Role Of Antioxidant Therapy In Management Of Type – 2 Diabetes Mellitus

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Abstracts: Introduction: Diabetes Mellitus is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. Free radical injury is important contributing factor for the development of Insulin resistance and impaired insulin secretion. Recently it has been suggested that glycation of antioxidant enzymes could alter the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals. Intake of vitamin E and vitamin C, due to antioxidant property, is associated with reduced risk of development of Diabetes Mellitus Type 2. With this background, this study was planned to explore the role of antioxidant therapy in the management of Diabetes Mellitus Type 2. Objectives: (1) To demonstrate increased oxidative stress in newly diagnosed Type 2 Diabetes Mellitus patients by measuring antioxidant enzymes activities. (2) To study the effect of oral hypoglycaemic agents on oxidative stress in Type 2 Diabetes Mellitus patients. (3) To evaluate the effects of vitamin C, vitamin E and their combination in patients having Type 2 Diabetes Mellitus managed with oral hypoglycaemic agents. Materials and Methods: The study included two groups consisting of 60 euglycemic healthy subjects and 64 newly diagnosed Diabetes Mellitus Type 2 patients. After 3 months of treatment with oral hypoglycaemic drug, the second group was divided into 4 subgroups with 16 subjects in each subgroup and were treated with oral hypoglycaemic agent alone, oral hypoglycaemic agent + vitamin C, oral hypoglycaemic agent + vitamin E, and oral hypoglycaemic agent + vitamin C + vitamin E respectively for further 3 months. Results : Hyperglycemia in patients with Diabetes Mellitus Type 2 is associated with reduced antioxidant status (superoxide dismutase and catalase activities, and reduced glutathione level). Treatment with oral hypoglycaemic agent for 3 months produced euglycemia with partial but statistically significant elevation of catalase activity and reduced glutathione level in blood. Following additional antioxidant therapy with vitamin C and vitamin E produced further significant increase in reduced glutathione level, however fasting plasma glucose level and activities of superoxide dismutase and catalase enzymes were found to be statistically non-significant.Conclusion: Antioxidant therapy with vitamin C and vitamin E in addition to oral hypoglycaemic agent reduces oxidative stress in patients having Type 2 Diabetes Mellitus [Desai N K et al NJIRM 2013; 4(2) : 128-133]

Key Words: Type 2 Diabetes Mellitus , Vitamin C, Vitamin E, Antioxidant

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Introduction: With an increasing incidence worldwide, Diabetes Mellitus will be a leading cause of morbidity and mortality for the foreseeable future[1]. Recently, it has been demonstrated that hyperglycemia may lead to glycation of antioxidant enzymes, which could alter the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals[2]. Increased oxidative stress plays a key role in the development of insulin resistance, β – cell dysfunction as well as other complications of Type 2 Diabetes Mellitus[3,4,5]. Strong evidence suggests that oxidative stress is the mediator of impaired endothelial function[6] which is found more commonly in patients with Diabetes Mellitus when compared to nondiabetic subjects[7]. Small scale clinical studies have reported that antioxidant therapy decreases oxidative stress[8] as well as improves glycemic control[9] in individuals with established Type 2 Diabetes Mellitus . Vitamin C and E have high antioxidant property. With this preliminary data, it was of interest to study the role of antioxidant therapy in the management of Type 2 Diabetes Mellitus.

Objectives: (1) To demonstrate increased oxidative stress in newly diagnosed Type 2 Diabetes Mellitus patients by measuring antioxidant enzymes activities. (2) To study the effect of oral hypoglycaemic agents on oxidative stress in Type 2 Diabetes Mellitus patients. (3) To evaluate the effects of vitamin C, vitamin E and their combination in patients having Type 2 Diabetes Mellitus managed with oral hypoglycaemic agents.

Material and Methods: This was a prospective study carried out at Shree Sayajirao General Hospital, Vadodara. The study had two groups- control group having 60 Euglycemic healthy subjects and test group having 64 newly diagnosed patients having Type – 2 diabetes mellitus (who were not on antidiabetic therapy) attending OPD at above mentioned hospital. Inclusion of subject in the study groups depended upon randomization carried out rand table. Time span of the study was January 2004 to February 2005.

