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A Comparative Study of Serum Antioxidant (Vitamin C) Level in People with and without Type 2 Diabetes in Narayanganj, Bangladesh

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Abstract

Diabetes is a group of metabolic disorders characterized by persistent hyperglycemia caused by reduced insulin secretion and/or impaired insulin sensitivity and has been correlated with oxidative stress. This study was designed to estimate the serum vitamin C level in type 2 diabetic patients and normal individuals in Narayanganj, Bangladesh and also to compare the blood levels of vitamin C, SGPT, SGOT, TC, TG, LDL, HDL and sugar in these groups for better comprehension of the role and status of antioxidants in diabetes patients. Pearson correlation of vitamin C level with other study parameters was also outlined. This case-control study included 23 diabetic patients (male 43.5% and female 56.5%) as cases and 24 healthy volunteers (male 29.2% and female 70.8%) as control. Every patient and control was informed of the investigation objective and written acquiescence was taken from them too. These participants had no prior history of hepatic, renal, retinal or cardiovascular disorders and were not taking any antioxidant (vitamin C). Anthropometric and clinical data were collected by visiting Nagbari Diabetic Hospital, Narayanganj, Bangladesh on a regular basis. Phenyl-hydrazine spectrophotometry method was used to estimate serum vitamin C level. Age (mean ± SEM) of the diabetic patient and controls were 65.39 ± 1.02and 64.13 ± 0.95 years respectively. The BMI (body mass index) value (mean ± SEM) of patient and controls were 24.90 ± 1.36 and 22.52 ± 2.13 kg/m^2 respectively. Serum level (mean ± SEM) of vitamin C in patient group was 4.69 ± 0.31(µmol/L) and that of control group was 7.83 ± 0.86 (µmol/L). Antioxidant level in patient group was significantly ($p<0.05$) lower than the control group. The Level (mean ± SEM) of SGOT and SGPT in patient group were 32.81 ± 2.48, 43.41 ± 5.61 µmol/L and those of control group were 22.54 ± 0.90, 24.46 ± 1.46 µmol/L respectively. The Level of TC, TG, HDL, LDL and sugar in patient group were 207.94 ± 6.42, 209.29 ± 13.91, 25.48 ± 2.04, 108.62 ± 6.17, 12.07 ± 0.91 mmol/L and those of control group 153.08 ± 6.03, 66.44 ± 3.2, 49.79 ± 1.29, 65.32 ± 3.80, 5.03 ± 0.24 mmol/L respectively. Pearson correlation analysis revealed opposite correlation of vitamin C level with all study parameters except HDL in both healthy and patient group. The results of the current study suggest a strong association of vitamin C depletion with diabetes and liver disorders. Antioxidant dietary supplements thus, may be favorable for the managing diabetes. However, in this context, more extensive research studies are required to perform.

Keywords: Diabetes, Vitamin C, Ascorbic acid, Liver disorders, Oxidative stress, Antioxidant

1. Introduction

Diabetes is a chronic disease outlined by persistent hyperglycemia caused by reduced insulin secretion and/or impaired insulin sensitivity (American Diabetes Association, 2014). When uncontrolled, this may lead to failure of several organs including the eyes, brain, heart and kidneys or may result in multiple metabolic disorders (WHO, 1999; Craig et al., 2009). Diabetic people are more likely to have peripheral arterial, cerebral, and cardiovascular atherosclerosis (American Diabetes Association, 2014).

Worldwide, diabetes is responsible for 3.2 million deaths annually (WHO, 2004). Only in 2014, there were 422 million diagnosed to have diabetes and the prevalence has been rising faster in low and middle-income nations than in high-income countries (WHO, 2022). High cholesterol, high triglycerides, low HDL, and raised levels of the liver enzymes SGPT and SGOT are also associated with diabetes (Ahmadieh & Azar, 2014).

Though, correlation between oxidative stress and diabetes have been reported (Mann, 1974;

Opara, 2004) and the importance of vitamin C has been mentioned in few journals (Christie- Dakhale et al. 2011; Chambial et al., 2013; Ahmadieh & Azar, 2014; Marin et al., 2018; Sun et al., 2022), there are insufficient studies on vitamin C blood levels and their effects in diabetic patients in Bangladesh. Thus, investigation has compared the blood levels of vitamin C, SGPT, SGOT, TC, TG, LDL, HDL and sugar in the diabetic patients with those of normal volunteers taking cases from Narayanganj, Bangladesh.

