



## VITAMINS E AND C CONCENTRATIONS IN ANAEMIA SUBJECTS ATTENDING BRAITHWAITE MEMORIAL SPECIALIST HOSPITAL PORT HARCOURT

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### ABSTRACT

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#### Keywords

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The study was carried out to determine Vitamins E and C concentrations in anaemic subjects attending Braithwaite memorial Specialist Hospital Port Harcourt. Three hundred (300) subjects made up of 150 anaemic patients (77 females and 73 males) age ranging between 15 – 67 years attending the Braithwaite Memorial Hospital (BMH) Port Harcourt and 150 apparently normal healthy individuals (78 females and 72 males), age ranging between 15 – 67 years were used as controls for this study. The subjects had their Haemoglobin, Vitamins E and C concentrations determined. The results were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) 22. The result of the study showed that there was significant difference ( $P < 0.05$ ) in haemoglobin concentrations (g/dl) of  $13.51 \pm 1.14$  in healthy subjects compared with  $6.77 \pm 1.82$  in anaemic subjects as shown in Table 1 below. Also the Vitamin E concentration (Mg/l) of  $7.82 \pm 2.38$  was significantly different from  $5.33 \pm 1.90$  in anaemic subjects ( $P < 0.05$ ). The Vitamin C concentration (Mg/dl) of  $0.61 \pm 0.29$  was not significantly different from  $0.28 \pm 0.16$  in anaemic subjects ( $P > 0.05$ ). This study showed that there was significant decrease in Vitamin E concentrations in anaemic subjects.

**Contribution/Originality:** The study was carried out to determine Vitamins E and C concentrations in anaemic subjects attending Braithwaite memorial Specialist Hospital Port Harcourt.

### 1. INTRODUCTION

Haemoglobin (Hb) outside its protective environment in the red blood cell is toxic. It undergoes unhindered oxidations process of its iron centre, which results in the transition of Hb from the ferrous (Hb Fe<sup>2+</sup>) functional form to the ferric nonfunctional form (Hb Fe<sup>3+</sup>) [1]. Autoxidation results in the production of superoxide (O<sub>2</sub><sup>-</sup>), and ferric haemoglobin (Hb Fe<sup>3+</sup>). The superoxide will by a process of dismutation, generate hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and other highly reactive species such as the hydroxyl radicals (OH<sup>-</sup>/OH). Therefore, haemoglobin is constantly exposed to an intracellular flux of H<sub>2</sub>O<sub>2</sub> (generated from its autooxidation or from other intracellular sources by a range of oxidase enzymes (glycollate and monoamine) as well as by the peroxisomal pathway for beta – oxidation [2]. It is evident that under appropriate conditions, damaging and potentially toxic species are formed, including ferryl protein (Hb Fe<sup>4+</sup>), a highly reactive Hb – associate oxidant which can peroxidize lipids, degrade carbohydrates and cross – link proteins [1].

Vitamin C, also known as ascorbic acid, is a water soluble vitamin unlike most mammals; humans do not have the ability to make their own vitamin C. Hence, Vitamin C has to be supplemented through diet. It has been reported that Vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implication for blood cholesterol levels and the incidence of gallstones [3]. It has been reported that smokers have lower blood levels of vitamin C. this has been attributed to increased oxidative stress from common cigarette smoke [4].

