ANTIOXIDANT VITAMIN STATUS OF NIGERIAN HOSPITALISED PATIENTS

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
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BY

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DECLARATION

I hereby declare that this thesis is a product of my own research work. No previous submission of it has been made for a degree at any University. The work of other researchers referred to have been duly acknowledge.

10 - 11 - 97

KABARA, HASSAN TIJJANI

DATE

CERTIFICATION

This thesis titled "Antioxidant vitamins status of Nigerian hospitalised patients" by **KABARA** Hassan Tijjani meets the regulations governing the award of the degree of M.Sc in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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To my wife (Rukayya Muhtar), daughters (Hafsa Hassan and Nafisa Hassan) and sisters (Aisha Tijjani and Halima Abdullahi).

ACKNOWLEDGMENT

It takes devotion and sacrifice to achieve a goal in every undertaking. In this way, I wish to express my gratitude to those whose devotion contributed immensely to the completion of this work.

First and foremost, my gratitude goes to Almighty Allah for His Loving provisions and care over me throughout the duration of this work.

I am very much indebted to my Supervisor, Dr. O. K. Obagaiye. Many thanks goes to her for her supervision, discussions, criticisms and suggestions which proved valuable during the moments of profound difficulties that were so frequently encountered throughout the duration of this work.

My profound gratitude also goes to my wife, colleagues and friends for their help and encouragement.

My appreciation goes to my parents, whose moral and financial support have helped me in forging ahead thus far.

KABARA HASSAN TIJJANI
MARCH, 1997.

ABSTRACT

The antioxidant vitamin status (vitamin A, C and E) *and* albumin concentrations of 20 apparently healthy controls *and* 73 hospitalised patients suffering from a variety of diseased conditions., namely cardiovascular, liver, renal, diabetes *and* GIT disorders were assessed. Our data revealed that control subject had significantly higher Antioxidant vitamins and albumin status than Diseased subjects with the exception *of* females GIT Subjects who had significant1y lower vitamin E levels than their male counterparts Age and Sex had no apparent effect, on the Antioxidant status of either the healthy or diseased population. Albumin concentration *in* control subjects was inversely ($r = -0.416$ $p < 0.05$) corre1at*ed* with renal patients.

The vitamin E levels of control was equally positively correlated with those for renal patients. vitamin A control was positively associated with baseline *value* ($r = 0.470$ $p < 0.05$). A re1ationships similar1y existe*d* between the vitamin A levels of renal and Cardiovascular patients ($r = 0.584$ $p < 0.05$).

Vitamin *C* levels of Cardiovascular *and* Diabetes renal or GIT patients ($p < 0.05$) were else inversely correlated. An interre1ationship was evident between the Antio*xida.*nt vitarnin status in Diseased co*nd*ition. A1bumin was *not* associated with Antioxidant vitamin sta*tu*s in diabetes. It

is applied from present data that regular diets of hospitalised patients can not maintain acceptable Antioxidant, vitamin status in\ these patients hence dietary supplementation of Antioxidant vitamin is recommended in the management of these disorder.

1t is postu1ated from present study that the most p1ausib1e mechanism for interrelationship evident amongst the Antioxidant vitamin is a rapid uti1ization of the Vitamin as a consequence of increased free radical, produced by drugs and the pathological effects of this diseased conditions.

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LIST OF ABREVIATIONS

1.	DNA	-	Deoxyribonucleic acid.
2.	HSA	-	Human Serum Albumin.
3.	RBP	-	Retinol Binding Protein.
4.	WHO	-	World Health Organisation.
5.	ICND	-	International Congress on Nutrition and Dietetics.
6.	A.B.U	-	Ahmadu Bello University.
7.	EDTA	-	*Ethyl Diamine Tetra Acetic acid.*
8.	PCV.	-	Packed Cell Volume.
9.	H.B.	-	Haemoglobin.
10	DT Mix	-	A mixture of 2,4 - Dinitrophenylhydrazine, thiourea and sulfuric acid.
11	TCA.	-	Trichloroacetic acid.
12	GIT.	-	Gastrointestinal Tract.
13	BCG.	-	Bromocresol green.
14	IUB.	-	International Union of Biochemist.

CHAPTER ONE

INTRODUCTION

Free radicals are highly reactive molecules that contain one or more unpaired electrons (Aruoma et al 1991). They originate endogenously from metabolic processes or exogenously as components of air pollution, cigarette smoke, radiation and infection. Free radicals can cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes, nucleotide in DNA and critical Sulphydryl bonds in protein (Machlin and Bendrich, 1987). There is increasing evidence (Halliwell, 1987) implicating free radicals in the pathogenesis of a number of infectious diseases such as malaria, tuberculosis, cardiovascular and renal disorders, and acute bacterial infections (Aruoma et al, 1991). *The body's major defence mechanism against free* radical damage are the antioxidants or free radical scavengers. These include the antioxidant vitamins, ceruloplasmin, uric acid, bilirubin, superoxide dimutase (EC1, 15, 11:1 and Glutathione peroxidase EC1, 11, 1:9).

The antioxidant Vitamins include B - carotene, the provitamin of Vitamin A (Wilson, 1975), ascorbic acid and the tocopherols (Vitamin E). Tocopherol is the major lipid soluble antioxidant breaking the chain of lipid peroxidation in cell membranes and preventing the formation of hydroperoxides (Wilson, 1975).

Vitamin C in addition to directly scavenging free radicals in the cytoplasm also participate in the regeneration of vitamin E and the antioxidant peptide Glutathione (Wilson, 1975).

The antioxidant status of the body can be considerably influenced by diet and should the normal defence mechanism be weakened by nutritional deficiencies, then pathogenic consequences may occur. Available evidence (Bristran et al, 1976) have indicated that Hospitalised patients are often severely malnourished as a result of anorexia and pathological wasting syndrome associated with such disorders.

Realizing that the effectiveness of the antioxidant defence system is dependent on an adequate dietary intakes of foods containing such antioxidant vitamins, which may be limiting in the diets of hospitalised patients, present study was embarked upon to assess the antioxidant vitamin status of Nigerian Hospitalised patients.

vitamin A adequacy was measured as an index of B-carotene adequacy as most of the Vitamin A of Nigerian hospitalised patients are derived predominantly from carotenoid precursors.

CHAPTER TWO
LITERATURE REVIEW

2:1 HISTORY:

Retinal was first isolated as an antivitamin and was called the anti infective Vitamin or axerophytol because it prevented the dryness of the eye (Moore, 1957). In 1930, Kaner established the structural formula of vitamin A and B - carotene. Isles and his colleagues also synthesized the pure form of the vitamin in 1947. Between 1910 and 1920, Zilver and his colleagues isolated ascorbic acid and subsequently synthesized the vitamin. Vitamin E was first isolated from wheat germ in 1916 (Moore, 1957).

2:2 STRUCTURES

The structure of vitamin A, tocopherol and ascorbic acid has been established. The IUB Commission (1971) proposed that vitamin A be considered as a generic term for description of all retinoids compounds, eliciting the biological activity of *Retinol. These include Retinol, 3 dehydroretinol and* their esters.

Vitamin E is also used as the generic names for nine trienols and the tocols eliciting the biological properties of the tocopherols, B-carotene, a provitamin of vitamin A is an asymmetrical molecule containing pro *B*-iohine rings connected by a conjugated chain. Chemically L-ascorbic acid is a simple compound with an empirical formula of C3 H8 O6.

The chemical structures of the vitamins are presented in table 1.

2:3 PROPERTIES

The chemical and physical properties of the naturally occurring and synthetic forms of the antioxidant vitamins are known (Murphy *et al*, 1990). Vitamin A exist as a Yellow oil, which is readily oxidized when exposed to heat, air or UV light.

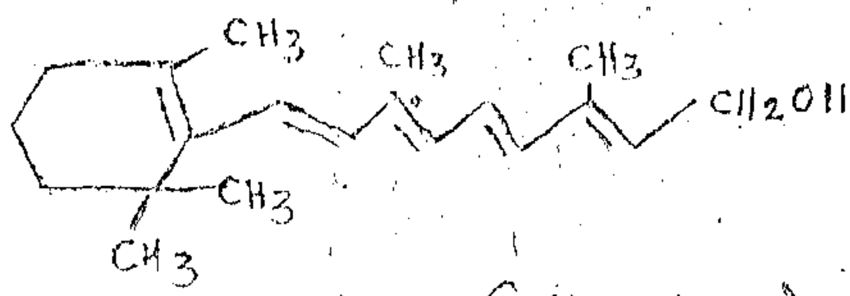
Ascorbic acid is a white crystalline powder which is readily destroyed by oxidation and alkali. It is however stable to acid. Tocopherols are fat soluble vitamins, which are relatively stable to alkali.

2:4 OCCURRENCE

Vitamin A is widely distributed in plant food sources as the provitamin carotenoids. Among the commonly occurring carotenoids such as &-carotene, B-carotene &-carotene and &-lycopene, B-carotene is the most abundant (Martin *et al*, 1985) B carotene is found in fruits and dark green leafy vegetables such as mangoes, carrot, pawpaw and Anaranth. Preformed dietary vitamin A is found in fatty fish mammalian livers and kidney. Murphy et al (1990) have confined that vitamin E is found principally in seed nuts, *vegetable oils and wheat germ oil. Other appreciable sources* of tocopherol exist in dairy process, eggs and vegetables. Ascorbic acid is widely distributed in citrus fruits, and vegetables such as tomatoes and carbage.

