### ANALYSIS OF PETROLEUM POLLUTION ON SERUM TRACE ELEMENT LEVEL, OXIDATIVE STATUS, AND BIOCHEMICAL INDICES IN THE RESIDENTS OF AN OIL HOST COMMUNITY (UGBEGUNGUN) IN DELTA STATE, NIGERIA

**By**

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**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc)**

**IN PHYSIOLOGY.**

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### CERTIFICATION

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### DEDICATION

I dedicate this work to Almighty God for his faithfulness, grace, favour, and love in my life and to my lovely parents; Mr. and Mrs. Emmanuel Kpotor, and to my siblings and friends for their love, prayer, and support to me.

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### ABSTRACT

Activities of crude oil refining disrupt natural ecosystems which expose residents to environmental pollution, and consequently pose threat to their health and wellness. This study was aimed to analyze serum trace elements levels, oxidative status, and biochemical indices in the residents of an oil host community (Ugbegungun) in Delta State, Nigeria. A total of 100 subjects were recruited for this study which comprised 50 individuals from Ugbegungun and 50 individuals from Okada, a non-oil host community. Serum levels of lead (Pb), zinc (Zn), and cadmium (Cd) were evaluated using Atomic Absorption Spectrophotometer. Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP) activities, and urea and creatinine were all evaluated with a commercially available enzymatic kit. The oxidative and antioxidative activities in the serum were estimated using the semi-automated spectrophotometric procedure. Enzyme-linked immunoassay was used to assess tumor necrotic factor-alpha (TNF-α) and 8-hydroxy-2-deoxyguanosine (8-OHdG). The results showed that serum levels of Pb, Cd, urea, creatinine, ALT, AST, ALP, malondialdehyde, TNF-α, and 8- OHdG were significantly higher (p<0.05) in female and male residents of Ugbegungun when compared with the female and male residents of the non-oil host community. The activities of superoxide dismutase, catalase, glutathione reductase, and total antioxidant capacity were significantly lower (p<0.05) in both female and male residents of Ugbegungun when compared with the residents of non-oil host community. In conclusion, the residents of an oil host community in Nigeria may be at high risk of kidney and liver toxicity through oxidative stress induction associated probably with heavy metal accumulation in the system.

### CHAPTER ONE

### INTRODUCTION

#### Background of the study

Environmental degradation is a major challenge in the oil host communities and this may affect the health of the residents in the area (Pfeiffer *et al.,* 2010). Petroleum-derived environmental pollution contaminants constitute one of the most prevalent sources of environmental degradation (Kautz and Topp, 2000). The hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms, including humans in large concentrations (Trench *et al.,* 2001). Petroleum also contains trace amounts of sulfur and nitrogen compounds, which are dangerous by themselves and produce secondary poisonous chemicals (Trench *et al.,* 2001). Petroleum can permanently contaminate large areas of soil, underground water, and sea water, making them economically useless as well as dangerous to human health (Trench *et al.,* 2001; Chiara *et al.,* 2009).

Refining petroleum creates air pollution which releases toxins into the atmosphere that are dangerous for human and ecosystem health. Effects of petroleum pollution on human health may vary depending on the concentration and duration of exposure (ATSDR*,* 2000). Inhaling petroleum vapors causes nervous system effects, liver tumors, and respiratory irritation, very high exposure may cause coma and death (Kponee *et al.,* 2015; Walsh *et al.,* 2016). A study carried out in the Niger Delta region of Nigeria, reported a very high frequency of neurological, haematological, and irritation symptoms in residents from an oil host community (Kponee *et al.,* 2015). Some studies reported fatigue, headaches, respiratory and eye irritation, and a high risk of spontaneous abortion among cleanup workers of Oil spillage (Sabastian *et al.,* 2001; 2002).

Trace elements such as nickel, lead, cadmium, and vanadium are also present in petroleum (Wang *et al.,* 2005; Li *et al.,* 2008; Zhao *et al.,* 2010; Wang *et al.,* 2012). Humans are exposed to these trace elements through ingestion, inhalation, food, and dermal contact (Cook et al., 2005). The adverse effects of trace elements on humans have long been documented by Jarup *et al.*, 2003; Bernard *et al.,* 2008; Kim *et al.,* 2015; Rahimzadeh *et al.,* 2017. An epidemiological research study reported an association between petroleum pollution and the number of cancers, cardiovascular and respiratory diseases (Pope *et al*., 2002). In another study carried out in the

Peruvian Amazon, a significantly high level of serum lead level among indigenous children and adults living close to an oil extraction site was established (Anticona *et al.,* 2011; 2012).

The environmental degradation in the oil host community is a major concern in Nigeria, which affects the everyday life of the residents. Thus, this study will bridge a gap and provide background information for further study, and provide a relationship between trace elements and alteration in the physiological process. This study will also offer possible roles of trace elements in related diseases which might lead to proper management, prevention, and effective policies control in the oil host communities in Nigeria.

#### Aim

This study was aimed to analyze serum trace elements levels, oxidative status, and biochemical indices in the residents of an oil host community (Ugbegungun) in Delta State, Nigeria.

#### Objectives of the study

The specific objectives of this study are:

* + 1. To estimate the serum trace elements level of an oil host community residents in Delta State, Nigeria.
    2. To evaluate the serum biochemical indices of an oil host community residents in Delta State, Nigeria.
    3. To determine the serum oxidative markers of an oil host community residents in Delta State, Nigeria.

### CHAPTER TWO

### LITERATURE REVIEW

#### Crude oil

Crude oil is made up of a vast number of hydrocarbon compounds that consist mainly of carbon and hydrogen in differing proportions. In addition, small amounts of organic compounds

containing sulfur, oxygen, nitrogen, and metals such as vanadium, nickel, cadmium, iron, and copper are also present (Bhatia *et al.,*2002).

Crude oil refineries activities can lead to disruption of sensitive environments and also threaten

the survival of indigenous people that live in these ecosystems (Rourke’O and Connolly, 2003). Its exploitation, drilling, and extraction from its factories may lead to a range of acute and

chronic health impacts (Paul and Selber, 2002; Chintan, and Mandalia, 2012), such as anaemia, delayed puberty in girls (Schoeters *et al.,* 2008), (Patrick, 2006) Fatigue, insomnia, headaches (Pearce, 2007), airway irritation (such as Coughing, wheezing and sore throat) (Rondini *et al.,*2010), gastrointestinal irritation (such as diarrhea, cramps, and nausea) (Goldfine *et al.,* 2000)., increased blood levels (Ścibior, 2005) and alveolar/bronchiolar carcinoma (National Toxicology Program, 2002) (International Agency for Research on Cancer, 2006)

The activities carried out both on-and off-shore (Oil companies) can have a detrimental influence on human health, ecosystems, and local life (Paul and Selber, 2002). The contaminations during these operations include the use of considerable quantities of water, which becomes contaminated with drilling muds (Rourke’O and Connolly, 2003) and cuttings (drilling wastes and associated wastes) before it is discharged into the environment (Chintan Pathak and Mandalia 2012).

### TRACE METALS

#### Cadmium

Cadmium (Cd) is a heavy metal that occurs as a natural constituent in the earth's crust (Sharma *et al.,* 2015). It is vastly used in batteries, coating, plating, alloys, and in various industries. Individuals are easily exposed to cadmium by inhalation and ingestion. It enters in air and binds to small particles where it can combine with water or soil causing contamination of fish, plants, and animals in nanoform. Site of a hazardous waste spill and improper waste disposal can cause

cadmium leakages in nearby habitats or environment (European Food Safety Authority, 2009). Cadmium is considered one of the hazardous metals to human health (Martin *et al.,* 2009). The organic matter in the soil absorbs cadmium increasing the risk of survival of various plants and also increasing the uptake of this toxic metal in food (Valko *et al.,* 2005).

Cadmium is a widely distributed toxic heavy metal that has been associated with many diseases including chronic renal dysfunction, osteomalacia, acute heart failure, secondary hypertension, atherosclerosis (Valko *et al.,* 2005; Lin *et al.,* 2021), infertility (Benoff *et al.,* 2004), damage to the central nervous system and immune system and sometimes death (Bell *et al.,* 2009). Also, Cd causes severe bone metabolism diseases such as osteoporosis, osteoarthritis, and osteomalacia

(Yonggang *et al.,* 2021). It has been reported that Cd induced osteoblast injury and oxidative

stress, which causes DNA damage, mitochondrial dysfunction, and endoplasmic reticulum stress, resulting in apoptosis (Yonggang *et al.,* 2021).

Although some studies have suggested that Cd may affect multiple systems by inducing lipid peroxidation in cells and disturbing the antioxidant system, the mechanism by which cadmium affects the cardiovascular system remains unclear (Houston *et al.,* 2007; López-Suárez *et al.,* 2013). A study carried out proved Cd to be the most mobile metal in oil-contaminated surface soil (Svetlana *et al.,* 2004).

Cadmium acts as a mitogen and promotes cancer in several tissues. It also stimulates cell proliferation, inhibits DNA repair, and apoptosis (Waalkes *et al.,* 2003). On the one hand, it induces necrosis which leads to tissue damage in the kidney (Waisberg *et al.,* 2003; Templeton *et al.,* 2010). It has been reported that Cd-induced elevation in lipid peroxidation was not only due to the inhibition of the activity of the superoxide dismutase (SOD) but also due to the direct action of Cd2+ on the peroxidation reaction (Tellez-Plaza *et al.,* 2013). Another study showed the role of cadmium in the induction of atherosclerosis in rabbits. It led to a total increase of lipids, cholesterol, free fatty acids and phospholipids, Triglyceride in the heart and kidney, and a decrease in serum and liver.

According to International Agency for Research on Cancer (IARC) (Luevano *et al.,*2014). Cadmium acute exposures may lead to inflammation followed by cough, dryness, and irritation of the nose and throat, headache, dizziness, chest pain, pneumonitis, and pulmonary edema (Roy *et al.,* 2013).

#### Lead

Lead (Pb) is the most important toxic heavy element in the environment (Wani *et al.,* 2015). Human exposure to lead and its compounds occur mostly in lead-related occupations with various sources like leaded gasoline, industrial processes such as smelting of lead and its combustion, pottery, boat building, lead-based painting, lead-containing pipes, battery recycling, grids, the arms industry, pigments, the printing of books (Rubin and Strayer, 2008). Lead is a highly poisonous metal affecting almost every organ in the body. Of all the organs, the nervous system is the most affected target in lead toxicity, both in children and adults. The toxicity in children is however of a greater impact than in adults. This is because their tissues, internal as well as external, are softer than in adults. Long-term exposure of adults can result in decreased performance in some tests of cognitive performance that measure functions of the nervous system. Infants and young children are especially sensitive to even low levels of lead, which may contribute to behavioural problems, learning deficits, and lowered intelligence quotient (Rubin and Strayer, 2008). Long-time exposure to Pb has been reported to cause anaemia, along with an increase in blood pressure, and that mainly in old and middle-aged people. Severe damage to the brain and kidneys, both in adults and children was found to be linked to exposure to heavy lead levels resulting in death.

Lead is toxic to multiple organ systems; even at low levels previously considered safe, it has been shown through a series of prospective epidemiological studies to produce adverse effects (Bruce *et al.,* 2005). Some of these are clinically evident, while others are discerned only through special testing and are thus defined as subclinical. The nervous system of the fetus and infant is especially susceptible to lead, which can cross the placenta and penetrate the blood-brain barrier. Lead interferes with neuronal migration, cell proliferation, and synapse formation during critical periods of early vulnerability. The consequences are loss of intelligence and disruption of behaviour. Because the brain has little capacity for repair, these effects are permanent and untreatable. The most recent research indicates that lead can damage the infant brain even at blood levels as low as 5 m/dl (Lanphear *et al.,* 2000).