Vitamin E also referred to, as d- alpha tocopherol is one of four fat-soluble vitamins. The names of all types of vitamin E begin with 'd' or 'dl', which refer to differences in chemical structure. The 'd' form is natural (also known as RRR- alpha tocopherol). And 'dl' is synthetic (known as -arc- alpha tocopherol). The natural form is more active and better absorbed. After the 'd' or 'dl' designation, often the Greek letter 'alpha' appears which also describes the structure. Synthetic "dl" vitamin E is found only in the alpha form – as in "dl-apha-tocopherol". Natural vitamin E may be found either as alpha- as in "d- alpha tocopherol", or in combination with beta, gamma, and delta, labeled "mixed" –as in mixed natural tocopherols . Arteriosclerosis is a disease of the arteries in which fatty, often calcified deposits develop on the inside of the arterial wall (intima) and eventually cause blockages that lead to cardiovascular disease (angina, heart attack and stroke). However, vitamin E in high doses can impair blood clotting and increase the risks of hemorrhage

The aim of this study is to determine the concentrations of vitamins E and C in anaemic Subjects attending Braithwaite Memorial Specialist Hospital Port Harcourt.

## 2. METHOD AND MATERIALS

### 2.1. Reagents

The reagents used for the determination of the biochemical parameters include vitamin E which were prepared reagents according to method previously described by Quaife, et al. [5] which uses Emmerie-Engel reaction and Vitamin C which were prepared reagents according to method of Omaye, et al. [6].

### 2.2. Subjects Selection

Three hundred (300) subjects divided into two groups were used in this study. The first group was the apparently normal healthy individuals. A total number of 150 individuals were used as controls for this study (78 females and 72 males), age ranging between 15 – 67 years. All controls for the study gave their consent in writing to participate in the study by filling questionnaires given to them and allowing blood samples to be taken from them. Subjects were picked randomly from all social classes. The control subjects were free from acute illnesses like malaria, typhoid, pneumonia, hepatitis, sinusitis; upper respiratory tract infection and none had history of concomitant illness like rheumatic heart diseases, diabetes mellitus, hypertension, leukaemia and others. Smokers and patients with history of blood transfusion were excluded from the study. 5mls of blood samples were collected by vene puncture into EDTA and plain sample bottles and taken to the laboratory for analysis.

The second group of the human subjects consisted of 150 anaemic patients (77 females and 73 males) age ranging between 15 – 67 years attending the Braithwaite Memorial Hospital (BMH) Port Harcourt. Patient's data, diagnosis and causes of illness were obtained from patients' folders. Blood samples were collected into EDTA and plain bottles for analysis and all cases of anaemia were confirmed by carrying out haemoglobin assay on in patients' samples

### 2.3. Biochemical Analysis

**Haemoglobin determination** was by Cyanmethaemoglobin method using Modified Drabkin's fluid. This fluid was prepared by dissolving 200mg potassium ferricyanide, 50mg potassium cyanide, 140mg potassium dihydrogen phosphate and 1ml of non ionic detergent. The dilution factor was 1:200. The potassium ferricyanide converts the haemoglobin to methaemoglobin, which is further converted to cyanmethaemoglobin by the action of potassium

cyanide. The absorbance of the solution was read at 540nm according to Cheesbrough [7]. Five millilitre (5ml) of Drabkins solution was pipetted into test tube labelled blank, test and control and 0.02ml of blood sample and control was added to tubes labelled test and control. It was incubated at 25°C for 5 minutes. The contents were read at 540nm using the content of the blank tube to Zero the spectrophotometer. The values of the unknown were extrapolated from a standard calibration curve.

Vitamin C in blood plasma was measured according to the method previously reported by Omaye, et al. [6]. Ascorbic acid in plasma is oxidized to form dehydroascorbic acid which reacts with acidic 2, 4 – dinitrophenyl hydrazine to form a yellow bishydrozone which was measured at 530nm. Standard used was L-Ascorbic acid. To 0.5ml of plasma in tube labelled test, 1.5ml of 6% TCA was added, centrifuged for 20mins at 1000g, 0.5ml of the supernatant was pipetted into separate clean tube and 0.5ml of DNPH reagent was added, incubated for 3hrs at room temperature. thereafter, 2.5ml of 85% H<sub>2</sub>SO<sub>4</sub> was added to test tube, allowed to stand for 30mins, and absorbance read at 530nm. Concentration of vitamin C was obtained from the calibration curve of vitamin C (stock 2mg/ml).

