Oral Hypoglycemic Agent and Ascorbic Acid Supplementation Therapy Synergistically Ameliorates Blood Glucose, Serum Lipid and Inflammatory Response in Type 2 Diabetes Mellitus Patients

Abstract

Background: Type 2 diabetes mellitus is a chronic metabolic disorder where its prevalence has steadily been on the increase all over the world. It is characterized by increased generation of free oxygen radicals leading to oxidative stress. Hence supplementation of dietary antioxidants especially in the hypoglycemic therapy presents good prospects in its management.

Aim: To evaluate the synergistic effect of ascorbic acid supplementation of a hypoglycemic agent on blood glucose, lipid profile and inflammatory response in Type 2 Diabetes Mellitus Patients.

Methods: A total of 160 patients with newly diagnosed type 2 diabetes mellitus were included in the study. Patients were divided into 4 groups of 40 patients each. Anthropometric characteristics of all patients were measured at baseline. Control group received randomly glibenclamide 5mg daily, the first experimental groups received 5mg of glibenclamide with 600mg (0.6g) of ascorbic acid orally daily, the second group received 5mg of glibenclamide with 1,200mg (1.2g) of ascorbic acid orally while the third group received 5mg of glibenclamide with 1,800mg (1.8g) of ascorbic acid. The treatment lasted for nine weeks after which serum biochemical parameters of all patients were determined and recorded.

Results: Our results showed that ascorbic acid supplementation caused a significant decrease ($p<0.05$) in TG, LDL and TC level in each experimental group at the end of the 9th week compared to the control but no significant difference ($p>0.05$) in between the groups. However, the HDL recorded a significant increase ($p<0.05$) in the treatment group supplemented with 1,800mg (1.8g) of ascorbic acid at the end of the 9th week compared to the control and the other ascorbic acid supplemented groups. In addition, there was a significant reduction in the serum level of C-reactive protein (CRP) in the 1.2g and 1.8g ascorbic acid supplemented groups at the end of the sixth week. Further progressive reduction at the end of the ninth week in all ascorbic acid supplemented group was also observed. Similar results were obtained for the other inflammatory biomarkers; leptin and adiponectin.

Conclusion: Ascorbic acid (vitamin C) in a dose of between 1.2g and 1.8g per day as add on therapy to oral hypoglycemic agent synergistically causes an improvement in blood glucose, lipid profile and inflammatory response to the desirable levels hence mitigating the risks of type 2 diabetes mellitus complications.

Keywords: Type 2 diabetes mellitus; Insulin; Glucose; Lipid profile; Hypoglycemia; Inflammatory response

Research Article

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Abbreviations: LDL: Low Density Lipoprotein; DM: Diabetes Mellitus; NADH: Nicotinamide Adenine Dinucleotide; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; ELISA: Enzyme-Linked Immunosorbent Assay; TG: Total Triglyceride; TC: Total Cholesterol; HDL: High Density Lipoprotein; ANOVA: Analysis of Variance

Introduction

Diabetes mellitus is one of the oldest diseases to common man. Diabetes mellitus has been defined as a disorder of carbohydrate metabolism which is marked by a continuous elevation of fasting blood glucose above 200mg/dl [1]. The distinction between type 1 and type 2 Diabetes Mellitus has been made. Type 1 diabetes,

which was formerly referred to as insulin-dependent or diabetes juvenile diabetes, is a chronic condition which is marked by the production of little or no insulin (a hormone needed to allow sugar known as glucose) to enter cells to produce energy by the pancreas [2]. Type 2 Diabetes Mellitus was explained to be one of the disorders of metabolic syndrome in 1988 [3]. Type 2 DM also hitherto referred to as non-insulin dependent Diabetes Mellitus is the most common form of Diabetes Mellitus characterized by insulin resistance, relative insulin deficiency and hyperglycemia [4]. Pathophysiologically, type 2 Diabetes Mellitus is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure [5,6]. This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells. There is an increase in the breakdown of fat with hyperglycemia. The involvement of impaired alpha-cell function has recently been recognized in the pathophysiology of type 2 DM [7]. As a result of this dysfunction, glucagon and hepatic glucose levels that rise during fasting are not suppressed with a meal. It has been discovered that people suffering from type 2 Diabetes Mellitus are often susceptible to different forms of both long term and short term complications which most times leads to their premature death. The increased morbidity and mortality rate as seen in patients suffering from type 2 Diabetes Mellitus is caused by the insidious onset of the disease as well as late recognition in very poor sub Saharan Africa [8].