Occupational exposure is a major source of lead poisoning in adults. According to estimates made by the National Institute of Occupational Safety and Health (NIOSH), more than 3 million

workers in the United States are potentially exposed to lead in the workplace (Needleman *et al.,*

2004).

#### Detection of lead poisoning

There are several methods known/used to detect elevated blood lead levels. The presence of changes in blood cells visible under the microscope or deletion of dense lines in the bones of children seen on X-ray are signs used for detecting lead poisoning. However, the main tool to detect elevated levels of body lead is to measure the level of lead in blood samples. This test gives however only an account of lead present in circulating blood but cannot show how much lead is stored in the body. As of 2012, the Centers for Disease Control and Prevention (USA) have set the standard elevated blood lead level for adults to be 10 μg/dL and for children 5 μg/dL of the whole blood (CDC, 2012). Previously, the standard lead level for children was 10 μg/dL. The appearance of clinical manifestations varies from individual to individual depending on other environmental factors.

#### Serum Trace Metals

The human body is composed of elements that can be roughly divided into abundant elements and trace elements. Abundant elements consist of the major elements that are involved in the formation of covalent bonds and are important constituents of tissues (oxygen, carbon, hydrogen, nitrogen, etc.), and semi-major elements, which often exist in the ionic state, and are involved in functions of the living body through maintenance of osmotic pressure and membrane potentials (potassium, sodium, etc.). Major elements account for 96% of the total body weight, and the semi-major elements account for 3 to 4% of the total body weight (Wada, 2001). The deficiency of major elements can lead to nutritional disorders, and their presence in excess can cause obesity. Deficiencies or excess states of semi-major elements often result in water and electrolyte abnormalities. Essential trace elements of the human body include zinc (Zn), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), and molybdenum (Mo) (Wada, 2001). Although these elements account for only 0.02% of the total body weight, they play significant roles, as active centers of enzymes or as trace bioactive substances. A major

outcome of trace element deficiencies is reduced activity of the concerned enzymes. However, since each trace element is related to so many enzymes, deficiency of a single trace element is often not associated with any specific clinical manifestations, but rather manifests as a combination of various symptoms (Wada, 2001). Deficiency and excess trace elements are either congenital or acquired. The deficiency state of trace elements is most frequently seen during high-calorie parenteral therapy or enteral nutrition. Zinc deficiency can develop within 2 weeks after the start of such therapies (Takagi *et al.,* 2001). Congenital abnormalities of trace element metabolism are rare. Abnormal intestinal absorption or disturbed transport of absorbed trace elements more often lead to the deficiency of trace elements such as “Acrodermatitis enteropathica” due to disturbed zinc absorption and Menkes disease due to abnormal copper transport through the intestinal mucosa (Okabe *et al.,* 2001), The site of uptake of trace elements into an active form is disturbed, the trace element becomes excess at that site. In cases of Wilson disease characterized by disturbed uptake of copper into ceruloplasmin (Okabe *et al.,* 2001), tissue damage and fibrosis due to copper occur in the liver and other sites. Although trace elements are the essential components of biological structures, they may show a toxic effect when they are more concentrated than the amount that is required for biological functions. In addition, the toxicity can be spread to other non-essential elements of very similar atomic characteristics which can mimic the reactivity of a trace element (Gecit *et al.,* 2011; Sayır *et al.,* 2011).

An epidemiological survey and animal study have shown that health problems can be caused by a deficiency of trace elements (International life science, Tokyo). It is shown that the deficiency of many trace elements, especially zinc, is associated with accelerated aging (International life science, Tokyo), immunodeficiency, accelerated progression of HIV infection (Patrick *et al.,* 2000) increased incidence of abnormal pregnancies (Shah *et al.,* 2001) developmental retardation in children (Bhandari *et al.,* 2001) and taste disorder (Tomita *et al.,* 2002). It has also been shown that deficiency of chromium is related to the development of diabetes mellitus and atherosclerosis and that selenium deficiency (Schrauzer *et al.,* 2000) is associated with the increase of cancer and ischemic heart disease.

A study carried out on renal cell carcinoma, shown that the level of lead (Pb) and cadmium (Cd) were higher than Zinc, Iron, and Manganese in the patient group (Pirincci *et al.,* 2013). Another

study carried out showed the increase in the level of serum lead and cadmium in workers exposed and an increase in the level of oxidative stress (Glutathione peroxidase) than in the control (not exposed) (Wojciech *et al.,* 2001).

Exposure to heavy metals is considered the main threat to human health and the biological

system. These metals have been widely studied and their effects on human health are regularly analyzed by international bodies (Tseten *et al.,* 2014). Cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), vanadium (V) and nickel (Ni) are among the metals that have potential adverse effects on human health (Kim *et al.,* 2013), and chronic exposures to them are nearly unavoidable in daily life, such as from airborne particles, soil, water, and subsequently food

(Callam *et al.,* 2013). The general population is primarily exposed to heavy metals through food;

fish being a major source of methylmercury exposure (Jarup *et al.,* 2003). These heavy metals as environmental pollutants have been recognized to have a role in the induction of malignant human growths. Recently, certain heavy metals such as Cd, Hg, Pb, chromium (Cr), and arsenic (As) showed a close association to breast cancer (Sutton *et al.,* 2012). An epidemiological study demonstrated that exposure to metals has toxic and carcinogenic effects on humans and animals (Chen *et al.,* 2008). Heavy metals are confirmed as human carcinogens; lead, cobalt, and iron are observed as potential carcinogens. Prostate cancer mortality was found to be strongly contributed to cadmium (Cd), followed by zinc (Zn) and chromium (Cr) (Downing *et al.,* 2007). Excess occupational and environmental exposure to metals is considered to be a major cause of metal- related cancer and is also associated with increased cancer risk (Wingren *et al.,* 2017).

#### Liver

Terminology related to the liver often starts in hepat- from ἡπατο-, from the Greek word for liver. The liver is an organ of the digestive system only found in vertebrates which detoxifies various metabolites, synthesizes proteins, and produces biochemicals necessary for digestion and growth (Abdel-Misih *et al.* 2010) (Canadian Cancer Society, 2015). In humans, it is located in the right upper quadrant of the abdomen, below the diaphragm. Its other roles in metabolism include the regulation of glycogen storage, decomposition of red blood cells, and the production of hormones.

The liver is an accessory digestive organ that produces bile, an alkaline fluid containing cholesterol and bile acids, which helps the breakdown of fat. The gallbladder, a small pouch that sits just under the liver, stores bile produced by the liver which is afterward moved to the small intestine to complete digestion (Tortora *et al.,* 2008). The liver's highly specialized tissue, consisting of mostly hepatocytes, regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Zakim *et al.,* 2002).

#### Structure

The liver is a reddish-brown, wedge-shaped organ with two lobes of unequal size and shape. A human liver normally weighs approximately 1.5 kg (3.3 lb) (Cotran *et al.,* 2005) and has a width of about 15 cm (6 in). There is considerable size variation between individuals, with the standard reference range for men being 970–1,860 g (2.14–4.10 lb) (Molina *et al.,* 2012) and for women 600–1,770 g (1.32–3.90 lb) (Molina *et al.,* 2015).

It is both the heaviest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlies the gallbladder (Tortora *et al.,* 2008). The liver is connected to two large blood vessels: the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta via the celiac trunk, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to lobules.

Lobules are the functional units of the liver. Each lobule is made up of millions of hepatic cells (hepatocytes), which are the basic metabolic cells. The lobules are held together by a fine, dense, irregular, fibroelastic connective tissue layer extending from the fibrous capsule covering the entire liver known as Glisson's capsule (Canadian Cancer Society, 2015). This extends into the structure of the liver by accompanying the blood vessels, ducts, and nerves at the hepatic hilum. The whole surface of the liver, except for the bare area, is covered in a serous coat derived from the peritoneum, and this firmly adheres to the inner Glisson's capsule.

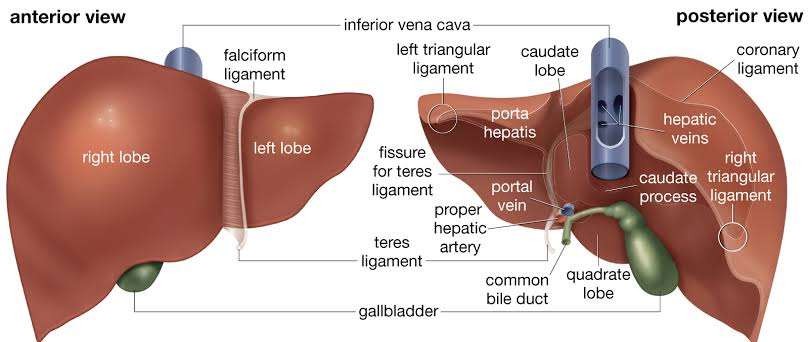


Figure 2.1: Structure of the liver (Encyclopedia Britannica)

### GROSS ANATOMY

#### Lobes

The liver is grossly divided into two parts when viewed from above – a right and a left lobe - and four parts when viewed from below (left, right, caudate, and quadrate lobes) (Liver, 2015).

The falciform ligament makes a superficial division of the liver into the left and right lobes. From below, the two additional lobes are located between the right and left lobes, one in front of the other. A line can be imagined running from the left of the vena cava and all the way forward to divide the liver and gallbladder into two halves (Renz *et al.,* 2014). This line is called Cantlie's line.

Other anatomical landmarks include the ligamentum venosum and the round ligament of the liver, which further divide the left side of the liver in two sections. An important anatomical landmark, the porta hepatis, divides this left portion into four segments, which can be numbered starting at the caudate lobe as I in an anticlockwise manner. From this parietal view, seven segments can be seen, because the eighth segment is only visible in the visceral view.

#### Surfaces

On the diaphragmatic surface, apart from a triangular bare area where it connects to the diaphragm, the liver is covered by a thin, double-layered membrane, the peritoneum, that helps to reduce friction against other organs (Singh, 2008). This surface covers the convex shape of the two lobes where it accommodates the shape of the diaphragm. The peritoneum folds back on itself to form the falciform ligament and the right and left triangular ligaments (McMinn, 2003).

These peritoneal ligaments are not related to the anatomic ligaments in joints, and the right and left triangular ligaments have no known functional importance, though they serve as surface landmarks (McMinn, 2003). The falciform ligament functions to attach the liver to the posterior portion of the anterior body wall.

The visceral surface or inferior surface is uneven and concave. It is covered in the peritoneum apart from where it attaches the gallbladder and the porta hepatis (Singh, 2008). The fossa of gall bladder lies to the right of the quadrate lobe, occupied by the gallbladder with its cystic duct close to the right end of the porta hepatis.

#### Impressions of the Liver

Several impressions on the surface of the liver accommodate the various adjacent structures and organs. Underneath the right lobe and to the right of the gallbladder fossa are two impressions, one behind the other and separated by a ridge. The one in front is a shallow colic impression, formed by the hepatic flexure, and the one behind is a deeper renal impression accommodating part of the right kidney and part of the suprarenal gland (Skandalakis *et al.,* 2009).

The suprarenal impression is a small, triangular, depressed area on the liver. It is located close to the right of the fossa, between the bare area and the caudate lobe, and immediately above the renal impression. The greater part of the suprarenal impression is devoid of peritoneum and it lodges the right suprarenal gland (Dorland's, 2012).