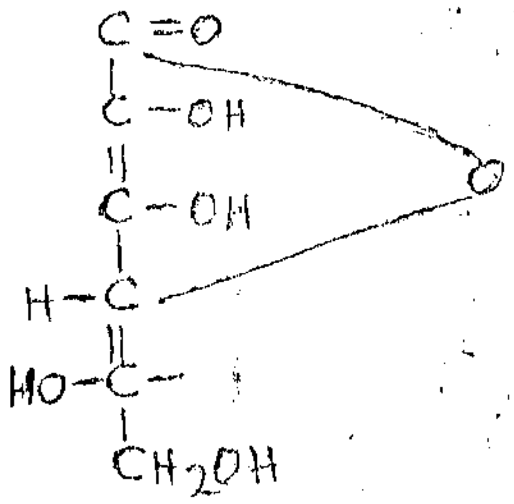
Table 1: CHEMICAL STRUCTURE OF THE VITAMIN A, C AND E

(1)



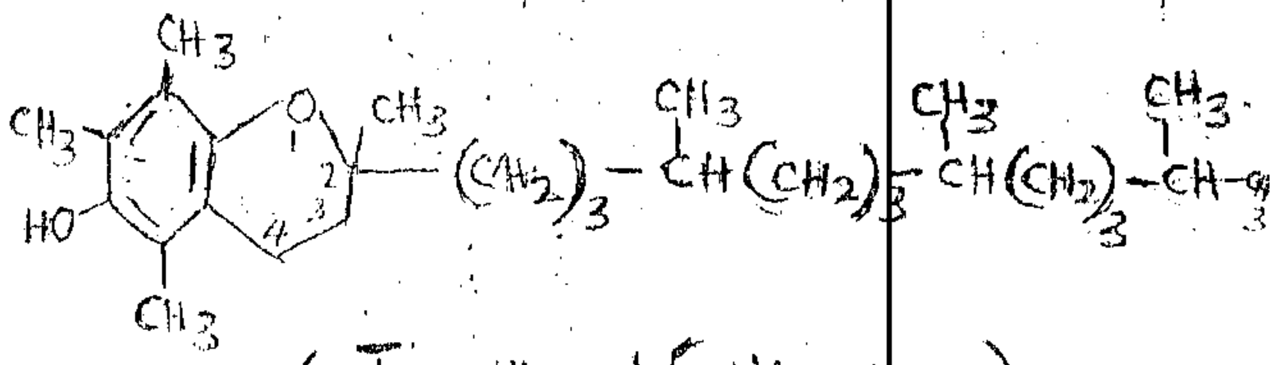
Retinol (Vitamin A)

(2)



Ascorbic acid (Vitamin C)

(3)



α-Tocopherol (Vitamin E)

[illegible] conditions in which Oxygen Free Radicals are thought to be involved.

TABLE 2.

BRAIN
Parkinson's disease
Neurotoxins
Vitamin E deficiency
Hyperbaric oxygen
Hypertensive cerebrovascular injury
Aluminium overload
Allergic encephalomyelitis (demyelinating diseases)
Potentiation of traumatic injury

EYE
Photic retinopathy
Ocular hemorrhage
Cataractogenesis
Degenerative retinal damage
Retinopathy of prematurity

HEART AND CARDIOVASCULAR SYSTEM
Atherosclerosis
Adriamycin cardiotoxicity
Keshan disease (selenium deficiency)
Alcohol cardiomyopathy

KIDNEY
Metal ion-mediated nephrotoxicity
Aminoglycoside nephrotoxicity
Autoimmune nephrotic syndromes

GASTROINTESTINAL TRACT
Non steroid-anti-inflammatory drug-induced GI tract lesions
Oral iron poisoning
Endotoxin liver injury
Diabetogenic actions of alloxan
Halogenated hydrocarbon liver injury
Free-fatty acid-induced pancreatitis

LUNG
Bronchopulmonary dysplasia
Mineral dust pneumoconiosis
Bleomycin toxicity
Hypoxia
Cigarette smoke effect
Emphysema
Adult respiratory distress syndrome (some forms)
Oxidant pollutants (ozone, SO_2, NO_2)

RED BLOOD CELLS
Fanconi's anemia
Sickle cell anemia
Favism
Malaria
Protoporphyrin photo-oxidation

CANCER

INFLAMMATORY-IMMUNE INJURY
Rheumatoid arthritis
Glomerulonephritis
Autoimmune diseases
Vasculitis (hepatitis B virus)

ALCOHOL

AGING

RADIATION INJURY

IRON OVERLOAD
Nutritional deficiencies (Kwashiorkor)
Thalassemia and other chronic anemias treated with multiple blood transfusions
Dietary iron overload (red wine, beer brewed in iron pots)
Idiopathic hemochromatosis

ISCHEMIA-REPERFUSION
Stroke/myocardial infarction
Organ transplantation

(skin injury due to solar radiation, porphyria, contact dermatitis, and photosensitizers may also involve free radical mechanisms.)

Source: Aruoma, Kaur & Halliwell. J Roy Soc Health 111(5): 172 (Oct 1991)

2:5 FUNCTION;

The role of the antioxidant vitamins in intermediary metabolism have been well established (Wolf, 1984; Sauberlich, 1974; Block, 1992). Vitamin A, for instance have been implicated in the maintenance of tissue integrity. Wolf (1984) in a review highlighted the role of vitamin A in the visual cycle, where it elicits its function as a Chromophore of the visual pigments in rods and cones as the most important biological function of the Vitamin. Page 11 presents the pathway proposed for the reaction. Pilts (1981) have also reported its role in mucopolysaccharide and glycogen synthesis.

Ascorbic acid has been shown (Basu and Schorah 1982) to function in hydrogen ion transfer systems and aid in the regulation of intracellular oxidation - reduction potentials (Kaller et al, 1981). Hallwell (1987) have reported the antioxidant potentials of tocopherol and ascorbic acid, and their involvement in the protection of cell membrane against oxidative stress initiated by free radicals. Zannan et al (1982) have implicated a role for ascorbic acid in the detoxification of certain poisonous compounds and drugs.

Ascorbic acid also functions in the conversions of folic acid to folinic acid and facilitate the intestinal absorption of iron (Sauberlich, 1974).

2:6 METABOLISM

The metabolism of B-carotene and Vitamin A is fairly established (Smith et al, 1972). Dietary retinyl esters and B-carotene are hydrolysed by pancreatic esterases in the intestinal lumen. This is absorbed into the mucosal wall where vitamin A is transported to the liver by the protein carrier, the retinol binding protein (RBP) (Ong, 1985).

Once retinol is delivered to the tissues, the RBP is catabolised in the proximal convoluted tubule in the Kidney. Vitamin A and carotenoids are excreted in the urine and bile as inactive metabolites. Vitamin E metabolism is similar to vitamin A as both require the presence of fat for absorption (Martin *et al*, 1985).

Metabolically, ascorbic acid (Baker et al, 1966; Ong, 1985) behaves like glucose. It is completely absorbed in the intestine. Jacob *et al* (1987) in a metabolic study reported that ascorbic is found in small amounts in the plasma but are concentrate in white blood cells and in the adrenal glands of the Kidney and the liver.

2:7 DEFICIENCY

The importance of vitamin A deficiency as the cause of blindness from xeropthalmia in many areas of the third world has been well recognised. Epidemological studies reported by WHO (1976) revealed that about 250,000 children go blind annually, of whom 50% die and 8 - 9 million develop non cornea xeropthalmia syndrome.

Chytill (1985) reported that in vitamin A deficiency states, there is stratification of cells followed by squamolar metaplasm and keratimyatin resulting into a destruction of the epithelial well. Vitamin A deficiency is also known to be associated with protein energy malnutrition (Whitehead 1970).

Summer *et al* (1974) reported increased risk of respiratory disease and diarrhea in children suffering from mild Vitamin A deficiency. A metabolic interrelationship between the onset of measles and vitamin A deficiency in children *have also been reported by* Barclay *et al* (1987). Experimental Derivation of Vitamin E in animals (Henning *et al*, 1987) is charactered by sterility in males and absorption in females. Haemorrhage and extensive lesions have also been reported. In man, muscular dystrophy (Stimson, 1975) *have been* associated with Vitamin E inadequacy. The main disorder associated with Hypovitaminosis C is scurvy, a disease which gained importance in the 16th century.

2:8 FREE RADICAL MECHANISM AND ANTIOXIDANT VITAMINS

Free radicals are molecules or molecular fragments with an unpaired electron. These radicals have been implicated in the etiology of many diseases, which include Tumor formation, toxic liver injury, neuromuscular disorders, arteries, non overload, gastrointestinal and liver disorders. Aruoma *et al* (1991) in a review tabulated thec linical condition in which oxygen free radicals are thought to be involved (Table 2).