Nutrients have been implicated in health and the prevention of diseases. Nutrients which include vitamins are vital to the maintenance of cardiovascular health (vitamin B1), erythrocytes synthesis (folate and vitamin B12), nerve function (vitamins B6 and B12) as well as coagulation (vitamin K) including other numerous functions [9].

Vitamin C which is also known as ascorbic acid has been described as a cofactor in multiple enzymatic reactions including collagen synthesis [9]. Humans are unable to produce vitamin C due to the absence of the enzyme, L-gulonolactone oxidase, which catalyzes the final step in the synthesis of ascorbic acid [10]; therefore, it is an essential nutrient in humans. Vitamin C acts as a reducing agent in free radical-mediated oxidation processes; therefore, it can act as an antioxidant [10]. Deficiency of vitamin C results in the defective formation of collagen and connective tissues in the skin, cartilage, dentine, bone, and blood vessels.

Vitamin C is an antioxidant, and the structural similarity between vitamin C and glucose makes it of interest in diabetes [11]. Oxidative stress can lead to disturbed glucose metabolism and hyperglycemia [12]. Therefore, a benefit of antioxidants to prevent diabetes or to achieve positive outcomes in type 2 diabetes mellitus (T2DM) is biologically plausible.

In as much as diabetes is not traditionally known as a risk factor for vitamin C deficiency, patients with diabetes should all receive dietary advice about healthy eating and vitamin C dietary sources, including fresh fruits and vegetables. The recommended dietary intake of vitamin C is 45 mg per day for adults [13]. Ascorbyl radical and dehydroascorbic acid which are the oxidized products of vitamin C are easily reconverted to ascorbic acid by NADH, NADPH or glutathione [14].

Therefore this study seeks to evaluate the synergistic effect of Ascorbic Acid as add on to Oral Hypoglycemic Agent in modulation of blood glucose, serum Lipid Levels and inflammatory biomarkers in Type 2 Diabetes Mellitus patients.

Materials and Method

Experimental population

The experimental subjects for the research was sourced from the Federal Medical Centre, Owerri, Imo State, Nigeria. This was a prospective, open label, randomized, comparative, controlled clinical trial. Newly diagnosed Type 2 Diabetes Mellitus male patients with fasting Blood glucose between 150mg/dl-250mg/dl within the age group of 35-70 years were included in the study.

Experimental design

Current medical history and diagnosis were noted during the first visit. 160 patients who were newly diagnosed with type 2 Diabetes Mellitus were randomly assigned into 4 groups with 40 patients in each group and given treatments respectively as indicated in the table below:

Group	Description
1	Oral administration of 5 mg of glibenclamide once a day for 9 weeks.
2	Oral administration of 5mg of glibenclamide once a day + 600mg Ascorbic Acid once a day for 9 weeks.
3	Oral administration of 5mg of glibenclamide once a day + 600mg Ascorbic Acid twice a day for 9 weeks.
4	Oral administration of 5mg of glibenclamide once a day + 600mg Ascorbic Acid thrice a day for 9 weeks.

After enrollment into the study, anthropometric characteristics for all patients were determined at baseline. Biochemical tests which include: fasting blood sugar, post prandial blood sugar as well as serum lipid profile covering low density lipoprotein (LDL), high density lipoprotein (HDL), total triglyceride (TG) and total cholesterol of type 2 experimental subjects were measured at the beginning of the study and at the end of the 3rd, 6th and 9th week. In addition, serum inflammatory biomarkers; C-Reactive Proteins, Leptin and Adiponectin were measured at the beginning of the study and at the end of the 3rd, 6th and 9th week. At each follow up visit, fasting, post prandial blood sugar, Lipid profile -LDL, HDL, Total triglyceride (TG), Total cholesterol (TC), C-Reactive Protein, Leptin and Adiponectin were recorded. A 2-hour postprandial blood glucose test was carried out using an auto-analyzer-AccuCheck Advantage II glucose kit exactly two hours after the type 2 diabetic experimental subjects under study ate their respective meals.