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Medial to the renal impression is a third and slightly marked impression, lying between it and the neck of the gall bladder. This is caused by the descending portion of the duodenum and is known as the duodenal impression (Dorland's, 2012).

The inferior surface of the left lobe of the liver presents behind and to the left of the gastric impression (Dorland's, 2012). This is moulded over the upper front surface of the stomach, and to the right of this is a rounded eminence, the tuber omentale, which fits into the concavity of the lesser curvature of the stomach and lies in front of the anterior layer of the lesser omentum.

#### Functions of the Livers

#### Carbohydrate Metabolism

The liver synthesizes and stores around 100g of glycogen via glycogenesis, the formation of glycogen from glucose. When needed, the liver releases glucose into the blood by performing glycogenolysis, the breakdown of glycogen into glucose (Human Anatomy and Physiology, 2012). The liver is also responsible for gluconeogenesis, which is the synthesis of glucose from certain amino acids, lactate, or glycerol. Adipose and liver cells produce glycerol by the breakdown of fat, which the liver uses for gluconeogenesis (Human Anatomy and Physiology, 2012). The liver also does glyconeogenesis which is the synthesis of glycogen from lactic acid.

#### Protein Metabolism

The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation. All plasma proteins except Gamma-globulins are synthesised in the liver. It is also responsible for a large part of amino acid synthesis. The liver plays a role in the production of clotting factors, as well as red blood cell production. Some of the proteins synthesized by the liver include coagulation factors I (fibrinogen), II (prothrombin), V, VII, VIII, IX, X, XI, XII, XIII, as well as protein C, protein S, and antithrombin. The liver is a major site of production for

thrombopoietin, a glycoprotein hormone that regulates the production of platelets by the bone marrow (Jelkmann, 2001).

#### Lipid Metabolism

The liver plays several roles in lipid metabolism: it performs cholesterol synthesis, lipogenesis, and the production of triglycerides, and a bulk of the body's lipoproteins are synthesized in the liver. The liver plays a key role in digestion, as it produces and excretes bile (a yellowish liquid) required for emulsifying fats and helps the absorption of vitamin K from the diet. Some of the bile drains directly into the duodenum, and some are stored in the gallbladder. The liver produces insulin-like growth factor 1, a polypeptide protein hormone that plays an important role in childhood growth and continues to have anabolic effects in adults.

#### Metabolism and Detoxification

The liver is responsible for the breakdown of insulin and other hormones. The liver breaks down bilirubin via glucuronidation, facilitating its excretion into bile. The liver is responsible for the breakdown and excretion of many waste products. It plays a key role in breaking down or modifying toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor. Preferably, the toxins are conjugated to avail excretion in bile or urine. The liver converts ammonia into urea as part of the ornithine cycle or the urea cycle, and the urea is excreted in the urine (Human Anatomy and Physiology, 2012).

#### Blood Reservoir

Because the liver is an expandable organ, large quantities of blood can be stored in its blood vessels. Its normal blood volume, including both that in the hepatic veins and that in the hepatic sinuses, is about 450 milliliters, or almost 10 percent of the body's total blood volume. When high pressure in the right atrium causes backpressure in the liver, the liver expands, and 0.5 to 1 liter of extra blood is occasionally stored in the hepatic veins and sinuses. This occurs especially

in cardiac failure with peripheral congestion. Thus, in effect, the liver is a large, expandable, venous organ capable of acting as a valuable blood reservoir in times of excess blood volume and capable of supplying extra blood in times of diminished blood volume (Liver, 2021).

#### Lymph production

Because the pores in the hepatic sinusoids are very permeable and allow ready passage of both fluid and proteins into the perisinusoidal space, the lymph draining from the liver usually has a protein concentration of about 6 g/dl, which is only slightly less than the protein concentration of plasma. Also, the high permeability of the liver sinusoid epithelium allows large quantities of lymph to form. Therefore, about half of all the lymph formed in the body under resting conditions arises in the liver.

#### Others

* + - * 1. The liver stores a multitude of substances, including vitamin A (1–2 years' supply), vitamin D (1–4 months' supply) (Liver, 2021), vitamin B12 (3–5 years' supply) (Vitamins, 2016), vitamin K, vitamin E, iron, copper, zinc, cobalt, molybdenum, etc.
        2. Haemopoiesis - The formation of blood cells is called haemopoiesis. In embryonic stage RBC and WBC are formed by liver. In the first trimester fetus, the liver is the main site of red blood cell production.
        3. Liver helps in purification of blood. The Kupffer cells of liver are phagocytic cells, helps in phagocytosis of dead blood cells and bacteria from the blood (Nguyen-Lefebvre *et al.,* 2015).
        4. The liver is responsible for immunological effects – the mononuclear phagocyte system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.
        5. The liver produces albumin, the most abundant protein in blood serum. It is essential in the maintenance of oncotic pressure and acts as a transport for fatty acids and steroid hormones.
        6. The liver synthesizes angiotensinogen, a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure.
        7. The liver produces the enzyme catalase to break down hydrogen peroxide, a toxic oxidising agent, into water and oxygen (Nguyen-Lefebvre *et al.,* 2015).

#### Liver function test

Liver function tests (LFTs or LFs), also referred to as a hepatic panel, are groups of blood tests that provide information about the state of a patient's liver (Nosek, 2016). These tests include prothrombin time (PT/INR), activated Partial Thromboplastin Time (aPTT), albumin, bilirubin (direct and indirect), and others. The liver transaminases aspartate transaminase (AST or SGOT) and alanine transaminase (ALT or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function (Abdel-Misih *et al.* 2010) (Canadian Cancer Society, 2015). Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed on a patient's blood sample. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Because some of these tests do not measure function, it is more accurate to call these liver chemistries or liver tests rather than liver function tests (Tortora *et al.,* 2008). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and monitor the response to treatment.

#### Total bilirubin

Measurement of total bilirubin includes both unconjugated (indirect) and conjugated (direct) bilirubin. Unconjugated bilirubin is a breakdown product of heme (a part of hemoglobin in red blood cells). The liver is responsible for clearing the blood of unconjugated bilirubin, by 'conjugating' it (modified to make it water-soluble) through an enzyme named UDP-glucuronyl- transferase. When the total bilirubin level exceeds 17 μmol/l, it indicates liver disease. When total bilirubin levels exceed 40 μmol/l, bilirubin deposition at the sclera, skin, and mucous membranes will give these areas a yellow colour, thus it is called jaundice (Zakim *et al.,* 2002).

The increase in predominantly unconjugated bilirubin is due to overproduction, reduced hepatic uptake of the unconjugated bilirubin, and reduced conjugation of bilirubin. Overproduction can be due to the reabsorption of a haematoma and ineffective erythropoiesis leading to increased red blood cell destruction. Gilbert's syndrome and Crigler–Najjar syndrome have defects in the UDP-glucuronyl-transferase enzyme, affecting bilirubin conjugation (Zakim *et al.,* 2002).

The degree of rising in conjugated bilirubin is directly proportional to the degree of hepatocyte injury. Viral hepatitis can also cause the rise in conjugated bilirubin. In parenchymal liver disease and incomplete extrahepatic obstruction, the rise in conjugated bilirubin is less than the complete common bile duct obstruction due to malignant causes. In Dubin–Johnson syndrome, a mutation in multiple drug-resistance proteins 2 (MRP2) causes a rise in conjugated bilirubin (Zakim *et al.,* 2002). In acute appendicitis, total bilirubin can rise from 20.52 μmol/l to 143 μmol/l.

In pregnant women, the total bilirubin level is low in all three trimesters (Zakim *et al.,* 2002). The measurement of bilirubin levels in the newborns is done through the use of bilimeter or transcutanoeus bilirubinometer instead of performing LFTs. When the total serum bilirubin increases over 95th percentile for age during the first week of life for high risk babies, it is known as hyperbilirubinemia of the newborn (neonatal jaundice) and requires light therapy to reduce the amount of bilirubin in the blood. Pathological jaundice in newborns should be suspected when the serum bilirubin level rises by more than 5 mg/dl per day, serum bilirubin more than the physiological range, clinical jaundice more than 2 weeks, and conjugated bilirubin (dark urine staining clothes). Haemolytic jaundice is the commonest cause of pathological jaundice. Those babies with Rh hemolytic disease, ABO incompatibility with the mother,

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, and minor blood group incompatibility are at increased risk of getting haemolytic jaundice.

#### Alanine aminotransferase

Alanine aminotransferase (ALT) is found in high concentrations in the liver, kidneys, heart, and muscles. This catalyzes the transamination reaction and only exists in a cytoplasmic form. Any kind of liver injury can cause a rise in ALT. A rise of up to 300 IU/l is not specific to the liver but can be due to the damage of other organs such as the kidneys or muscles. When ALT rises to more than 500 iu/l, the causes are usually from the liver. It can be due to hepatitis, ischemic liver injury, and toxins that cause liver damage. The ALT levels in Hepatitis C rise more than in Hepatitis A and B. Persistent ALT elevation for more than 6 months is known as chronic hepatitis. Alcoholic liver disease, Non-alcoholic fatty liver disease (NAFLD), fat accumulation in the liver during childhood obesity, steatohepatitis (inflammation of fatty liver disease) are associated with a rise in ALT. A rise in ALT is also associated with reduced insulin response, reduced glucose tolerance, and increased free fatty acids and triglycerides. Bright liver syndrome (bright liver on ultrasound suggestive of the fatty liver) with raised ALT is suggestive of metabolic syndrome (Zakim *et al.,* 2002).

In pregnancy, ALT levels would rise during the second trimester. In one of the studies, measured ALT levels in pregnancy-related conditions such as hyperemesis gravidarum was 103.5 iu/l, pre- eclampsia was 115, HELLP syndrome was 149. ALT levels would reduce by greater than 50% in three days after child delivery. Another study also shows that caffeine consumption can reduce the risk of ALT elevation in those who consume alcohol, overweight people, impaired glucose metabolism, and viral hepatitis (Zakim *et al.,* 2002).

#### Aspartate aminotransferase

Aspartate aminotransferase (AST) exists in two isoenzymes namely mitochondrial form and cytoplasmic form. It is found in the highest concentration in the liver, followed by the heart, muscle, kidney, brain, pancreas, and lungs (Cotran *et al.,* 2005). This wide range of AST-

containing organs makes it a relatively less specific indicator of liver damage compared to ALT. An increase of mitochondrial AST in the blood is highly suggestive of tissue necrosis in myocardial infarction and chronic liver disease. More than 80% of the liver AST activity is contributed by mitochondrial form of the isoenzymes, while the circulating AST in the blood is contributed by cytoplasmic form of AST. AST is especially markedly raised in those with liver cirrhosis (Zakim *et al.,* 2002). AST can be released from a variety of other tissues and if the elevation is less than two times the normal AST then no further workup needs to be performed if a patient is proceeding to surgery.

In certain pregnancy conditions such as hyperemesis gravidarum, AST can reach as high as 73 IU/L, 66 IU/L in pre-eclampsia, and 81 IU/L in HELLP syndrome.

#### AST/ALT ratio

The AST/ALT ratio increases in liver functional impairment. In alcoholic liver disease, the mean ratio is 1.45, and the mean ratio is 1.33 in post necrotic liver cirrhosis. The ratio is greater than

1.17 in viral cirrhosis, greater than 2.0 in alcoholic hepatitis, and 0.9 in non-alcoholic hepatitis. Ratio is greater than 4.5 in Wilson disease or hyperthyroidism (Zakim *et al.,* 2002).