Machlin L. Y and Bendrich A. (1987) reported that free radicals elicit their action by promoting tissue injury to DNA, cell membrane, Enzymes and other cell components. The antioxidant vitamins have been indicated (Machlin and Bendrich, 1987) to have a protective effect against free radicals production in Health and Disease. Block (1992) for example, have reported the beneficial effect of ascorbic acid supplementation in mortality from cardiovascular disease. Schwartz and weiss (1994) have also recently identified the beneficial effect of vitamin C in the prevention of pulmonary diseases and in lowering blood pressure (Hemula, 1991).

Mayer *et al* (1994) also highlighted the protective effect of Vitamin E and B carotene in the incidence of lung cancer.

2:9 ANTIOXIDANT VITAMIN IN DISEASE

Available evidence (Jayalakshmi *et al*, 1958) indicate that cancer, tuberculosis and chronic infections, particularly pneumonia, chronic nephritis, urinary tract infections and prostrate diseases result to increased excretion of vitamin

A. Serum vitamin A levels are also depressed in patients with celiac disease, sprue, obstructive jaundice, cystic fibrosis and giardiasis.

Dubick *et al* (1987) and Ginter *et al* (1979) have also independently reported decreased plasma vitamin C concentrations in atherosclerotic ocular diseases.

Gey *et al* (1987) identified an inverse correlation between plasma vitamin E and Vitamin A levels to the incidence and mortality from ischaemic heart disease. Ginger *et al* (1979), also reported decreased plasma and leukocyte ascorbate in diabetics.

Vitamin E deficiency has equally been implicated in nearly all gastrointestinal tract disorder (Machlin, 1980).

2:10 ASSESSMENT OF NUTRITIONAL STATUS

Various diagnostic methods have been proposed (Sauberlich 1974, Olson 1984, Jacob et al, 1987) for the estimation of vitamin A, E and C in biological fluids. These methods include enzymatic assays, microbiological, colorimetric and photometric chemical assays (Sauberlich et al, 1974). Definitive guideline for accessing these vitamin status have also been formulated. Sauberlich et al (1974) have proposed a plasma ascorbate concentration of 0.3 - 0.8 mg/dl as indicative of acceptable ascorbic acid status while the ICND have proposed a plasma retinol level of < 10 mg/dl as indicative of deficient vitamin A status. Other specialised technique that have been proposed in the evaluation for the clinical signs of vitamin A deficiency include the *measurement of retinal function* (Vinton *and* Russel 1981) *and* conjuctival integrity by impression cytology technique (Wiltpen *et al*, 1986).

2:11 ASSESSMENT OF THE NUTRITIONAL STATUS OF HOSPITALISED PATIENTS

It is well established (Bistran *et al*, 1975, Bistran *et al*, 1976) that protein energy malnutrition often exist in adult hospitalised patients as a consequence of anorexia or the pathological wasting phenomenon associated with the disorders.

Rostchild *et al* 1973) noticed that under these conditions, serum albumin concentrations and other plasma protein may alter considerably. Bistran *et al* (1975) therefore, proposed that the measurement of serum albumin be used to detect malnutrition in hospitalised patients.

Fleck *et al* (1985) noted that serum albumin levels are *affected by changes in capillary permeability and by* external losses and by alteration in lymphatic rehim.

Stress (Kinney 1976); sepsis (Mealer, 1976) and cancer (cray and waterhune, 1957) have all been indicated and lead to decrease serum albumin concentration. Albumin concentrations have also been indicated to fall considerably in liver disorders.

2:12 CARDIOVASCULAR DISEASE AND ANTIOXIDANT VITAMINS

Medically cardiovascular Disease is a generic name use to describe all diseases of the heart. Many investigators have classified the disorders of the heart into four broad categories namely congenital heart Disease or failure, atherosclerosis, hypertension and rheumatic fever. The term heart failure refers to myocardial failure, manifested by various symptoms and physical signs produced by an underlying disease process in which cardiac output does not meet the demands of the body.

Atherosclerosis is a lesion of large and medium arteries charactered by yellowish material and lipophages or fat-injesting cells in the intimal layer of the arteries, hypertension on the other head refer to a condition in which constriction of the arteries produces excess or high blood pressure in the heart, while rheumatic fever refers to a disease, usually affecting children and young adults, often characterized by fever, pains in the joints, and a tendency to evolve the vaives of the heart.

Akinkugbe (1969) have reported arteriod hypertension and cardiac failure as the most important cardiovascular disorder in the Nigerian populace. The role of free radicals in the etiology of cardiovascular disease have been established (Hallwell, 1987).

The consequent effect of antioxidant vitamins in informing the outcome of disease condition have also been documented (Eristrian et al 1992). Block (1992) for instance reported a relationship between Vitamin C consumption and cardiovascular mortality. Gey (1991) also reported an increase relationship between plasma vitamin E and mortality from ischaemic heart disease in Grnopea.

2:13 LIVER DISEASES AND ANTIOXIDANT VITAMINS

Sheila and summerfeild (1979) defined liver diseases as all diseases affecting the liver, some of which include viral and Drug hepatitis, liver cirrhosis, acute liver failure Hepatoma and Amoebic liver absess. Other forms of liver diseases include iron storage diseases, Wilson disease, Alpha-antitrypsin deficiency, Glycogen storage diseases and cystic fibrosis. The predominant form of liver disease in the Nigerian population (Ayoola, 1983) are viral hepatitis, cirrhosis, sickle cell, hepatic disease and Hepatoma.

Available evidence (Sheila and Summerfielld, 1979) indicate that viral hepatitis can be classified into two types, type

A virus which causes acute hepatitis in young adults and children and the type B virus which causes both acute and chronic hepatitis.

Cirrhosis which is a pathological liver disease, occurs as a result of prolonged liver cell necrosis followed by fibrosis in the liver. Cholestasis is however a disorder which occur due to the inability or failure of bile flow from the hepatocyte to the duodenum.

Existing studies (Aruoma *et al*, 1991) have implicated free radical injury as a potential mechanism for the etiology of some liver Disorders. A deficiency of most antioxidants vitamin have equally been reported in liver injuries (caroll *et al*, 1970).

2:14 RENAL DISEASE AND ANTIOXIDANT VITAMINS

Kagan (1979) described renal disease as all disorders associated with Kidney failure. He described the following conditions as falling under Kidney disorders. They are bilharzia, acute and chronic nephritis, chronic phyelonephritis and chronic renal disease.

The predominant form of renal disease found in most Nigerian hospitals as reported by Katchy and Khwaja (1983) include ureteric obstruction due to bilharzial infection. Previous investigators have identified decreased plasma ascorbate levels in hemodialysed renal disease patients.

2:15 GIT DISEASE AND ANTIOXIDANT VITAMINS

GIT disease are disorders affecting the gastrointestinal tract (Jones *et al*, 1968). They include diarrhoca, dysphagia, dyspepsia and abdominal pain.

Schloelmerich et al (1984) have found that patients with crohn's disease have been shown to be vitamin A deficient.Decreased absorption of fat soluble Vitamins has been suggested as cause of this deficiency. Carotene conversion is also limited in diseases of intestine, hence the low levels of Vitamin A in serum (Mikhail *et al*, 1982).