Determination of vitamin E was performed according to the method previously described by Quaife, et al. [5] using Emmerie-Engel reaction. Emmerie-Engel reaction was used for color reaction of tocopherol (vitamin E). Ferric chloride and α<sup>1</sup> α<sup>1</sup> dipyridyl (Emmerie-Engel) reagents. The Principle of the reaction is based on the reduction by tocopherol of ferric to ferrous ions which then forms a red complex with α<sup>1</sup> α<sup>1</sup> dipyridyl. Tocopherols and carotenes were first extracted into xylene and extinction read at 460nm to measure the carotenes. A correction was made for these by the addition of ferric chloride and absorbance read at 520nm. Concentration of vitamin E was obtained using the formula:

$$\text{Vitamin E} = \frac{\text{Readings at 520nm} - \text{Reading at 460nm} \times 0.29}{\text{Readings of standard at 520 nm}} \times 10$$

Test tubes were labelled test, standard and blank. Plasma (1.5ml) was pipetted in tube labelled test, while 1.5ml of vitamin E standard ( D-α tocopherol, 10mg in ethanol), was pipetted into tube labelled standard, and 1.5ml of water was pipetted in tube labelled blank. Absolute ethanol and xylene, 1.5ml each was pipetted into all tubes, mixed and centrifuged at 1000g for 10mins. Thereafter, 1ml of xylene layer was transferred into separate test tubes labelled test, standard, and blank. Then 1ml α<sup>1</sup> α<sup>1</sup> dipyridyl reagent was added to all tubes, mixed and absorbance read at 460nm. Finally, 0.33ml of ferric chloride was finally added to all tubes, mixed and absorbance read at 520nm after 10mins. Concentration of vitamin E was obtained using the above formula.

#### 2.4. Statistical Analysis

All statistical analysis of the results were performed using Statistical Package for Social Sciences (SPSS) version 16.0 software produced by SPSS Inc., Chicago, IL. Results are expressed as Mean ± SD, and they were compared using students' t test. Values of P < 0.05 indicate statistical significant difference in the mean values while P > 0.05 means no significant difference.

### 3. RESULT

The result of the study showed that there was significant difference (P < 0.05) in haemoglobin concentrations (g/dl) of 13.51 ± 1.14 in healthy subjects compared with 6.77 ± 1.82 in anaemic subjects as shown in Table 1 below. Also the Vitamin E concentration (Mg/l) of 7.82 ± 2.38 was significantly different from 5.33 ± 1.90 in anaemic subjects (P < 0.05). The Vitamin C concentration (Mg/dl) of 0.61 ± 0.29 was not significantly different from 0.28 ± 0.16 in anaemic subjects (P > 0.05) as shown in Table 1.

**Table-1.** Vitamin E and C concentrations in anaemia.

Parameters	Apparently Normal Individuals	Anaemic subjects	P Value
Haemoglobin (g/dl)	13.51±1.14	6.77±1.82	P<0.05
Vitamin E (mg/l)	7.82±2.38	5.33±1.90	P<0.05
Vitamin C (mg/dl)	0.61±0.29	0.28±0.16	P>0.05

The result of the study showed that there was significant difference ( $P < 0.05$ ) in haemoglobin concentrations (g/dl) of  $13.79 \pm 1.61$  in healthy male subjects compared with  $6.75 \pm 1.95$  in male anaemic subjects as shown in Table 2 below. Also the Vitamin E concentration (Mg/l) of  $7.99 \pm 2.58$  was significantly different from  $5.32 \pm 1.75$  in male anaemic subjects ( $P < 0.05$ ). The Vitamin C concentration (Mg/dl) of  $0.63 \pm 0.37$  was not significantly different from  $0.29 \pm 0.17$  in male anaemic subjects ( $P > 0.05$ ) as shown in Table 2.

