SERUM LEVELS OF ANTIOXIDANT MINERALS AND ENZYMES AMONG CANCER PATIENTS IN ZARIA

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May, 2015

DECLARATION

I declare that this research work entitled **"Serum levels of antioxidant minerals and enzymes among cancer patients in Zaria"** was carried out by me in the Department of Chemical pathology, Faculty of Medicine, Ahmadu Bello University Zaria. The information derived from the literature was duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

JibrinJibrin …….

CERTIFICATION

This dissertation titled**; "Serum levels of antioxidant minerals and enzymes among cancer patients in Zaria"** by JIBRIN JIBRIN meets the regulations governing the award of the degree of MSc Chemical Pathology of the Ahmadu Bello University and is approved for its contribution to knowledge and literacy presentation.

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ABSTRACT

Numerous studies have shown a depletion of antioxidants and increased amount of ROS (reactive oxygen species) in patients with malignancies but no such study have been carried out on the levels of antioxidants in cancer patients in Zariawhich necessitated this study. The overall objective of this study was to measure the serum antioxidant minerals and enzymes in cancer patients and controls.

One hundred cancer patients who were histologically or cytologically confirmed and an equal number of apparently healthy aged and sex-matched controls were recruited. Their serum antioxidant minerals (selenium; Se, copper; Cu and zinc; Zn) and enzymes (glutathione peroxidase; Gpx, superoxide dismutase; SOD and catalase; Cat) were measured using Atomic Absorption Spectrophotometry and direct colorimetric methods.

The mean serum concentrations of Cu, Zn, SOD and GPx were significantly lower in cancer patients compared to the controls while the mean concentrations of Se and Cat were similar in both the patients and controls. There was a significant and negative correlation between the duration of cancer and the serum levels of Zn, Se, and GPx. There was no significant difference in the serum concentration of the analytes between cancer patients aged greater than 65 years and those less than 65 years.

It was therefore concluded from this study that the serumlevels of antioxidant minerals (Cu and Zn) and enzymes (SOD and GPx) were lower in patients with cancer than the controls and that there was a decrease in the levels of antioxidant minerals (Zn and Se) and enzyme (Gpx) in the serum of cancer patients as the duration of cancer progresses. It was recommended that a routine evaluation of antioxidant minerals and enzymes be considered in the management of cancer patients in Nigerian hospitals.

TABLE OF CONTENTS

Title page……..ii

Declaration ……..iii

Certification……..….iv

Acknowledgement……v

Abstract……vi

Table of contents……..viii

List of tables……x

List of figures……..xi

List of appendices……..xii

Abbreviations/symbols used……..xiii

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study……..1

1.2 Statement of problem……4

1.3 Justification……...4

1.4 Aim and objectives of the Study.....4

CHAPTR TWO

2.0 LITERATURE REVIEW……6

2.1 Cancer……...6

2.1.1 Epidemiology……..…6

2.1.2 Aetiology……7

2.1.3 Clinical aspects of neoplasia……14

2.1.4 Laboratory diagnosis of cancer…….21

2.2 Antioxidants……24

2.2.1 Sources and Origins of Antioxidants……..….25

2.2.2 Classification of Antioxidants……..26

2.2.3 Antioxidants and Cancer……..28

2.2.4 Cancer therapy and Antioxidant status…….31

CHAPTER THREE

3.0 MATERIALS AND METHOD……...34

3.1Study Area…….34

3.2 Study Design…….34

3.3 Study population ……...34

3.4 Study Protocol…….……..37

3.5 Specimen collection and processing……..37

3.6 Chemicals and Reagents……38

3.7 Equipment's……38

3.8 Analytical methods……38

3.9 Quality control……46

3.10 Statistical analysis……..…47

CHAPTER IV

4.0 RESULTS……48

CHAPTER V

5.0 DISCUSSION……..60

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION……..……63

6.1 Summary…….63

6.2 Conclusion…….63

6.3 Recommendation……64

References…….65

Appendices…….71

LIST OF TABLES

1. Table 2.1: Inherited Predisposition to Cancer …….....10

2. Table 2.2: Table 2.2. Major Chemical Carcinogens……..……12

3. Table 2.3: Paraneoplastic syndromes……18

4. Table 3.1: Glutathione Peroxidase Reaction Scheme……43

5. Table 4.1: Biodata and clinical description of study population……..…….50

6. Table 4.2: Serum levels of Antioxidant Minerals and Enzymes among cancer patients and controls……..…….51

7. Table 4.3: Reference intervals of antioxidant minerals and enzymes in healthy controls….52

8. Table 4.4: Prevalence of abnormal antioxidant enzymes and minerals in patients……53

9. Table 4.5: Serum antioxidant enzymes and minerals in cancer patients according to age…54

10. Table 4.6: Serum antioxidant enzymes and minerals in controls according to age…….55

11. Table 4.7: Correlation analysis between Cancer duration and Analyte concentrations …….56

LIST OF FIGURES

1). Figure 4.1:Relationship between cancer duration and serum zinc concentration (r = -252, p = 0.011)……...57

2). Figure 4.2**:** Relationship between cancer duration and serum selenium concentration (r = -346, p = 0.001)…….….58

3). Figure 4.3: Relationship between cancer duration and serum glutathione peroxidase (GPx) concentration (r = 830, p = 0.001)…….59

LIST OF APPENDICES

1. Appendix I: Patients information form……..71

2. Appendix II: Informed written consent…….....72

3. Appendix III: Proforma …….73

3. Appendix IV: Ethical Approval ……..……74

ABBREVIATIONS/ SYMBOLS USED

A	Absorbance
Astd	Absorbance of standard
ABUTH	Ahmadu Bello University Teaching Hospital
ACEIs	Angiotensin converting enzyme inhibitors
ARBs	Angiotensin II receptor blockers
α	Alpha
β	Beta
BP	Blood pressure
BTP	Beta trace protein
CAT	Catalase
CDC	Centre for Disease Control
CRF	Chronic renal failure
CKD	Chronic kidney disease
C	Concentration
Cstd	Concentration of standard
Cr	Creatinine
CrCl	Creatinine Clearance
CVD	Cardiovascular disease
oC	Degree Celsius
δ	Delta
DM	Diabetes mellitus
eCrCl	Estimated creatinine clearance
e.g	For example

ELISA	Enzyme-linked immunosorbent assay
ESRD	End stage renal disease
ESRF	End stage renal failure
et al	and others
G	Gauge
GPx	Gluthathione peroxidase
g	Gramme
GFR	Glomerular filtration rate
>	Greater than
<	Less than
≥	Greater than or equal
≤	Less than or equal
H	Hydrogen
HIV	Human immunodeficiency virus
HIVAN	HIV- associated nephropathy
Ht	Height
K/DOQI	Kidney Disease Outcome Quality Initiative
kg	Kilogram
kg/m2	Kilogram per metre square
µL	Micro litre
µmol	Micromole
µmol/L	Micromole per litre
MDRD	Modification of Diet in Renal Disease
ml	Milliliter
ml/min	Milliliter per minute

mmolMilli mole

mmol/LMilli mole per litre

min	Minute
ngNanograms	
%	Per cent
±	Plus or minus
ROS	Reactive oxygen species
rpm	Revolution per minute
RRT	Renal replacement therapy
SPSS	Statistical package for the social sciences
std	Standard
SOD	Superoxide dismutase
USA	United States of America
WHO	World Health Organisation
Wt	Weight
yr	Year

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Cancer is the malignant form of uncontrolled growth of cells and tissues (*Stricker et al, 2008*). It is the most dreaded non-communicable disease in developing countries where it is invariably fatal, due to lack of adequate preventive and curative services, unlike in developed countries which have policy, strategies and programmes for cancer prevention and management (*WHO, 2002; Nnodu, 2010*). Consequently, although the incidence of cancer is rising globally, the developing countries account for 52% of this increase (*Parkin, 2003*) and for 70% of cancer deaths (*Kolawole, 2011*), while only possessing 5% of global funds for cancer control and very few human and material resources (*Kolawole, 2011*). It is the second most common cause of death in developed countries; constituting 12% of all deaths after cardiovascular disease (*Kolawole, 2011*). It accounts for the death of more people than tuberculosis, HIV/AIDS and Malaria combined (*Kolawole, 2011*).

Endogenous antioxidants (for example, glutathione) that neutralize or trap reactive oxygen species act as cancer preventive agents. Human observational studies provide further support, showing on one hand that oxidative stress increases with clinical progression of breast cancer and on the other hand that a diet rich in antioxidant containing foods reduce the risk of certain cancers (*Hereberg, 2005*).

New data have showed that some dietary antioxidants may have potential as adjuvants in cancer therapy by their ability to induce programmed cell death (apoptosis) (*Sun, 1990*). Studies

in cell cultures show that vitamins C and E, selenium and some phytochemicals selectively induce apoptosis in cancer cells while sparing normal cells. Other findings in a model of metastatic growth show that vitamin C is an angiostatic factor and may have potential in aiding host resistance to tumour growth and invasiveness (*Sun, 1990*). Antioxidants also show promise in cancer therapy by their palliative action in reducing painful side effects associated with the treatment.

Plasma contains a large number of antioxidants, some of which prevent the initiation of the process of oxidation (preventive antioxidants) (*Sies, 1997*), while others inhibit the further progression of the cascade of reactions (chain breaking antioxidants). Vitamin E is the main lipid-soluble chain-breaking antioxidant in membranes and plasma. Vitamin C is a major, extremely versatile antioxidant of human plasma. It effectively scavenges a wide variety of free radicals in plasma or cytosol and is the main reductant of oxidized vitamin E. It is capable of preventing initiation of lipid peroxidation, while other water-soluble antioxidants such as betacarotene, bilirubin, uric acid and thiol-compounds (mainly plasma proteins) are only effective in decreasing the rate of lipid peroxidation. Ceruloplasmin acts as a preventive antioxidant in plasma, by tightly binding the plasma copper and inhibiting iron-dependent lipid peroxidation and hydroxyl radical formation. Selenium is an essential part of glutathione peroxidase, an important intracellular antioxidant.

Antioxidants have been shown to decrease chronic inflammation in patients with solid tumours and may also have other actions beyond their effect on free radicals *(Subapriya et al, 2002*). Vitamin C, for instance, was shown to stabilize p53 and enhance, in a cell culture medium, the sensitivity of cervical cancer cells to cisplatinum. Vitamin C, in conjunction with vitamin K_3, has been shown to enhance cell killing, used alone and in conjunction with

chemotherapy. Numerous studies showed enhanced efficacy when 5-FU is used in conjunction with different antioxidants. The issue of multidrug resistance is of great importance. Factors such as increased concentration of glutathione and upregulation of glutathione peroxidase and transferase, as well as increased production of nuclear transcription factor (NF-kB) have been considered of central importance. In addition, the proteosome (ubiquitin) pathway, which is important for degradating intracellular proteins is involved. Various flavonoids, including especially curcumin have been shown to have potential beneficial effects in these areas. Curcumin was shown to increase sensitivity to doxorubicin and act as a radiosensitizer in a prostate cancer cell line by effecting NF-kB, GSH and the ubiquitin pathways*(Antunes, 2001*).

In complementary oncology, the administration of vitamins and other micronutrients tailored to individual needs can help to reduce the frequency of side effects from antineoplastic treatment and improve its effectiveness and therapeutic index through better compliance, fewer therapy dropouts and higher possible dosages, and improve quality of life for the tumour patient (*Hereberg, 2005*). Numerous studies have shown a depletion of antioxidants and an increased amount of reactive oxygen species in patients with malignancies (*Manoharan, 1994*).