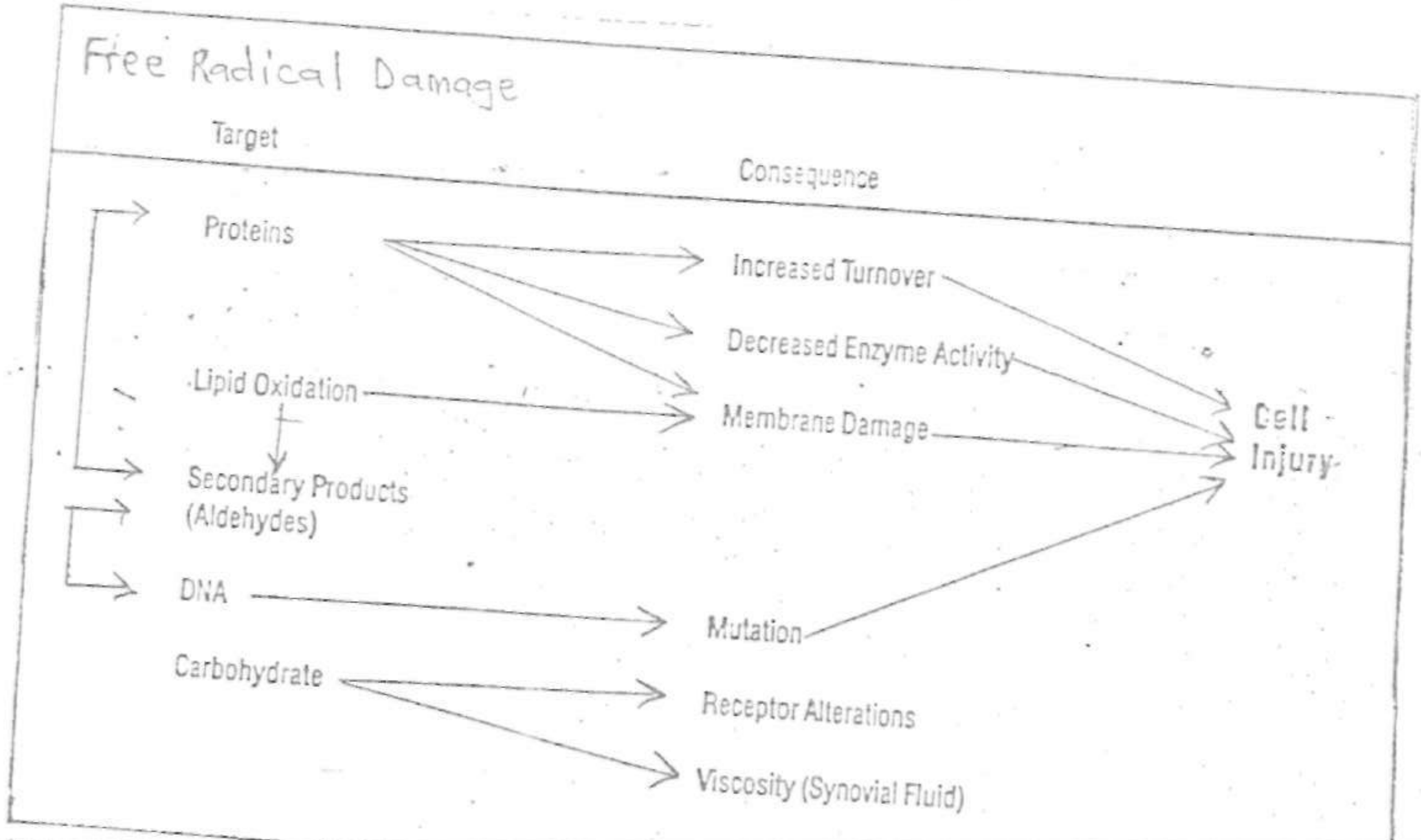
Cater and Cook (1963) carried out studies on the serum total carotenoids, vitamin A and serum colour in Nigerian Soldiers, 22 patients, in most, gastroenteritis or schistosomiasis and 20 healthy as controls. Serum vitamin A was low and carotenoids levels was high from the subjects.

Low Vitamin C reserves have also been reported in gastrointestinal diseases (Hughes and Williams, 1978).

2:16 *DIABETIC DISEASE AND ANTIOXIDANT VITAMINS*

Diabetic mellitus is a disease in which the metabolism (body utilization) of sugars is greatly impaired due to the faulty secretion of insulin by the pancreas (Schmidt's, 1981).

Low vitamin A levels have also been reported for diabetic patients (Moore, 1957). This has been attributed to an impairment in metabolic conversion of carotene to the vitamin in presence of lowered vitamin A intakes. Ginter *et al* (1979) reported, decreased plasma and leukocytes ascorbic acid in diabetic.



Source: Hoffmann-La Roche Inc. Nutley, New Jersey

CHAPTER THREE

METHODOLOGY

3:1 SUBJECTS:

Seventy eight hospitalised patients (aged 10 - 65) were drawn from three hospitals within Zaria locality namely, A.B.U. Teaching Hospital, Wusasa General Hospital and Anna - Kitz Hospital. These subjects include liver disease renal disease,diabetes, gastrointestinal disorder and cardiovascular patients.

The diagnosis of disease were done by a medical doctor and blood collection was carried out under supervision of a medical Doctor.

3:2 NUTRITIONAL STATUS

The anthropometric and demographic data were also collected. The data were collected using questionnaire for each patient. The questionnaire carried information such as age, sex, type of disease occupation etc.

3:3 BLOOD SAMPLING

Twenty milliliters of venous blood were collected from each patient. An aliquot of blood sample (5 ml) was dispensed into EDTA container and then used immediately for the assay of Haemoglobin and packed cell volume. The remaining blood aliquot (15 ml) were separated as serum and frozen at -20oC for 4 four weeks until used for vitamin A, C and E and serum albumin assays.

3:4 HAEMOGLOBIN DETERMINATION METHOD (Drabkins, 1935).

0.02 ml of the patient's blood was dispensed into a tube containing 5 ml of Drabkin solution. The mixture was mixed well and allowed to stand for ten minutes.

The blank and standard tubes containing 5 ml of Drabkin solution and an appropriate known concentration of cyanomethaemoglobin was simultaneously set up. The absorbance of contents of tubes for standard test was read at 540 nm.

Hb concentrations was calculated thus;

$$Hb = \frac{T \times C \times 250}{A \times 1000} \text{ g/100ml}$$

Where T is the test absorbance at 540nm, A the standard absorbaNce at haemoglobin standard (mg/100 ml), and 250 the dilution factor; 1000 convert from mg/100ml to g/100ml.

3:5 PACKED CELL VOLUME ESTIMATION METHOD (Microcapillary)

A plain capillary tubes was filled to 3/4 length with venous blood and sealed with a plastacine. The capillary tubes were the subsequently arranged in the haematocrit centrifuge and spin for 5 minutes. The PCV is determined by measuring the height of the red cell column and expressing this as a ration of the height of the total blood column as follows:

$$PCV = \frac{\text{Height of red cell column}}{\text{Height of total blood column}}$$

3:6 MEASUREMENT OF SERUM ALBUMIN WITH BROMCRESOL GREEN

(Doumas et al, 1971)

25 ml of serum or working standard solutions was added 5.0 ml of working dye solution. It was mixed and allowed to stand for 10 minutes at 25%c. The absorbance was then measured at 628 nm after adjusting the instrument to zero absorbance with the working dye solution. It was mixed and allowed to stand for 1o minutes at 25oC.

3.7 VITAMIN C ESTIMATION METHOD (ROE AND KEUTHER 1942)

To 2.0ml of serum was pipetted 6.0 ml of 5% trichloro acetic acid (TCA. The resulting protein precipitate was centrifuge and decanted. 2.0 ml of the above supernatant was then pipetted into tube labelled sample and sample Blank.

2.0 ml of working ascorbate standard was pipetted into tube for the ascorbate standard and 2.0 ml of 5% TCA was pipetted into the tube for the ascorbate Blank.

One drop of indophenol reagent was then added to all tubes and mixed well. 0.5 ml of a mixture of 2,4 -

Dinitrophenydrazine, thiourea, and sulphuric acid in water (DT mix) was added to Ascorbate standard and to all samples *except the blank. All tubes were incubated for one hour in* a 60oC water bath and cooled in ice water. 0.5 ml of DT mix was added to sample blank and ascorbate blank only.

Why samples remained in ice bath, 2.5 ml of 85% sulphuric acid was adjusted to zero using ascorbate blank. Optical density of ascorbate standard was read and recorded. The spectrophotometer was adjusted to zero using the sample blank for each corresponding sample.

Calculations: The serum ascorbate was calculated using the formula below;-

$$\frac{\text{OD of Sample}}{\text{OD of Standard}} \times 2 = \text{mg of total ascorbate for 100 ml.}$$

3.8 A FLUOROMETRIC MICROMETHOD FOR SERUM VITAMINS A AND E

(Hansen and Warwick 1969).

To three separate tubes containing 2.2 ml aliquot of serum, water and standard respectively was added 1 ml of distilled water. The tubes were mixed in a vortex mixer for 30 seconds.

Two milliliters of distilled ethanol were then added to each tube after which 5 ml of n were added and the tubes were capped and mixed for 30 seconds. They were then centrifuged at 900 x g for 5 minutes.

The supernatant or the Organic layer of disposable pipette. The solutions are then read in a fluorometer. The fluorometric readings were obtained following the calibration of the instrument by blank sample. The florescence and excitation units for unknown and standard were obtained.

Calculations: Vitamin A and Vitamin E (free) concentrations in the serum were calculated using the following formulas:-

$$\text{Vitamin A} = \frac{(uf)h}{(sf)} \text{ mg/100 ml}$$

$$\text{Free Vitamin E} = \frac{(uf)h}{(sf)} \text{ mg/ml}$$

Where uf was the fluorescence of the unknown, sf was the fluorescence of the standard concentration in mg per 100ml respectively.

RESULT AND DISCUSSION

4.1 ANTIOXIDANT VITAMIN STATUS AND ALBUMIN CONCENTRATION OF THE CONTROL POPULATION

In this study, the antioxidant vitmin and albumin concentrations of 20 apparently healthy Nigerian subjects were evaluated by serum and dietary measurement. Our data revealed that 80% of the subjects derived vitamin A predominantly from plant sources. While foods from animal origin supplied 7% of the total vitamin A intake. All the subjects consumed vitamin A above the 750ug RE/day RDA proposed for such subjects.

On Table 3 and 4 is presented the vitamin A, C and E; and albumin concentrations of apparently healthy subjects ,Age and Sex had no significant effect ($p<0.05$) on the level of the nutrients. A serum vitamin A concentration of 30-50ug/dl(1.0-1.7umol/l) has been reported (Olson 1987). Adopting this criteria for the control population, all the subjects evaluated had acceptable vitamin A status.