Blood samples were collected and centrifuged to separate serum for estimation of lipid profile. Total cholesterol was estimated using a method reported by Liebermann Burchard Reaction [15]. Low density lipoprotein (LDL) was indirectly measured using Friedwald's method [16]. Triglycerides were measured using Hantzsch condensation method [17]. High density lipoprotein was measured by Maddison et al. [18]. C-reactive protein was determined by a highly-sensitive Hitchi 917 autoanalyzer from hsCRP, Roche Diagnostics. Adiponectin and leptin were measured by ELISA from Bender Medsystems.

Statistical analysis

All the data were presented as mean±SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P<0.05$ was considered to be significant.

Results

Table 1 shows the anthropometric characteristics of patients with type 2 Diabetes Mellitus at the beginning of the study. There was no significant difference (P>0.05) in the duration of the disease amongst the patients in between the groups. There was no significant difference (P>0.05) in the age, body mass index, weight and waist circumference respectively in between the groups.

Table 2 shows the blood glucose measurements before the administration of glibenclamide and ascorbic acid at baseline. There was no significant difference (p>0.05) in the fasting blood sugar and post prandial blood levels of patients in between the groups.

Table 3 shows the blood glucose measurements following the administration of glibenclamide and ascorbic acid at the end of the third week. There was no significant difference (p>0.05) in the fasting blood sugar and post prandial blood in between the groups.

Table 4 shows the blood glucose measurements following the administration of glibenclamide and ascorbic acid at the end of the sixth week. There was no significant difference (p>0.05) in the fasting blood sugar and post prandial blood in between the groups.

Table 5 shows the blood glucose measurements following the administration of glibenclamide and ascorbic acid at the end of the ninth week. There was a significant increase (p<0.05) in the fasting blood sugar in the control group compared to the groups. There was no significant increase (p>0.05) in the post prandial blood sugar in between groups 1,2 and 3 while there was a significant decrease (p<0.05) in the level of post prandial blood in group 4 compared to the other three groups.

Table 6 shows the serum lipid and inflammation profiles of the experimental subjects at the beginning of the study. There was no significant difference (p>0.05) in the serum levels of the lipid biomarkers (triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein) in between all the experimental groups. There was no significant difference (p>0.05) in the serum levels of the inflammation biomarkers (C-reactive protein, leptin and adiponectin) in between all the experimental groups.

Table 7 shows the serum lipid and inflammation profiles of the experimental subjects at the end of the third week of the study. There was no significant difference (p>0.05) in the serum levels of the lipid biomarkers (triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein) in between all the experimental groups. There was no significant difference (p>0.05) in the serum levels of the inflammation biomarkers (C-reactive protein, leptin and adiponectin) in between all the experimental groups.

Table 8 shows the serum lipid and inflammation profiles of the experimental subjects at the end of the sixth week of the study. There was no significant difference (p>0.05) in the serum levels of the lipid biomarkers (triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein) in between all the experimental groups. There was a significant decrease (p<0.05) in the serum levels of the inflammation biomarkers (C-reactive protein, leptin and adiponectin) in all the ascorbic acid supplemented groups compared to the control group.

Table 9 shows the serum lipid and inflammation profiles of the experimental subjects at the end of the ninth week of the study. There was no significant difference (p>0.05) in the serum levels of triglycerides in between all the experimental groups. There was no significant difference (p>0.05) in the serum levels of total cholesterol in each of groups 2 and 3 compared to the control group while the total cholesterol level of group 4 decreased significantly (p<0.05) compared to each of the control group as well as groups 2 and 3. This was observed similarly for low density lipoprotein and high density lipoprotein. There was a significant decrease (p<0.05) in the serum levels of the inflammation biomarkers (C-reactive protein, leptin and adiponectin) in all the ascorbic acid supplemented groups compared to the control group.