Reactive oxygen species (ROS) are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) control their accumulation *(*Mates, 1999). Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radicals and its accumulation. These ROS are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage. They are also responsible for oxidation of bases in cellular DNA making them mutagenic, cytotoxic and crosslinking agents, which in

turn causes uncontrolled expression of certain genes causing increased multiplication of cells leading to cancer *(Mates, 1999*).

1.2 STATEMENT OF PROBLEM

Antioxidants have protective role in cancer by preventing the accumulation of ROS that may cause lipid peroxidation of tissues. Although several studies have implicated overproduction of lipid peroxidation byproducts and disturbances in antioxidant defense system in the pathogenesis of cancer, no such study have been carried out on the level of lipid peroxidation and antioxidants levels in cancer patients in Zaria. The present study therefore, was undertaken to evaluate the status of antioxidant minerals and enzymes in serum of patients with various cancers.

1.3 JUSTIFICATION OF THE STUDY

Evaluating cancer patients in Zaria for serum antioxidant levels could provide a scientifically grounded application of antioxidant supplements which could improve treatment outcomes and significantly contribute to the nation's health.

1.4 AIM AND OBJECTIVES OF THE STUDY

1.4.1 General Objective

To evaluate serum antioxidant minerals and enzymes in cancer patients.

1.4.2 Specific Objectives

1. To measure the serum antioxidant minerals (selenium, copper and zinc) and enzymes (glutathione peroxidase, superoxide dismutase and catalase) in cancer patients and healthy controls in ABUTH Zaria.

2. To compare the values of antioxidant minerals and enzymes obtained from cancer patients and those of healthy controls.
3. To compare the results of serum antioxidant minerals and enzymes among different age groups of cancer patients and healthy controls.
4. To evaluate the relationship between the duration of cancer and serum levels of antioxidant minerals and enzymes in the patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CANCER

Cancer is the malignant form of uncontrolled growth of cells and tissues (*Stricker et al, 2008*). It is the most dreaded non-communicable disease in developing countries where it is invariably fatal, due to lack of adequate preventive and curative services, unlike in developed countries which have policy, strategies and programs for cancer prevention and management (*WHO, 2002; Nnodu, 2010*). Consequently although the incidence of cancer is rising globally, the developing countries account for 52% of this increase (*Parkin, 2003*) and for 70% of cancer deaths (*Kolawole, 2011*) while only possessing 5% of global funds for cancer control and very few human and material resources (*Kolawole, 2011*). It is the second most common cause of death; constituting 12% of all deaths after cardiovascular disease (*Kolawole, 2011*). It accounts for the death of more people than Tuberculosis, HIV/AIDS and Malaria combined (*Kolawole, 2011*).

2.1.1 Epidemiology of Cancer

Cancer is a public health problem worldwide affecting all categories of persons. It is the second common cause of death in developed countries and among the three leading causes of death in developing countries. WHO (2008) reported that about 24.6 million people live with cancer world wide and 12.5% of all deaths are attributable to cancer and if the trend continues, it is estimated that by 2020, 16 million new cases will be diagnosed per annum out of which 70%

will be in developing countries. Parkin et al in 2003 reported that in indigenous Africans, 650,000 people of estimated 965 million are diagnosed of cancer annually and lifetime risk of dying from cancer in African women is 2 times higher than in developed countries.

Annually, there are about 100,000 new cancer cases in Nigeria, this is estimated to increase to 500,000 in 2020 (*Durosinmi, 2008*, *WHO 2008*). A study by GLOBOCAN 2012 estimated the five year prevalence of cancer in Nigeria to be about 243.6/100,000 (0.24%) (Ferlay et al, 2012). Cancer currently accounts for 4.4% of all deaths and is likely to increase to 6.8% in 2030 (*WHO 2008*). Out of 89,000 cancer deaths in 2005; 54,000 of these were younger than 70 years. The commonest cancers of Nigerian men are cancers of prostate, liver and lymphomas (*Parkin, 2003; Globocan, 2008; Awodele et al., 2011*). In women, cancer of cervix and breast are commonest (*Adebamowo, 2007*) with minimal regional variation. While cervical cancer is commonest in Zaria, Northern Nigeria (*Adewuyi, 2010*) as in rest of Africa (*Ngoma, 2006*), breast cancer has become the commonest cancer of women in Ibadan, Southern Nigeria (*Awodele et al., 2011*) Nigeria.

2.1.2 Aetiology of Cancer

The aetiology for many cancers are still unknown, however there are risk factors which are either modifiable or non-modifiable (Stricker*et al*., 2008). The modifiable factors include tobacco use, physical inactivity, unhealthy diet, obesity, ultraviolet radiation and infectious agents like Human Papilloma Virus (HPV), Hepatitis Viruses (HBV, HCV) and Helicobacter pylori. The non-modifiable factors include heredity, sex, ethnicity, immunosuppression and ageing (WHO, 2002; Nnodu, 2010).

2.1.2.1 Geographic and Environmental Variables

Although many impressive advances in understanding the molecular pathogenesis of cancer have been made by analyzing hereditary cancers, environmental factors that give rise to somatic mutations are the predominant cause of the most common sporadic cancers (WHO, 2009). This notion is supported by the geographic differences in death rates from specific forms of cancer. For example, death rates from breast cancer are about fourfold to fivefold higher in the United States and Europe compared with Japan. Conversely, the death rate for stomach carcinoma in men and women is about seven times higher in Japan than in the United States (Ferlay*et al*, 2013). Liver cell carcinoma is relatively infrequent in the United States but is the most lethal cancer among many African populations. Nearly all the evidence indicates that these geographic differences are environmental rather than genetic in origin.

2.1.2.2 Age

In general, the frequency of cancer increases with age (WHO, 2009). Most cancer mortality occurs between ages 55 and 75; the rate declines, along with the population base, after age 75. The rising incidence with age may be explained by the accumulation of somatic mutations associated with the emergence of malignant neoplasms. The decline in immune competence that accompanies aging also may be a factor. Cancer causes slightly more than 10% of all deaths among children younger than 15 years. The major lethal cancers in children are leukemia, tumors of the central nervous system,lymphomas, soft tissue sarcomas, and bone sarcomas age (WHO, 2010).

2.1.2.3 Heredity

There is evidence now to indicate that for many types of cancer there exist not only environmental influences but also hereditary predispositions (Notani, 2001). Hereditary forms of cancer can be divided into three categories as shown in Table 2.1.

2.1.2.4 Familial Cancers

Virtually all the common types of cancers that occur sporadically have been reported to occur in familial forms (Notani, 2001). Examples include carcinomas of colon, breast, ovary, and brain. Features that characterize familial cancers include early age at onset, tumors arising in two or more close relatives of the index case, and sometimes multiple or bilateral tumors. The transmission pattern of familial cancers is not clear. In general, siblings have a relative risk between 2 and 3. Segregation analysis of large families usually reveals that predisposition to the tumors is dominant, but multifactorial inheritance cannot be easily ruled out. Certain familial cancers can be linked to the inheritance of mutant genes (Notani, 2001).

2.1.2.5 Carcinogenic Agents

Genetic damage lies at the heart of carcinogenesis. Three classes of carcinogenic agents can be identified (Stricker*et al*, 2008): (1) chemicals, (2) radiant energy, and (3) microbial agents. Chemicals and radiant energy are documented causes of cancer in humans, and oncogenic viruses are involved in the pathogenesis of tumors in several animal models and at least in some human tumors.

1) Chemical carcinogens

More than 200 years ago, the London surgeon Sir Percival Pott correctly attributed scrotal skin cancer in chimney sweeps to chronic exposure to soot. Based on this observation, the Danish Chimney Sweeps Guild ruled that its members must bathe daily. No public health

measure since that time has achieved so much in the control of a form of cancer (Strickeret *al*, 2008). Subsequently, hundreds of chemicals have been shown to be carcinogenic in animals (Table 2.2).

Table 2.1: Inherited Predisposition to Cancer (Autosomal Dominant).

Source: Robbins and Cotran, (7th ed., ch 7, pg287)

Gene	**Inherited Predisposition**
RB	Retinoblastoma
p53	Li-Fraumeni syndrome (various tumors)
p16INK4A	Melanoma
APC	Familial adenomatous polyposis/colon cancer
NF1, NF2	Neurofibromatosis 1 and 2
BRCA1, BRCA2	Breast and ovarian tumors
MEN1, RET	Multiple endocrine neoplasia 1 and 2
MSH2, MLH1, MSH6	Hereditary nonpolyposis colon cancer
PATCH	Nevoid basal cell carcinoma syndrome

i) Direct-acting agents

Direct-acting agents require no metabolic conversion to become carcinogenic. They are in general weak carcinogens but are important because some of them are cancer chemotherapeutic drugs (e.g., alkylating agents) that have successfully cured, controlled, or delayed recurrence of certain types of cancer (e.g., leukemia, lymphoma, Hodgkin lymphoma, and ovarian carcinoma), only to evoke later a second form of cancer, usually leukemia (Stricker*et al*, 2008)..

ii) Indirect-Acting Agents

The designation *indirect-acting agent* refers to chemicals that require metabolic conversion to an *ultimate carcinogen* before they become active (Stricker et al, 2008). Some of the most potent indirect chemical carcinogens-the polycyclic hydrocarbons-are present in fossil fuels. For example, benzo*[a]*pyrene and other carcinogens are formed in the high-temperature combustion of tobacco in cigarette smoking. These products are implicated in the causation of lung cancer in cigarette smokers. Polycyclic hydrocarbons may also be produced from animal fats during the process of broiling meats and are present in smoked meats and fish. The principal active products in many hydrocarbons are epoxides, which form covalent adducts (addition products) with molecules in the cell, principally DNA, but also with RNA and proteins (WHO, 2009).

The aromatic amines and azo dyes are another class of indirect-acting carcinogens. Before its carcinogenicity was recognized, β-naphthylamine was responsible for a 50-fold increased incidence of bladder cancers in heavily exposed workers in the aniline dye and rubber industries (WHO, 2009).

Table 2.2. Major Chemical Carcinogens (Direct-Acting Carcinogens).

Source: Robbins and Cotran, (7th ed., ch 7, pg321)

ALKYLATING AGENTS	β-Propiolactone
	Dimethyl sulfate
Diepoxybutane	
	Anticancer drugs (cyclophosphamide , chlorambucil
,	nitrosoureas and others
ACYLATING AGENTS	1-Acetyl-imidazole
Dimethylcarbamyl chloride	
Procarcinogens That Require Metabolic Activation	
POLYCYCLIC AND HETEROCYCLIC AROMATIC HYDROCARBONS	
Benz(*a*)anthracene	
Benzo(*a*)pyrene	
Dibenz(*a*, *h*)anthracene	
	3-Methylcholanthrene
	7, 12-Dimethylbenz(*a*)anthracene
AROMATIC AMINES, AMIDES, AZO DYES	
2-Naphthylamine (β-naphthylamine)	
Benzidine	
	2-Acetylaminofluorene
Dimethylaminoazobenzene (butter yellow)	
NATURAL PLANT AND MICROBIAL PRODUCTS	
Aflatoxin B_1	
Griseofulvin	
Cycasin	
Safrole	
	Betel nuts
OTHERS	Nitrosamine and amides
	Vinyl chloride, nickel, chromium
	Insecticides, fungicides
	Polychlorinated biphenyls

2.1.2.6 Radiation Carcinogenesis

Radiation, whatever its source (UV rays of sunlight, x-rays, nuclear fission, radionuclides) is an established carcinogen (Robbins *et al,* 2010). Unprotected miners of radioactive elements have a 10-fold increased incidence of lung cancers. Follow-up of survivors of the atomic bombs dropped on Hiroshima and Nagasaki disclosed a markedly increased incidence of leukemia-principally acute and chronic myeloid leukemia-after an average latent period of about 7 years, as well as an increased mortality rate from thyroid, breast, colon, and lung carcinomas (Robbins *et al,* 2010). The nuclear power accident at Chernobyl in the former Soviet Union continues to exact its toll in the form of high cancer incidence in the surrounding areas. Therapeutic irradiation of the head and neck can give rise to papillary thyroid cancers years later. The oncogenic properties of ionizing radiation are related to its mutagenic effects; it causes chromosome breakage, translocations, and, less frequently, point mutations. Biologically, double-stranded DNA breaks seem to be the most important form of DNA damage caused by radiation. There is also some evidence that nonlethal doses of radiation may induce genomic instability, favoring carcinogenesis (Robbins *et al,* 2010).