Schorah (1979) proposed that lower limit of normality for establishing adequate vitamin C status should be a plasma ascorbic acid of between 0.2-0.3mg/dl (11.4-17.0umol/l). In present study among the control subjects, no subjects suffered from biochemical hypovitaminosis C.

TABLE 3: VITAMIN A AND E STATUS OF HEALTHY AND DISEASED SUBJECTS:

			CONTROL	CARDIC	LIVER	G.I.T.	DIABETES	RENAL
VITAMIN A µmol/L	MALE	MEAN ± SD	2.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.2
		RANGE	(1.4 - 2.5	(0.6 - 1.4	(0.5 - 1.4	(0.6 - 1.2	(0.7 - 0.9	(0.6 - 0.9)
	FEMALE	MEAN ± SD	1.9 ± 0.4	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
		RANGE	(1.2 - 2.4)	(0.5 - 1.2)	(0.5 - 1.2)	(0.5 - 0.8)	(0.6 - 0.9)	(0.7 - 0.9)
VITAMIN E µmol/L	MALE	MEAN ± SD	23.2 ± 2.8	19.7 ± 9.3	16.0 ± 6.5	14.6 ± 3.5	14.9 ± 1.4	13.9 ± 2.3
		RANGE	(18.6 -27.9)	(11.5 -41.8)	(9.3 -39.2)	(11.6 -20.9	(13.0 -16.3)	(11.6 -16.3
	FEMALE	MEAN ± SD	22.8 ± 2.3	15.0 ± 6.3	16.3 ± 5.0	12.3 ± 1.2	13.2 ± 1.4	12.8 ± 1.5
		RANGE	(16.6 -25.5)	(9.3 -30.2)	(11.6 -27.9)	(11.6 -13.9)	(11.6 -13.9)	(11.6 -13.9)

TABLE 4: VITAMIN C AND ALBUMIN CONCENTRATIONS OF CONTROL AND HOSPITALISED SUBJECTS.

			CONTROL	CARDIO	LIVER	G.I.T.	DIABETES	RENAL
VITAMIN C µMol/L	MALE	MEAN ± SD	51.1 ± 17.0	17.0 ± 5.7	17.0 ± 5.7	17.0 ± 5.7	17.0 ± 5.7	17.0 ± 5.7
		RANGE	(28.4 - 79.5)	(11.4-28.4)	(5.7-28.4)	(11.4-22.7)	(11.4-22.7)	(17.0 - 22
	FEMALE	MEAN ± SD	39.7 ± 11.4	17.0 ± 5.7	17.0 ± 5.7	17.0 ± 0.0	17.0 ± 5.7	17.0 ± 0.0
		RANGE	(28.4-56.8)	(11.4-22.7)	(11.4-22.7)	(11.4-17.0)	(11.4-17.0)	(11.4 - 17
ALBUMIN g/l	MALE	MEAN ± SD	46.0 ± 4.0	30.0 ± 7.0	33.0 ± 8.0	28.0 ± 7.0	33.0 ± 9.0	31.0 ± 3.0
		RANGE	(40 - 52)	(20 - 40)	(20 - 50)	(20 - 35)	(24 - 47)	(26 - 41)
	FEMALE	MEAN ± SD	45.0 ± 3.0	30.0 ± 8.0	30 ± 11.0	24.0 ± 3.0	27.0 ± 6.0	26.0 ± 6.0
		RANGE	(40 - 50)	(20 - 36)	(20 - 51)	(20 - 27)	(20 - 32)	(21 - 30)

The Vitamin E and albumin concentrations of the subjects were also within these earlier reported for a similar population (Natta and Machlin 1979)

4:2 ANTIOXIDANT STATUS OF DISEASED POPULATION RELATIVE TO CONTROL POPULATION

In the present data, the serum Vitamin A, Vitamin E and ascorbic concentrations of apparently healthy controls were significantly ($p < 0.05$) higher than those observed for Diseased subjects (Table 3 & 4). Although a slight increase in serum ascorbic acid concentration ($p > 0.05$) was observed for male subjects compared to female subjects, Age and Sex had no apparent ($p > 0.05$) effect on the Antioxidants evaluated for the Diseased subjects (table 3 and 4). Albumin concentration of Diseased subjects was however slightly higher ($p > 0.05$) than those observed for control subjects. Females also had significantly lower albumin ($p < 0.05$) concentrations for subjects suffering from either GIT, Diabetes or renal disorders than their male counterparts.

Dietary data failed to show a difference in the dietary intake of the Hospitalised patients and control patients. Only the dietary data for diabetic subjects revealed a concise reduction in their caloric intake. This finding suggests that dietary management of nutritional disorders are not strictly adhered to by Nigerian Hospitalised patients. The decreased Antioxidant Vitamin concentrations evident in the present study compared to the control subjects comfirm previous findings (caroll et al, 1970 etc) and attest that regular diets can not maintain Antioxidants Vitamins status in hospitalised patients. In light of this data it is postulated that Antioxidants Vitamin supplementation be introduced as a regimen in the management of this common Disorders.

4:3 INTERRELATIONSHIPS BETWEEN ANTIOXIDANT VITAMINS AND ALBUMIN STATUS OF CONTROL AND DISEASED POPULATION

In order to see, whether we might be able to postulate by regression data, the antioxidant concentration levels of the Hospitalised population if the baseline data of the apparently control population are known, present study was equally designed to evaluate by multiple regression data the intra correlations evident between Antioxidant Vitamin and Albumin in control and Diseased conditions.

Our data revealed that albumin concentration in control subjects was negatively but significantly associated with Albumin of renal subjects ($r = - 0.416$ $p < 0.05$). There was however no significant relationship ($p > 0.05$) between Albumin concentration in the control population and other diseased states (Table 5).

Available evidence (Adams and Maegraith, 1984) have shown that Hypoproteinemia is a common finding in chronic renal failure and cannot be entirely explained on the basis of loss of protein intake. The disturbance in protein metabolism in untreated renal disorder which often leads to negative Nitrogen balance may therefore be due to negative protein turnover or degradation with defective synthesis.

Coler et al (1969) studied albumin synthesis in 27 patients with terminal renal failure and attributed the low serum albumin levels to reduced albumin synthesis and not to increased albumin catabolism. We extend previous finding, in this regard postulating for the Nigerian Renal Patients that Albumin (control population) = 5.24 - 0.25 (Albumin Renal Population).

More definitive studies however need to be conducted to ascertain the molecular basis of this interrelationship. When Vitamin E (control) was used as the independent variable, a significant inverse association was evident between control and renal patients (Table 6). A positive but significant association ($r = 0.04 - 0.09$ $p < 0.05$) also existed between the Vitamin E of control patients and cardiovascular disease patients. With the exception of the positive association evident between Vitamin A of control and Gastrointestinal Tract ($r=0.470$ $p < 0.005$) all other Disease condition were unassociated with control or baseline values (Table 5). Renal Vitamin C was however inversely correlated with Vitamin C concentration of the healthy subject ($p < 0.005$) (Table 8).

TABLE 5: REGRESSION DATA INTER RELATIONSHIP OF ALBUMIN CONTROL VS DISORDERS

Dep. Variable	Ind. Variable	Correlat. Equation	Correlation Coefficient	Level Of Sign
Albumin (Control)	Albumin (Renal)	Y=5.24-0.25x	-0.416	P<o.0
Albumin (Control)	Albumin (Liver)	Y=4.40+0.05x	0.119	P<0.05
Albumin (Control)	Albumin (Card.)	Y=4.41+0.05x	0.100	P<0.05
Albumin (Control)	Albumin (G I T)	Y=4.04+0.20x	0.304	P<0.05
Albumin (Control)	Albumin Diabetes	Y=4.66-0.06x	-0.132	P<0.05

TABLE 6: REGRESSION DATA INTER RELATIONSHIP OF VITAMIN E CONTROL VS DISORDERS

Dep. Variable	Ind. Variable	Correla. Equation	Correlation Coefficient	Level Of Sign
Vitamin E (Control)	Vitamin E (Renal)	Y=14.36-0.79x	-0.443	P<0.05
Vitsmin E (Control)	Vitamin E (Liver)	Y=4.42-0.92x	-0.392	P<0.05
Vitamin E (Control)	Vitamin E (Cardio)	Y=10.24+0.04x	-0.146	P>0.05
Vitamin E (Control)	Vitamin E (G I T)	Y=7.68+0.41x	0.409	P<0.05
Vitamin E (Control)	Vitamin E (Diabetes)	Y=7.09+0.43x	0.239	P>0.05

4:4 INTERRELATIONSHIP IN THE ANTIOXIDANT VITAMIN STATUS AND ALBUMIN CONCENTRATION OF DISEASED SUBJECTS

The mean concentrations of the Vitamin A, C, E and Albumin levels of the Diseased subjects are presented on Table 3 and 4. There was no significant variations of Diseased subjects, Vitamin E was however significantly lower in Gastrointestinal Disorders ($p < 0.05$) than in all other Diseased conditions.

For cardiovascular subjects, it was observed that individuals with atherosclerosis had higher antioxidant Vitamin status ($p > 0.05$) than those patients suffering from terminal Heart failure.