Table 1: Anthropometric characteristics of patients with type 2 Diabetes Mellitus at baseline according to their experimental groupings at baseline.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Diabetes Duration	11.34±0.08^a	11.62±0.15^a	11.22±0.26^a	11.09±0.32^a
Age	51.70±0.02^a	52.50±0.09^a	51.20±0.01^a	51.40±0.05^a
Body Mass Index	29.20±0.02^a	29.90±0.02^a	29.50±0.02^a	29.20±0.02^a
Weight	81.50±0.32^a	81.80±0.32^a	81.60±0.32^a	81.40±0.32^a
Waist Circumference	102.10±0.06^a	102.10±0.06^a	102.10±0.06^a	102.10±0.06^a

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 2: Blood glucose measurements before the administration of glibenclamide and ascorbic acid at baseline.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Fasting Blood Sugar (mg/dl)	187.20±0.02[a]	187.40±0.09[a]	187.10±0.01[a]	187.60±0.05[a]
Post Prandial Blood Sugar (mg/dl)	215.11±0.02[a]	214.2±0.02[a]	214.50±0.02[a]	214.70±0.02[a]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 3: Blood glucose measurements at the end of the third week of glibenclamide and ascorbic acid administration.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Fasting Blood Sugar (mg/dl)	174.31±0.02[a]	174.39±0.09[a]	174.12±0.01[a]	174.50±0.05[a]
Post Prandial Blood Sugar (mg/dl)	211.11±0.02[a]	211.2±0.02[a]	211.50±0.02[a]	211.90±0.02[a]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 4: Blood glucose measurements at the end of the sixth week of glibenclamide and ascorbic acid administration.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Fasting Blood Sugar (mg/dl)	169.19±0.02[a]	169.40±0.09[a]	169.10±0.01[a]	169.60±0.05[a]
Post Prandial Blood Sugar (mg/dl)	202.11±0.02[a]	200.2±0.02[a]	201.50±0.02[a]	200.70±0.02[a]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 5: Blood glucose measurements at the end of the ninth week of glibenclamide and ascorbic acid administration.

Parameters	Group 1	Group 2	Group 3	Group 4
Fasting Blood Sugar (mg/dl)	156.20±0.02[a]	152.40±0.09[b]	147.10±0.01[c]	144.60±0.05[d]
Post Prandial Blood Sugar (mg/dl)	195.47±0.02[a]	194.21±0.02[a]	193.91±0.02[a]	184.70±0.02[b]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 6: Serum lipid and inflammation profiles at baseline.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Triglycerides (mg/dl)	195.17±0.09[a]	194.86±0.13[a]	194.70±0.62[a]	195.16±0.07[a]
Total Cholesterol (mg/dl)	211.67±0.63[a]	211.59±0.12[a]	211.40±0.35[a]	211.79±0.82[a]
Low Density Lipoprotein (mg/dl)	136.52±0.29[a]	135.97±0.27[a]	136.16±0.81[a]	135.89±0.81[a]
High Density Lipoprotein (mg/dl)	39.12±0.49[a]	39.25±0.03[a]	40.13±0.56[a]	39.29±0.71[a]
C-reactive protein (mg/l)	3.42±0.02[a]	3.35±0.01[a]	3.37±0.22[a]	3.36±0.11[a]
Leptin (ng/ml)	74.94±0.23[a]	74.90±0.54[a]	74.91±0.32[a]	74.92±0.15[a]
Adiponectin (ng/ml)	35.61±0.55[a]	35.59±0.41[a]	35.60±0.21[a]	35.59±0.12[a]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 7: Serum lipid and inflammation profiles at the end of the third week.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Triglycerides (mg/dl)	179.22±0.32[a]	178.40±0.01[a]	178.10±0.21[a]	178.60±0.85[a]
Total Cholesterol (mg/dl)	195.11±0.02[a]	194.92±0.02[a]	194.80±0.06[a]	195.17±0.01[a]
Low Density Lipoprotein (mg/dl)	119.72±0.62[a]	120.01±0.06[a]	119.31±0.08[a]	119.78±0.24[a]
High Density Lipoprotein (mg/dl)	39.02±0.62[a]	39.19±0.18[a]	39.12±0.32[a]	39.72±0.57[a]
C-reactive protein (mg/l)	3.41±0.02[a]	3.33±0.01[a]	3.35±0.01[a]	3.35±0.17[a]
Leptin (ng/l)	73.54±0.23[a]	74.45±0.21[a]	74.72±0.58[a]	74.61±0.04[a]
Adiponectin (ng/l)	35.21±0.41[a]	35.31±0.14[a]	35.32±0.05[a]	35.38±0.12[a]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 8: Serum lipid and inflammation profiles at the end of the sixth week.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Triglycerides (mg/dl)	171.20±0.12[a]	170.40±0.08[a]	169.10±0.01[a]	170.92±0.15[a]
Total Cholesterol (mg/dl)	180.11±0.02[a]	180.32±0.02[a]	181.01±0.02[a]	179.70±0.02[a]
Low Density Lipoprotein (mg/dl)	110.71±0.12[a]	111.81±0.02[a]	111.81±0.02[a]	110.91±0.02[a]
High Density Lipoprotein (mg/dl)	39.22±0.24[a]	39.24±0.19[a]	39.09±0.42[a]	39.72±0.67[a]
C-reactive protein (mg/l)	3.11±0.09[a]	2.92±0.01[a]	1.98±0.01[b]	1.76.35±0.17[c]
Leptin (ng/l)	73.54±0.23[a]	51.45±0.21[b]	44.72±0.58[c]	35.61±0.04[c]
Adiponectin (ng/l)	35.21±0.41[a]	22.31±0.14[b]	18.32±0.05[c]	14.38±0.12[c]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Table 9: Serum lipid and inflammation profiles at the end of the ninth week.