#### Alkaline Phosphatase

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. It can also be found on the mucosal epithelium of the small intestine, proximal convoluted tubule of the kidneys, bone, liver, and placenta. It plays an important role in lipid transposition in small intestines and calcification of bones. 50% of all the serum ALP activities in the blood are contributed by bone. Acute viral hepatitis usually has normal or increased ALP. For example, hepatitis A has increased ALP due to cholestasis (impaired bile formation or bile flow obstruction) and would have the feature of prolonged itching. Other causes include infiltrative liver diseases, granulomatous liver disease, abscess, amyloidosis of the liver, and peripheral arterial disease. Mild elevation of ALP can be seen in liver cirrhosis, hepatitis, and congestive cardiac failure. Transient hyperphosphataemia is a benign condition in infants and can reach a

normal level in 4 months. In contrast, low levels of ALP are found in hypothyroidism, pernicious anemia, zinc deficiency, and hypophosphatasia (Zakim *et al.,* 2002).

ALP activity is significantly increased in the third trimester due to increased synthesis from the placenta. In pregnancy conditions such as hyperemesis gravdirum, ALP levels can reach 215 iu/l, meanwhile, in pre-eclampsia, ALP can reach 14 iu/l, and in HELLP syndrome ALP levels can reach 15 iu/l (Zakim *et al.,* 2002)

#### Gamma-Glutamyltransferase

Gamma-glutamyltransferase (GGT) is a microsomal enzyme found in hepatocytes, biliary epithelial cells, renal tubules, pancreas, and intestines. It helps in glutathione metabolism by transporting peptides across the cell membrane. Much like ALP, GGT measurements are usually elevated if cholestasis is present (Cotran *et al.,* 2005). In acute viral hepatitis, the GGT levels can peak at the 2nd and 3rd week of illness, and remained elevated at 6 weeks of illness. GGT is also elevated in 30% of the hepatitis C patients. GGT can increase by 10 times in alcoholism. GGT can increase by 2 to 3 times in 50% of the patients with non-alcoholic liver disease. When GGT levels are elevated, the triglyceride level is elevated also. With insulin treatment, the GGT level can reduce. Other causes of elevated GGT are diabetes mellitus, acute pancreatitis, myocardial infarction, anorexia nervosa, Guillain–Barré syndrome, hyperthyroidism, obesity, and myotonic dystrophy (Zakim *et al.,* 2002).

#### Albumin

Albumin is a protein made specifically by the liver and can be measured cheaply and easily. It is the main constituent of total protein (the remaining constituents are primarily globulins). Albumin levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephrotic syndrome, where it is lost through the urine. The consequence of low albumin can be edema since the intravascular oncotic pressure becomes lower than the extravascular space. An alternative to albumin measurement is prealbumin, which is better at detecting acute changes

(half-life of albumin and prealbumin is about 2 weeks and about 2 days, respectively) (Molina *et al.,* 2012).

#### Kidney

The word “renal” is an adjective meaning “relating to the kidneys”, and its roots are French or late Latin (Kalantar-Zadeh *et al.,* 2021). The kidneys are two reddish-brown bean-shaped organs found in vertebrates. They are located on the left and right in the retroperitoneal space, and in adults, humans are about 12 centimeters (4+1⁄2 inches) in length (Lote *et al.,* 2012). They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder. The nephron is the structural and functional unit of the kidney. Each adult human kidney contains around 1 million nephrons, while a mouse kidney contains only about 12,500 nephrons.

#### The structure of the kidney

In humans, the kidneys are located high in the abdominal cavity, one on each side of the spine, and lie in a retroperitoneal position at a slightly oblique angle. The asymmetry within the abdominal cavity, caused by the position of the liver, typically results in the right kidney being slightly lower and smaller than the left, and being placed slightly more to the middle than the left kidney (Glodny *et al.,* 2009). The left kidney is approximately at the vertebral level T12 to L3 (Molina *et al.,*2012), and the right is slightly lower. The right kidney sits just below the diaphragm and posterior to the liver. The left kidney sits below the diaphragm and posterior to the spleen. On top of each kidney is an adrenal gland. The upper parts of the kidneys are partially protected by the 11th and 12th ribs. Each kidney, with its adrenal gland is surrounded by two layers of fat: the perirenal fat present between renal fascia and renal capsule and para renal fat superior to the renal fascia.

The kidney is a bean-shaped structure with a convex and a concave border. A recessed area on the concave border is the renal hilum, where the renal artery enters the kidney and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perirenal fat, renal fascia, and Para renal fat. The anterior (front) surface of these tissues is the peritoneum, while the posterior (rear) surface is the transversalis fascia.

The superior pole of the right kidney is adjacent to the liver. For the left kidney, it is next to the spleen. Both, therefore, move down upon inhalation.

A Danish study measured the median renal length to be 11.2 cm (4+7⁄16 in) on the left side and

10.9 cm (4+5⁄16 in) on the right side in adults. Median renal volumes were 146 cm3 (8+15⁄16 cu in) on the left and 134 cm3 (8+3⁄16 cu in) on the right (Walter, 2004).

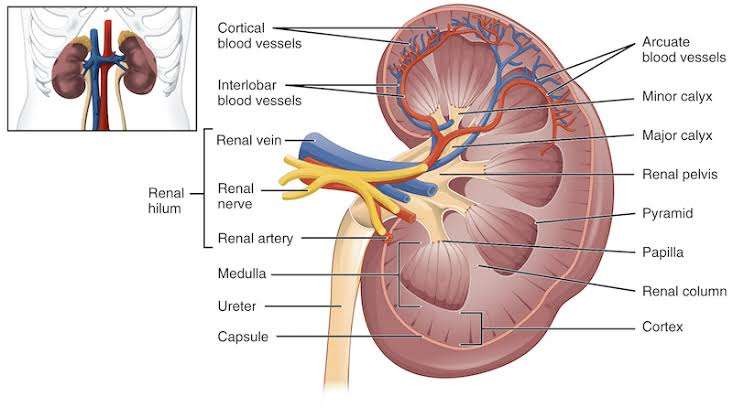


Figure 2.2: Structure of the kidney (Sunshine Community Health Center)

#### Gross anatomy of the kidney

The functional substance, or parenchyma, of the kidney is divided into two major structures: the outer renal cortex and the inner renal medulla. Grossly, these structures take the shape of eight to 18 cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (Clapp, 2009), between the renal pyramids are projections of cortex called renal columns. Nephrons, the urine-producing functional structures of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the renal corpuscle, which is located in the

cortex. This is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct (Clapp, 2009).

The tip, or papilla, of each pyramid empties urine into a minor calyx; minor calyces empty into major calyces, and major calyces empty into the renal pelvis. This becomes the ureter. At the hilum, the ureter and renal vein exit the kidney and the renal artery enters. Hilar fat and lymphatic tissue with lymph nodes surround these structures. The hilar fat is contiguous with a fat-filled cavity called the renal sinus. The renal sinus collectively contains the renal pelvis and calyces and separates these structures from the renal medullary tissue. The kidneys possess no overtly moving structures (Kalantar-Zadeh *et al.,* 2021).

#### Nerve supply

The kidney and nervous system communicate via the renal plexus, whose fibers course along the renal arteries to reach each kidney (Bard *et al.,* 2003). Input from the sympathetic nervous system triggers vasoconstriction in the kidney, thereby reducing renal blood flow (Bard *et al.,* 2003). The kidney also receives input from the parasympathetic nervous system, by way of the renal branches of the vagus nerve; the function of this is yet unclear (Bard *et al.,* 2003). Sensory input from the kidney travels to the T10-11 levels of the spinal cord and is sensed in the corresponding dermatome, the pain in the flank region may be referred from the corresponding kidney (Bard *et al.,* 2003).

#### Functions of the kidney

The kidneys excrete a variety of waste products produced by metabolism into the urine. The microscopic structural and functional unit of the kidney is the nephron. It processes the blood supplied to it via filtration, reabsorption, secretion, and excretion; the consequence of those processes is the production of urine. These include the nitrogenous wastes urea, from protein catabolism, and uric acid, from nucleic acid metabolism. The ability of mammals and some birds to concentrate wastes into a volume of urine much smaller than the volume of blood from which

the wastes were extracted is dependent on an elaborate countercurrent multiplication mechanism. This requires several independent nephron characteristics to operate: a tight hairpin configuration of the tubules, water and ion permeability in the descending limb of the loop, water impermeability in the ascending loop, and active ion transport out of most of the ascending limb. In addition, passive countercurrent exchange by the vessels carrying the blood supply to the nephron is essential for enabling this function.

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II, aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others.

1. Formation of urine
2. Hormone secretion
3. Blood pressure regulation
4. Acid-base balance
5. Regulation of osmolality (Le *et al.,* 2013)

#### Kidney Injury and Failure

Generally, humans can live normally with just one kidney, as one has more functioning renal tissue than is needed to survive. Only when the amount of functioning kidney tissue is greatly diminished does one develop chronic kidney disease. Renal replacement therapy, in the form of dialysis or kidney transplantation, is indicated when the glomerular filtration rate has fallen very low or if the renal dysfunction leads to severe symptoms (Kalantar-Zadeh *et al.,* 2021).

#### Congenital disease

1. Congenital hydronephrosis
2. Congenital obstruction of urinary tract
3. Duplex kidneys, or double kidneys, occur in approximately 1% of the population. This occurrence normally causes no complications, but can occasionally cause urinary tract infections (Ian, 2008).
4. Duplicated ureter occurs in approximately one in 100 live births
5. Horseshoe kidney occurs in approximately one in 400 live births
6. Nephroblastoma (Syndromic Wilm's tumour)
7. Nutcracker syndrome
8. Polycystic kidney disease
   * Autosomal dominant polycystic kidney disease afflicts patients later in life. Approximately one in 1000 people will develop this condition
   * Autosomal recessive polycystic kidney disease is far less common but more severe, than the dominant condition. It is apparent in utero or at birth.
9. Renal agenesis. Failure of one kidney to form occurs in approximately one in 750 live births. Failure of both kidneys to form used to be fatal; however, medical advances such as amnioinfusion therapy during pregnancy and peritoneal dialysis have made it possible to stay alive until a transplant can occur.
10. Renal dysplasia
11. Unilateral small kidney
12. Multicystic dysplastic kidney occurs in approximately one in every 2400 live births
13. Ureteropelvic Junction Obstruction or UPJO; although most cases are congenital, some are acquired (Stephen *et al*., 2006).

#### Diagnosis of kidney disease

Many renal diseases are diagnosed based on a detailed medical history, and physical examination (Gaitonde, 2017). The medical history takes into account present and past symptoms, especially those of kidney disease; recent infections; exposure to substances toxic to the kidney; and family history of kidney disease.

Kidney function is tested by using blood tests and urine tests. The most common blood test is creatinine, urea, and electrolytes. Urine tests such as urinalysis can evaluate for pH, protein, glucose, and the presence of blood. Microscopic analysis can also identify the presence of urinary casts and crystals (Rose *et al.,* 2012). The glomerular filtration rate (GFR) can be directly measured ("measured GFR", or mGFR) but this is rarely done in everyday practice. Instead, special equations are used to calculate GFR ("estimated GFR", or eGFR) (Rose *et al.,* 2012; Kidney Disease, 2012).

#### Blood test

A blood test is a laboratory analysis performed on a blood sample that is usually extracted from a vein in the arm using a hypodermic needle, or via fingerprick. Blood tests are often used in health care to determine physiological and biochemical states, such as disease, mineral content, pharmaceutical drug effectiveness, and organ function. A venipuncture is useful as it is a minimally invasive way to obtain cells and extracellular fluid (plasma) from the body for analysis. Blood flows throughout the body, acting as a medium that provides oxygen and nutrients to tissues and carries waste products back to the excretory systems for disposal. Consequently, the state of the bloodstream affects or is affected by, many medical conditions. For these reasons, blood tests are the most commonly performed medical tests (Lote, 2012). If only a few drops of blood are needed, a fingerstick is performed instead of a venipuncture (Mescher, 2016).