Study recruited study or test subjects having age between 30 to 60 years and both gender. Study participants were screened for the basic biochemical and haematological parameters i.e. haemoglobin, total and differential count of white blood cells, erythrocyte sedimentation rate, blood urea, serum creatinine and serum glutamic oxaloacetic transaminase and urine sample for routine and microscopic examination. Subjects having systolic blood pressure > 140 mmHg and/ or diastolic blood pressore > 90mmHg, taking steroids or thyroxine, history of any chronic infection like TB, Leprosy, recent trauma or surgery, having renal, cardiac, liver disorder, alcohol addiction or smoking, hypersensitivity to vitamin C or vitamin E as well as pregnant and lactating female were excluded from the study. After explaining the nature and the purpose of the study consent was obtained from all the subjects before screening and including them in the study. Selection of oral hypoglycaemic agent in the test group was done according to body mass index [BMI] of the patient. If BMI is 25 or more than 25 then metformin (n=32) was prescribed and if less then 25 then glibenclamide (n=32) was prescribed.

Patients were followed up at every 15 days throughout the study period. Compliance was checked by capsules or tablets counts and reinforcement was done at each visit. Dose of oral hypoglycaemic agent was adjusted in first month according to fasting plasma glucose. At the end of 3 months fasting plasma glucose {colorimetric end point test - Glucose Oxidase Peroxide [GOD – POD] method[10]} and antioxidant enzymes activities i.e. superoxide dismutase, catalase, reduced glutathione were measured. After 3 months of treatment with oral hypoglycaemic agent, group II was further divided into four subgroups of 16 subjects in each group as follows,

Group II_A (n=16) Oral hypoglycaemic agent treatment for 3 months.

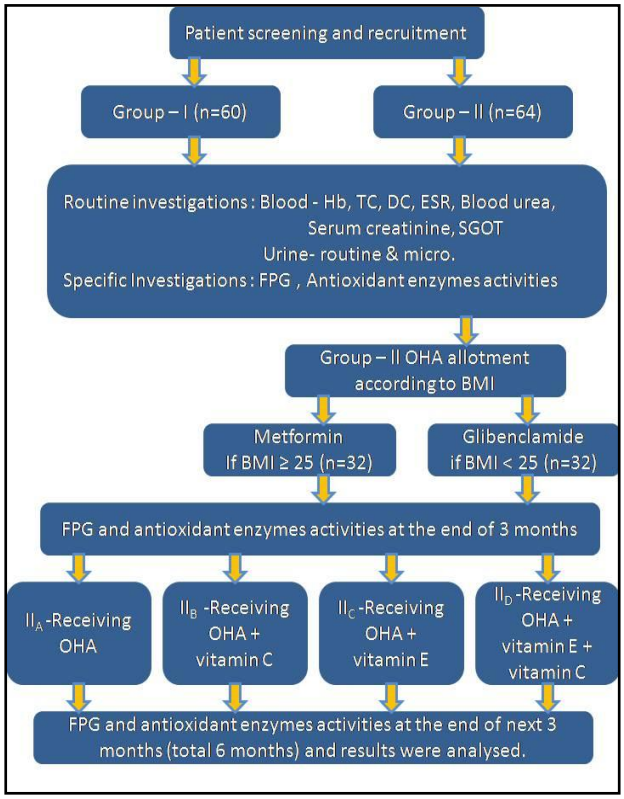
Group II_B (n=16) Oral hypoglycaemic agent treatment + vitamin C (500mg once a day daily) for 3 months.

Group II_C (n=16) Oral hypoglycaemic agent treatment + vitamin E (800mg once a day daily) for 3 months.

Group II_D (n=16) Receiving oral hypoglycaemic agent treatment + vitamin C (500mg once a day daily) therapy + vitamin E (400mg once a day daily) for 3 months.

At the end of next 3 months (total 6 months) fasting plasma glucose and antioxidant enzymes activities were measured.

Figure 1. Showing flowchart of study design along with conduct.



Statistical Analysis: Data were analyzed by applying appropriate paired and unpaired "t" test by Sigmastat V 4.0. (Systat Software Inc.,

California) Values expressed as Mean ± SD and $p < 0.05$ was considered as statistically significant.

