Plasma Ascorbic Acid In Hypercholestrlaemic Subjects

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Citation

Abstract

Plasma Ascorbic acid, total cholesterol, High density lipoprotein cholesterol levels and Total cholesterol/High density lipoprotein cholesterol ratio were determined in 52 newly diagnosed hypercholesterolaemic subjects and 48 normocholesterolaemic subjects. Hypercholesterolaemic subjects were defined as those with plasma total cholesterol ≥200mg/dl.
The results showed that the mean plasma ascorbic acid levels were significantly lower in hypercholesterolaemic subjects when compared to normocholesterolaemics (P< 0.005) while total cholesterol , high density lipoprotein cholesterol and total cholesterol/high density lipoprotein cholesterol ratio were higher in hypercholesterolaemics than control (P<0.005).
The low ascorbic acid levels observed in hypercholesterolaemics may be due to increased lipid peroxidation and hence low ascorbic acid levels may play a role in the aetiology of hypercholesterolaemia and therefore vitamin C supplements maybe useful in the management of Hypercholesterolaemia.

INTRODUCTION

Plasma lipids have many physiological functions and they have been incriminated as aetiological factors in atherosclerosis and coronary heart disease [1] . Hypercholesterolaemia is due to an increase in both free and esterified cholesterol. It could be genetic, acquired or may be secondary to other disorders or to dietary factors [2] .

The beneficial effect of lowering serum cholesterol have been demonstrated in several experiments and results show that reducing serum cholesterol on average by just 1 mmol/l (34.6 mg/dl) with diet or drugs resulted in significant decreases in the incidence of coronary heart disease and rate of progression of coronary lesions [3] .

Ascorbic acid has antioxidant properties protecting cellular membranes [4] and could therefore protect against atherosclerosis [5] ; of which hypercholesterolaemia is a risk factor. This study is aimed at determining the level of ascorbic acid in hypercholesterolaemics.

MATERIALS AND METHODS
SUBJECTS AND SAMPLES

Patients attending the medical outpatient of the University of Benin Teaching Hospital, Benin City, Nigeria, had their plasma total and high density lipoprotein cholesterol estimated and those with total cholesterol levels ≥ 200 mg/dl were selected for the study as hypercholesterolaemic

subjects. 52 newly diagnosed subjects were selected for this study and 48 apparently healthy normocholesterolaemic subjects were used as control. Prior to the study, the subjects were not on Ascorbic acid supplement and were also not on treatment as they had no knowledge of their cholesterol levels.

10 ml of venous blood sample were collected in appropriately labeled potassium EDTA anticoagulated containers. These were centrifuged and plasma separated into appropriately labeled containers. The cholesterol was assayed immediately while the plasma was stored frozen and the Ascorbic acid assayed within 48 hours.

ESTIMATION OF PLASMA ASCORBIC ACID REAGENTS

- Trichloroacetic acid (TCA) 5 %(w/v)

- Indophenol reagent

- Sulfuric acid 9N

- Sulfuric acid,85%(v/v)

- Dinitrophenylhydrazine - Thiourea reagent

- Ascorbic acid stock 50 mg/100 ml

- Ascorbic acid working standard 0.5 mg/100ml

METHOD OF ANALYSIS

The 2, 4-dinitrophenylhydrazine method by Roe and kuether as modified by Daphne was used in estimating plasma ascorbic acid [6]

PRINCIPLE

Ascorbic acid is oxidized to dehydroascorbate by the action of 2,6-dichlorophenolindophenol. The dehydrate is hydrolysed to diketogulonic acid in the strongly acid medium. This forms an Osazone with 2, 4 dinitrophenyl hydrazine. The Osazone rearranges to a stable reddish brown product which can be measured photometrically at 500 nm.

PROCEDURE

2 ml plasma was pipetted into centrifuge tube. 6 ml of 5% TCA was added and mixed thoroughly. This was centrifuged and the protein free supernatant was used for the estimation. 2 ml of supernatant were pipetted into tubes marked 'sample' and 'sample blank'.

2 ml ascorbic acid standard was pipetted into tubes marked 'standard' and 2 ml of 5%TCA into a tube marked standard blank.

0.1 ml of indophenol was added to all tubes and mixed thoroughly.

0.5 ml Dinitrophenylhydrazine-Thiourea reagent was added to the standard and samples. All tubes were incubated for 1 hour in a 60°C water bath.0.5 ml Dinitrophenylhydrazine-Thiourea reagent was added to standard blank and all sample blanks. While all the tubes remain in ice water, 2.5 ml 85% sulfuric acid was added to all tubes and mixed thoroughly. The absorbance of were read at 500 nm using a spectrophotometer.

