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# Relationship Between Serum Ascorbate, Cholesterol and Total Protein Levels in Patients with Non-insulin Dependent Diabetes Mellitus (NIDDM)

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Serum ascorbate, cholesterol and total protein levels were estimated in 25 newly diagnosed non-insulin dependent diabetes mellitus (NIDDM) patients. They were between 24 and 73 years of age and were age and sex- matched. The findings were compared with those of control subjects. Plasma ascorbate concentrations were found to be significantly lower ($P<0.005$) in NIDDM patients than in healthy subjects. Cholesterol level was significantly higher ($P<0.005$) in the diabetic than in the control subjects while there was no difference in protein level. A significant ($P<0.005$) effect of fruit was observed consumption on plasma ascorbate levels. Urinary ascorbate was significantly higher ($P<0.005$) in the non-diabetic subjects than in the diabetic patients.

**Key words:** Ascorbate, non-insulin dependent diabetes, cholesterol, diabetes mellitus

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## Introduction

Diabetes mellitus is a heterogenous syndrome with different modes of presentation and variable complexity of metabolic disturbances in the body. It is characterized by an elevation of blood glucose (hyperglycemia), glycosuria, osmotic diuresis, as well as alteration in carbohydrate, protein and lipid metabolism and is known to be caused by a faulty production of, or sub-normal tissue response to insulin (Withby *et al.*, 1984; WHO, 1985).

Ascorbate insufficiency/deficiency in patients with non-insulin dependent diabetes mellitus (NIDDM) has been reported in developed as well as certain developing countries.

However, there is no report on the ascorbate status of Nigerians with NIDDM. There have also been scientific evidence linking vitamin C insufficiency with mortality and morbidity and the possible prevention of diabetic complications through the use of vitamin C (Alpert and Braunwald, 1984).

In mammals, vitamin C, is physiologically related to glucose physiology. The status of both may be abnormal in persons with diabetes mellitus. In a population whose dietary vitamin C intake is marginal, diabetic individuals become vitamin C deficient (Stankoya *et al.*, 1984). Vitamin C levels in diabetics, especially those with severe micro-angiopathy or micro-vascular complications are low, probably as a result of vitamin C utilization during free-radical scavenging (Antia, 1986)). Of particular significance is the role of vitamin C in human nutrition and its association with diabetes mellitus (Tietz, 1987).

Several studies have revealed, the pleni-potential roles of this water soluble vitamin, particularly as an outstanding anti-oxidant in the human body (Stankoya *et al.*, 1984). A nutrition survey in 1965 noted that there was no significant evidence of vitamin C deficiency in the population group studied in Nigeria (Adenaike *et al.*, 1989). From 1965 to date, fruit intake has become more of a luxury than a necessity in Nigeria. Citrus fruits usually preferred by most people are seasonal and harvesting and storage techniques favour the destruction of the ascorbic acid content of the fruits. Since the vitamin is highly labile to oxidation and intense heat, the leafy vegetables which also furnish this vitamin are deprived of it by traditional cooking methods (Fafunso and Basir, 1976, 1977). Some studies have claimed that the average adult Nigerian does not ingest enough fruits (Adenaike, 1989). They found that only 13.1% of the studied group of adult population were found to ingest adequate quantities of vitamin C. Ascorbic acid has been found to have a hypocholesterolemic effect (Ginter *et al.*, 1978). Ramirez and Flowers (1980) has demonstrated that subjects with either marginal or acute vitamin C deficiency presented with higher plasma levels of cholesterol and normalization of cholesterol level was achieved with 20 mg $dl^{-1}$ daily intake of vitamin C. The role of ascorbic acid in the metabolism of proteins is reflected by the imbalance of serum proteins and enhanced catabolism and reduced protein synthesis under scorbutic conditions (Chalopin and Cooper, 1955). The aim of this study was to:

(a) Identify the base-line values for ascorbic acid and other nutritional parameters such as cholesterol and protein in newly diagnosed NIDDM and non-diabetic individuals.
(b) Determine the urinary ascorbate level in both groups.
(c) Identify the relationship between vitamin C, fruit consumption, total cholesterol and total protein in Nigerian diabetics.