2.1.2.7 Viral and Microbial Oncogenesis

Many DNA and RNA viruses have proved to be oncogenic in animals but only a few viruses have been linked with human cancer. There is also a role of the bacterium *H. pylori* in gastric cancer (Stricker*et al*, 2008).

i) Oncogenic RNA Viruses

The study of oncogenic retroviruses in animals has provided spectacular insights into the

genetic basis of cancer. However, human T-cell leukemia virus-1 (HTLV-1) is the only retrovirus that has been demonstrated to cause cancer in humans. HTLV-1 is associated with a form of T-cell leukemia/lymphoma that is endemic in certain parts of Japan and the Caribbean basin but is found sporadically elsewhere, including the United States. Similar to the human immunodeficiency virus (HIV), HTLV-1 has tropism for CD4+ T cells, and this subset of T cells is the major target for neoplastic transformation. Human infection requires transmission of infected T cells via sexual intercourse, blood products, or breastfeeding. Leukemia develops only in about 3% to 5% of infected individuals after a long latent period of 20 to 50 years. There is little doubt that HTLV-1 infection of T lymphocytes is necessary for leukemogenesis, but the molecular mechanisms of transformation are not clear (Robbins *et al*, 2010).

i) Oncogenic DNA Viruses

As with RNA viruses, several oncogenic DNA viruses that cause tumors in animals have been identified. Four DNA viruses-human papillomavirus (HPV), Epstein-Barr virus (EBV), Kaposi sarcoma herpesvirus (KSHV, also called human herpesvirus 8), and hepatitis B virus (HBV)-are of special interest, because they are strongly associated with human cancer (Robbins et al, 2010).

2.1.3 Clinical aspects of Neoplasia

Ultimately the importance of neoplasms lies in their effects on patients. Although malignant tumuors are more threatening than benign tumors, any tumor, even a benign one, may cause morbidity and mortality. Both malignant and benign tumors may cause problems because of (1) location and impingement on adjacent structures, (2) functional activity such as hormone synthesis or the development of paraneoplastic syndromes(3) bleeding and infections when the

tumor ulcerates through adjacent surfaces, (4) symptoms that result from rupture or infarction, (5) cachexia or wasting. The following discussion considers the effects of a tumor on the host, the grading and clinical staging of cancer, and the laboratory diagnosis of neoplasms (Robbins et al, 2010).

a) Effects of Tumor on Host

Location is crucial in both benign and malignant tumors. A small (1-cm) pituitary adenoma can compress and destroy the surrounding normal gland and give rise to hypopituitarism. A 0.5-cm leiomyoma in the wall of the renal artery may lead to renal ischemia and serious hypertension. A comparably small carcinoma within the common bile duct may induce fatal biliary tract obstruction (Stricker et al, 2008).

Hormone production is seen with benign and malignant neoplasms arising in endocrine glands. Adenomas and carcinomas arising in the β-cells of the islets of the pancreas can produce hyperinsulinism, sometimes fatal. Analogously, some adenomas and carcinomas of the adrenal cortex elaborate corticosteroids that affect the patient (e.g., aldosterone, which induces sodium retention, hypertension, and hypokalemia). Such hormonal activity is more likely with a well-differentiated benign tumor than with a corresponding carcinoma.

A tumor may ulcerate through a surface, with consequent bleeding or secondary infection. Benign or malignant neoplasms that protrude into the gut lumen may become caught in the peristaltic pull of the gut, causing intussusception and intestinal obstruction or infarction.

b) Cancer Cachexia

Many cancer patients suffer progressive loss of body fat and lean body mass,

accompanied by profound weakness, anorexia, and anemia, referred to as *cachexia.* There is some correlation between the size and extent of spread of the cancer and the severity of the cachexia. However, cachexia is not caused by the nutritional demands of the tumor. Although patients with cancer are often anorexic, current evidence indicates that cachexia results from the action of soluble factors such as cytokines produced by the tumor and the host rather than reduced food intake. In patients with cancer, calorie expenditure remains high, and basal metabolic rate is increased, despite reduced food intake. The basis of these metabolic abnormalities is not fully understood. It is suspected that TNF produced by macrophages in response to tumor cells or by the tumor cells themselves mediates cachexia. TNF suppresses appetite and inhibits the action of lipoprotein lipase, inhibiting the release of free fatty acids from lipoproteins (Robbins et al, 2010).

c) Paraneoplastic Syndromes

Symptom complexes that occur in patients with cancer and that cannot be readily explained by local or distant spread of the tumor or by the elaboration of hormones indigenous to the tissue of origin of the tumor are referred to as *paraneoplastic syndromes.* They appear in 10% to 15% of patients with cancer, and it is important to recognize them for several reasons: They may represent the earliest manifestation of an occult neoplasm. In affected patients, they may represent significant clinical problems and may even be lethal.They may mimic metastatic disease and confound treatment (Table 2.4).

c) Grading and Staging of Cancer

Methods to quantify the probable clinical aggressiveness of a given neoplasm and its apparent extent and spread in the individual patient are necessary for making accurate prognosis and for comparing end results of various treatment protocols. For instance, the results of treating

extremely small, highly differentiated thyroid adenocarcinomas that are localized to the thyroid gland are likely to be different from those obtained from treating highly anaplastic thyroid cancers that have invaded the neck organs (Robbins et al, 2010).

The *grading* of a cancer attempts to establish some estimate of its aggressiveness or level of malignancy based on the cytologic differentiation of tumor cells and the number of mitoses within the tumor. The cancer may be classified as grade I, II, III, or IV, in order of increasing anaplasia.

Staging of cancers is based on the size of the primary lesion, its extent of spread to regional lymph nodes, and the presence or absence of metastases. This assessment is usually based on clinical and radiographic examination (computed tomography and magnetic resonance imaging) and in some cases surgical exploration. Two methods of staging are currently in use: the TNM system (*T*, primary tumor; *N*, regional lymph node involvement; *M*, metastases) and the AJC (American Joint Committee) system. In the TNM system, T1, T2, T3, and T4 describe the increasing size of the primary lesion; N0, N1, N2, and N3 indicate progressively advancing node involvement; and M0 and M1 reflect the absence or presence of distant metastases. In the AJC method, the cancers are divided into stages 0 to IV, incorporating the size of primary lesions and the presence of nodal spread and of distant metastases (Robbins et al, 2010).

Table 2.3. Paraneoplastic syndromes

Source:Robbins and Cotran, (7th ed., ch 7, pg334)

CLINICAL SYNDROMES ENDOCRINOPATHIES	MAJOR FORMS OF UNDERLYING CANCER	CAUSAL MECHANISM
Cushing syndrome	Small-cell carcinoma of lung Pancreatic carcinoma Neural tumors	ACTH or ACTH-like substance
Syndrome of inappropriate antidiuretic hormone secretion	Small-cell carcinoma of lung; intracranial neoplasms	Antidiuretic hormone or atrial natriuretic hormones
Hypercalcemia	Squamous cell carcinoma of lung Breast carcinoma Renal carcinoma Adult T-cell leukemia/lymphoma Ovarian carcinoma	Parathyroid hormone-related protein, TGF-α, TNF, IL-1
Hypoglycemia	Fibrosarcoma Other mesenchymal sarcomas Hepatocellular carcinoma	Insulin or insulin-like substance
Carcinoid syndrome	Bronchial adenoma	Serotonin, bradykinin

	(carcinoid) Pancreatic carcinoma Gastric carcinoma	
Polycythemia	Renal carcinoma Cerebellar hemangioma Hepatocellular carcinoma	Erythropoietin
NERVE AND MUSCLE SYNDROME		
Myasthenia, Disorders of the central and peripheral nervous systems	Bronchogenic carcinoma, Breast carcinoma	Immunologic
DERMATOLOGIC DISORDERS		
Acanthosisnigricans	Gastric carcinoma Lung carcinoma Uterine carcinoma	Immunologic; secretion of epidermal growth factor
Dermatomyositis	Bronchogenic, breast carcinoma	Immunologic
OSSEOUS, ARTICULAR, AND SOFT-TISSUE CHANGES		
Hypertrophic	Bronchogenic carcinoma	Unknown

osteoarthropathy and clubbing of the fingers		
VASCULAR AND HEMATOLOGIC CHANGES		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma Bronchogenic carcinoma Other cancers	Tumor products (mucins that activate clotting)
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Anemia	Thymic neoplasms	Unkown
OTHERS		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

KEY; ACTH, adrenocorticotropic hormone; TGF, transforming growth factor; TNF, tumor necrosis factor; IL, interleukin

2.1.4 Laboratory Diagnosis of Cancer

a) Morphologic Methods

Clinical data are invaluable for optimal pathologic diagnosis. Radiation-induced changes in the skin or mucosa can be similar to those of cancer. Sections taken from a healing fracture can mimic an osteosarcoma. The laboratory evaluation of a lesion can be only as good as the specimen submitted for examination. The specimen must be adequate, representative, and properly preserved (Notani, 2001).

Several sampling approaches are available, including excision or biopsy, fine-needle aspiration, and cytologic smears.

Fine-needle aspiration of tumuors is an approach that is widely used. It involves aspiration of cells from a mass, followed by cytologic examination of the smear. This procedure is used most commonly with readily palpable lesions affecting the breast, thyroid, lymph nodes, and salivary glands. Modern imaging techniques permit extension of the method to deeper structures, such as the liver, pancreas, and pelvic lymph nodes. It obviates surgery and its attendant risks. Although it entails some difficulties, such as small sample size and sampling errors, in experienced hands it can be extremely reliable, rapid, and useful (Robbins *et al*, 2010).

Cytologic smears provide another method for the detection of cancer. Historically, this approach has been used widely for the discovery of carcinoma of the cervix, often at an in situ stage, but now it is used with many other forms of suspected malignancy, such as endometrial carcinoma, bronchogenic carcinoma, bladder and prostate tumors, and gastric carcinomas; for the identification of tumor cells in abdominal, pleural, joint, and cerebrospinal fluids; and, less commonly, with other forms of neoplasia(Robbins *et al*, 2010).