2. Materials and methods

2.1 Chemicals and reagents

Analytical grade of Thiourea, Trichloroacetic acid (Merck Life Science Ltd., Mumbai, India), Acetic acid (Merck KGaA, Darmstadt, Germany), Copper sulfate (PT. Smart Lab, Indonesia), Sulfuric acid (Merck KGaA, Darmstadt, Germany), Metaphosphoric acid, Dinitrophenylhydrazine (Research Lab Fine Chem Industries, Mumbai, India) were used in this study.

2.2 Study design

This case-control study was carried out in the Department of Pharmacy, R. P. Shaha University, collaborating with Nagbari Diabetic Hospital, Narayanganj, from June 2019 to December 2019. Moral consent was taken from the concerned committee of the diabetic hospital. This research enrolled 23 diabetic patients (male 43.5% and female 56.5%) as cases and 24 healthy volunteers (male 29.2% and female 70.8%) as control. Every patient and control was informed about the research motive and authorization was taken as well. These participants had no prior history of hepatic, renal, retinal or cardiovascular disorders and were not taking any antioxidants.

2.3 Data collection

A thorough patient history was gathered by regularly visiting Nagbari Diabetic Hospital. The collected date included age, gender, body mass index, serum level of glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride level (TG), and total cholesterol level (TC), SGPT and SGOT.

2.4 Blood sample collection for determination of serum level of vitamin C

Each participant's antecubital vein was used to collect five milliliters of blood after an overnight fasting. After about 30 minutes waiting at room temperature to permit clotting, the samples were centrifuged at 3000 rpm for 15 minutes. The serum was then collected and preserved at -20°C until the serum ascorbic acid (Vitamin C) content was determined.

2.5 Determination of serum ascorbic acid

Phenyl-hydrazine spectrophotometry method was used to determine serum ascorbic acid. Sample and standard absorbance were recorded in a UV-Visible Spectrophotometer (C-7200, Peak Instruments, USA) against reagent blanks at 520 nm. The ascorbic acid content in the serum was determined according to Sarwar et al. (2015).

2.6 Preparation of standard solution

After being heated to 105°C for 60 minutes, 100 mg of standard ascorbic acid was stored in a desiccator for 16 hours. Ascorbic acid solution (50 mg/dL) was prepared by dissolving it in metaphosphoric acid, which was then kept at 4oC. For working standard solution, 100 µL stock solution was diluted to 5 mL with trichloroacetic acid or metaphosphoric acid solution (1 mg/dL) immediately before used.

2.7 Procedure for serum analysis

A 300 µL serum and 1.2 mL trichloroacetic acid solution was centrifuged for ten minutes at 3000 rpm. After treating with 0.4 mL of DTC solution (a mixture of 2,4-dinitro phenyl hydrazine, thiourea, and copper sulphate solutions at a ratio of 20:1:1), 0.96 mL of clear supernatant was heated at 60 °C for 60 minutes in a water bath. The sample was then cooled and 1.6 mL sulfuric acid solution (65%) was added gradually. The whole procedure was repeated using 0.3 mL of the working standard solution of ascorbic acid and 0.3 mL of the reagent blank. By using a UV Visible spectrophotometer, the absorbance of both the samples and the standard were measured at 520 nm, in contrast to the reagent blank.

2.8 Statistical analysis

All values were presented as mean ± SEM. Using an independent sample t-test, the degree of significance of several factors between the case and control group was evaluated. Lastly, a correlation between the study parameters was carried out using Pearson's correlation analysis.

The data was analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA).

3. Results and Discussion

3.1 Anthropometric and demographic profile of the study population

The study group consisted of 23 diabetes patients having 10 males and 13 females, whereas the control group included 24 volunteers having 7 males and 17 females. All the data have been expressed as mean ± SEM. Table 1 lists the anthropometric and demographic characteristics of the patients and the controls. It was observed that mean age of the diabetic patient (n = 23) and controls (n = 24) were 65.4 ± 1.0 and 64.1 ± 0.9 years respectively. The average value of BMI (body mass index) of the diabetic patients and controls were 24.90 ± 1.36 and 22.52 ± 2.13 kg/m2 respectively. These two groups' age and BMI differences were not determined to be statistically significant.