## Materials and Methods

**Selection of patients:** The subjects selected were twenty-five newly diagnosed untreated diabetics attending the diabetic clinic of the University College Hospital Ibadan, Nigeria. Twenty-five apparently healthy individuals who were age and sex-matched represented the control group. The geographic distribution of the subjects covers major ethnic zones in the country (Hausa, Ibo and Yoruba) with majority being of the Yoruba ethnic origin tribe. The ascorbic acid intakes through fruits and vegetables were assessed by ordinary ranking as frequent, occasional and rare fruit consumers.

**Sample collection:** Fasting blood samples were collected from the subjects by vene-puncture. Ten ml of blood was drawn from each subject into appropriate sample tubes for ascorbic acid, cholesterol, total protein and glucose analysis. Samples were analyzed less than one hour after blood collection. Plasma and urinary ascorbate levels were determined spectrophotometrically, using the method of Aye (1978). Cholesterol and glucose were determined by enzymatic method (Allain *et al.*, 1974; Som *et al.*, 1980 and Salmenpera, 1984) and total protein by Biuret method.

**Characteristics of subjects:** Thirteen (52%) of the diabetic patients had no formal education or only attended primary school, while 9 (28%) and 5 (20%) had secondary and tertiary education respectively. Eleven (44%) of the diabetics with informal education were also engaged in menial jobs and earned about six hundred Naira ($6.00) or less per month. By using a scoring system, the subjects were classified into three socio-economic classes (SEC): lower: Thirteen (52%), 15 (64%), middle: 9 (36%), 7 (28%), upper: 3 (12%), 2 (8%) for the diabetic and control groups respectively.

## Results

**Fruit consumption of study group:** There was a significant difference ($P<0.005$) between the serum ascorbate levels in the diabetic and control subjects ($P<0.005$), being higher in the control (Table 1). This is also true for the urinary ascorbate levels in both groups ($P<0.005$). The values were higher ($P<0.005$) in both male and female control subjects than in the diabetic patients. The ranges for total cholesterol were 110 to 250 mg $dl^{-1}$ in the diabetics against 69-138 in the control subjects. The total cholesterol level is considered significant ($P<0.005$) when compared with the control subjects. The diabetic patients had a mean total protein of $6.08 \pm 0.16$ mg $dl^{-1}$, showing non-significant difference between the control group with a mean value of $6.51 \pm 0.2$ mg $dl^{-1}$ (Table 2).

Forty percent of all patients claimed that they had frequent intake of fruits. There was non significant difference with regards to fruit consumption when both groups were compared. However, differences ($P<0.005$) were significant within each group with reference to frequency of fruits consumption (Table 3). Also a comparison of the diabetic group with the control group showed an appreciable increase in fruits consumption among the controls than the diabetics: 40% (frequent), 24% (occasional), 36% (rare) in diabetic patients compared to 48% (frequent), 32% (occasional) and 20% (rare) in control subjects.

This result may not be unconnected with the sample size.

There was a non-significant effect of age on the parameters investigated except for glucose levels within the age range of 54-63 and 64-73 ($P<0.005$) and cholesterol levels within the age range of 24-33 and 34-43 ($P<0.005$).

Table 1: Biochemical parameters of diabetic and control subjects at presentation

| Biochemical indices | Diabetic | Control | P value |
|---|---|---|---|
| Age (years) | 45.00±2.8 | 43.00±1.5 | P>0.005 |
| Fasting blood glucose (FBG) | 288.00±6.40 | 86.00±2.40 | P<0.005 |
| Serum ascorbate (mg $dl^{-1}$) | 0.40±0.03 | 1.00±0.05 | P<0.005 |
| Urinary ascorbate (mg $dl^{-1}$) | 0.72±0.06 | 1.60±0.10 | P<0.005 |
| Total cholesterol (mg $dl^{-1}$) | 178.00±8.2 | 96.00±6.04 | P<0.005 |
| Total protein (mg $dl^{-1}$) | 6.08±0.16 | 6.51±.17 | P>0.005 |