Phlebotomists, laboratory practitioners, and nurses are those in charge of extracting blood from a patient. However, in special circumstances, and emergencies, paramedics and physicians extract the blood. Also, respiratory therapists are trained to extract arterial blood to examine arterial blood gases (Kalantar-Zadeh *et al.,* 2021).

#### Creatinine test

Serum creatinine (a blood measurement) is an important indicator of kidney health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine is produced through a biological system involving creatinine, phosphocreatine (also known as creatinine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply). Creatinine is synthesized primarily in the liver from the methylation of glycocyamine (guanidinoacetate, synthesized in the kidney from the amino acids arginine and glycine) by S- Adenosyl methionine. It is then transported through the blood to the other organs, muscles, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine. Creatinine conversion to phosphocreatine is catalyzed by creatinine kinase; spontaneous formation of creatinine occurs during the reaction (Glodny *et al.,* 2009).

It is removed from the blood primarily by the kidneys, through glomerular filtration, and also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. If the filtration in the kidney is deficient, blood creatinine concentrations rise. Therefore, creatinine concentrations in blood and urine may be used to calculate the creatinine clearance (CrCl), which correlates approximately with the glomerular filtration rate (GFR). Blood creatinine concentrations may also be used alone to calculate the estimated GFR (eGFR) (Glodny *et al.,* 2009).

The GFR is clinically important because it is a measurement of kidney function. However, in cases of severe kidney dysfunction, the CrCl rate will overestimate the GFR because hypersecretion of creatinine by the proximal tubules will account for a larger fraction of the total creatinine cleared (Glodny *et al.,* 2009). Ketoacids, cimetidine, and trimethoprim reduce creatinine tubular secretion and, therefore, increase the accuracy of the GFR estimate, in

particular in severe kidney dysfunction. (In the absence of secretion, creatinine behaves like inulin.)

An alternate estimation of kidney function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. BUN-to-creatinine ratio (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea concentration raised out of proportion to the creatinine may indicate a prerenal problem such as volume depletion, about 1% to 2% of muscle creatine is converted to creatinine daily. The conversion is nonenzymatic and irreversible (Molina *et al.,* 2012). Men tend to have a higher concentration of creatinine than women because, they have a greater mass of skeletal muscle. An increase in dietary intake of creatinine or eating a lot of protein (like meat) can increase daily creatinine excretion (Kidneys Location Stock Illustration, 2013).

Serum creatinine is the most commonly used indicator (but not direct measure) of renal function. Elevated creatinine is not always representative of a true reduction in GFR. A high reading may be due to increased production of creatinine not due to decreased kidney function, interference with the assay, or decreased tubular secretion of creatinine. An increase in serum creatinine can be due to increased ingestion of cooked meat (which contains creatinine converted from creatinine by the heat from cooking) or excessive intake of protein and creatinine supplements, taken to enhance athletic performance. Intense exercise can increase creatinine by increasing muscle breakdown. Creatinine secretion by the tubules can be blocked by some medications, again increasing measured creatinine (Uhlén *et al.,* 2015).

#### Urea test

Urea serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. It is a colorless, odorless solid, highly soluble in water, and practically non-toxic (LD50 is 15 g/kg for rats). Dissolved in water, it is neither acidic nor alkaline. The body uses it in many processes, most notably nitrogen excretion. The liver forms it by combining two ammonia molecules (NH3) with a carbon dioxide (CO2) molecule in the urea cycle. Urea is widely used in fertilizers as a source of nitrogen (N) and is an important raw material for the chemical industry (Gaitonde, 2017).

Friedrich Wöhler discovered that urea can be produced from inorganic starting materials, which was an important conceptual milestone in chemistry in 1828. It showed for the first time that a substance previously known only as a byproduct of life could be synthesized in the laboratory without biological starting materials, thereby contradicting the widely held doctrine of vitalism, which stated that only living things could produce the chemicals of life (Gaitonde, 2017).

Amino acids from ingested food that are used for the synthesis of proteins and other biological substances or produced from catabolism of muscle protein are oxidized by the body as an alternative source of energy, yielding urea and carbon dioxide (Stephen *et al.,* 2006). The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle. The first step in the conversion of amino acids from protein into metabolic waste in the liver is the removal of the alpha-amino nitrogen, which results in ammonia. Because ammonia is toxic, it is excreted immediately by fish, converted into uric acid by birds, and converted into urea by mammals (Gaitonde, 2017).

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L- arginine act as intermediates. Urea production occurs in the liver and is regulated by N-acetyl glutamate. Urea is then dissolved into the blood (in the reference range of 2.5 to 6.7 mmol/liter) and further transported and excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat. In water, the amine groups undergo slow displacement by water molecules, producing ammonia, ammonium ion, and bicarbonate ion. For this reason, old, stale urine has a stronger odor than fresh urine.

The cycling of and excretion of urea by the kidneys is a vital part of mammalian metabolism. Besides its role as a carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons, which allows for the re-absorption of water and critical ions from the formation of urine. The body uses this mechanism, which is controlled by the antidiuretic hormone, to create hyperosmotic urine (urine with a higher concentration of dissolved substances than the blood plasma). This mechanism is important to prevent the loss of water, maintain blood pressure, and maintain a suitable concentration of sodium ions in the blood plasma. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons (Rose *et al.,*

2012), thereby raising the osmolarity in the medullary interstitium surrounding the thin descending limb of the loop of Henle, which makes the water reabsorb. By action of the urea transporter 2, some of this reabsorbed urea eventually flows back into the thin descending limb of the tubule via the collecting ducts, and into the excreted urine (Rose *et al.,* 2012).

The equivalent nitrogen content (in gram) of urea (in mmol) can be estimated by the conversion factor 0.028 g/mmol (Hansen *et al.,* 2015). 1 gram of nitrogen is equivalent to about 6.25 grams of protein, and 1 gram of protein is roughly equivalent to 5 grams of muscle tissue. In situations such as muscle wasting, 1 mmol of excessive urea in the urine (as measured by a urine volume in litres multiplied by urea concentration in mmol/l) roughly corresponds to a muscle loss of 0.67 gram.

#### Electrolytes

The primary ions of electrolytes are sodium (Na+), potassium (K+), calcium (Ca2+), magnesium (Mg2+), chloride (Cl−), hydrogen phosphate (HPO42−), and hydrogen carbonate (HCO3−) (Glodny *et al.,* 2009). The electric charge symbols of plus (+) and minus (−) indicate that the substance is ionic and has an imbalanced distribution of electrons, the result of chemical dissociation. Sodium is the main electrolyte found in extracellular fluid and potassium is the main intracellular electrolyte, both are involved in fluid balance and blood pressure control (Molina *et al.,* 2012).

All known multicellular lifeforms require a subtle and complex electrolyte balance between the intracellular and extracellular environments (Molina *et al.,* 2015). In particular, the maintenance of precise osmotic gradients of electrolytes is important. Such gradients affect and regulate the hydration of the body as well as blood pH, and are critical for nerve and muscle function. Various mechanisms exist in living species that keep the concentrations of different electrolytes under tight control (Glodny *et al.,* 2009).

Both muscle tissue and neurons are considered electric tissues of the body. Muscles and neurons are activated by electrolyte activity between the extracellular fluid or interstitial fluid, and intracellular fluid. Electrolytes may enter or leave the cell membrane through specialized protein structures embedded in the plasma membrane called "ion channels". For example, muscle

contraction is dependent upon the presence of calcium (Ca2+), sodium (Na+), and potassium (K+). Without sufficient levels of these key electrolytes, muscle weakness or severe muscle contractions may occur (Molina *et al.,* 2012).

Electrolyte balance is maintained by oral, or in emergencies, intravenous (IV) intake of electrolyte-containing substances, and is regulated by hormones, in general with the kidneys flushing out excess levels. In humans, electrolyte homeostasis is regulated by hormones such as antidiuretic hormones, aldosterone, and parathyroid hormones. Serious electrolyte disturbances, such as dehydration and overhydration, may lead to cardiac and neurological complications and, unless they are rapidly resolved, will result in a medical emergency (Molina *et al.,* 2012).

Measurement of electrolytes is a commonly performed diagnostic procedure, performed via blood testing with ion-selective electrodes or urinalysis by medical technologists. The interpretation of these values is somewhat meaningless without analysis of the clinical history and is often impossible without parallel measurements of renal function. The electrolytes measured most often are sodium and potassium. Chloride levels are rarely measured except for arterial blood gas interpretations since they are inherently linked to sodium levels. One important test conducted on urine is the specific gravity test to determine the occurrence of an electrolyte imbalance.

#### Tumor Necrotic Factor-alpha (TNF-ɑ)

One of the best-characterized cytokines is tumor necrotic factor-alpha (TNF-ɑ) which is known also as a cachectin or differentiation-inducing factor (DIF) and secreted mostly by monocytes, macrophages, T lymphocytes, and mast cells. It is a pro-inflammatory cytokine and one of the 22 proteins which belong to the TNF family (Baowska-Kozakiewicz, 2013). This pro-inflammatory cytokine is present in two forms. First is a membrane (precursor) form with a molecular weight of 26 kDa and the second is a 17kDa secretory form after the enzyme modification (Korobowicz, 2006; Hochiriu *et al.,* 2013).

A report showed that TNF-ɑ is useful as a potential marker. It was noted that changes in serum concentration of TNF-ɑ may be a potential marker in multiple sclerosis (MS) which is an inflammatory and neurodegenerative disease (Kacperska *et al.,* 2014). The possibility of using

this cytokine as a marker was observed in inflammatory bowel disease (IBD) although another report showed the opposite information (Eder *et al.,* 2007).

### CHAPTER THREE

#### Methodology

This chapter presents the analysis of petroleum pollution on serum trace element level, oxidative Status, and biochemical indices in the residents of an oil host community (Ugbegungun) in Delta State, Nigeria. The methods employed to undertake this study were encapsulated into the research design, setting, target population, sample size, sampling technique, instrument for data collection, validity/reliability of instrument, method of data analysis, and ethical considerations.

#### Design

In conducting this study, an experimental design was used to evaluate the serum trace metals, oxidative status, and biochemical alteration among indigenes of an oil host community in Delta State. The collection of data from respondents was done through the administration of a well- structured questionnaire and blood collection (5ml sample collected).

#### Settings

The residents of Ugbegungun community which is a riverine Itsekiri village with a boundary to Escravos which lies between latitude 5.5991ºNorth and longitude 5.2020ºEast, located in Warri South East Local Government Area in Delta State, Nigeria. It is an oil-rich community which host oil-producing companies. The volunteer from Okada, Edo State, Nigeria served as control. Okada is located in the North-East Local Government Area (LGA) of Edo State, which lies between latitude 5º401 North and longitude 5º001 East.

#### Ethical consideration

Ethical approval was obtained from Delta state Ministry of Health (HM/596/T/156) which mandated the confidentiality and privacy of the respondents. Before data collection commenced, careful explanation of the purpose, consent and implication were made known to participants, the participants were given assurance of confidentiality, by so doing; there would be no disclosure of

information such as names to the other students as the information obtained was personal and private.