Immunohistochemistry offers a powerful adjunct to routine histology (Robbins et al, 2010). Detection of cytokeratin by specific monoclonal antibodies labeled with peroxidase points to a diagnosis of undifferentiated carcinoma rather than large-cell lymphoma. Similarly, detection of prostate-specific antigen (PSA) in metastatic deposits by immunohistochemistry allows definitive diagnosis of a primary tumor in the prostate. Immunohistochemical detection of estrogen receptors allows prognostication and directs therapeutic intervention in breast cancers. *Flow cytometry* is used routinely in the classification of leukemias and lymphomas. In this method, fluorescent antibodies against cell surface molecules and differentiation antigens are used to obtain the phenotype of malignant cells (Robbins et al, 2010).

b) Tumor Markers

Biochemical assays for tumor-associated enzymes, hormones, and other tumor markers in the blood cannot be utilized for definitive diagnosis of cancer; however, they contribute to finding cases and in some instances are useful in determining the effectiveness of therapy or the appearance of a recurrence. PSA, used to screen for prostatic adenocarcinoma, may be one of the most used, and most successful, tumor markers in clinical practice. Prostatic carcinoma can be suspected when elevated levels of PSA are found in the blood (Stricker et al, 2008).

c) Molecular Diagnosis

An increasing number of molecular techniques are being used for the diagnosis of tumors and for predicting their behavior (Stricker et al, 2008).

1. *Diagnosis of malignancy.* Because each T and B cell has unique rearrangement of its antigen receptor genes, polymerase chain reaction (PCR)-based detection of T-cell receptor or immunoglobulin genes allows distinction between monoclonal (neoplastic)

and polyclonal (reactive) proliferations. Many hematopoietic neoplasms, and a few solid tumors, are defined by particular translocations, and thus the diagnosis can be made by detection of such translocations. For example, fluorescence in situ hybridization (FISH) or PCR can be used to detect translocations characteristic of Ewing sarcoma and several leukemias and lymphomas. PCR-based detection of *BCR-ABL* transcripts provides the molecular diagnosis of chronic myeloid leukemia (Robbins et al, 2010).

2. *Prognosis and behavior.* Certain genetic alterations are associated with a poor prognosis, and thus the presence of these alterations determines the patient's subsequent therapy. FISH and PCR methods can be used to detect amplification of oncogenes such as *Her-2/Neu* and *N-MYC,* which provide prognostic and therapeutic information for breast cancers and neuroblastomas.
3. *Detection of minimal residual disease.* Another emerging use of molecular techniques is detection of minimal residual disease after treatment. For example, detection of *BCR-ABL* transcripts by PCR gives a measure of residual disease, in patients treated for chronic myeloid leukemia.
4. *Diagnosis of hereditary predisposition to cancer.* Germ-line mutation of several tumor suppressor genes, such as *BRCA1,* increases a patient's risk of developing certain types of cancer. Thus, detection of these mutated alleles may allow the patient and physician to devise an aggressive screening protocol, as well as to consider prophylactic surgery. In addition, such detection allows genetic counseling of relatives at risk.

d) Molecular Profiling of Tumors

One of the most exciting advances in the molecular analysis of tumors has been made

possible by DNA-microarray analysis. This technique allows simultaneous measurements of the expression levels of several thousand genes. mRNA is extracted from the samples, reverse transcribed to cDNA, and labeled with fluorescent molecules (Robbins et al, 2010).

2.2 ANTIOXIDANTS

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances (Ansari *et al*, 1996).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997).

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied,

particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline. For many years chemists have known that free radicals cause oxidation which can be controlled or prevented by a range of antioxidants substances (Bjelakovic*et al*., 2007). It is vital that lubrication oils should remain stable and liquid should not dry up like paints. For this reason, such oil usually has small quantities of antioxidants such as phenol or amine derivatives, added to them. Although plastics are often formed by free radical action, they can also be broken down by the same process, so they too, require protection by antioxidants like phenols or naphthol. Low density polythene is also of protected by carbon black which absorbs the ultraviolet light which causes radical production (Sies, 1997).

2.2.1 Sources and Origin of Antioxidants

Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish. The list below describes food sources of common antioxidants. Beta-carotene is found in many foods that are orange in color, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin and mangoes. Some green, leafy

vegetables, including collard greens, spinach and kale, are also rich in betacarotene (Borek, 1991). Lutein, best known for its association with healthy eyes, is abundant in green, leafy vegetables such as collard greens, spinach, and kale. Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges and other foods. Estimates suggest 85% of American dietary intake of lycopene comes from tomatoes and tomato products (Rodriguez-Amaya, 2003; Xianquan et al., 2005).

Selenium is a component of antioxidant enzymes. Plant foods like rice and wheat are the major dietary sources of selenium in most countries, so also meats, nuts and bread.

Vitamin A is found in three main forms: retinol (Vitamin A1), 3,4-didehydroretinol (Vitamin A2), and 3-hydroxyretinol (Vitamin A3). Foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and mozzarella

cheese (Baublis*et al*., 2000). Vitamin C is also called ascorbic acid and can be found in high abundance in many fruits and vegetables and is also found in cereals, beef, poultry, and fish.

Vitamin E, also known as alpha-tocopherol, is found in almonds, in many oils including wheat germ, safflower, corn and soybean oils, and is also found in mangoes, nuts, broccoli, and other foods (Herrera *et al*, 2001).

2.2.2 Classification of Antioxidants

Antioxidants are grouped into two namely;

(1) Primary or natural antioxidants.

(2) Secondary or synthetic antioxidants.

Primary or natural antioxidants: They are the chain breaking antioxidants which react withlipid radicals and convert them into more stable products.Antioxidants of this group are mainly phenolic instructures and include the following (Hurrell, 2003).

(1) Antioxidants minerals - These are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc and manganese.

(2) Antioxidants vitamins – It is needed for most body metabolic functions. They include vitamin C, vitamin E, vitamin B.

(3) Phytochemicals - These are phenolic compounds that are neither vitamins nor minerals. These include:

Flavonoids: These are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark their colours. Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble colour in fruits and vegetables. Beta carotene, which is rich in carrot and converted to vitamin A when the body lacks enough of the vitamin. Lycopene, high in tomatoes and zeaxantin is high in spinach and other dark greens. Herbs and spices-source include Diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives.

Secondary or synthetic antioxidants

These are phenolic compounds that perform the function of capturing free radicals and stopping

the chain reactions, the compound include (Hurrell, 2003).

i. Butylated hydroxyl anisole (BHA).

ii. Butylatedhydroxyrotoluene (BHT).

iii. Propyl gallate (PG) and metal chelating agent (EDTA).

iv. Tertiary butyl hydroquinone (TBHQ).

v. Nordihydroguaretic acid (NDGA).

2.2.3 Antioxidants and Cancer

Among the possible causes of cancer, damage to DNA and other cellular molecules by reactive oxygen species ranks high as a major factor in the onset and development of the disease (*Manoharanet al, 1994; Subapriya et al, 2002*). Reactive oxygen species are by-products of normal metabolism which increases in inflammation and exposure to exogenous agents including nitrogen oxide pollutants, smoking, certain drugs (for example, acetaminophen, bleomycin) and radiation. Reactive oxygen species can induce cancer by causing mutations, oxidize lipids and proteins, and alter signal transduction pathways that enhance cancer risk. Experimental studies support the role of reactive oxygen species in cancer, in part by showing that dietary antioxidants (for example, vitamins C and E, selenium, β-carotene, and other phytochemicals) as well as endogenous antioxidants (for example, glutathione) that neutralize or trap reactive oxygen species act as cancer preventive agents. Human observational studies provide further support, showing on one hand, that oxidative stress increases with clinical progression of breast cancer and on the other hand, that a diet rich in antioxidant containing

foods reduce the risk of certain cancers (*Hereberg, 2005*).

Once cancer has occurred however, treatment with radiation and chemotherapeutic agents that generate free radicals in cells largely relies on their oxidative damage to eradicate the cancerous cells. Thus, the question arises whether antioxidants that protect normal cells from acute and long-term free radical damage may afford the same protection to tumor cells and hinder the overall outcome of cancer therapy (*Sun, 1990*). Clinicians treating cancer patients are faced with this puzzle, and while rarely restricting the intake of antioxidant-rich food, they may advise patients to refrain from taking antioxidant supplements, lest they interfere with the course of treatment. New data, however, show that some dietary antioxidants may have potential as adjuvants in cancer therapy by their ability to induce programmed cell death (apoptosis). Studies in cell cultures show that vitamins C and E, selenium and some phytochemicals selectively induce apoptosis in cancer cells while sparing normal cells. Other findings in a model of metastatic growth show that vitamin C is an angiostatic factor and may have potential in aiding host resistance to tumour growth and invasiveness. Antioxidants also show promise in cancer therapy by their palliative action, reducing painful side effects associated with treatment (Baublis et al., 2000).

Antioxidants have been shown to decrease chronic inflammation in patients with solid tumors and may also have other actions beyond their effect on free radicals. vitamin C, for instance, was shown to stabilize p53 and enhance, in a cell culture medium, the sensitivity of cervical cancer cells to cisplatinum(*Hereberg, 2005*). Numerous studies show that vitamin C decreases the concentration of glutathione (GSH). GSH as well as being an antioxidant is a detoxifying agent. A high level of GSH is associated with resistance to multiple chemotherapeutic agents. This might be because the presence of GSH within the malignant cell protects the cell,

as well as promoting the detoxification of the chemotherapeutic agent itself. Therefore, through the mechanism of vitamin C depleting GSH, this might explain the enhanced efficacy of certain chemotherapeutic agents used in conjunction with vitamin C, as has been shown in different studies (*Hereberg, 2005*). Vitamin C, in conjunction with vitamin K_3, has been shown to enhance cell killing, used alone and in conjunction with chemotherapy. With some chemotherapeutic agents, such as 5-flourouracil (5-FU), the production of reactive oxygen species is not a significant contributor to their efficacy. Numerous studies showed enhanced efficacy when 5-FU is used in conjunction with different antioxidants. The issue of multidrug resistance is of great importance. Factors such as increased concentration of glutathione and upregulation of glutathione peroxidase and transferase, as well as increased production of nuclear transcription factor (NF-kB) have been considered of central importance. In addition, the proteosome (ubiquitin) pathway, which is important for degradating intracellular proteins is involved. Various flavonoids, including especially curcumin have been shown to have potential beneficial effects in these areas. Curcumin was shown to increase sensitivity to doxorubicin and act as a radiosensitizer in a prostate cancer cell line by effecting NF-kB, GSH and the ubiquitin pathways*(Antunes, 2001*).

In complementary oncology, the administration of vitamins and other micronutrients tailored to individual needs can help to reduce the frequency of side effects from antineoplastic treatment and improve its effectiveness and therapeutic index through better compliance, fewer therapy dropouts and higher possible dosages, and improve quality of life for the tumour patient (*Hereberg, 2005*). Numerous studies have shown a depletion of antioxidants and an increased amount of reactive oxygen species in patients with malignancies (*Manoharan, 1994*).

Reactive oxygen species (ROS) are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and gluthathione peroxidase (GPX) control their accumulation *(*Mates, 1999). Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radicals and its accumulation. These ROS are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage. They are also responsible for oxidation of bases in cellular DNA making them mutagenic, cytotoxic and crosslinking agents, which in turn causes uncontrolled expression of certain genes causing increased multiplication of cells leading to cancer *(Mates, 1999*).

2.2.4 Cancer Therapy and Antioxidant Status

Reactive oxygen species (ROS) are constantly produced in animal and human cells but excessive ROS can induce oxidative damage in cell constituents and promote a number of degenerative diseases, cancer and aging. Cellular antioxidants protect against the damaging effects of ROS. Chemotherapy and radiation therapy are associated with increased formation of reactive oxygen species and depletion of critical plasma and tissue antioxidants, including concentrations of vitamin C, α-tocopherol, and β-carotene. In addition to micronutrient antioxidants, the tripeptide glutathione (γ-glutamyl-cysteine-glycine; GSH) is a critical endogenous antioxidant that acts directly as a free radical scavenger and in conjunction with the detoxification enzymes glutathione peroxidase (GSHPx) and glutathione-*S*-transferase. Studies in animal models and in humans showed that plasma and hepatic GSH concentrations are markedly reduced by chemotherapeutic agents, such as busulfan, cermustine (BCNU), and cisplatin.