Results: Majority of the control subjects (51.6%) and Type 2 Diabetes Mellitus (46.9%) patients belongs to the age group of 41 – 50 years ; while 33.3 % control subjects and 35.9 % Type 2 Diabetes Mellitus patients belongs to the age group of 51 – 60 years (Table 1). Average age and body mass index were comparable in both the groups. Number of female subjects were nearly equal i.e. 30 (50%) and 30 (46.9%) in the control group and Type 2 Diabetes Mellitus group respectively.

As compared to control group fasting plasma glucose was and remained significantly higher in test group patients before treatment with oral hypoglycemic drug however there was substantial reduction of fasting plasma glucose in post treatment groups (Table 3). Activity of superoxide dismutase was significantly low in test group as compared to control group (Table 3). It increased significantly only in the groups which were treated with vitamin E *i.e.* group IIc and IId as compared to untreated levels of diabetic patients but did not achieved the comparable levels to the control group(Table 3). Treatment with oral hypoglycaemic agent alone or in combination with vitamin C or vitamin E or both did not produced any alteration in activity of catalase as compared to value observed before the treatment or in control group (Table 3). Treatment with vitamin C or vitamin E or both significantly increased reduced glutathione levels as compared to value observed before the treatment or in control group (Table 3)

Table 1 – Showing Age Distribution of the Study Subjects of Both Groups. (Control n=60, Test n=64)

Age group (years)	Control Group	Test Group	Total n=124
30 – 40	9 (15%)	11 (17.2%)	20
41 – 50	31 (51.6%)	30 (46.9%)	61
51 – 60	20 (33.3%)	23 (35.9%)	43
Total	60	64	124

Table 2 – Showing General Characteristic Data of Both Groups. (Control n=60, Test n=64)

Characteristics	Control Group	Test Group
Age (Years)	46.63 ± 6.97	46.96 ± 6.32
Female	30 (50%)	30 (46.9%)
Body mass index (BMI) (kg/m^2)	24.38 ± 3.25	25.15 ± 2.08

Data shown as mean ± SD

Table 3 – Showing Fasting Plasma Glucose and Antioxidant Enzymes Activity of Control Group and Test Group.

Treatments	Fasting Plasma Glucose (mg/dl)	SOD (EU)	Catalase (µ mol H_2O_2/ mg/sec)	Reduced Glutathione (gm/Hb %)
Control (n=60)	90.46 ± 10.57	1.336 ± 0.01	8.076 ±0.94	11.579 ±1.42
Before OHA (n=64)	201.2 ±19.16^	1.15 ±0.088^	7.731 ±1.04*	7.644 ±1.04^
After OHA alone (n=16)	98.0 ±6.86^	1.164 ±0.088	7.738 ±1.24	9.728 ±0.624^
After OHA + vitamin C (n=16)	94.87 ±7.032^	1.183 ±0.11	7.983 ±1.04	12.4 ±1.38#^
After OHA + vitamin E (n=16)	95.06 ±6.64^	1.208 ±0.096*	7.921 ±1.10	13.379 ±1.73# ^ $
After OHA + vitamin C + vitamin E (n=16)	94.62 ±8.68^	1.214 ±0.088#	7.942 ±1.10	15.15 ±0.1.50#^ @ ?

n= 64 in before treatment with OHA group, n= 16 in each after treatment group, values are expressed as Mean ± SD, * $P < 0.05$, # $P < 0.01$, ^ $P < 0.001$ as compared to its corresponding value of control, $ $P < 0.05$, @ $P < 0.001$ as compared to its corresponding value of treatment with OHA + vitamin C. ? $P < 0.01$ has compared to its corresponding value of treatment with OHA + vitamin E.

The raise observed was significantly high in group treated with oral hypoglycemic drugs along with vitamin C and E as compared to oral hypoglycemic drug alone or its combination with vitamin C or E (Table 3). None of the subjects receiving antioxidant therapy reported any clinical adverse effects during or till 2 weeks after the study period.

Discussion: Red cell superoxide dismutase (superoxide dismutase) and catalase activities and blood reduced glutathione level were decreased in subjects with impaired glucose tolerance and in Type 2 Diabetes Mellitus patients[10]. Reduction in superoxide dismutase and catalase activities is due to glycation of protein which leads to functional changes of antioxidant enzymes and changes in the concentration of cofactors. Factors generating oxidative stress in Type 2 Diabetes Mellitus are hyperglycemia, hyperinsulinemia and increased hyperglycaemic index. Impaired antioxidant activity in patients having Diabetes Mellitus contributes to development of microvascular complication by precipitating endothelial dysfunction. Dietary antioxidants have been hypothesized to have a protective effect against the development of diabetes by inhibiting oxidative chain reactions [11].