Due to the similarity in Antioxidant Vitamin and insignificant positive difference in albumin concentration evident among the Diseased condition observed in present data (Table 3 and 4), an attempt was made to evaluate the possible interrelationship that may be evident, in the antioxidant status in the Disorders.

Our Data revealed that the Vitamin A levels of cardiovascular patient was correlated with renal patients (0.584 $p < 0.05$). The relationship between other variable was not significant (Table 9). Although previous investigators have not reported such correlations, Smith et al (1972) have ascribed the reduction in Vitamin A levels of cardiovascular patients to a possible defect in retinol transport system.

Carol et al (1970) have also attributed subnormal vitamin A levels in liver disease patients to a concomitant release of Vitamin A from the liver thus facilitating the excretion of the vitamin.

Other investigators (Moore, 1957) have postulated low vitamin A in diabetics to be due to an impairement in themetabolic conversion of carotene to vitamin A in the presence of decreased intake of the vitamin. It appears from present study that a similar mechanism may be available for promoting decreased vitamin A levels in both cardiovascular and renal diseased patients.

This findings appears to find metabolic basis, since etiological disorders predisposing individuals to renal disease include hypertension and other cardiovascular diseases (Kagan et al 1979).

The vitamin C levels among the disease subjects was however not significantly correlated (Table 10). Vitamin C for cardiovascular disease subjects was inversely correlated with the vitamin levels for Diabetes, renal and GIT subjects ($p < 0.05$).

Ascorbic acid depletion in cardiovascular disorders have been attributed to the deliberation of the integrity of the capillaries of the ground state. Ginter et al (1979) ascribed vitamin C depletion in diabetes to an inability of the patients.

It appears safe to postulate that vitamin C depletion evident in these disorders may be due to a similar mechanism.

For Albumin (Table 11) a positive intercorrelations existed between serum albumin concentration in cardiovascular and GIT order. A similar relationship was also evident between cardiovascular and renal disease patients.

Vitamin E was however unassociated among the disease conditions (Table 12). Keter et al (1983) have however described the decreased serum tocopherol concentration lipoprotein synthesis while Schomarch et al (1984) have reported decreased absorption of that soluble vitamins especially tocopherol as the biological basis for vitamin E deficiency in GIT disorders.

TABLE 7: REGRESSION DATA INTER RELATIONSHIP OF VITAMIN A CONTROL VS DISORDERS

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitsmin A (Control)	Vitamin A (Renal)	Y=58.96-0.14x	-0.342	P<0.05
Vitsmin A (Control)	Vitamin A (Liver)	Y=68.76-6.51x	-0.351	P>0.05
Vitamin A (Control)	Vitamin A (Cardio)	Y=52.38+0.16x	0.121	P>0.05
Vitamin A (Control)	Vitamin A (G I T)	Y=39.53+0.84x	0.470	P<0.05
Vitamin A (Control)	Vitamin A (Diabetes)	Y=39.40+0.70x	0.229	P>0.05

TABLE 8: REGRESSION DATA INTER RELATIONSHIP OF VITAMIN C CONTROL VS DISORDERS

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of sign
Vitamin C (Control)	Vitamin C (Renal)	Y=1.01-0.71x	-0.452	P<0.05
Vitamin C (Control)	Vitamin C (Liver)	Y=1.02-0.75x	-0.253	P>0.05
Vitamin C (Control)	Vitamin C (Cardio)	Y=0.96-0.50x	-0.169	P>0.05
Vitamin C (Control)	Vitamin C (G I T)	Y=0.51+1.22x	0.324	P>0.05
Vitamin C (Control)	Vitamin C (Diabetes)	Y=0.87-0.38x	-0.285	P>0.05

TABLE 9: REGRESSION DATA INTER RELATIONSHIP OF VITAMIN A CARDIOVASCULAR VS OTHER DISORDERS

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin A (Cardio)	Vitamin A (Liver)	Y=26.32+32.51x	0.184	P>0.05
Vitamin A (Cardio)	Vitamin A (G I T)	Y=41.30+55.10x	1.147	P>0.05
Vitamin A (Cardio)	Vitamin A (Diabetes)	Y=27.56+35.41x	0.309	P>0.05
Vitamin A (Cardio)	Viatmin A (Renal)	Y=31.61+28.56x	0.584	P<0.05

TABLE 10: REGRESSION DATA INTER RELATIONSHIP OF VITAMIN C CARDIOVASCULAR VS OTHER DISORDERS

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin C (Cardio)	Vitamin C (Liver)	Y=0.72+0.03x	0.236	P>0.05
Vitamin C (Cardio)	Vitamin C (G I T)	Y=0.85-0.24x	-0.486	P<0.05
Vitamin C (Cardio)	Vitamin C (Diabetes)	Y=0.93-0.34x	-0.634	P<0.05
Vitamin C (Cardio)	Vitamin C (Renal)	Y=0.79-0.24x	-0.964	P<0.05

TABLE 11: REGRESSION DATA INTER RELATIONSHIP OF ALBUMIN CARDIOVASCULAR VS OTHER DISORDERS

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Albumin (Cardio)	Albumin (Liver)	$Y=3.85+0.13x$	0.156	$P>0.05$
Albumin (Cardio)	Albumin (G I T)	$Y=4.04-0.20x$	-0.532	$P<0.05$
Albumin (Cardio)	Albumin (Diabete)	$Y=5.22-0.24x$	-0.393	$P>0.05$
Albumin (Cardio)	Albumin (Renal)	$Y=5.62-0.55x$	-0.709	$P<0.05$

TABLE 12: REGRESSION DATA INTER RELATIONSHIP OF VITAMIN E CARDIOVASCULAR VS OTHER DISORDERS

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin E (Cardio)	Vitamin E (Liver)	$Y=10.42+8.67x$	0.014	$P>0.05$
Vitamin E (Cardio)	Vitamin E (G I T)	$Y=9.84-0.69x$	-0.269	$P>0.05$
Vitamin E (Cardio)	Vitamin E (Diabetes)	$Y=4.42-0.97x$	-0.197	$P>0.05$
Vitamin E (Cardio)	Vitamin E (Renal)	$Y=7.87-0.11x$	-0.060	$P>0.05$

4:5 ANTIOXIDANT VITAMIN STATUS AND ALBUMIN RELATIONSHIP WITHIN EACH DISEASE CONDITION

It was the intent of present study to identify whether any inter-relationship may exist between antioxidant vitamin depletion in diseased conditions. To this end, the vitamin A, C, E and albumin concentrations of each disease subject were subjected to multiple Regression. Our data revealed a significant positive association between all variable, Vitamin A, Vitamin C, Vitamin E and Albumin in Cardiovascular, renal disease or Gastrointestinal patients (*Table 13 - 17*).

This finding appears to be of Clinical significance, especially as free radical tissue injury have been implicated in these disorders. It appears that antioxidant vitamin *may be readily depleted by the same mechanism.*

For vitamin A which is formed from the antioxidant B-carotene is readily utilised in arresting the free radicals formed as a result of injury of these metabolic Disorders.

In the present study, Albumin concentration of both healthy and disease subjects were evaluated, because malnutrition, arising from semi starvation resulting from anorexia of the disease is often evident among hospitalised patients. Such malnutrition states have been shown to be accompanied by increased impairment in the development of viscera function and hypo-albumin (Bistrain 1977) and white head et al 1970).

It is important to state that then slightly lower values observed for diseased subjects relative to control may be due to similarity in diets.

The interrelationship that however occur between vitamin A and Vitamin E may however suggest a possible involvement of albumin in the transport of these vitamins in diseased conditions.

Previous investigators have reported rapid decrease in all other transport proteins following malnutrition but although for diabetic mellitus (Table 5), an interrelationship was evident in the antioxidant vitamins, starvation, noticed these albumin concentrations are often maintained under poor dietary conditions.

It was clear that albumin was unassociated ($p > 0.05$) with vitamin A (Table 16) vitamin E or ascorbic acid.

In conclusion, it is suggested that more studies be conducted to a certain these metabolic relationships reported in the present study.