All values are mean ± standard error of mean (SEM)

Table 2: Biochemical parameters of the diabetic and control subjects according to sex

| Biochemical indices | Diabetic male | Diabetic female | Control male | Control female | P value |
|---|---|---|---|---|---|
| FBG | 258.00±18.6 | 184.20±10.80 | 94.00±2.80 | 89.20±3.9 | P<0.005 |
| Serum ascorbate | 0.30±0.01 | 0.50±0.03 | 0.81±0.05 | 1.22±0.04 | P<0.005 |
| Urinary ascorbate | 0.53±0.03 | 1.00±0.09 | 1.28±0.10 | 2.04±0.08 | P<0.005 |
| Total cholesterol | 199.00±9.06 | 150.00±8.6 | 103.00±4.3 | 86.00±3.5 | P<0.005 |
| Total protein | 6.00± 0.24 | 6.24±0.19 | 6.52±0.30 | 6.50±0.22 | P>0.005 |

Table 3: Relationship between fruits consumption and ascorbate levels in newly diagnosed NIDDM patients and control subjects

| Biochemical indices | Diabetic plasma ascorbate | Control plasma ascorbate |
|---|---|---|
| Regular consumers | 0.51±0.04 | 1.20±0.2 |
| Occasional consumers | 0.35±0.04 | 0.90±0.03 |
| Rare consumers | 0.03±0.02 | 0.72±0.02 |

P<0.005

## Discussion

About 50 years ago, vitamins, particularly vitamin C, were of interest only to a small group of scientists. Recent knowledge about vitamin C and its functions has been derived from diverse lines of investigations with each providing findings that cumulatively furnished a comprehensive biochemical understanding about this vitamin especially its role in human health. Recently too, there has been a report linking vitamin C deficiency and development of diabetic complications (Stanloya *et al.*, 1984). In such patho-physiological conditions, acute or chronic de-saturation could occur due to increased demand for vitamin.

Vitamin C (ascorbate) is known to be involved in the pathogenesis and evolution of diabetes mellitus (Stankoya *et al.*, 1984; Antia, 1986; Som *et al.*, 1980). A critical analysis of the data presented in this study will enable diabetologists to define a reliable programme for the prophylaxis and treatment of diabetic conditions and recognize the need for supplementation with vitamin C for diabetic patients. It should be noted that plasma ascorbate would be a reflection of ascorbate metabolism in tissues and erythrocytes, particularly with respect to the oxidation of ascorbate to dehydroascorbate.

In this study, plasma ascorbate was found to be significantly lower than the control subjects. Values obtained in this study for control subjects are significantly lower than the values reported in other studies. Allain *et al.* (1974) reported a mean value of 1.47±0.06 in healthy adults in the United States of America. Salmenpera (1984) also reported a mean plasma value of 1.24 mg $dl^{-1}$ in healthy and lactating finnish women compared to a mean of 1±0.05 reported in this study. This result thus confirms the reported inadequate intake of fruits by an average Nigerian and can be said to reflect a sub-optimal level of vitamin C of the general Nigerian population to a certain extent. Results also revealed sex difference with higher values in females. The reason for this is not known.

From this study, there exists a relationship between fruit consumption and ascorbate levels, showing higher values in the regular consumers, followed by occasional consumers and then the rare consumers. These findings reinforce the view of Ojo (1991 unpublished work) in which he observed differences in ascorbate level and fruits consumption. Low fruits consumption especially citrus fruits could partly explain the low ascorbic acid status in the group of diabetic patients studied. This also might be responsible for the marginally low levels in the non-diabetic control subjects. Though it has been suggested that biochemical measurement of serum ascorbate represents the most objective assessment of vitamin C nutritional status of an individual, however, some scientists have equally encouraged urinary ascorbate determination as a supplementary method of indirectly assessing vitamin C status.