#### Inclusive and exclusive criteria

#### Inclusion criteria

Individuals between 20 and 50 years old, that were physically, mentally healthy, willing to participate, and that have lived in the community for more than 10 years were recruited for the study.

#### Exclusion criteria

Individuals that are above 50 years of age, people with underlying illnesses such as cardiovascular disease, poor eyesight, memory, and sleep disorder were not included in this study.

#### Enrollment visit

We had contact with the participants before the scheduled enrollment visit to provide information about pollution risks. We also confirmed that all the participants have been informed of the study during the visit.

#### Research enrollment guideline

The guideline used for the enrollment of subjects were:

* The participants signed an informed consent form.
* The participants met all of the inclusion and none of the exclusion criteria.
* The subjects were enrolled once the written consent was obtained and subject eligibility assessed.

#### Sample size determination

The sample size to be used was determined with the G\*Power 3.1.9.2 software. The number of study participants were 100. Fifty (50) participants were the resident of an oil host community

(Ugbegungun, Delta state) and fifty (50) participants (control) were residents of a non-oil host community (Okada, Edo State) Nigeria.

#### Sample collection

Each participant signed an informed consent form, met all the inclusion and none of the exclusion criteria before samples were collected.

Also, 5mL of blood sample was collected from the cubital fossa (medial cubital vein, basilica vein, or cephalic vein) of each participant into a vial without anticoagulant/additives (plain bottle) and was allowed to clot, after which it was centrifuged at 5,000 resolution per minute for 5 minutes to obtain the serum. The serum was used to determine different variables. The data collections were closely monitored to ensure the quality and accuracy of the data.

#### Estimation of Serum cadmium, lead, and zinc

Atomic absorption spectrophotometry (AAS) is an analytical technique used to measure metals (ICE 3000, Thermo Fisher Scientific, Waltham, MA). The sample size needed is very small (typically about 10 mg - i.e. 100 of a gram) and its removal causes little damage. The sample was accurately weighed and then dissolved, using nitric acids. The resulting solution is sprayed into the flame of the instrument and atomized. Light of a suitable wavelength (Lead with wavelength 217.0, Cadmium with wavelength 228.8, and Zinc with wavelength 213.9) is shone through the flame, and some of this light is absorbed by the atoms of the sample. The amount of light absorbed is proportional to the concentration of the element in the solution, and hence in the original object. Measurements were made separately for each element in turn to achieve a complete analysis.

#### Kidney function test

#### Electrolytes

The measurement for the electrolytes was done using the ISE series operator (V2.0) Analyser. This series of electrolyte analyzers are automated, microprocessor-controlled analytic instruments that apply ISE (Ion Selective Electrode) technology to the measurement of the contents of potassium (K+), sodium (Na+), chloride (Cl-), and HCO3- in human body liquid.

#### Urea Principle

Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

Urea + H2 Urease----->O2NH3 + CO2

NH3 + hypochlorite + phenol > indophenol

(blue compound)

#### Assay Procedure

Assay procedure and protocol for Urea

|  |  |  |  |
| --- | --- | --- | --- |
|  | Blank | Standard | Sample |
| Sample | - | - | 5 µL |
| Standard | - | 5 µL | - |
| Distilled water | 5 µL | - | - |
| Reagent 1 | 50 µL | 50 µL | 50 µL |

Wavelength - 546

#### Calculations

Urea Concentration = Change in sample absorbance × conc. of standard (mg/dL)

change in standard absorbance

#### Creatinine

**Principle**

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

#### Assay Procedure

Assay procedure and protocol for Urea

|  |  |  |  |
| --- | --- | --- | --- |
|  | Reagent Blank | Standard | Sample |
| ddH2O | 50 µL | - | - |
| Standard | - | 50 µL | - |
| Sample | - | - | 50 µL |
| Working Reagent | 500 µL | 500 µL | 500 µL |

Change in standard absorbance

Wavelength = 492

**Calculations**

Creatinine Concentration = Change in sample absorbance × conc. of standard (mg/dL)

#### Liver function test

* + 1. **Assay for Alanine aminotransferase (ALT) Principle**

Alanine aminotransferase was estimated with the method of Reitman *et al.* (1957). It was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4- dinitrophenylhydrazine.

α-oxoglutarate + L-alanine → L-glutamate + pyruvate

#### Procedure

The serum sample (0.1mL) was mixed with 0.5 mL of phosphate buffer (L alanine) and incubated for 30 minutes at 37 0C. 2, 4-dinitrophenylhydrazine (0.5 mL) was added to the mixture, mixed, and allowed to stand for 20 minutes at 25 0C. 5 mL of 0.4mol/l sodium hydroxide was added to the mixture and the absorbance of the solution was read after 5 minutes at a wavelength of 546nm. The serum concentration of ALT was expressed as IU/L.

ALT (IU/L) = absorbance of unknown × value of Standard

Absorbance of standard

#### Assay for Aspartate aminotransferase (AST) Principle

Aspartate aminotransferase was measured with the Reitman *et al.* (1957) method. The AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4- dinitrophenylhydrazine

α – oxoglutarate + L-glutamate  L-glutamate + Oxaloacetate

Serum (0.1mL) was mixed with 0.5 mL of phosphate buffer (L aspartate) and incubated for 30 minutes at 37 0C and then 2,4-dinitrophenylhydrazine was mixed with supernatant for 20 minutes at 25 0C (at room temperature) and left to stand for 10 minutes. The absorbance of the solution was read at a wavelength of 546nm. The serum concentration of AST was expressed as IU/L.

AST (IU/L) = absorbance of unknown × value of Standard

Absorbance of standard

#### Assay for Alkaline phosphatase Principle

The alkaline phosphate acts upon the AMP buffered sodium thymolphthalcin monophosphate. The addition of an alkaline reagent tops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

#### Procedure

For each serum sample, 0. 25 mL of alkaline phosphatase substrate was dispensed into labelled test tubes and equilibrated to 37 0C for three (3) minutes. At timed intervals, 0. 05 mL of each standard or control and the sample was added to their respective test tubes and mixed gently. Ionized water was used as blank. The mixtures were incubated for ten (10) minutes at 37 0C. 1.25 mL alkaline phosphatase colour developer was added at timed intervals and mix well. The wavelength of the spectrophotometer was set at 590nm and zero with a reagent blank. Sample and standard absorbance were read, ALP value was evaluated expressed as IU/L.

#### Calculation

ALP (IU/L) = absorbance of unknown × value of Standard

Absorbance of standard

#### Oxidative status

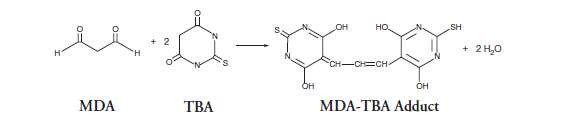
#### Malondialdehyde

Malondialdehyde (MDA) was determined using the method of Varshney and Kale (1990)

#### Principle

MDA which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient marker for the determination of the extent of lipid peroxidation. The assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-2TBA adduct (Pink-red coloured complex) that absorbs light at 535nm.

Reaction equation;



#### Procedure

* + - 1. 50 µL of serum sample was dispensed into a clean test tube
      2. 100 µL of TCA/TBA working solution was added
      3. 1.85 mL of distilled water was added
      4. The mixture was placed in a boiling water bath for 15 minutes
      5. This was centrifuged after cooling
      6. The absorbance of the supernatant fluid was read at 535nm using reagent blank.

|  |  |  |
| --- | --- | --- |
|  | Sample | Blank |

|  |  |  |
| --- | --- | --- |
| Serum | 50 µL | - |
| TBA/TCA Solution | 100 µL | 100 µL |
| Distilled water | 1850 µL | 1900 µL |

#### Calculation

TBARS activity (mmol/mL) = Absorbance of sample × V x 1000

A × v × L × Y

Where;

V = Total volume to which test was diluted

A = Molar extinction coefficient of product = 1.56 x 105M-1cm-1 v = Volume of serum sample used

Y = Total protein estimated for a particular sample L = Light path = 1cm.

#### Superoxide dismutase Assay Principle

Superoxide Dismutase (SOD) activity was determined by the kinetic method of Misra and Fridovich, (1972). Adrenaline auto-oxidises rapidly in an aqueous solution to adrenochrome, whose concentration can be determined at 420nm. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme, SOD inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anions. The degree of inhibition is thus a reflection of the activity of SOD.

#### Assay procedure and protocol

Assay procedure and protocol for assay of Superoxide Dismutase

|  |  |  |  |
| --- | --- | --- | --- |
|  | Blank | Reference | Test |
| Distilled water | 3.0 mL | 0.2 mL | - |
| Sample | - | - | 0.2 mL |
| Phosphate buffer | - | 2.5 mL | 2.5 mL |
| Adrenaline | - | 0.3 mL | 0.3 mL |

Solutions were mixed properly and the absorbances were read at 420nm.

#### Calculation

% Inhibition = ABSref **-** ABStest X 100

ABSref

Thus, SOD (Unit/ml) = % Inhibition

50 x S

Where:

ABSref = Absorbance of reference ABStest = Absorbance of Test

S = Total protein (g/dL) for each sample

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL

#### Quality control measures

The spectrophotometer was put on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

#### Catalase

The activity of the enzyme Catalase was determined by the kinetic method of Cohen *et al*., 1970.

#### Principle

Catalase reacts with and catalyses the breakdown of reagent hydrogen peroxide to water and oxygen. The absorbance of hydrogen peroxide at 480nm is measured directly to calculate the reaction rate since water and oxygen do not absorb at this wavelength. In the presence of

Catalase, the reaction rate is proportionally (linearly) enhanced.

2H2O2

Catalase

2H2O + O2

Test procedure and protocol for catalase assay

|  |  |  |
| --- | --- | --- |
|  | Blank | Test |
| 0.34M H2O2 | 5.0 mL | 5.0 mL |
| Distilled water | 0.5 mL | - |
| 6M H2SO4 | 1.0 mL | 1.0 mL |
| 0.01M KMnO4 | 1.0 mL | 1.0 mL |
| Sample | - | 0.5 mL |

Absorbances of test samples were read at 480nm, and at 0sec, 20sec, 40sec, 60sec, and 80sec for each sample.

#### Calculation:

The activity of serum Catalase was calculated using the formula:

Catalase (Unit/ml serum) = ΔABS of test x V x 1000

m x v x L x y

Where; ΔOD = Mean of the differences in the absorbance for each test V = Total volume of the reaction mixture

m = molar extinction coefficient for H2O2 = 40 M-1cm-1

L = Light path = 1cm

v = Volume of sample used

y = Total protein (g/dl) for the respective sample

The activity; Unit/g = mole of H2O2 consumed per minute. **Conversion**: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL **Quality control:**

The assay was done in a cold water bath using an ice block to prevent the undesirable deterioration of H2O2.

The spectrophotometer was on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

#### Glutathione Peroxidase (GPx)

Glutathione Peroxidase activity (GPx) was determined by the kinetic method (Flohe and Gunzler, 1984).

#### Principle

GPx catalyses the reaction of pyrogallol with hydrogen peroxide to form purpurogallin (purple to black coloured) whose absorbance is read at 420nm.

2pyrogallol + 3H2O2 + purpurogallen + 5H2O + CO2

#### Procedure and Protocol

|  |  |  |
| --- | --- | --- |
|  | Blank | Sample |
| Sample | - | 200 µL |
| Phosphate buffer | 2.5 mL | 2.5 mL |
| H2O2 | 2.5 mL | 2.5 mL |
| Distilled water | 1.7 mL | 1.5 mL |
| Pyrogallol solution | 2.5 mL | 2.5 mL |

The absorbance of each sample was read at 420nm and 0sec, 20sec, 40sec, 60sec, 80sec, and 100sec.