TABLE 13: REGRESSION DATA INTER RELATIONSHIP OF CARDIOVASCULAR DISORDER

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin A (Cardio)	Vitamin E (Cardio)	Y=55.47+3.86x	0.879	P(0.05
Vitamin A (Cardio)	Vitamin C (Cardio)	Y=58.96+8.57x	0.791	P(0.05
Vitamin A (Cardio)	Vitamin (Cardio)	Y=28.42+0.69x	0.659	P(0.05
Vitamin E (Cardio)	Vitamin C (Cardio)	Y=12.63+0.65x	0.681	P(0.05
Vitamin E (Cardio)	Albumin (Cardio)	Y=10.26+0.86x	0.580	P(0.05
Vitamin C (Cardio)	Albumin (Cardio)	Y=1.32+0.67x	0.716	P(0.05

TABLE 14: REGRESSION DATA INTER RELATIONSHIP OF LIVER DISORDER

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin A (Liver)	Vitamin E (Liver)	Y=38.26+0.18x	0.876	P(0.05
Vitamin A (Liver)	Vitamin C (Liver)	Y=58.27+0.85x	0.875	P(0.05
Vitamin A (Liver)	Albumin (Liver)	Y=28.97+0.52x	0.678	P(0.05
Vitamin E (Liver)	Vitamin C (Liver)	Y=8.96+0.84x	0.824	P(0.05
Vitamin E (Liver)	Vitamin (Liver)	Y=7.86+0.05x	0.705	P(0.05
Vitamin E (Liver)	Vitamin E (Liver)	Y=0.74+0.03x	0.784	P(0.05

TABLE 15: REGRESSION DATA INTER RELATIONSHIP OF RENAL DISORDER

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin A (Renal)	Vitamin E (Renal)	Y=52.02+0.81x	0.821	P<0.05
Vitamin A (Renal)	Vitamin C (Renal)	Y=28.54+0.45x	0.485	P<0.05
Vitamin A (Renal)	Albumin (Renal)	Y=38.46+0.68x	0.658	P<0.05
Vitamin E (Renal)	Vitamin C (Renal)	Y=10.34+0.43x	0.643	P<0.05
Vitamin E (Renal)	Albumin (Renal)	Y=12.82+0.82x	0.882	P<0.05
Vitamin C (Renal)	Albumin (Renal)	Y=0.72+0.96x	0.923	P<0.05

TABLE 16: REGRESSION DATA INTER RELATIONSHIP OF DIABETICS DISORDER

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin A (Diabetes)	Vitamin E (Diabetes)	Y=24.82+0.49x	0.499	P<0.05
Vitamin A (Diabetes)	Vitamin C (Diabetes)	Y=32.12+0.24x	0.714	P<0.05
Vitamin A (Diabetes)	Albumin Diabetes)	Y=22.21+0.34x	0.348	P>0.05
Vitamin E (Diabetes)	Vitamin C (Diabetes)	Y=12.50+0.63x	0.626	P<0.05
Vitamin E (Diabetes)	Albumin (Diabetes)	Y=4.54+0.32x	0.310	P>0.05
Vitamin C (Diabetes)	Albumin (Diabetes)	Y=0.46+0.12x	0.172	P>0.05

TABLE 17: REGRESSION DATA INTER RELATIONSHIP OF GASTROINTESTINAL TRACT DISORDER

Dep. Variable	Ind. Variable	Correla Equation	Correlation Coefficient	Level Of Sign
Vitamin A (G I T)	Vitamin E (G I T)	Y=42.12+0.64x	0.864	P<0.05
Vitamin A (G I T)	Vitamin C (G I T)	Y=26.98+0.57x	0.733	P<0.05
Vitamin A (G I T)	Albumin (G I T)	Y=35.26+0.16x	0.787	P<0.05
Vitamin E (G I T)	Vitamin C (G I T)	Y=7.87+0.42x	0.799	P<0.05
Vitamin E (G I T)	Albumin (G I T)	Y=8.65+0.87x	0.817	P<0.05
Vitamin C (G I T)	Albumin (G I T)	Y=0.88+0.27x	0.970	P<0.05

Conditions. It was clear that albumin was unassociated (p > 0.05) with vitamin A (Table 16) vitamin E or ascorbic acid.

In concclusion, it is suggested that more studies be conducted to a certain these metabolic relationships reported in the pesent study.

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APPENDIX I

VALUES OF DISEASED SUBJECT WITH SEX AND AGE

S/N	Sex	Age	DISEASE	(µg/100ml) Vitamin A	(µg/ml) Vitamin E	(mg/100ml) Vitamin C	(g/dl) Albumin	(g/dl) Haemo-globin	(Percentage) PCV
1.	M	35	Cardiovascular	22	6	0.25	2.4	13.7	44
2.	M	45	G.I.T.	25	7	0.40	3.6	8.7	32
3.	M	35	Cardiovascular	18	6	0.35	3.2	12.7	35
4.	M	60	Liver	14	5	0.20	2.0	4.3	15
5.	M	40	Renal	15	5	0.30	2.7	6.3	20
6.	M	23	G.I.T.	20	5	0.20	2.4	8.2	30
7.	M	50	Cardiovascular	22	6	0.35	3.0	12.3	42
8.	F	35	Diabetes M.	18	6	0.25	3.2	12.3	36
9.	F	30	Cardiovascular	14	4	0.15	2.3	5.0	15
10.	F	28	Liver	18	5	0.20	2.0	5.0	24
11.	M	27	Cardiovascular	25	7	0.30	2.7	12.7	43
12.	M	43	G.I.T.	20	6	0.25	2.3	8.3	30
13.	M	27	Liver	30	9	0.35	3.0	12.0	41
14.	M	30	Cardiovascular	21	6	0.25	2.4	13.0	42

S/N	Sex	Age	DISEASE	(µg/100ml) Vitamin A	(µg/ml) Vitamin E	(mg/100ml) Vitamin C	(g/dl) Albumin	(g/dl) Haemo-globin	(Percentage) PCV
15.	M	60	Cardiovascular	18	5	0.20	2.0	11.0	35
16.	F	55	G.I.T	17	5	0.30	2.7	7.7	28
17.	F	50	Cardiovascular	25	7	0.40	3.6	14.0	41
18.	F	50	Cardiovascular	18	5	0.20	2.0	10.0	22
19.	F	45	Cardiovascular	25	7	0.40	3.2	10.3	25
20.	M	60	Renal	20	6	0.25	2.6	9.7	25
21.	M	27	G.I.T.	22	6	0.35	3.0	14.3	35
22.	M	50	Liver	36	9	0.30	2.7	10.7	33
23.	M	59	Cardiovascular	20	6	0.20	2.0	10.0	32
24.	M	40	Liver	18	4	0.10	2.4	8.3	30
25.	M	32	Liver	14	4	0.15	2.7	4.3	20
26.	F	45	Diabetes M.	18	5	0.20	2.0	10.0	28
27.	F	65	Liver	23	6	0.25	2.4	12.0	38
28.	F	55	Cardiovascular	25	7	0.30	2.7	16.7	57
29.	F	50	Cardiovascular	18	5	0.20	3.0	10.7	30
30.	F	56	Cardiovascular	22	6	0.30	2.7	5.7	19

S/N	Sex	Age	DISEASE	(µg/100ml) Vitamin A	(µg/ml) Vitamin E	(mg/100ml) Vitamin C	(g/dl) Albumin	(g/dl) Haemo-globin	(Percent) PCV
31.	F	16	Sickle Cell	17	4	0.20	2.1	5.7	25
32.	F	55	Cardiovascular	20	5	0.20	3.0	8.7	33
33.	F	30	Cardiovascular	22	6	0.30	2.7	12.3	36
34.	F	25	G.I.T.	18	5	0.20	2.0	9.0	28
35.	M	42	Cardiovascular	30	8	0.30	2.7	12.0	42
36.	M	50	Cardiovascular	17	5	0.20	3.2	10.7	39
37.	M	30	Diabetes M.	21	7	0.25	2.4	12.0	33
38.	M	30	G.I.T	35	9	0.40	3.6	12.7	43
39.	M	38	G.I.T.	17	5	0.20	2.0	9.0	34
40.	F	34	G.I.T.	15	6	0.25	2.4	12.0	41
41.	F	52	Cardiovascular	35	12	0.40	5.0	12.0	33
42.	M	40	Cardiovascular	30	10	0.30	3.0	14.0	41
43.	M	45	Diabetes M.	25	6	0.25	2.7	13.0	42
44.	M	60	Cardiovascular	30	10	0.30	3.6	15.0	43
45.	M	20	Diabetes M.	22	6	0.20	4.7	12.0	40
46.	F	50	Cardiovascular	32	13	0.30	3.0	9.0	30
47.	M	65	Cardiovascular	34	15	0.35	2.7	13.0	42
48.	F	30	Diabetes M.	25	6	0.30	3.0	10.0	28

S/N	Sex	Age	DISEASE	(µg/100ml) Vitamin A	(µg/ml) Vitamin E	(mg/100ml) Vitamin C	(g/dl) Albumin	(g/dl) Haemo-globin	(Percentage) PCV
49	F	30	Renal	20	5	0.25	2.1	12.0	41
50.	M	25	Diabetes M.	26	7	0.35	3.9	16.0	45
51.	M	70	Cardiovascular	16	5	0.20	3.0	14.0	42
52.	M	68	Cardiovascular	38	9	0.40	4.3	13.0	42
53.	F	65	Cardiovasulcar	23	6	0.25	2.0	10.0	35
54.	M	18	Sickle Cell	21	5	0.30	2.0	8.0	28
55.	M	35	Cardiovascular	35	15	0.40	4.7	12.3	41
56.	F	77	Cardiovasoular	26	7	0.35	3.6	8.7	32
57.	F.	60	G.I.T.	22	5	0.25	2.4	9.0	32
58.	M	43	Cardiovascular	39	18	0.45	3.9	12.7	43
59.	M	54	Cardiovascular	27	7	0.30	2.7	13.7	45
60.	M	60	Diabetes M.	20	6	0.20	3.0	14.3	46
61.	M	50	Liver	26	7	0.35	3.6	15.0	45
62.	M	54	Liver	25	6	0.30	3.7	13.0	44
63.	M	47	Liver	28	7	0.35	4.3	15.0	43
64.	F	28	Liver	21	5	0.25	3.0	12.3	41
65.	F.	33	Liver	30	10	0.40	3.9	10.0	36
66.	M	45	Renal	26	7	0.35	4.1	16.0	46