Thus chemotherapy and radiation therapy are effective through varied mechanisms. The production of ROS is one of the ways that radiation therapy, and some chemotherapeutic agents(for example, alkylating agents, platinum agents) work. It is not clear that this is the most important mechanism for their action. On the other hand, the production of ROS leads to many of the side effects associated with these treatments. As there is concern that antioxidants can neutralize ROS, and therefore interfere with chemo/radiation therapy, considerable controversy has emerged over the issue if patients should refrain from using antioxidants while receiving chemotherapy or radiation therapy. The great majority of oncologists and radiation therapists will advise their patients against using antioxidants during these treatment phases, particularly in earlier stages of treatment, when these treatments have the most likelihood of benefit. This is probably a prudent recommendation, as the bottom line is that we don't know if there is any decreasing of the efficacy of chemotherapy and radiation therapy if antioxidants are used concurrently. However, the issue is very complex, and most of the literature does not support this position. In fact, many studies suggest that concurrent use of antioxidants not only decreases adverse reactions, but also might increase the efficacy of chemo/radiation therapy.

As noted above, antioxidants have actions beyond their effect on free radicals.Vitamin C, for instance, was shown to stabilize p53 and enhance, in a cell culture medium, the sensitivity of cervical cancer cells to cisplatinum. Vitamin C, in conjunction with vitamin K3, has been shown to enhance cell killing, used alone and in conjunction with chemotherapy. Numerous studies showed enhanced efficacy when 5-FU is used in conjunction with different antioxidants *(Antunes, 2001*). Various flavonoids, including especially curcumin, have been shown to have potential beneficial effects in these areas. Curcumin was shown to increase sensitivity to

doxorubicin and act as a radiosensitizer in a prostate cancer cell line, by effecting NFkB,AP-1, GSH, and the ubiquitin pathways (*Hereberg, 2005*).

Various clinical studies have looked at this issue. Protection of hearing loss was looked at with patients receiving cisplatinum and a combination of Vitamins C, E and selenium. A partial protection was noted in patients who achieved the highest level of these nutrients (Baublis et al., 2000). Another study noted protection against bone marrow suppression and nephrotoxicity in patients treated with cisplatinum who also received selenium, compared to controls. A non randomized study looked at patients with non small cell lung cancer who were treated with conventional approaches as well as a combination of antioxidants, trace elements, and fatty acids. A significant improvement was noted compared to historical controls, and patients were also noted to tolerate chemo/radiation therapy better (Baublis*et al*., 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

Zaria. Zaria is a heterogeneous city with a population of about 1.490,000 people, located on the high plains of Northern Nigeria, 652.6 meters above the sea level, some 950km away from the coast (112 031N 7Q 42E) (Zaria Online, 2014). Farming is the predominant occupation of the people of Zaria but businesses and civil service form a substantial part of the population. The climate in Zaria is mostly tropical with the temperature range from 15.3-36.2 ^{0}C and a rainfall in the range of 0-816 mm/month. There are two main seasons in Zaria; a rainy season from April to October and a dry season from November to March (Zaria Online, 2014).

3.2 STUY DESIGN

This was a cross-sectional study conducted at Ahmadu Bello University Teaching Hospital Shika,

3.3 STUDY POPULATION

A total number of 100 cancer patients with histologically or cytologically confirmed diagnosis of cancer were recruited for this study. A total number of 100 apparently healthy aged and sex-matched subjects were also recruited as controls. Serum antioxidant minerals (selenium, copper and zinc) and enzymes (glutathione peroxidase, superoxide dismutase and catalase) were assessed on both cancer patients and controls.

3.3.1 Sample size determination

The sample size (n) for this study was determined using the formula for the calculation of minimum sample size (Oyejide, 1992; Singha, 1996):

$$n = \frac{Z^2 (p) (q)}{d^2}$$

Where, n = sample size, Z^2 = the value of standard normal deviation which at 95% confidence level is 1.96, d = level of precision, 0.01, q = 1-p, and p = prevalence of cancer in Nigeria = 0.24% (Ferlay*et al*, 2012). Therefore, the sample size (n) is calculated as:

$$n = \frac{(1.96)^2 (0.0024) (0.9976)}{(0.01)^2}$$

n = 82

The calculated sample size for the study was approximately 82 patients. However, a total of 100 patients were used to take care of possible attrition.

3.3.2 Inclusion Criteria for Patients

i). All cancer patients with histologically or cytologically confirmed diagnosis and who agreed to participate were included in the study.

ii). All cancer patients not on chemotherapy, radiotherapy or any other forms of treatment (for example, traditional medications) likely to generate reactive oxygen species were also recruited.

3.3.3 Exclusion Criteria for Patients

i). All cancer patients on chemotherapy, radiotherapy or traditional medicationslikely to generate reactive oxygen species were excluded from the study.

ii). All patients who have other conditions likely to affect serum antioxidant levels (for example, malnutrition) were excluded from the study.

iii). All those who declined to give consent for inclusion were also excluded from the study.

3.3.4 Inclusion Criteria for Controls

i). All apparently healthy individuals of similar age and sex as the patients who agreed to participate in the study were recruited as controls.

ii). All apparently healthy individuals not on traditional medications or supplements likely to generate reactive oxygen species were recruited as controls.

3.3.5 Exclusion Criteria for Controls

i). All apparently healthy individuals on traditional medications or supplements likely to generate reactive oxygen species were excluded from the study.

ii). Allapparently healthy individuals who declined to give consent for inclusion were excluded from the study.

3.3.6 Informed Consent

Informed written consent was obtained from all subjects before inclusion in the study

using a standard protocol (Appendices I and II). In patients who are too ill to respond, consent was obtained from the patient's care giver.

3.3.7 Ethical Approval

This was sought and obtained from the Health Research Ethics Committee of ABUTH Zaria before embarking on the study (Appendix IV).

3.4 STUDY PROTOCOL

Patients were recruited from the oncology clinics. Subjects who satisfy the study inclusion criteria were selected. A proforma (Appendix III) was administered to the study population to include the biodata, family history of disease, laboratory results and drug history of the study subjects.

The weight (kg) of all subjects was recoded to the nearest 0.5 kg using Hansons standard bathroom (standing) weighing scale with subjects wearing light clothing. The following biochemical investigations were performed on all the study subjects, viz; serum antioxidant minerals (selenium, copper and zinc) and enzymes (glutathione peroxidase, superoxide dismutase and catalase).

3.5 SPECIMEN COLLECTION AND PROCESSING

Blood specimen (about 5ml) for biochemical analysis was collected from a peripheral vein (antecubitalvenepuncture). In this case, the antecubital fossa was cleaned with methylated spirit and allowed to dry. A torniquet was applied a few centimeters above the antecubital fossa to distend the veins. Blood was then taken using a sterilized 5ml syringe and 21G needle and

transferred into a plain bottle and allowed to clot. It was then centrifuged for 10 minutes at 4000 rpm. The serum was separated from the cells and transferred into sample bottle and then frozen at -20^{0}C until the time for analysis.

3.6 CHEMICALS/REAGENTS

The chemicals and the reagent kits used for the determination of antioxidant enzymes and minerals were procured from Wako Co. Ltd, Japan, and Merck, Darmstadt, Germany, respectively. All the chemicals and the reagent kits were of analytical grade.

3.7 EQUIPMENT

Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. Beckman Coulter DU-20 General purpose UV/VIS spectrophotometer (Germany), Shimadzu model AA-670 Flameless (Graphite-Furnace) Atomic Absorption Spectrophotometer (GFAAS) and Shimadzu model AA-670 Flame Atomic Absorption Spectrophotometer (FAAS) were used for the measurement of the of the analytes.

3.8 ANALYTICAL METHODS

3.8.1. Measurement of Serum Zinc (AAS, Smith, 1979)

Serum concentration of Zinc was measured using method of Smith et al. (1979).

Principle

AAS procedure is based upon the fact that metal atoms absorb light energy strongly at discrete characteristic wavelength which coincides with the emission spectra line of that element.

For estimation of serum zinc, a hollow cathode lamp of zinc was used. The hollow cathode lamp emits a spectrum where the beam traverses the flame and is focused on the entrance slit of a monochromator which is set to read the intensity of a spectral line at wavelength 213.9 nm. Light with this wavelength is absorbed by the flame.

Procedure

The stored serum samples were allowed to come to room temperature (25^0C) mixed gently and then a 1:5 dilution-1 ml of serum in 4 ml of 1% nitric acid for deproteinization was made. Working standard solutions of zinc, 0.1, 0.2, 0.3, 0.4 and 1.0 ppm, were prepared by diluting with water from the stock standard solution and each standard solution was run one by one in AAS (Shimadzu model AA-670) at wavelength 213.9 nm and absorbance was recorded. Serum sample 0.5 ml was pipetted into a plastic test tube and 2.0 mL of de-ionized water added and the solution immediately mixed for 30 sec. The control sample of pooled serum was similarly prepared. Standard curves for zinc was obtained by plotting the absorbance of standards against their concentrations.

Calculation

Then concentrations of zinc in the serum and control sample were calculated from their respective regression equations (r = 0.9985)

A = (0.1499485) [Zn conc.] (µg/ml)+ 0.0001701.

3.8.2. Measurement of Serum Copper (AAS, Dawson, 1968)

Serum copper concentrations were measured by method of Dawson et al. (1968).

Principle:

AAS procedure is based upon the fact that metal atoms absorb light energy strongly at discrete characteristic wavelength which coincides with the emission spectra line of that element. For estimation of serum copper, a hollow cathode lamp of copper was used. The hollow cathode lamp emits a spectrum where the beam traverses the flame and is focused on the entrance slit of a monochromator which is set to read the intensity of a spectral line at wavelength 324.8 nm. Light with this wavelength is absorbed by the flame.

Procedure:

The stored serum samples were allowed to come to room temperature (25^0C) mixed gently and then a 1:5 dilution-1 ml of serum in 4 ml of 1% nitric acid for deproteinization was made. Working standard solutions of copper, 0, 0.25, 0.5, 0.75 and 1.0µg/ml, were prepared by diluting with water from the stock standard solution and each standard solution was run one by one in AAS (Shimadzu model AA-670) at wavelength 324.8 nm and absorbance was recorded. Serum sample 0.5 ml was pipetted into a plastic test tube and 2.0 mL of de-ionized water added and the solution immediately mixed for 30 sec. The control sample of pooled serum was similarly prepared. Standard curves for zinc was obtained by plotting the absorbance of standards against their concentrations.

Calculations:

The concentrations of copper in the serum and control samples were calculated from their respective regression equations (r = 0.0.9967).

A = (0.06) [Cu conc.] (µg/ml) + 0.0002174.

3.8.3. Measurement of Serum Selenium (AAS, Lloyd, 1982)

Serum selenium concentrations were measured by method of Lloyd et al. (1982)

Principle:

AAS procedure is based upon the fact that metal atoms absorb light energy strongly at discrete characteristic wavelength which coincides with the emission spectra line of that element. For estimation of serum selenium, a hollow cathode lamp of selenium was used. The hollow cathode lamp emits a spectrum where the beam traverses the flame and is focused on the entrance slit of a monochromator which is set to read the intensity of a spectral line at wavelength 196.0 nm. Light with this wavelength is absorbed by the flame.