With that in mind, urinary ascorbate levels were determined in both diabetic patients and control subjects. The results showed lower values in diabetic patients than the control subjects. It is believed that once the ascorbate level exceeds the range of 1.4 to 1.6 mg $dl^{-1}$, its threshold in blood would be exceeded and is consequently excreted in the urine. By inference, the higher the urinary ascorbate, the higher the vitamin C content in the ingested diet in normal individuals. The urinary ascorbate level obtained in this study is not sufficient to be used as an indicator or index of vitamin C intake especially among the diabetics. Research studies have indicated that low plasma ascorbate and the accumulation of a significant level of dehydroascorbate in the diabetic patients may be due to: increased turn-over of ascorbate into dehydroascorbate and decreased rate of reduction of dehydroascorbate to ascorbate within the body system. In the diabetics, there is a rapid conversion of ascorbate into dehydroascorbate. This is assumed to be partly responsible for the low plasma ascorbate. This rapid conversion may also partly explain the urinary ascorbate status in the diabetic patients; thus for the diabetic patients, urinary ascorbate may not accurately assess the vitamin C intake since there is more dehydroascorbate than ascorbate excreted in the urine.

The mean total cholesterol obtained for the diabetic is higher than the control. Many factors are known to influence plasma cholesterol concentration. Dietary cholesterol and carbohydrate metabolism increase plasma concentration of cholesterol. Sex, age and geographic location also influence cholesterol level (Lee *et al.*, 1988). Male and female diabetics of all age groups have significantly higher level of total serum cholesterol than those of sex and age-matched non-diabetic subjects (Aduba *et al.*, 1978). This finding agrees with the present study. Ramirez and Flowers (1980) demonstrated that subjects with either marginal or acute vitamin C deficiency presented with higher level of cholesterol and observed a normalization of cholesterol level following the administration of vitamin C. Low vitamin C level may have contributed in some way to the high level of cholesterol observed in the diabetic patients studied.

Recent clinical investigations have demonstrated the link between dietary protein intakes and progression of diabetic nephropathy with an influence on glycaemic control. Pomerleau and Lombardi (1993) found that moderate and adequate protein intakes reduce urinary albumin excretion and positively affect the glycaemic control. The values obtained for plasma protein level in the diabetic females and in control females did not differ significantly, but differ significantly between the male diabetics and male controls, being higher in the male control. The reason for this is not clear but dietary intakes could partly explain the difference in plasma protein concentrations between diabetic and control subjects with regards to sex. Therefore the estimation of total protein should form part of a routine laboratory in the diabetics particularly among Nigerian diabetics.

There was a significant difference between the glucose level in diabetic and control subjects, being higher in the diabetics than the control. In this study the effect of vitamin C supplementation on blood glucose level was not assessed.

The results of the present study indicated the need to regular monitoring of ascorbic acid status in diabetic patients so as to determine when supplementation is necessary in order to prevent complications that could probably arise from its deficiency. At present Nigerian diabetic patients are not supplemented with vitamin C. This study then is a step in the right direction as it furnishes information on the current vitamin C status of diabetic patients and equally gives a reflection of the vitamin C status of an average adult.

Although, diabetic mellitus may not be as alarming as some of the

terminal diseases, its consequences may be equally devastating in view of its impact on the society with regards to decreased productivity, social stigma and sudden death. While efforts being made in using therapy to effectively manage diabetes are welcomed, there is a need to look beyond the drug therapy for effective management of the syndrome. The importance of this lies in the fact that diabetes requires a life-long management. Moreover, with the ever-increasing cost of drugs and the difficulty in affording them, many diabetic patients may not be able to adhere to their therapeutic regime and therefore deteriorate in health. It is shown from this study that natural nutrients especially vitamin C is significantly low in diabetic patients. At present, there is scanty information on the current vitamin C status in the Nigerian population. Our findings would therefore, alert the patients, the health professionals, the public and the government of the inherent danger in its inadequate intakes and the attendant complications that may result from such. Also, since the ascorbate levels in healthy subjects has been found to be marginally low, the national health policy should incorporate public education on the need for individuals to increase their fruits intakes as there is a generally poor culture of fruit consumption.

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