Table 1: The study population's anthropometric and demographic characteristics

Variables	Patient group	Control group	p value
	Value ± SEM		
Age (years)	65.4 ±1.0	64.1 ±0.9	0.373^{NS}
BMI (kg/m^2)	24.90 ±1.36	22.52 ± 2.13	0.571^{NS}

NS = Not significant

3.2 Antioxidant level (Vitamin C)

As shown in table 2, the average serum levels of vitamin C in the patient in the patient group was 4.69 ± 0.31 and that of control group was 7.83 ± 0.86 (μmol/L). Thus it was obvious that the level of antioxidant in patient group was significantly ($p < 0.05$) lower than that of control group.

Table 2: Vitamin C serum levels in the study population

Parameters	Values (Mean ± SEM)		
	Patient group	Control group	p value
Vitamin C (μmol/L)	4.69±0.31	7.83 ±0.86	0.002^{*}

*p < 0.05 (Significant difference between patient and control groups at 95% confidence interval)

3.3 Other parameters

Diabetes is related to some other major diseases such as cardiovascular disease (CVS) (Leon and Maddox, 2015) and liver diseases (Ahmadieh & Azar, 2014). When comparing diabetic patients to control subjects, the mean blood levels of variables linked to CVS and liver disease, such as TG, TC, HDL, LDL, sugar, serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT), were significantly ($p < 0.05$) higher in the former group. On the other hand, these diabetes individuals had much reduced HDL levels. (table 3).

Table 3: Serum level of other parameters (SGOT, SGPT, TC, TG, HDL, LDL, sugar) of the study objects.

Parameters	Values (Mean ± SEM)		
	Patient group	Control group	p value
SGOT (µmol/L)	32.81 ± 2.48	22.54± 0.90	0.000*
SGPT (µmol/L)	43.41 ± 5.61	24.46 ± 1.46	0.002*
TC (mmol/L)	207.94 ± 6.42	153.08 ± 6.03	0.000*
TG (mmol/L)	209.29 ± 13.91	66.44 ± 3.21	0.000*
HDL ((mmol/L)	25.48 ± 2.04	49.79 ± 1.29	0.000*
LDL (mmol/L)	108.62 ± 6.17	65.32 ± 3.80	0.000*
Sugar (mmol/L)	12.07 ± 0.91	5.03 ± 0.24	0.000*

*P< 0.05 (significant difference at 95% confidence interval between patient and control groups)

3.4 Correlation between vitamin C and the levels of SGOT, SGPT, TC, TG, HDL and sugar

Both the patient and control groups underwent Pearson's correlation analysis to ascertain relationships between the relevant variables. Table 4 indicates that there was a positive association between vitamin C and HDL and a negative relationship between vitamin C and other variables, including SGOT, SGPT, TC, TG, LDL, and sugar.

Table 4: Correlation between vitamin C and the levels of SGOT, SGPT, TC, TG, HDL, and sugar

Correlation Parameters	Patient group		Control group	
	R	p	r	p
Vitamin C and SGPT	-0.052	0.814	-0.005	0.983
Vitamin C with SGOT	-0.142	0.517	-0.414*	0.044
Vitamin C with TC	-0.140	0.524	-0.026	0.903
Vitamin C with TG	-0.298	0.168	-0.294	0.163
Vitamin C with HDL	0.039	0.861	0.354	0.090
Vitamin C with LDL	-0.166	0.450	-0.171	0.423
Vitamin C with sugar	-0.091	0.679	-0.060	0.779

Values with a negative sign imply an inverse correlation; r = Correlation co-efficient; p = Significance; *Correlation is significant at 0.05 level (two-tailed).

According to our present research, vitamin C levels are lower in diabetics than in healthy participants, indicating a major role for oxidation in the pathophysiology of hepatic diseases and diabetes. Consequently, vitamin C supplements may be useful in treating the complications arising from these long-term conditions. Nevertheless, further research is necessary before a definitive conclusion can be established.

4. Conclusion

This investigation gave the idea of levels of vitamin C in diabetes patients as well as association of depletion of vitamin C with serum level of sugar, TC, LDL, HDL, and SGPT and SGOT, which are the parameters usually checked in diabetes and liver disorders. Vitamin C supplements thus might be helpful in managing; though extensive research findings are required in this regard. 5.

5. Ethical statement

The Nagbari Diabetic Centre in Narayanganj, Bangladesh and R. P. Shaha University both gave their approval for the study's overall design, protocol, and volunteer permission forms.

6. Informed approval

All individuals taking part in the study gave their informed consent.

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Competing interest

The authors have no conflicts of interest.

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