Procedure:

The stored serum samples were allowed to come to room temperature (25^0C) and mixed gently, then 500 µL is pipetted into a test tube and diluted with 1% nitric acid (1:2) and 0.1% Triton X-100 and 10 µL were introduced into graphite furnace with appropriate volume of palladium modifier (1.2 µg; 2 µg; 2.4 µg; 5 µg and 10 µg) and iridium modifier (0.1 µg; 0.5 µg and 1 µg). Working standard solutions containing 4, 15, 30, 45, and 60µm/ml of selenium were prepared from stock standard solution in nitric acid-Triton X-100 mixture spiked with palladium or iridium solution as matrix modifier. Each standard solution was run one by one in Flameless Atomic Absorption Spectrophotometer (Shimadzu model AA-670) at wavelength 196.0 nm and absorbance was recorded. Standard curves for selenium was obtained by plotting the absorbance of standards against their concentrations. The control sample of pooled serum was similarly prepared as the serum sample.

Calculation:

The concentrations of selenium in the serum and control sample were calculated from their respective regression equations (r = 0.0.9991).

A = (0.00163) [Se conc.] (ng/ml)+ 0.002699.

3.8.4. Measurement of Serum Glutathione Peroxidase (colorimetric,Mannervik, 1985)

Serum Glutathione Peroxidase was measured by method of Mannervik (1985)

Principle:

This kit uses an indirect determination method. It is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (b-Nicotinamide Adenine Dinucleotide Phosphate, reduced),(Mannervik, B. 1985). The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPx activity, since GPx is the rate limiting factor of the coupled reactions.

Procedure:

Glutathione Peroxidase Assay Buffer 940µl was pipetted into a 1 ml quartz cuvette while keeping the temperature of the assay buffer in the spectrophotometer at 25 °C by using a thermostated cell holder. NADPH Assay Reagent 50µl, 10 µl of sample and 20 µl of enzyme were added to the cuvette and mixed by inversion. The reaction was started by adding 10 ml of the 30 mMtert-Butyl Hydroperoxide solution and mixed by inversion. The decrease in

absorbance at 340 nm was followed using a kinetic programme. A total of 6 readings were taken separated by an interval of 10seconds after initial delay of 15seconds. The amount of enzyme in the sample was then calculated.

Table 3.1: Glutathione Peroxidase Reaction Scheme

	GPx Assay Buffer (ml)	NADPH Assay Reagent (ml)	Enzyme (0.25 units/ml)(ml)	Sample (ml)	30 Mm t-Bu OOH (ml)
Blank	940	50	-	-	10
Positive control	920	50	20	-	10
Sample	930	50	-	10	10

Calculation:

The activity of Glutathione Peroxidase in the sample was calculated using the formula:

Activity per extract (mmol/min/ml = Units/ml) $\Delta A_{340} \times DF/6.22 \times V$

where,

$\Delta A_{340} = A_{340}/min_{(blank)} - A_{340}/min_{(sample)}$,

$6.22 = \boldsymbol{\varepsilon}^{mM}$ for NADP

DF = dilution factor of sample before adding to reaction

V = sample volume in ml

Unit definition: 1 unit of glutathione peroxidase will cause the formation of 1.0 mmol of $NADP^+$ from NADPH per minute at pH 8.0 at 25 °C in a coupled reaction in the presence of reduced glutathione, glutathione reductase, and tert-butyl hydroperoxide.

3.8.5. Measurement of SerumSuperoxide Dismutase (colorimetric,Kakkar, 1984)

Serum superoxide dismutase was measured by method ofKakkar et al (1984)

Principle:

It is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide. One unit is defined as that amount of enzyme causing half the maximum inhibition of NBT reduction. The reaction velocity depends on variable assay conditions such as light intensity and reaction temperature.

Procedure:

Into a series of test tubes was pipetted 0.2ml EDTA/Cyanide, 0.1ml NBT, 3ml Phosphate buffer and 10µg of enzyme. Two test tubes with no enzyme was included as control. The tubes were placed in a light box providing uniform light intensity and incubated for 5-8 minutes to achieve a standard temperature. At zero time and at 1min timed intervals 0.05 ml riboflavin was added. All tubes in the light box were incubated for 12 minutes and at timed intervals of 1min the absorbance read at 560nm.The percent inhibition of NBT reduction was then determined. The percent inhibition was plotted versus amount of enzyme in test.

Calculation:

The amount of enzyme resulting in one half of maximum inhibition was then determined as follows

Units/mg = 1000/µg enzyme resulting in ½ max.inhibition

3.8.6. Measurement of SerumCatalase (colorimetric, Beers, 1952)

Serum catalase was measured by method of Beers et al (1952)

Principle:

Catalase activity was measured by the method described by Beers and Sizer (1952) in which the disappearance of peroxide is followed spectrophotometrically at 240 nm (Continuous Spectrophotometric Rate determination). One Unit decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.0 under the specified conditions.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

CONDITIONS: T = 25℃, pH = 7.0, A240nm, Light Path = 1 cm

Procedure:

Into two test tubes labeled test (T) and blank (B), 3ml of reagent Awas pipetted into tube A and 2.9ml of reagent B into tube B respectively. These were equilirated to 25℃ and the absorbance recorded. Then 0.1ml of reagent C was added into the tube labeled T and mixed immediately by

inversion and the time required for the A240nm to decrease from 0.45 to 0.40 absorbance units was recorded.

Calculation:

The results were calculated as follows:

Units/ml enzyme = (3.45)(df)/(min)(0.1)

Where,

3.45 = Corresponds to the decomposition of 3.45 micromoles of hydrogen peroxide in a 3.0 ml reaction mixture producing a decrease in the A240nm from 0.45 to 0.40 absorbance units

df = Dilution factor

min = Time in minutes required for the A240nm to decrease from 0.45 to 0.40 absorbance units

0.1 = Volume (in milliliter) of enzyme used.

3.9 QUALITY CONTROL

Adequate quality control measures were observed when carrying out the analysis of the samples to ensure that the results obtained are reliable.

3.10 STATISTICAL ANALYSIS

The data obtained was statistically treated accordingly using Statistical Program for the Social Sciences (SPSS 16.0) for windows (IBM). The results of serum antioxidant minerals (selenium, copper and zinc) and enzymes (glutathione peroxidase, superoxide dismutase and catalase) obtained from cancer patients were compared with those of apparently healthy individuals (controls) using the two tailed student's t-test. The two tailed student's t-test was also employed to compare the results of different age groups of cancer patients as well as controls. Correlations between serum antioxidants and the duration of cancer and between antioxidants and the different age groups of cancer patients were carried out using Pearsons linear correlation analysis. A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered as statistically significant.

CHAPTER FOUR

4.0 RESULTS

The Biodata and Clinical characteristics of both the 100 cancer patients and healthy controls respectively are as shown in table 4.1. The age range is 30 to 90years and 31 to 91years for both the patients and the healthy controls respectively. The mean age is 58.20 (± 11.47) years and ≥58.86 (± 11.47) years for both the patients and healthy controls respectively. Majority of the subjects were Hausa/Fulani constituting 65% and 70% of the patients and the healthy controls respectively. The sex ratio is equal (1.16) but with a female predominance (65%). The mean cancer duration is 2.99years with a range of 0.4 to 11years.

Table 4.2 shows the serum levels of antioxidant minerals and enzymes in both patients and controls. It showed that cancer patients have lower values of Cu, Zn, SOD and GPO compared to the controls and this was found to be statistically significant ($p<0.05$). Serum levels of Se and Cat in cancer patients and controls were similar ($p>0.05$).

Table 4.3 shows the reference interval of the serum antioxidant minerals and enzymes using the controls. To determine the reference interval of the serum antioxidant minerals and enzymes in the study population the mean and 2standard deviation of the healthy controls was utilized.

Table 4.4 shows the prevalence of abnormal serum antioxidant enzymes and minerals in cancer patients with serum levels seen in 82, 92, 17, 54, 0, and 65 percent for Cu, Zn, Se, SOD, Cat, and GPO respectively.

Tables 4.5 and 4.6 show the results of serum antioxidant enzymes and minerals according to age groups in cancer patients and controls respectively. The results in table 4.5 showed that the differences in the values of Se, SOD, Cat, and GPO between cancer patient of different age groups were significant ($p<0.002$, $p<0.001$, $p<0.001$and $p<0.035$ respectively). The results in table 4.6 also showed that the values of Se, SOD, Cat and GPx in the two different age groups of controls were significant ($p<0.001$, $p<0.001$, $p<0.001$and $p<0.001$ respectively). However, the results of Cu and Zn in the two different age groups of cancer patients and controls were not statistically significant ($p>0.907$, $p>0.226$) and ($p>0.255$, $p>0.092$) respectively.

Tables 4.7 show the results of the relationship between cancer duration and serum antioxidant enzymes and minerals in patients. The results in this table show that there a is significant negative correlations between the duration of cancer and each of Zn, Se, and GPO ($r= -0.252$, $p<0.011$; $r = -0.346$, $p<0.001$; $r = -0.830$, $p<0.001$ respectively). This is shown in figures 4.1 to 4.3.

Table 4.1: Biodata and Clinical description of Study Population.

Biodata and Clinical Characteristics	Number of patients% (**n=100**)	Number of controls% (**n=100**)	P-value
AGE(years)			
Mean ± SD	58.20±(11.47)	58.86±(11.47)	0.66
Range	30-90	31-91	
Age groups			
<65	75	71	
≥65	25	29	
SEX			
M:F Ratio	1:16	1:16	
Male	35	35	
Female	65	65	
ETHNICITY			
Hausa/Fulani	65	70	
Others	35	30	
DURATION OF CANCER			
Range (yeas)	0.4-11		
Mean ± SD (years)	2.99 (1.38)		

Table 4.2: Serum levels of Antioxidant Minerals and Enzymes among cancer patients and controls.

ANALYTE	PATIENTS (n=100) Mean ± SD	CONTROLS (n-100) Mean ± SD	p-values
Cu (µmol/l)	4.02 ± 1.57	7.50 ± 1.27	0.001
Zn (µmol/l)	1.38 ± 1.64	4.63 ± 1.35	0.001
Se (µmol/l)	0.14 ± 0.04	0.04 ± 0.02	0.96
SOD (µmol/min/ml)	0.78 ± 0.48	1.09 ± 0.12	0.001
Cat (µmol/min/ml)	12.32 ± 0.76	12.34 ± 0.88	0.809
GPX (µmol/min/ml)	1.50 ± 0.54	2.45 ± 0.37	0.001

*Cu=copper, Zn=zinc, Se=selenium, SOD=superoxide dismutase, Cat=catalase, GPX=glutathione peroxidase.

Table 4.3: Reference Intervals of Antioxidant Minerals and Enzymes in healthy Controls in this Study.

Analyte	Mean	SD	Reference Interval (Mean ± 2SD)
Cu (µmol/l)	7.5	1.27	5.41 - 10.1
Zn (µmol/l)	4.63	1.35	2.46 - 5.91
Se (µmol/l)	0.14	0.02	0.11 - 0.18
SOD (µmol/min/ml)	1.09	0.125	0.9 - 1.36
Cat (µmol/min/ml)	12.34	0.88	11.01 - 14.64
GPX (µmol/min/ml)	2.45	0.37	1.86 - 2.90

*Cu=copper, Zn=zinc, Se=selenium, SOD=supperoxide dismutase, Cat=catalase, GPX=glutathione peroxidase.

Table 4.4: Prevalence of abnormal antioxidant enzymes and minerals in patients.

