seems to be predictive in assessing the magnitude of oxidative stress. The higher levels of MDA and DHAA ran parallelly in the diabetic groups in the initial stage, but the DHAA level takes the upper hand in the long-standing cases, leading to complications. It seems that ascorbic acid is a better antioxidant in comparison to Vitamin E and mixed carotenoids in normalizing insulin resistance and glucose utilization. Mixed carotenoids and Vitamin E could spare the action of ascorbic acid up to a certain extent. It was evident that probably the duration of ill-treated diabetes state was an important factor in the pathophysiology of diabetes mellitus. Prolonged insulin resistance could be the main factor in the caution of diabetic retinopathy. The magnitude of oxidative stress was found to be directly proportional to hyperinsulinemia and hyperglycemia.

All the antioxidants more or less could improve the glucose tolerance and insulin response to glucose load probably by combating oxidative stress, it has been suggested that the presence of insulin resistance is the important predictor of Type II diabetes and coronary heart disease (Kalofoutis et al. 2007).

Ethical Approval:

Ethical Committee clearance was obtained from Institutional Ethical Reviewed Board of Hospital before commencement of study. Informed consent was obtained from all individual patients included in this study.

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Nil

Competing Interests:

The authors declare that they have no competing interests.

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