Parameters	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Group 4 (n=40)
Triglycerides (mg/dl)	187.20±0.02[a]	187.40±0.09[a]	187.10±0.01[a]	187.60±0.05[a]
Total Cholesterol (mg/dl)	165.37±0.07[a]	164.20±0.08[a]	164.81±0.02[a]	156.70±0.02[b]
Low Density Lipoprotein (mg/dl)	110.71±0.12[a]	110.71±0.12[a]	109.71±0.12[a]	101.71±0.19[b]
High Density Lipoprotein (mg/dl)	39.26±0.52[a]	39.29±0.24[a]	39.22±0.51[a]	42.22±0.24[b]
C-reactive protein (mg/l)	3.08±0.09[a]	1.92±0.01[b]	1.23±0.01[c]	1.05±0.17[d]
Leptin (ng/l)	73.54±0.23[a]	31.45±0.11[b]	22.15±0.51[c]	15.15±0.01[c]
Adiponectin (ng/l)	35.21±0.41[a]	17.31±0.14[b]	12.11±0.04[c]	9.01±0.14[d]

Results represented as mean±S.E.M, n=40, Values with the same superscripts across the same row are not significantly different (P>0.05).

Discussion

The effects of ascorbic acid in diabetes have been a subject of interest spanning over 50 years. Diabetic patients are characterized by levels of free radicals hence reduced level of antioxidants most especially Ascorbic acid. A study of 23 observational studies examining the ascorbic acid status of people with diabetes published between 1935 and 1996 revealed that people with diabetes have at least 30% lower ascorbic acid concentrations than do people without diabetes [19]. However, the validity of the research results is a bit questionable as there was heterogeneity in the studies with regards to the ascorbic acid status measurement method including the fact that subjects were unmatched with regards to important covariates such as sex, smoking status, ascorbic acid intake as well as acute illness.

Anthropometric evaluation of type 2 diabetes mellitus experimental patients

In the current study, the anthropometric characteristics of patients with type 2 diabetes mellitus at baseline, the body

mass index, waist circumference as well as body weights of the experimental subjects were measured at the beginning of the study. There was no significant difference in all the experimental groups with regards to the duration of type 2 diabetes. Group 1 patients who received 5mg of glibenclamide had a diabetes duration of 11.34±0.08 years which was not significantly different *(P>0.05)* from the group 2 patients who received 5mg of glibenclamide plus 600mg of ascorbic acid daily as well as other two groups (group 3 and group 4).The age of group 4 type 2 diabetic subjects was 51.40±0.05 years and this was not significantly different *(P>0.05)* from the other three groups. The body mass index (BMI) which is an important anthropometric parameter for evaluation of type 2 diabetic patient was 29.90±0.02 years for group 3 patients who received 5mg of glibenclamide plus 1.2g of ascorbic acid daily and this was not significantly *(P>0.05)* different for the other three groups (group 1, group 2 and group 4).