ANALYTE	Cu	Zn	Se	SOD	Cat	GPX
LOW	82	92	17	54	0	65
NORMAL	8	8	83	46	100	35
TOTAL	100	100	100	100	100	100

*Cu=copper, Zn=zinc, Se=selenium, SOD=supper oxide dismutase, Cat=catalase, GPX=glutathione peroxidase.

Table 4.5: Serum antioxidant enzymes and minerals in cancer patients according to age.

ANALYTE	PT<65 (n=75) Mean +SD	PT≥65 (n=25) Mean + SD	p-value
Cu (µmol/l)	4.01 ± 1.66	7.52 ± 1.35	0.907
Zn (µmol/l)	1.42 ±1.79	4.75 ± 1.45	0.226
Se (µmol/l)	0.15 ±0.05	0.15 ± 0.02	0.002
SOD (µmol/min/ml)	0.77 ± 0.49	1.12 ± 0.12	0.001
Cat (µmol/min/ml)	11.94 ± 0.77	12.56 ± 0.87	0.001
GPX (µmol/min/ml)	2.35 ± 0.39	2.51 ± 0.36	0.035

Cu=copper, Zn=zinc, Se=selenium, SOD=superoxide dismutase, Cat=catalase, GPX=glutathione,PT = patient

Table 4.6: Serum antioxidant enzymes and minerals in controls according to age.

ANALYTE	CT<65 (n=75) Mean +SD	CT≥65 (n=25) Mean + SD	p-value
Cu (µmol/l)	7.60 ± 1.28	7.28 ± 1.24	0.255
Zn (µmol/l)	4.78 ±1.42	4.28 ± 1.11	0.092
Se (µmol/l)	0.15 ± 0.02	0.12 ± 0.01	0.001
SOD (µmol/min/ml)	1.15 ± 0.10	0.96± 0.06	0.001
Cat (µmol/min/ml)	12.53 ± 0.90	11.91 ± 0.67	0.001
GPX (µmol/min/ml)	2.56 ± 0.33	2.20 ± 0.38	0.001

Cu=copper, Zn=zinc, Se=selenium, SOD=superoxide dismutase, Cat=catalase, GPX=glutathione, CT = control

Table 4.7: Correlation analysis between Cancer duration and Analyte concentrations

ANALYYTE	r-value	p-value
Cu (µmol/l)	-0.087	0.390
Zn (µmol/l)	-0.252	0.011
Se (µmol/l)	-0.346	0.001
SOD (µmol/min/ml)	-0.146	0.147
Cat (µmol/min/ml)	0.146	0.147
GPX (µmol/min/ml)	-0.830	0.001

Cu=copper, Zn=zinc, Se=selenium, SOD=superoxide dismutase, Cat=catalase, GPX=glutathione peroxidase, SD= standard deviation,

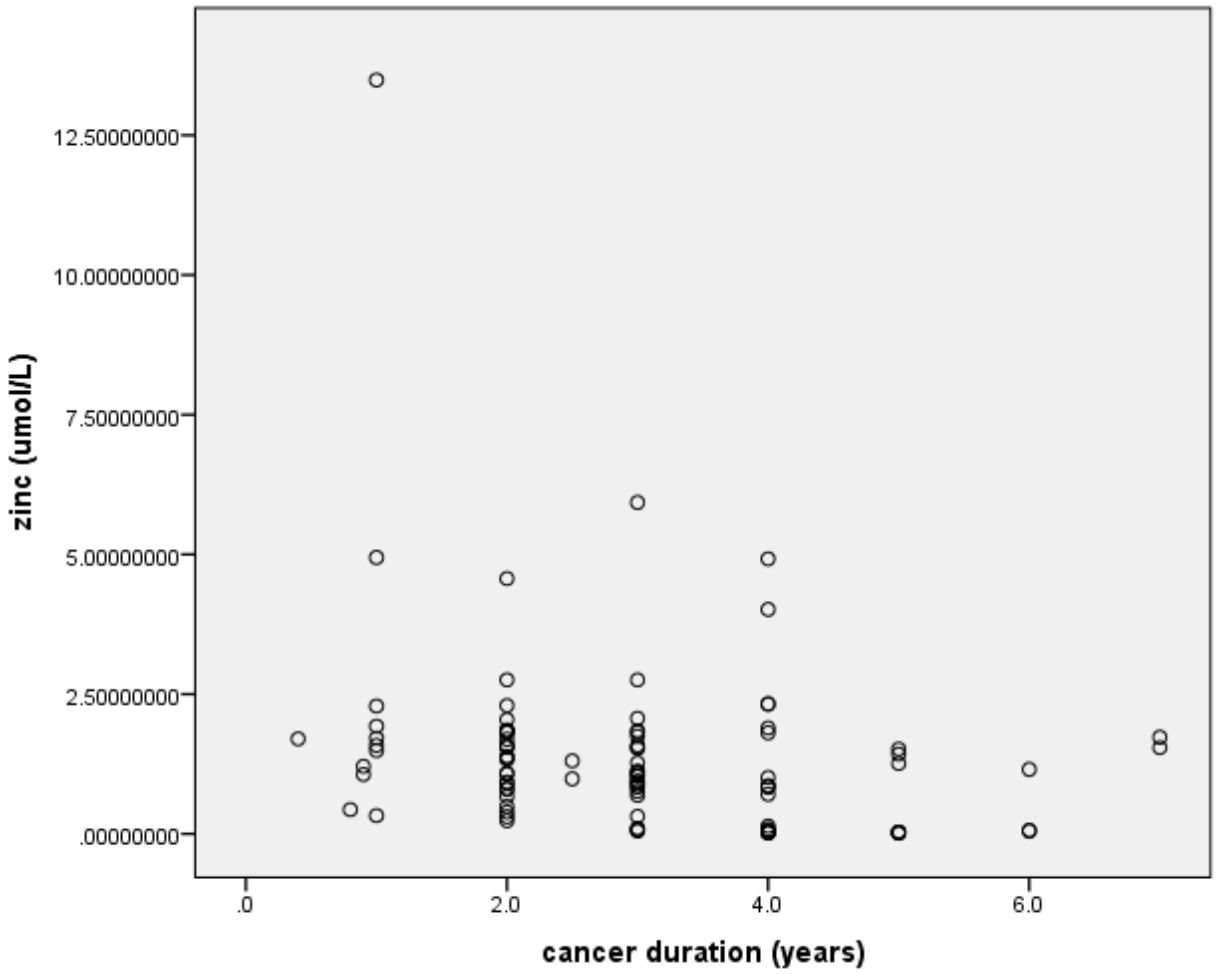


Figure 4.1: Relationship between cancer duration and serum zinc concentration (r = -252, p = 0.011).

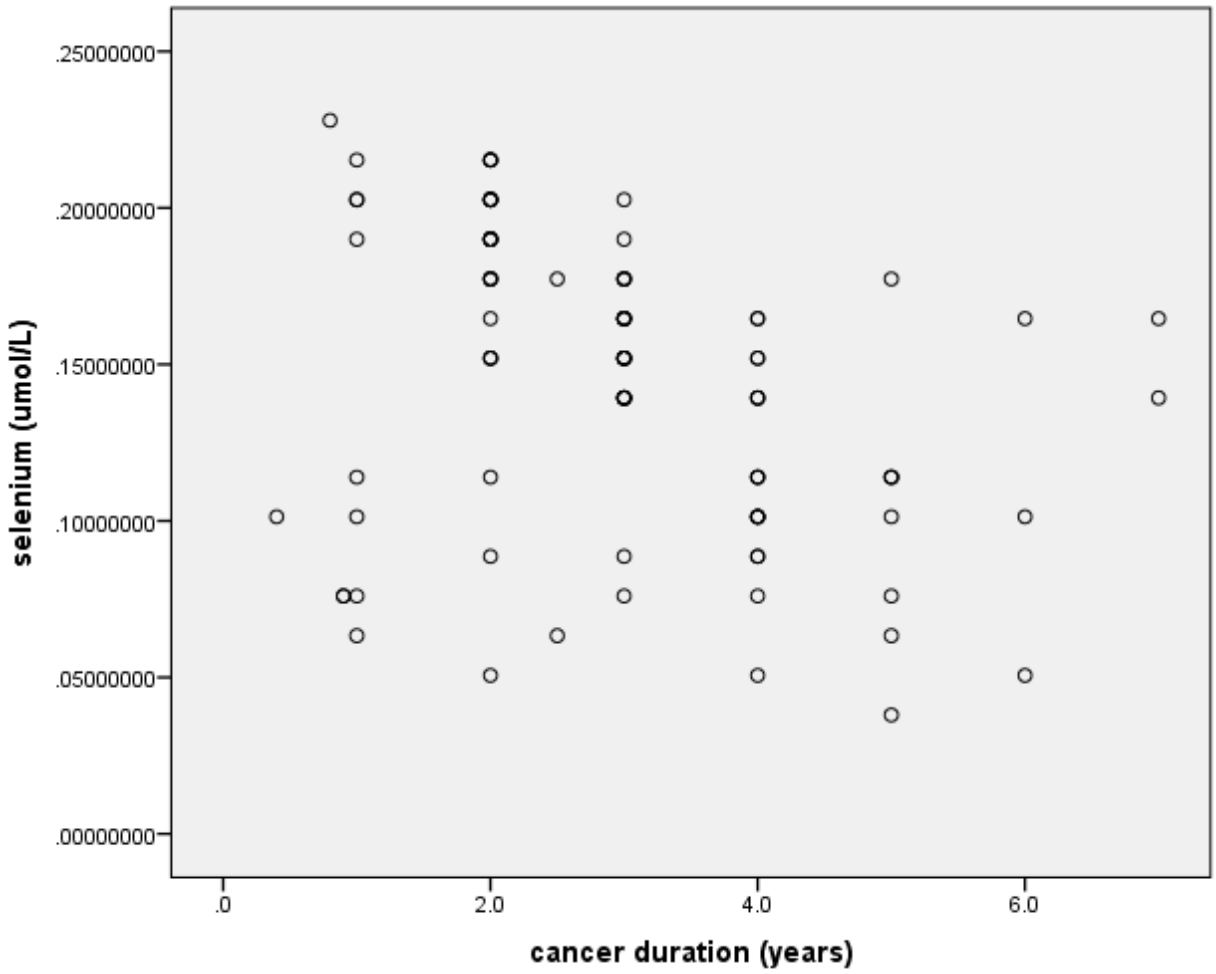


Figure 4.2: Relationship between cancer duration and serum selenium concentration (r = -346, p = 0.001).