#### Calculation

Concentration of GPx was calculated using the formula below GPx (Unit/ml) = ΔABS x V x Df

A x v x L x Y

Where;

ΔABS = Mean of the differences in the absorbance V = Total volume of the reaction mixture

Df = Dilution factor

A = Molar extinction coefficient of purpurogallin = 12.0 M-1cm-1 v = Volume of serum sample used

L = Light path = 1cm

Y = Total protein (g/dl) for each sample.

Expression of result: The result was expressed in Units/ml of serum, where 1 unit = mole of pyrogallol oxidized per minute.

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL

#### Quality control measures

The pyrogallol solution was kept in a brown bottle to avoid degeneration from reaction with light. The spectrophotometer was on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

#### Glutathione reductase (GR)

Dithionitrobenzoic acid; 5, 51-dithiobis (2-nitrobenzoic acid) reacted with the GSH generated from the reduction of GSSG by the GR in a sample which formed a yellow product 3-thio-6- nitrobenzoate (TNB2-). The rate of change in the optical density, measured at 412 nm, is directly proportional to GR activities in the sample.

Assay procedure and protocol

|  |  |  |  |
| --- | --- | --- | --- |
|  | Blank (µL) | Standard (µL) | Sample (µL) |
| Sample |  |  | 500 |
| TCA |  |  | 2000 |
| Was mixed properly and allowed to stand for 5 min at room temperature and was spun for 10  mins at 4000rpm | | | |
| Supernatant |  |  | 500 |
| Ellman’s reagent | 500 | 500 | 500 |
| Distilled water | 500 |  |  |
| Standard |  | 500 |  |
| Buffer | 3000 | 3000 | 3000 |

The absorbance was read at 412 nm.

#### Calculation:

Glutathione reductase (U/g/dl) = ABSample × Standard conc. (g/dl)

ABSstd

#### Quality control measures

The spectrophotometer was put on and allowed to run for 15 mins before use as a quality control measure according to the manufacturer.

#### Total antixiodant capacity

The 2,2-diphenyl-1-picrylhdrazyl (DPPH) method was used in the determination of total antioxidant capacity (TAC). DPPH is a stable free radical, due to the delocalization of the spare electron on the whole molecule (Molyneux, 2004).

#### Procedure

Each Trolox standard and sample was assayed in duplicate or triplicate. A freshly prepared standard curve was used in each time the assay was performed.

* + - 1. 25 µl of the diluted Trolox standard or samples was added to a 96-well microtiter plate.
      2. Diluted the 50X ABTS Reagent 1:50 in either 1X Assay Buffer (Hydrophilic samples) or 75% ethanol (Lipophilic sample).
      3. 0Added 150 µl of the diluted ABTS reagent to each well using either a multichannel pipette or a plate reader liquid handling system. Mixed thoroughly. Immediately began timing the reaction
      4. Incubated for 5 mins on an orbital shaker
      5. Read the plate at 415 nm immediately. The green colour faded over time.

#### Serum tumor necrotic factor-alpha

The quantitative sandwich enzyme immunoassay technique was used to determine serum tumor necrotic factor-alpha (TNF-α) levels. The Quantikine HS, human TNF-ɑ ELISA kit was uesd.

#### Assay Principle

This was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for TNF-α pre-coated onto 96-well plates. Standards and test samples were added to the wells, a biotinylated detection polyclonal antibody from goat specific for TNF-α was subsequently added and then followed by washing with Tris-buffered saline (TBS) buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with TBS buffer. Horseradish peroxidase substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalysed by HRP to produce a blue colour product that changed into yellow after adding an acidic stop solution. The density of yellow is proportional to the human TNF-α amount of sample captured in the plate.

#### Assay Procedure

0.1ml of 1000 pg/ml, 500 pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml and 15.6pg/ml of TNF-α standard solutions were aliquoted into the precoated wells. 0.1ml of the sample diluent buffer was also aliquoted into the control well (zero well). 0.1ml of the prediluted serum was added subsequently into the empty wells. The plate was sealed by an adhesive cover and

incubated at 37oC for 90minutes. The cover was then removed, content discarded, and blotted onto paper towels. 0.1ml of biotinylated anti-human TNF-α antibody working solution was added to each well. This was then sealed with a new adhesive cover and incubated at 37oC for 60minutes. This was then washed 3 times by adding 0.3ml 0.01M TBS into each well leaving the buffer each time for 1 minute. The wash buffer was then discarded and blotted onto paper towels. 0.1ml of prepared ABC working solution was added to each well. This was then sealed with a new adhesive cover and incubated at 37oC for 30minutes. This was then washed 5 times by adding 0.3ml 0.01M TBS into each well leaving the buffer each time for 1 minute. The wash buffer was then discarded and blotted onto paper towels. 90µl of prepared TMB colour developing agent was added into each well. This was then sealed with a new adhesive cover and incubated at 37oC in dark for 25minutes. The different shades of blue can be seen in the well at varying concentrations. 0.1ml of the TMB stop solution was then added to each well. The blue colour changes to yellow immediately. The absorbance is then read at 450nm in a microplate reader within 30minutes.

The relative O.D450= O.D450 of each well-O.D450 of the zero well. The standard curve was plotted by using the relative O.D450 of each standard solution (Y) versus the respective concentration of the standard solution (X). The human TNF-α concentration of the samples were interpolated from the standard curve after multiplication by the dilution factor.

#### 8-hydroxy-2-deoxyguanosine Sample preparation

A quantitative sandwich enzyme immunoassay technique was used to determine serum 8- hydroxy-2-deoxyguanosine levels. The Quantikine HS, human TNF-ɑ ELISA kit was uesd.

#### Principle

The ELISA kit uses Sandwich-ELISA as the method. The micro ELISA strip plate provided in this kit has been pre-coated with an antibody specific to 8-OHdG. Standards or samples are added to the appropriate micro ELISA strip plate wells and combined with the specific antibody. Then a Horseradish 8-OHdG (HRP)-conjugated antibody specific for 8-OHdG was added to each

micro ELISA strip plate well and incubated. Free components were washed away. The TMB substrate solution was added to each well. Only those wells that contain 8-OHdG and HRP conjugated 8-OHdG antibody will appear blue and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm.

#### Procedure

1. Dilution of standards: diluted the standard by small tubes first, then pipetted the volume of 50 µL from each tube to microplate well, each tube use two wells, a total of ten wells.

|  |  |  |
| --- | --- | --- |
| 240ng/L | Standard No. 1 | 300µl Oringinal Standard + 150µl Standard diluents |
| 160ng/L | Standard No. 2 | 300µl Standard No. 1 + 150 µl Standard diluents |
| 80ng/L | Standard No. 3 | 150µl Standard No. 2 + 150µl Standard diluents |
| 40ng/L | Standard No. 4 | 150µl Standard No. 3 + 150µl Standard diluents |
| 20ng/L | Standard No. 5 | 150µl Standard No. 4 + 150µl Standard diluents |

1. In the micro ELISA strip plate, a well empty was left as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample were added (dilution factor is 5). Samples were loaded onto the bottom without touching the well. Mixed well with gentle shaking.
2. Incubation: incubated for 30 minutes at 370C after being sealed with a closure plate membrane.
3. Dilution: diluted the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T0).
4. Washing: carefully peel off the closure plate membrane, aspirate, and refill with the wash solution. Discard the wash solution after resting for 30secs. The washing procedure was repeated 5 times.
5. 50µl HRP-Conjugate reagent was added to each well except the blank control well.
6. Incubated as described in Step 3.
7. Washed as described in Step 5.
8. Coloring: added 50µl Chromogen Solution A and 50µl Chromogen Solution B to each well, mixed with gentle shaking, and incubate at 370C for 15min. please avoid light during coloring.
9. Termination: added 50µl stop solution to each well to terminate the reaction. The colour in the well should change from blue to yellow.
10. Read absorbance O.D at 450nm using a microliter plate reader. The OD value of the blank control well is set as zero. The assay was carried out within 15mins after adding the stop solution.

Assay range: 10ng/L - 300ng/L

#### Statistical Analysis

Statistical analysis was done with GraphPad Prism version 8.0.1 (244). Data was analyzed with Student’s independent t-test and a p-value less than 0.05 (p<0.05) was considered significant. Data are presented in Mean ± Standard Error of Mean (SEM)

### CHAPTER FOUR

### RESULTS

#### Body mass index

Figure 4.1 shows that no significant difference (p>0.05) in the body mass index of male (25.4 ± 1.45 kg/m2) and female (24.6 ± 2.23 kg/m2) residents of an oil host community when compared with male (22.2 ± 0.91 kg/m2) and female (22.6 ± 0.75 kg/m2) residents of a non-oil host Community.

**30** Oil Host Residents Non-Oil Host Residents



**25**

**20**

**Body Mass Index (kg/m2)**

**15**

**10**

**5**

**0**

**Male Female**

Figure 4.1: Body mass index of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

#### Serum zinc, lead, and cadmium levels

Figure 4.2 shows that the serum zinc level was not significant (p<0.05) in the male (32.9 ± 2.00 µg/L) and female (32.2 ± 0.76 µg/L) residents of an oil host community when compared with the male (33.4 ± 6.79 µg/L) and female (39.9 ± 4.23µg/L) residents of a non-oil host Community.

Figure 4.3 shows that the serum lead level was significantly higher (p<0.05) in male (4.3 ± 0.17 µg/L) and female (4.2 ± 0.10 µg/L) residents of an oil host community when compared with male (2.9 ± 0.17 µg/L) and female (3.6 ± 0.20µg/L) of a non-oil host Community.

Figure 4.4 shows that the serum cadmium level was significantly increased (p<0.05) in male (1.4

± 0.02 µg/L) and female (1.4 ± 0.01 µg/L) residents of an oil host community when compared with male (1.1 ± 0.08 µg/L) and female (1.1 ± 0.06 µg/L) of a non-oil host Community.

Oil Host Residents

Non-Oil Host Residents

**Male Female**



**50**

**45**

**40**

**35**

**30**

**25**

**20**

**15**

**10**

**5**

**0**

**Zinc concentration (** **g/dL)**

Figure 4.2: Serum zinc level of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**5.0**



Non-Oil Host Residents

**\***

**\***

Oil Host Residents

**4.5**

**4.0**

**3.5**

**Lead concentration (** **g/dL)**

**3.0**

**2.5**

**2.0**

**1.5**

**1.0**

**0.5**

**0.0**

**Male Female**

Figure 4.3: Serum lead level of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**1.50**



Oil Host Residents

**\***

Non-Oil Host Residents

**\***

**1.25**

**1.00**

**Cadmium concentration (** **g/dl)**

**0.75**

**0.50**

**0.25**

**0.00**

**Male Female**

Figure 4.4: Serum cadmium level of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Serum chlorine, bicarbonate, potassium, and sodium ions levels

Figure 4.5 shows that the serum chlorine ion was significantly reduced (p<0.05) in female (104.6

± 0.69 mmol/L) residents of an oil host community when compared with the female (106.5 ± 0.51 mmol/L) residents of a non-oil host community.

Figure 4.6 shows that the serum bicarbonate ion was significantly reduced (p<0.05) in male (20.4

± 0.68 mmol/L) and female (19.2 ± 0.41 mmol/L) residents of an oil host community when compared with male (22.0 ± 054 mmol/L) and female (22.1 ± 0.46 mmol/L) residents of a non- oil host community.