Effect of ascorbic acid supplementation on fasting blood and post prandial blood sugar levels in type 2 diabetes mellitus experimental patients

There was a progressive reduction in the fasting blood sugar as well as postprandial blood sugar levels across glibenclamide treated only (group 1) as well as both glibenclamide treatment supplemented with ascorbic acid in groups 2,3 and 4 at the end of 3rd,6th and 9th compared to baseline though there was no significant difference *(P>0.05)* in between the treatment groups as regards fasting blood sugar and postprandial blood sugar level at the end of the third week. The same observation was made at the end of the sixth and ninth weeks.

The results of the present study correlate with the results from previous research [20]. In a study to determine the link between vitamin C levels and glycemic control in diabetic subjects, it was revealed that there was a weak, negative correlation between vitamin C levels and hemoglobin A_{1c} (HbA_{1c}) [20]. The vitamin C and glucose levels association was also studied in a large US adults population without a diabetes history from NHANES 2003–2006. It was found that the serum vitamin C concentrations were inversely associated with HbA_{1c} levels [21]. Another study involving the use of vitamin C supplements resulted to a significantly lower risk of diabetes in diabetic patients with the use of daily vitamin C supplements as compared to vitamin C non supplemented groups [22]. Also in the study, the potential usefulness of a vitamin C supplement was limited to those who did not take a multivitamin or those who had a lower dietary intake of vitamin C. However, the observational nature could not demonstrate a cause-and-effect relation, nor could confounding be excluded, such as health-conscious users of supplements being less likely to develop disease.

In another study, the vitamin C supplementation to standard hypoglycemic therapy was evaluated in type 2 diabetic patients treated with metformin randomized to 500 mg twice daily of vitamin C or placebo for 12 weeks [23]. Those given vitamin C were identified to have lower fasting and post prandial meal blood glucose levels as well asHbA_{1c} when compared to the placebo group in as much as all patients were treated with metformin.

Postulation of mechanism of action of hypoglycemic activity of ascorbic acid

Hypothetically, the mechanism by which ascorbic acid brings about its hypoglycemic effect could be by the inhibition of the cellular uptake of dehydroascorbic acid (DHA) which transportable form of vitamin C in its oxidized state. Glucose strongly causes the inhibition of the DHA uptake in the erythrocytes hence it is very likely that hyperglycemia resulting from diabetes will lead to an elevated vitamin C level in erythrocytes and this could be reversed by the large intake of vitamin C. Summarily, the biochemical mechanisms underlying reduced ascorbic acid levels in diabetic patients includes elevated metabolic turnover, increase in urinary losses, reduced cellular uptake [23].

Effect of ascorbic acid supplementation on lipid profile in type 2 diabetes mellitus experimental patients

Vitamin C supplementation decreases oxidative stress and also aids in lipid metabolism regulation. Hence the current study also evaluated the impact of ascorbic acid supplementation on lipid profile in type 2 diabetic subjects. Important lipid biomarkers include triglycerides, total cholesterol, low density lipoproteins as well as high density lipoproteins [24]. In the present study, we observed a significant progressive reduction in the serum level of the triglycerides, total cholesterol and low density lipoproteins across glibenclamide treated only (group 1) as well as both glibenclamide treatment supplemented with ascorbic acid in groups 2, 3 and 4 at the end of 3rd, 6th and 9th compared to baseline though there was no significant difference *(P>0.05)* in between the treatment groups as regards the three lipid biomarkers. However, the observed results was not applicable to high density lipoproteins as there was no significant difference (P>0.05) in the serum level of all experimental subjects at the end of the third and sixth weeks compared to baseline. Group 4 patients who received 1.8g (1,500mg) of ascorbic acid had a significantly reduced (P<0.05) serum HDL compared to the three other treatment groups at the end of the ninth week.