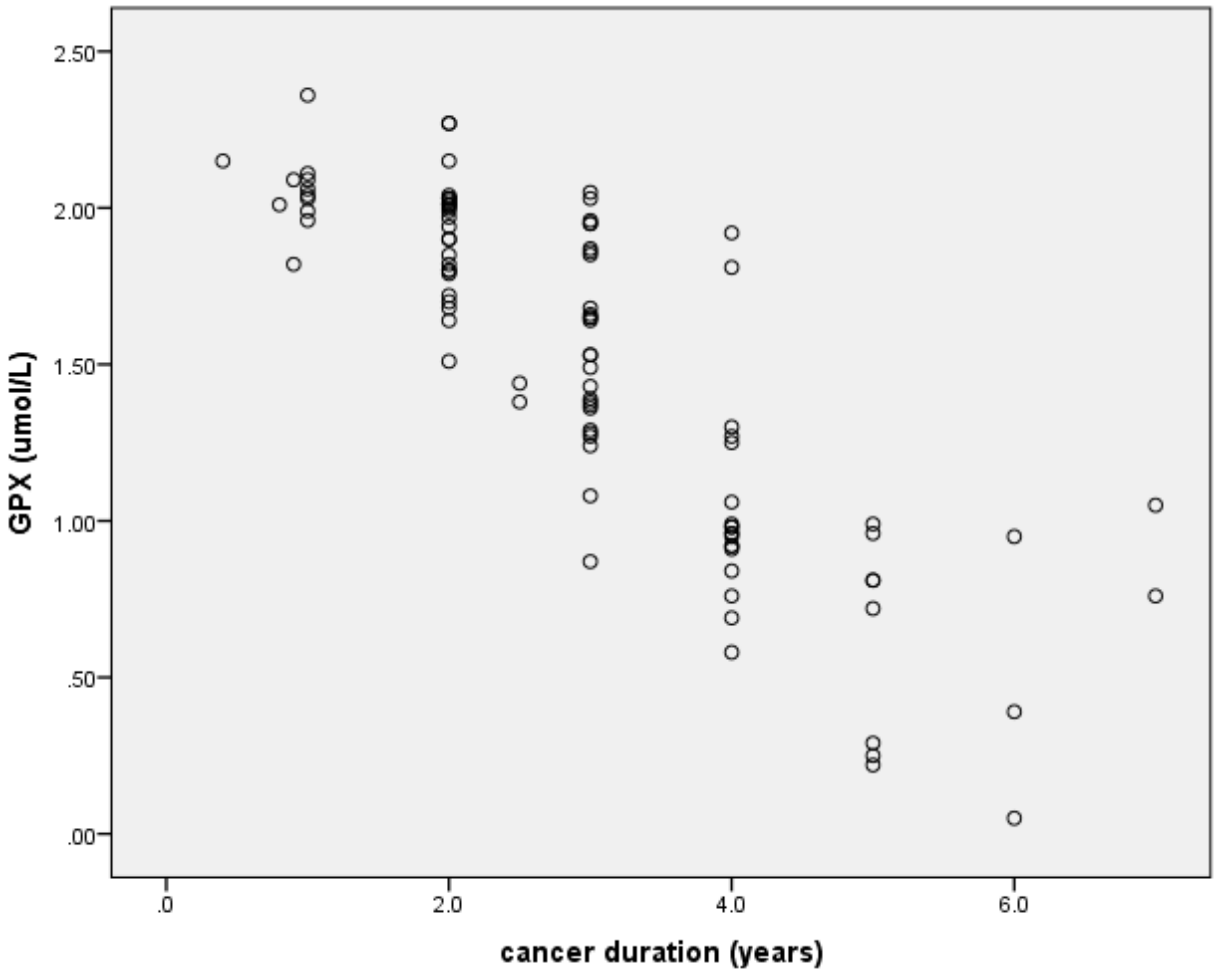


Figure 4.3: Relationship between cancer duration and serum glutathione peroxidase (GPx) concentration (r = 830, p = 0.001).

CHAPTER FIVE

4.0 DISCUSSION

Antioxidants have protective role in cancer by preventing the accumulation of ROS (reactive oxygen species) that may cause lipid peroxidation of tissues, oxidation of DNA bases resulting in DNA crosslinking, uncontrolled expression of genes, increased multiplication of cells and cancer. Numerous studies have shown a depletion of antioxidants and increased amount of ROS in patients with malignancies *(Manoharan, 1994;Mates, 1999)*. This probably results from consumption of antioxidants caused by high levels of ROS and oxidative stress in cancer patients as well as renal loss of water-soluble, small molecular weight antioxidants such as uric acid *(Mates, 1999)*.

In the present study, the serum concentrations of Cu, Zn, SOD and GPx in cancer patients were significantly lower compared to the controls ($p<0.05$).This is in agreement with the studies carried out by other researchers such as *Manoharan (1994) and* Mates (1999). The significant reduction in the serum concentrations of Cu, Zn, SOD, and GPx demonstrates that there was increased amount of ROS which led to the depletion of antioxidant enzymes and minerals in the cancer patients. ROS are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) control their accumulation *(*Mates, 1999). Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radicals and resulting in its accumulation. The accumulation of these free radicals is responsible for oxidation of tissues leading to lipid peroxidation and tissue damage. They are also responsible for oxidation of bases in cellular DNA making them mutagenic,

cytotoxic and crosslinking agents, which in turn causes uncontrolled expression of certain genes causing increased multiplication of cells leading to cancer *(Mates, 1999*). However, the serum concentrations of se and Cat were similar in both the patients and controls. The relative difference between the results of the present study and those in the literature in relation to Se and Cat may be due to variations in the rate at which some of the antioxidants are generated and also the rate they are subsequently utilized.

The present study indicates a significant and negative correlation between the duration of cancer and the serum levels of Zn, Se, and GPx. This demonstrates that as cancer duration increases the serum concentrations of Zn, Se and GPx decreases. This is in agreement with the study by Hereberg (2005) which demonstrates that oxidative stress increases with clinical progression of cancer causing more consumption of antioxidants. It is also in agreement with the studies by *Manoharan, (1994) and* Mates (1999) which showed a depletion of antioxidants and increased in the amount of ROS in patients with malignancies. This probably results from increased consumption of antioxidants caused by high levels of ROS and oxidative stress in cancer patients as well as renal loss of water-soluble, small molecular weight antioxidants such as uric acid *(Mates, 1999).*However, the serum concentrations of SOD and Cat were similar in both the patients and controls. The relative difference between the results of the present study and those in the literature in relation to SOD and Cat may be due to variations in the rate at which some of the antioxidants are generated and also the rate they are subsequently lost or utilized.

The present study also demonstrates that there is a significant difference in the serum concentrations of Se, SOD, Cat, and GPx between control subjects aged greater than 65 years

and those aged less than 65 years, while in cancer patients there was no significant difference in the serum concentrations of these analytes. While some studies (Akila et a*l*., 2007) have reported that antioxidant activities are affected with ageing and there was age related lipid peroxidation and oxidative damage increases with aging process, in the present study no such findings were observed.The relative difference between the results of the present study and those in the literature regarding the antioxidants status in relation to age in cancer patients may be associated with several factors like sample size variations, application of various methods and genetic diversities of each population. In addition, the composition and antioxidant capacity may vary with dietary and nutrient deficiency.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The present study showed that the serum concentrations of Cu, Zn, SOD and GPx in cancer patients were significantly lower compared to the controls which demonstrates that there was increased amount of ROS which led to the depletion of antioxidant enzymes and minerals in the cancer patients. The study also indicates a significant and negative correlation between the duration of cancer and the serum levels of Zn, Se, and GPx indicating that as cancer duration increases the serum concentrations of Zn, Se and GPx decreases. Lastly, the present study also demonstrated no significant difference in the serum concentrations of any analytebetween the two age group of cancer patients.

6.2 CONCLUSION

It could be concluded from the findings of the present study that:

1. There is a decrease in the serum levels of antioxidant minerals(Cu and Zn) and enzymes (SOD and GPx) in cancer patients.
2. There is a decrease in the levels of antioxidants minerals (Zn and Se) and enzymes (GPx) in the serum of cancer patients as the duration of cancer progresses.
3. The decrease in the levels of antioxidants minerals and enzymes in the serum of cancer patients observed in the present study indicates the oxidant nature of the disease and emphasizes the involvement of oxidants and antioxidants in the pathogenesis of cancer.

6.3 RECOMMENDATIONS

It could be recommended from the findings of the present study that:

1. A routine evaluation of antioxidant minerals and enzymes be considered in the management of cancer patients in Nigerian hospitals. The results may be very useful in identifying new treatments or preventive options using antioxidant supplementation or nutritional prescriptions that might elevate antioxidant levels in this group of patients
2. Adjunct antioxidant mineral and enzyme supplements could be utilized as it decreases the oxidative stress and also increases the serum levels of this antioxidants.
3. Further prospective studies with larger sample size are required in this direction.

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APPENDIX I

PATIENT INFORMATION FORM

Serial No- ------ Hospital No-------- Age ------- Phone No---------- Date----------

This study is on "**Serum levels of Antioxidant Minerals and Enzymes among Cancer patients in Zaria**" will be carried out by me, JibrinJibrin an MSc candidate of the Department of Chemical Pathology of ABU, Zaria

It involves collection of blood from cancer patients brought to ABUTH, Zaria to analyzeantioxidant minerals and enzymes and a physical examination including your weight and blood pressure will also be taken.

You are free to participate or decline from the study without any consequences.

Your safety shall be given utmost priority.

It is hoped that the study will help in improving the active management of patients with cancer.

The data from the study will be treated with strict confidentiality.

The test will be at no cost to you and if any abnormality is detected you shall be contacted through the phone numbers provided.

If you desire to participate in this study kindly sign the attached form.

Thank you.

JibrinJibrin

Phone contact: 08034634449 (Email address: jibrinndana@yahoo.com).

APPENDIX II

INFORMED WRITTEN CONSENT

S/ No…… Date……

I----------

of----------(address) agree to participate in this study, measurement of antioxidants in cancer patients. The full procedure before and after the test and the probable benefits were explained to me. I understand that a sample of my blood will be taken for the test and that if I so wish the results will be communicated to me in confidence and at no extra costs to me. I make this consent willingly without being subjected to any pressure.

Participant's Name----------Signature----------

Witness Name----------Signature----------

Researchers Name----------Signature----------

APPENDIX III

PROFORMA FOR THE STUDY OF ANTIOXIDANTS MINERALS AND ENZYMES

Name---------- S/No------ Hospital number---------- Phone No----------

Religion----------Marital Status: Married □ Single □ Divorced □ i. Widowed □

Education Primary □ Secondary □ Tertiary □Quranic □ None □

Age ----------Sex----------Ethnic group----------

Occupation----------Address ----------

Weight (kg)---------- Height(m)----------BMI---------- Blood pressure----------

Diagnosis----------Duration of cancer----------

Drugs. a) cytotxics----------

b) multivitamins/minerals----------

RESULTSa) Catalase ----------

b) Glutathione peroxidase----------

c) Superoxide dismutase----------

d) Selenium ----------

e) Copper ----------

f) Zinc ----------

APPENDIX IV

HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuth@yahoo.com *Website: www.abuth.org*

Chairman of Board: **Chief. Shuaib Oyedokun Afolabi** *Fnil*
Chief Medical Director: **Prof. Lawal Khalid,** *MBBS, FMCS, FWACS, FRCS(ED) mnl*
Chairman, Medical Advisory Committee: **Prof. Abdullahi Mohammed,** *MBBS, FWACP, FICS*
Director of Administration: **Barr. Ishak Bello,** *LL.B, BL., LL.M, PGDM, AHAN, FCAI*

Our Ref: ABUTH/HREC/TRG /36

Your Ref: _____ *Date:* 6th August, 2014

ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

Serum levels of antioxidant minerals and enzymes among cancer patient in Zaria.

ABUTH Ethics Committee assigned number:	-	**ABUTH/HREC/K50/2014**
Name of the principal Investigator:	-	**Dr. Jibrin Jibrin,**
Address of the Principal Investigator:	-	**Department of Chemical Pathology, ABUTH-Zaria**
Date of receipt of valid application:	-	**7th April, 2014**
Date of meeting when final determination on ethical approval was made:	-	**20th & 21st May, 2014**

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and ***given full approval by the Health Research Ethics Committee.***

Please note: this approval dates from **7th August, 2014– 7th August, 2015.**

No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: http://www.nhrec.net.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.

Prof. A. I. Mamman
Chairman, ABUTH HREC