CALCULATION

Figure 1

$$\text{Conc of ascorbic (mg/100ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{conc of ascorbic acid in acid standard}$$

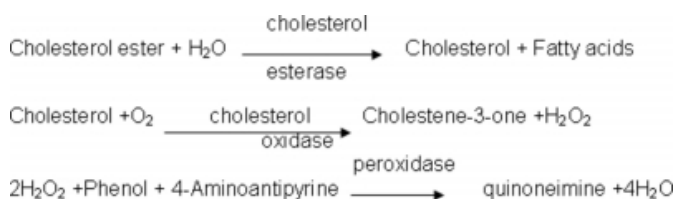## ESTIMATION OF PLASMA TOTAL CHOLESTEROL

The enzymatic colorimetric method by Tinder was used [7].

REAGENTS

4-Aminoantipyrine 0.5 mM, Phenol 6mM, 3,5-dichlorophenol 0.2mM, Cholesterol esterase ≥ 500kµ/l, Cholesterol oxidase ≥ 300kµ/l, Peroxidase ≥ 1200kµ/l , Phosphate buffer (pH 6.7) 70mM.

PRINCIPLE

Figure 2

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_2O$$

PROCEDURE

To 1000ul of reagent in tubes meant for samples, standard and blank, 10µl of samples, standard and distilled water were added respectively. The content was mixed and incubated for 5mins at 37°C. The absorbance of the samples and standard was measured against reagent blank at 546nm

CALCULATION

Figure 3

$$\text{Plasma cholesterol Conc (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc of cholesterol in standard}$$

ESTIMATION OF HDL-CHOLESTEROL
REAGENTS

HDL-cholesterol precipitant (Phosphotungstic 4.8%, Magnesium chloride 3M)

PRINCIPLE

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of acid phosphotungstic in the presence of magnesium ions. After centrifugation the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant is determined.

PROCEDURE

Into centrifuged tubes, 500µl of sample and 100µl of HDL-cholesterol precipitant was added, mixed and allowed to stand for 10mins at room temperature .The tubes were centrifuged at 4,000rpm.100µl of supernatant were assayed for cholesterol estimation with the procedure given above for total cholesterol estimation.

Figure 4

$$\text{Concentration of HDL(mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

STATISTICAL ANALYSIS

All measures of central tendencies and dispersion were

analyzed using descriptive statistics in statistical packages for social sciences (SPSS) statistical package. The non-parametric Kruskal-Wallis test was used to test for the significant difference.

RESULTS

The results showed that there was a significant decrease in the plasma ascorbic acid level of hypercholesterolaemic subjects. The total cholesterol, HDL-cholesterol and Total cholesterol / HDL-cholesterol ratio increased significantly when compared with control. (P<0.05) TC/HDL-C ratio is the most effective lipid profile for predicting coronary heart disease. Progression of growth of coronary lesion is associated with relatively high values for TC/HDL-C ratio while low values have no disease progression.

Figure 5

Table 1: Plasma Ascorbic Acid, Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C) Levels And TC/HDL-C Ratio In Normocholesterolaemic And Hypercholesterolaemic Subjects

Subjects	Ascorbic acid (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	TC/HDL-C Ratio
Normocholesterolaemics(n=48)	0.66±0.25	151±22	34±12.4	5.1±2.3
Hypercholesterolaemics (n=52)	0.32±0.19	218±36	41±17	6.2±2.7
P-Value	P<0.05	P<0.05	P<0.05	P<0.05

DISCUSSION

Hypercholesterolaemia is defined as cholesterol level greater than 200mg/dl [8]. The hypothesis that elevated plasma cholesterol may induce endothelial dysfunction and thus contribute to atherosclerosis has been a particular focus of attention. It has been demonstrated that lipid peroxidation products are damaging to the cells and can initiate a sequence of events leading to atherosclerotic lesion [9].

Ascorbic acid is an antioxidant, protecting cellular membrane thus preventing oxidative stress and damage [10]. It reduces lipid peroxidation thus preventing the development of atherosclerotic plaques. Therefore the findings of elevated total cholesterol levels and low levels of plasma ascorbic acid in hypercholesterolaemic subjects is an indication of

predisposition to risk of developing atherosclerosis. Ascorbic acid deficiency leads to defective formation of intracellular substances. The low levels as observed in the hypercholesterolaemic subjects can amplify tissue damage caused by lipid peroxidation thereby resulting in the development of atherosclerosis.

CONCLUSION

We conclude that hypercholesterolaemics have lower levels of plasma ascorbic acid compared with normocholesterolaemics. This low level of Ascorbic acid may not be dietary but may be due to the increased demand for ascorbic acid due to high lipid peroxidation in hypercholesterolaemics, Ascorbic acid supplements may be beneficial in the management of hypercholesterolaemia.

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