In a similar study, 500mg and 1000mg of vitamin C were administered on type 2 diabetes mellitus patients for six weeks. Those patients who received 1000mg of vitamin C daily in the study recorded no significant decrease in the serum triglyceride level, while there was a significant decrease in the serum LDL level of the same patients [25].

Effect of ascorbic acid supplementation on inflammatory biomarkers in type 2 diabetes mellitus experimental patients

Chronic inflammation is closely related to insulin resistance in type 2 diabetes [26]. Therefore, with attention to extensive anti-inflammatory effects of ascorbic acid on downstream markers of inflammation, we used the effect of ascorbic acid supplementation on attenuation of inflammation in type 2 diabetes patients. We used inflammatory biomarkers like C - reactive protein, leptin and Adiponectin.

Increasing level of C Reactive protein as an inflammatory marker is known to be associated with great cardio-vascular diseases risks. In addition, CRP is often suggested as a reliable

laboratory biomarker for risk of cardio-vascular disorders in patients with diabetes mellitus [27].

In the present study, we observed no significant difference in the serum level of C - reactive protein in both the ascorbic acid supplemented and non supplemented groups at the end of the third week compared to their respective baseline values. However, there was a significant reduction ($P<0.05$) in the serum level of c-reactive protein in the patients administered 1200mg (1.2g) and 1800mg (1.8g) of ascorbic acid supplementation at the end of the 6th and 9th weeks compared to the other patients who received only 5mg of glibenclamide (group 1) and 600mg of ascorbic acid orally daily. With regards to the higher risk of CVD in persons with diabetes mellitus (nearly two folds in comparison to healthy ones), control of inflammatory factors such as CRP is critical [28].

Leptin is a peptide hormone which is released by adipocytes and could inhibit obesity by stimulating satiety centers in brain [29]. Most of obese peoples exhibit leptin receptor deficiency, which consequently lead to leptin resistance condition [30].

In the present study, we observed no significant difference ($P>0.05$) in the serum level of leptin in both the ascorbic acid supplemented and non supplemented groups at the end of the third week compared to their respective base line values. However, there was a significant reduction ($P<0.05$) in the serum level of leptin in the patients administered with oral ascorbic acid supplementation at the end of the 6th and 9th weeks compared to the other patients who received only 5mg of glibenclamide (group 1) daily for the 6th and 9th weeks respectively. The works by Fischer et al. [31] showed that leptin level in patients with type 2 diabetes is higher than normal. They confirmed a positive correlation between fasting leptin level and insulin resistance independent of body fat mass. We showed for the first time that oral administration of alpha-tocopherol or ascorbic acid could decrease serum leptin level in diabetic subjects.

A new adipocyte-specific protein called adiponectin has been implicated to play an important role in the development of atherosclerosis and insulin resistance. In as much as adiponectin are found in high concentrations, its levels are reduced in obese subjects compared to lean subjects. Adiponectin levels are correlated correlations with measures of adiposity [32]. Adiponectin levels have been reported to also be lowered in patients with insulin resistance and type 2 diabetes [32]. In the present study, we observed no significant difference ($P>0.05$) in the serum level of adiponectin in both the ascorbic acid supplemented and non supplemented groups at the end of the third week compared to their respective base line values. However, there was a significant reduction ($P<0.05$) in the serum level of adiponectin protein in the patients administered with oral ascorbic acid supplementation at the end of the 6th and 9th weeks compared to the other patients who received only 5mg of glibenclamide (group 1) daily for the 6th and 9th weeks respectively. The hypothetical mechanism by which adiponectin improves insulin sensitivity could be linked to increase in fatty acid oxidation as well as inhibition of glucose synthesis in the liver.

Conclusion

Based on the results of our research, it is evident ascorbic acid plays a major synergistic role in the therapeutic management of type 2 diabetes mellitus with an oral hypoglycemic agent by significantly reducing the blood glucose as well as improving lipid profile and inflammatory response. Consequently, the dietary requirements for ascorbic acid (vitamin C) supplementation may be greater in people with diabetes.

Consent

A detailed medical and general physical examination was performed. Patients were enrolled after informed and written consent as per the inclusion and exclusion criteria.

Ethical Approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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