**Table-2.** Vitamin E and C concentrations in male anaemic subjects.

Parameters	Apparently Normal males Individuals	males Anaemic subjects	P Value
Haemoglobin(g/dl)	13.79±1.61	6.75±1.95	P<0.05
Vitamin E(mg/l)	7.99±2.58	5.32±1.75	P<0.05
Vitamin C(mg/dl)	0.63±0.37	0.29±0.17	P>0.05

The result of the study showed that there was significant difference ( $P < 0.05$ ) in haemoglobin concentrations (g/dl) of  $13.12 \pm 0.85$  in healthy female subjects compared with  $6.74 \pm 1.65$  in male anaemic subjects as shown in Table 3 below. Also the Vitamin E concentration (Mg/l) of  $8.19 \pm 4.66$  was significantly different from  $5.38 \pm 2.08$  in female anaemic subjects ( $P < 0.05$ ). The Vitamin C concentration (Mg/dl) of  $0.61 \pm 0.31$  was not significantly different from  $0.31 \pm 0.37$  in female anaemic subjects ( $P > 0.05$ ) as shown in Table 3.

**Table-3.** Vitamin E and C concentrations in female anaemic subjects.

Parameters	Apparently Normal females Individuals	Females Anaemic subjects	P Value
Haemoglobin(g/dl)	13.12±0.85	6.74±1.65	P<0.05
Vitamin E(mg/l)	8.19±4.66	5.38±2.08	P<0.05
Vitamin C(mg/dl)	0.61±0.31	0.31±0.37	P>0.05

#### 4. DISCUSSION

Haemoglobin is normally intracellular protein and it is packaged within the red cells, which are rich in antioxidant defence enzymes. Hence, haemoglobin outside its normal location is a potentially damaging molecule. Haem and iron so released can stimulate lipid peroxidation [8].

The results of the present study showed that vitamin E was significantly reduced in anaemic patients compared to healthy controls. This finding is also suggestive of the existence of low antioxidant defence in anaemic patients. The decreased levels of antioxidant status may be due to lower dietary intake of antioxidant vitamins such as vitamin E and sulphur-containing amino acids such as methionine in the diet or like albumin, more utilization of the vitamin to remove excess free radicals produced by anaemic patients. The result of this study is in agreement with Das, et al. [9]; Jain and Williams [10]; Thomas, et al. [11]; Titus, et al. [12] and Nwanjo [13]. Furthermore, vitamin E as well as cholesterol is transported in plasma almost exclusively by lipoproteins. Thus decrease levels of lipoproteins carriers may affect this vitamin [14, 15]. It is generally known that vitamin E is a lipid-soluble antioxidant in cell membrane, functioning as a scavenger of peroxy radicals, it is probably the most important inhibitor of the free-radical chain reaction of lipid peroxidation [8]. In addition to its antioxidant function, tocopherols are capable of quenching singlet molecular oxygen, thus protecting membranes against light induced oxidative damage. Tilton, et al. [16] have reported signs of decrease levels of vitamin E in haemolytic anaemia.

The results of this study showed no significant difference in vitamin C concentrations in anaemic subjects compared to healthy controls. Vitamins C and E play an excellent role in protecting cells from oxidative damage [17]. They are both potent antioxidants and they provide a buffer against oxidative stress that results to cell

damage. Both in health and diseases, increasing interest is being directed to the significance of optimal vitamin and antioxidant status. Antioxidant status evidently varies considerably among populations. Moreover, changes over time in dietary habits affect not only the intake of fruits and vegetables but also the consumption of polyunsaturated fatty acids, all of which influences the oxidant- antioxidant balance. The decrease levels of these vitamins observed in this study could also be as a result of increase in free radical scavenging activities of vitamins C and E in quenching excess ROS production in the anaemic patients.

## 5. CONCLUSION

The study has shown decrease in Vitamin E concentrations in anaemic subjects attending BMSH.

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