S/N	Sex	Age	DISEASE	(μg/100ml) Vitamin A	(μg/ml) Vitamin E	(mg/100ml) Vitamin C	(g/dl) Albumin	(g/dl) Haemo-globin	Percen-tage) PCV
67.	M	52	Liver	22	6	0.30	3.7	13.0	44
68.	M	40	Liver	18	5	0.20	3.0	14.0	45
69.	F	49	Liver	25	6	0.25	2.1	.9.0	38
70.	M	45	Liver	29	7	0.35	3.6	14.0	42
71.	M	37	Liver	40	16	0.45	5.0	13.0	44
72.	F	32	Liver	15	5	0.15	2.4	11.0	37
73.	M	50	Liver	26	7	0.30	3.0	15.0	44
74.	M	48	Liver	21	5	0.20	2.0	13.0	46
75.	F	70	Liver	27	7	0.35	2.7	11.0	40
76.	M	40	Liver	25	6	0.25	3.0	16.0	48
77.	M	28	Liver	28	7	0.30	3.6	12.7	43
78.	F	30	Liver	34	12	0.40	5.1	10.3	41
79.	F	35	Renal	25	6	0.30	3.0	11.0	42
80.	M	75	Liver	28	7	0.35	4.6	15.0	45
			TOTAL	1900.0	560.0	22.60	236.6	915.0	2926.0

APPENDIX II

VALUES OF CONTROL SUBJECTS WITH SEX AND AGE

S/N	Sex	Age	DISEASE	(μg/100ml) Vitamin A	(μg/ml) Vitamin E	(mg/100ml) Vitamin C	(g/dl) Albumin	(g/dl) haemo-globin	(Percen-tage) PCV
1.	M	31	Normal	55	10	0.70	4.5	15	43
2.	M	35	Normal	53	9	0.60	4.3	14	45
3.	M	28	Normal	40	8	0.50	4.0	13	42
4.	M	40	Normal	60	10	0.80	4.6	15	45
5.	M	46	Normal	72	12	1.40	5.2	16	48
6.	M	29	Normal	50	10	0.65	4.4	14	42
7.	M	26	Normal	47	9	0.70	4.1	13	43
8.	M	32	Normal	61	10	0.85	4.7	14	44
9.	M	60	Normal	65	11	1.10	4.9	15	45
10.	M	55	Normal	70	12	1.25	5.0	16	50
11.	M	48	Normal	62	10	1.05	4.8	15	47
12.	M	38	Normal	55	9	0.75	4.5	14	44
13.	F	30	Normal	50	10	0.65	4.4	14	41
14.	F	28	Normal	51	9	0.60	4.3	13	40
15.	F	35	Normal	62	10	0.95	4.8	15	45
16.	F	25	Normal	48	9	0.55	4.2	12	39
17.	F	40	Normal	68	11	1.00	5.0	16	47
18.	F	48	Normal	63	11	0.90	4.7	15	46
19.	F	37	Normal	58	10	0.80	4.6	14	43
20.	F	22	Normal	35	8	0.50	4.0	12	38
			TOTAL	1,125	198	16.30	91.0	285	877

APPENDIX III

Questionnaire on the study of Vitamin Status of

Hospitalised Patients in Zaria Area

INSTRUCTION:

Please Tick ☐ the correct response and supply answers where necessary.

Personal Information

1. Name...

2. Age..

3. Sex: Male ☐ Female ☐

4. Home Address...

....

5. Hospital Name..

6. Ward Number..

7. Height.......... 8. Weight..... .... 9. Tribe.............

10. Religion................. 11. Marital Status.............

12. Parity...

Socioeconomic Status

13. Income per annum..

14. How many wives..

15. How many children...

16. Occupation...

Clinical History

17. Do you suffer from any of the following diseases:

a) Diabetes mellitus ☐

b) Chronic liver disease ☐

c) Cardiovascular disease ☐

d) Renal disease ☐

e) Sickle cell disease ☐

f) G I F disorder ☐

g) High blood pressure ☐

19. Duration of the disease..............................

20. Severity of the disease acute ☐ chronic ☐

21. Any family history of the above named diseases?

Yes ☐ No ☐

22. If yes name the relation .... Sex...... Age.....

23. What is your relationship (Father, Mother, Brothers, Uncle)............................

24. Do you smoke? Yes ☐ No ☐

a) If yes how many stick per day.......................

25. Do you take alcohol? Yes ☐ No ☐

a) If yes is it weekly, daily, monthly or Occasionally.........

26. Hospital Centre previously visited.................................

27. Dietary Management

Day	Breakfast	Lunch	Dinner	Snack Items
Monday				
Tuesday				
Wednesday				
Thursday				
Friday				
Saturday				
Sunday				

28. Laboratory Measurement

Blood Group	Genotype	Na^+	K^+	Cl^-
Total Bilirubin	Fasting Blood Sugar	Urea	WBC	Blood Pressure
Total Protein	Albumin	Hb	PCV	RBC

29. Date of collection of Blood sample.................

APPENDIX IV

I Haemoglobin Estimation

Materials:

1. Potassium ferricyanide
2. Potassium cyanide
3. Test-tubes
4. 1000ml volumetric flask
5. Colorimeter EEL
6. Haemoglobin pipette (ML 32)
7. Haemoglobin Standard
8. Drabkins solution was made by dissolving 200mg (one-fifth of a gram) of potassium ferricyanide and 500mg (half a gram) of potassium cyanide in 1000mls of water

II Packed Cell Volume Estimation

Materials:

1. Plain capillary tubes
2. Cotton wool swab
3. Bunsen burner
4. Microhaematocrit machine
5. Microhaematocrit reader

III Estimation of Vitamin C

Materials

1. Klett-Summerson photoelectric colorimeter with a green filter and Klett tubes

2. 5% Trichloroacetic acid (TCA), store in cold.

3. A 60^{o} water bath

4. 2,6-Dichlorophenolindophenol, NaSalt (Eastman, Rochester, N.Y., no. 3463).

5. 2,4 Dinitrophenylhydrazine (Eastman, no. 1921).

6. Thiourea

7. 9N Sulphuric acid (3 parts water plus 1 part concentrated acid).

8. Dissolve 2g of 5 and 1g of 6 in 100ml of 7: this is the "DT Mix" - filter when necessary.

9. 85% Sulphuric acid (85ml of concentrated acid plus 15ml of distilled water).

10. Ascorbic acid - "Stock standard" - 50mg in 100ml of 5% TCA. Store in cold.

11. Ascorbic acid - "working standard" - 1ml of 10 to 100ml with 5% TCA. Make fresh with every run.

IV Estimation of serum albumin

Materials:

1. Spectrophotometer or a colorimeter equiped with a 630-nm interference filter.
2. Succinate buffer, 0.10M, PH 4.0: Dissolve 11.9g of succinic acid in about 800ml of water, adjust PH to 4.0 with NaOH and dilute to 1 litre with water. Store at 4°c.
3. Bromcresol green solution stock; 0.60mM. Dissolve 419mg of BCG in 10ml of 0.1N NaOH in a 1 litre volumetric flask. Dilute to volume with water and store at 4°c.
4. Working dye solution: Dilute one volume of stock BCG solution with three volumes of 0.10M succinate buffer, add 4.0ml 30% Brij-35 per litre and carefully adjust PH to 4.20 $\pm$ 0.05. Store at 4°c.
5. Brij-35 solution, 30%. This solution is available from Technicon Instrument Company, Tarrytown, New York - Brij-35 is obtainable from Fisher Scientific Company

6. Albumin standard solution stock; 10.00 g/dl: Dissolve 10.00g of HSA - V, corrected for moisture content and 50mg of sodium azide in water in 100-ml volumetric flask. Dilute to volume with water and store at $4^{\circ}c$.

7. Albumin standard solutions. Working; 2.0, 3.0. 4.0, 5.0., and 6.0g/dl: Appropriate dilutions of the stock albumin standard solution are made with 50mg/dl aqueous Sodium azide solution. Store at $4^{\circ}c$. Albumin solutions containing 50mg/dl sodium azide show no evidence of bacterial growth after storage for 6 months at $4^{\circ}c$.

Estimation of serum Vitamin A and E

Materials:

1. Flurometer model III with high sensitivity conversion kit.
2. Special interference filters.
3. A 4-watt General Electric No. F_4T_4/BL lamp with major wavelength emission at 360m u
4. Beckman potentiometer recorder No. 93500.

5. Glass centrifuge tubes, 15 by 110mm, 15ml capacity, with teflon lined plastic screw caps.

6. Round bottom pyrex cuvettes, 12 by 75mm with minimal volume.

7. Cuvette washer.

8. Vortex mixer model K-500-J

9. Absolute Ethanol

10. Hexane

11. Water, double distilled

12. Vitamin A acetate

13. Vitamin E acetate

14. Potassium hydroxide