Vitamin E (Paolisso et al., 1993) and vitamin C (Paolisso et al., 1994) have been demonstrated to reduce oxidative stress in Type 2 Diabetes Mellitus patients[12.13]. These findings are in coherence with present study. In present study we found that supplementation of vitamin C + vitamin E along with oral hypoglycaemic agent further significantly increased reduced glutathione level as compared to all the other groups. Thus combined therapy consisting oral hypoglycaemic agent with vitamin C + vitamin E produced additive antioxidant effect especially on reduced glutathione as well as on superoxide dismutase levels. However there was no change in activity of catalase in the group receiving oral hypoglycaemic agent with vitamin C + vitamin E as compared to the group receiving oral hypoglycaemic agent therapy alone.

Few clinical studies demonstrated that vitamin C supplementation improves glycemic control in Type 2 Diabetes Mellitus patients[13]. But the present study shows that there was no further improvement in glycemic control after addition of vitamin C along with oral hypoglycaemic agent.

Vitamin E has a number of effects at the cellular level that are not dependent on its antioxidant activity and may potentially contribute to improved insulin action. Vitamin E accelerates diacylglycerol kinase activity, thereby decreasing levels of diacylglycerol, which is an allosteric activator of protein kinase C [14] which apparently impairs insulin action by phosphorylating serine or threonine residues on insulin receptor and insulin receptor substrate – I proteins[15]. In present study, there was no further improvement in glycemic control after supplementation of vitamin E therapy along with oral hypoglycaemic agent for 3 months.

Several reports have shown that as might logically be anticipated, a combination of different antioxidants may be superior to monotherapy [16]. In the groups treated with oral hypoglycaemic agent with either of the antioxidant alone the fasting plasma glucose level was significantly high as compare to that observed in euglycemic healthy subjects. However, in oral hypoglycaemic agent with vitamin C + vitamin E treated group the fasting plasma glucose level was almost equivalent to that observed in the euglycemic healthy subjects. Thus it is tempting to suggests that combinations consisting oral hypoglycaemic agent with vitamin C + vitamin E may produce better glycemic control then that produced by any of the antioxidants given alone along with oral hypoglycaemic agent.

Atherosclerotic cardiovascular diseases are a major source of morbidity and mortality in people with diabetes. Oxidative modification of low density lipoprotein is an important step in the development and progression of atherosclerosis in experimental studies[17]. In addition to its antioxidant properties, vitamin E has been also reported to reduce the cytotoxic effect of oxidized

lipoproteins, smooth muscle cell proliferation, platelet adherence and aggregation, inflammation and it improves endothelial function[18]. Vitamin E has been found to prevent microvascular complications of diabetes. Indeed in animal models it decreases hyperglycemia induced protein kinase C activation and D – acetyl glycerol levels, which have been associated with abnormalities in the retinal, renal and vascular tissues in diabetes[19]. Evidence on the role of oxidative stress on diabetes complications suggest that free radical production is clearly increased as the result of glucose or free fatty acid metabolism via multiple pathways. It is likely that oxidative stress may accelerate the basic pathogenic process of diabetic complications. However, oxidative stress may not be playing leading role in the microvascular complications because these complications are not evident in patients with insulin resistance without diabetes, even though increase in oxidative stress also exist to a similar extent in both. Conversely the beneficial effects of antioxidants seem to be present in animal models of diabetes. Hence if oxidative stress plays only a supportive role in diabetic complications then antioxidants may be helpful only when paired with other treatment of diabetic complications[20]. It is suggested that addition of antioxidant therapy along with oral hypoglycaemic agent therapy in the management of Type 2 Diabetes Mellitus patients may prevent diabetic complications. As the sample size of the study was less with limited duration, further randomized, double blind, large scale, long term studies are warranted to establish their use in clinical practice for better glycemic control and prevention of diabetic complications.

Conclusion:- Additional antioxidant therapy with vitamin C and vitamin E reduces oxidative stress in Type 2 Diabetes Mellitus patients by significantly increasing blood reduced glutathione and superoxide dismutase level; and together they improve glycaemic control as well.

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