Figure 4.7 shows that the serum potassium ion was significantly higher (p<0.05) in male (4.6 ± 0.26 mmol/L) and female (4.8 ± 0.18 mmol/L) residents of an oil host community when compared with male (4.0 ± 0.13 mmol/L) and female (4.2 ± 0.12 mmol/L) residents of a non-oil host community.

Figure 4.8 shows that the sodium ion was significantly lower (p<0.05) in male (133.7 ± 0.50 mmol/L) and female (134.9 ± 0.57mmol/L) residents of an oil host community when compared with male (140.3 ± 1.10 mmol/L) and female (143.0 ± 0.95 mmol/L) residents of a non-oil host community.

**150**



**\***

Oil Host Residents

Non-Oil Host Residents

**125**

**100**

**Chlorine ion (mmol/L)**

**75**

**50**

**25**

**0**

**Male Female**

Figure 4.5: Serum chlorine ions of the residents of an oil host community in Delta State and non- oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**25**



**\***

**\***

Oil Host Residents

Non-Oil Host Residents

**20**

**15**

**Bicarbonate ion (mmol/L)**

**10**

**5**

**0**

**Male Female**

Figure 4.6: Serum bicarbonate ions of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**8**



**\***

**\***

Oil Host Residents

Non-Oil Host Residents

**6**

**Potassium ion (mmol/L)**

**4**

**2**

**0**

**Male Female**

Figure 4.7: Serum potassium ions of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**Sodium ion (mmol/L)**

Oil Host Residents

Non-Oil Host Residents

**Male Female**



**150**

**\***

**\***

**125**

**100**

**75**

**50**

**25**

**0**

Figure 4.8: Serum sodium ions of the residents of an oil host community in Delta State and non- oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Serum creatinine and urea level

Figure 4.9 shows that the serum creatinine level was significantly higher (p<0.05) in male (0.96

± 0.05 mg/dL) and female (0.96 ± 0.07 mg/dL) residents of an oil host community when compared with male (0.65 ± 0.05 mg/dL) and female (0.65 ± 0.04 mg/dL) of a non-oil host community.

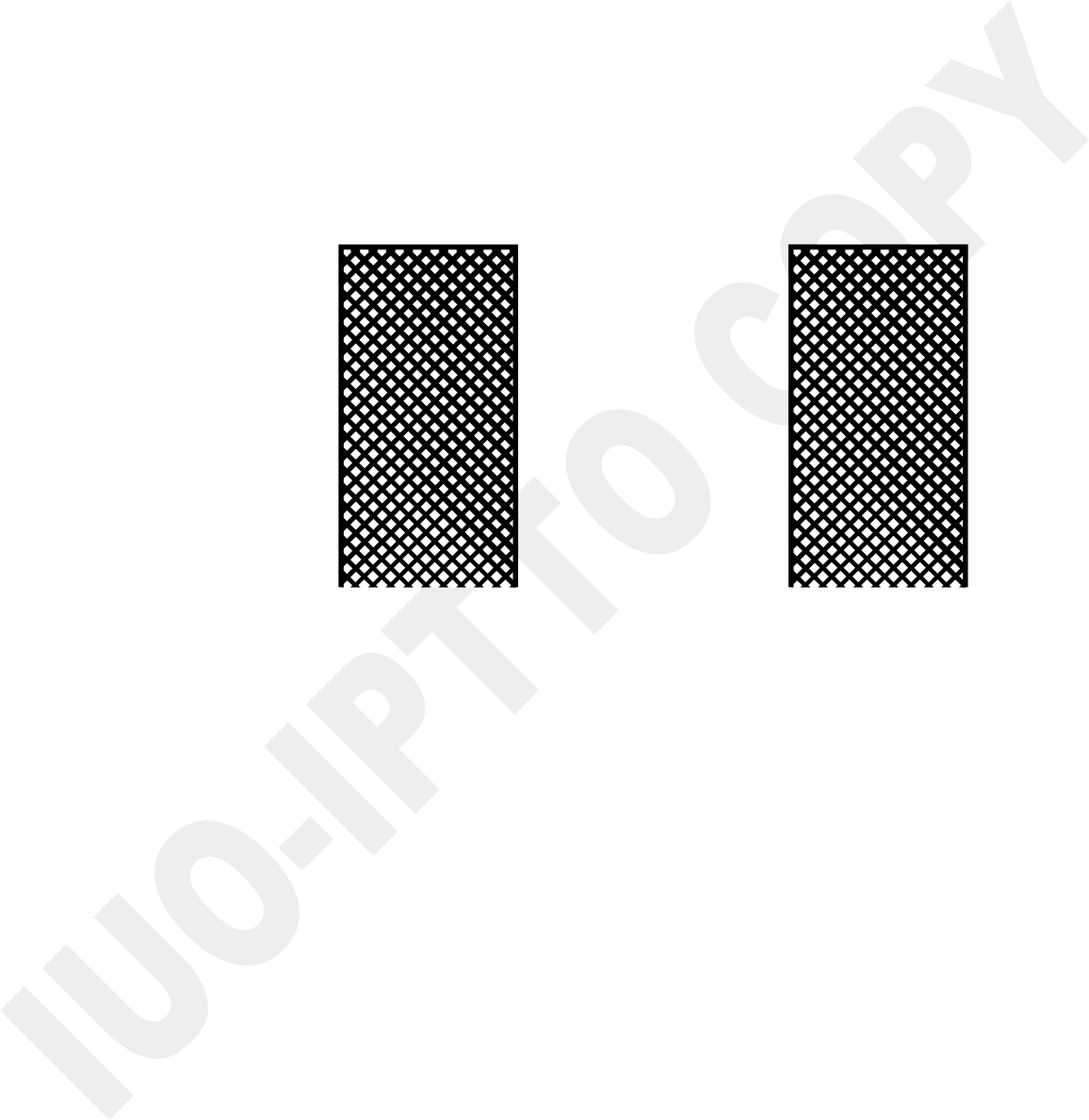
Figure 4.10 shows that the serum urea level was significantly higher (p<0.05) in male (41.0 ± 2.19 mg/dL) and female (42.8 ± 1.84 mg/dL) residents of an oil host community when compared with male (18.8 ± 2.01 mg/dL) and female (18.4 ± 2.21 mg/dL) of a non-oil host community.

**1.5**



## Oil Host Residents Non-Oil Host Residents

**1.0 \* \***



**Creatinine (mg/dL)**

**0.5**

**0.0**

**Male Female**

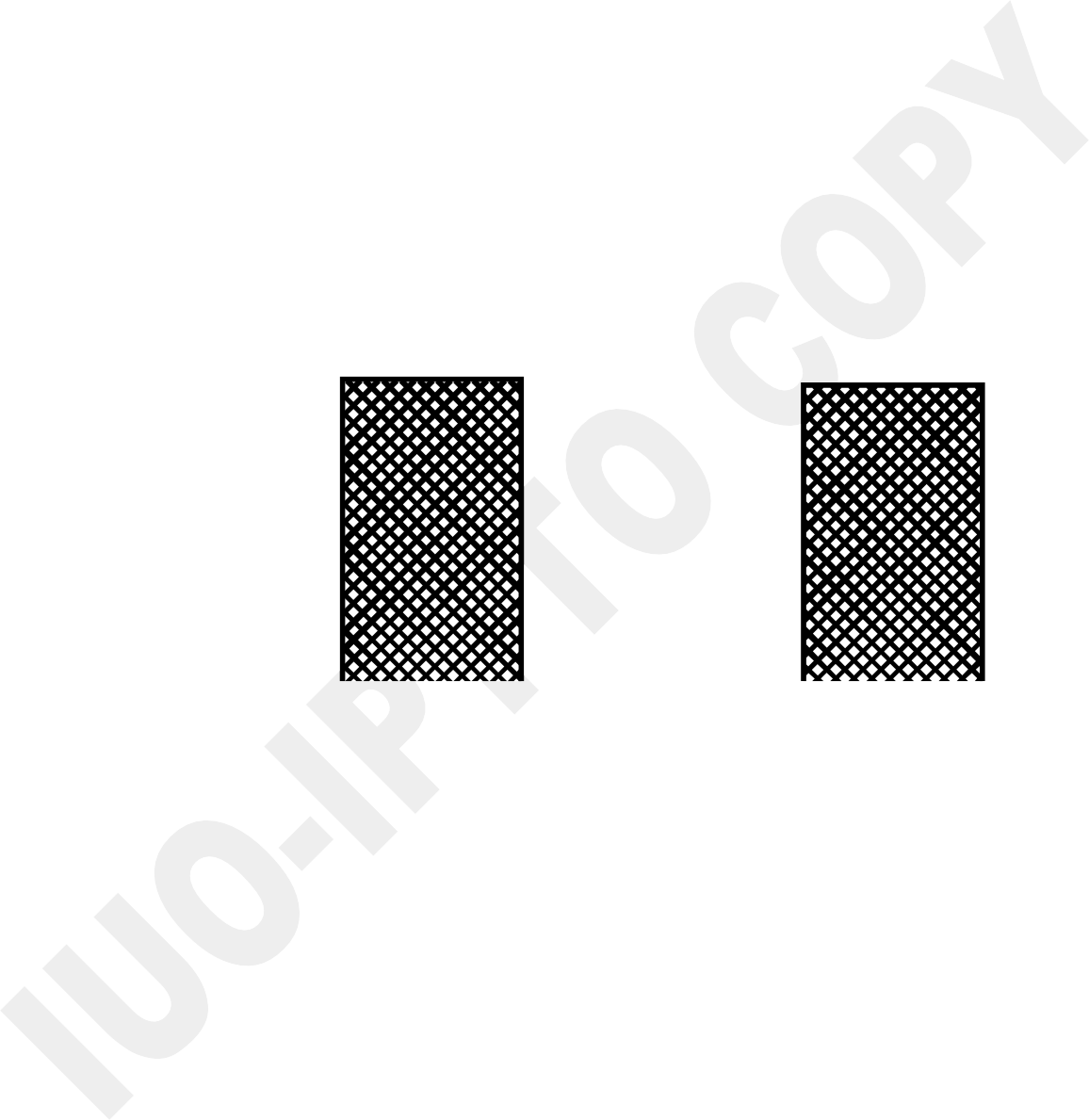
Figure 4.9: Serum creatinine level of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

# **50** Oil Host Residents Non-Oil Host Residents



**\* \***



**40**

**30**

**Urea (mg/dL)**

**20**

**10**

**0**

**Male Female**

Figure 4.10: Serum urea level of the residents of an oil host community in Delta State and non- oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Alanine transaminase, aspartate transaminase, and alkaline phosphate activities

Figure 4.11 shows that the alanine transaminase activity was significantly higher (p<0.05) in male (59.6 ± 2.91 IU/L) and female (44.4 ± 4.09 IU/L) residents of an oil host community when compared with male (32.0 ± 3.26 IU/L) and female (25.6 ± 1.91 IU/L) residents of a non-oil host community.

Figure 4.12 shows that the aspartate transaminase activity was significantly higher (p<0.05) in male (42.3 ± 3.71 IU/L) and female (35.8 ± 3.08 IU/L) residents of an oil host community when compared with male (25.9 ± 2.99 IU/L) and female (25.7 ± 2.63 IU/L) residents of a non-oil host community.

Figure 4.13 shows that the alkaline phosphatase activity was significantly higher (p<0.05) in male (147.4 ± 3.42 IU/L) and female (139.9 ± 3.93 IU/L) residents of an oil host community when compared with male (110.6 ± 5.98 IU/L) and female (112.9 ± 6.82 IU/L) residents of a non-oil host community.

**80**

Oil Host Residents

Non-Oil Host Residents

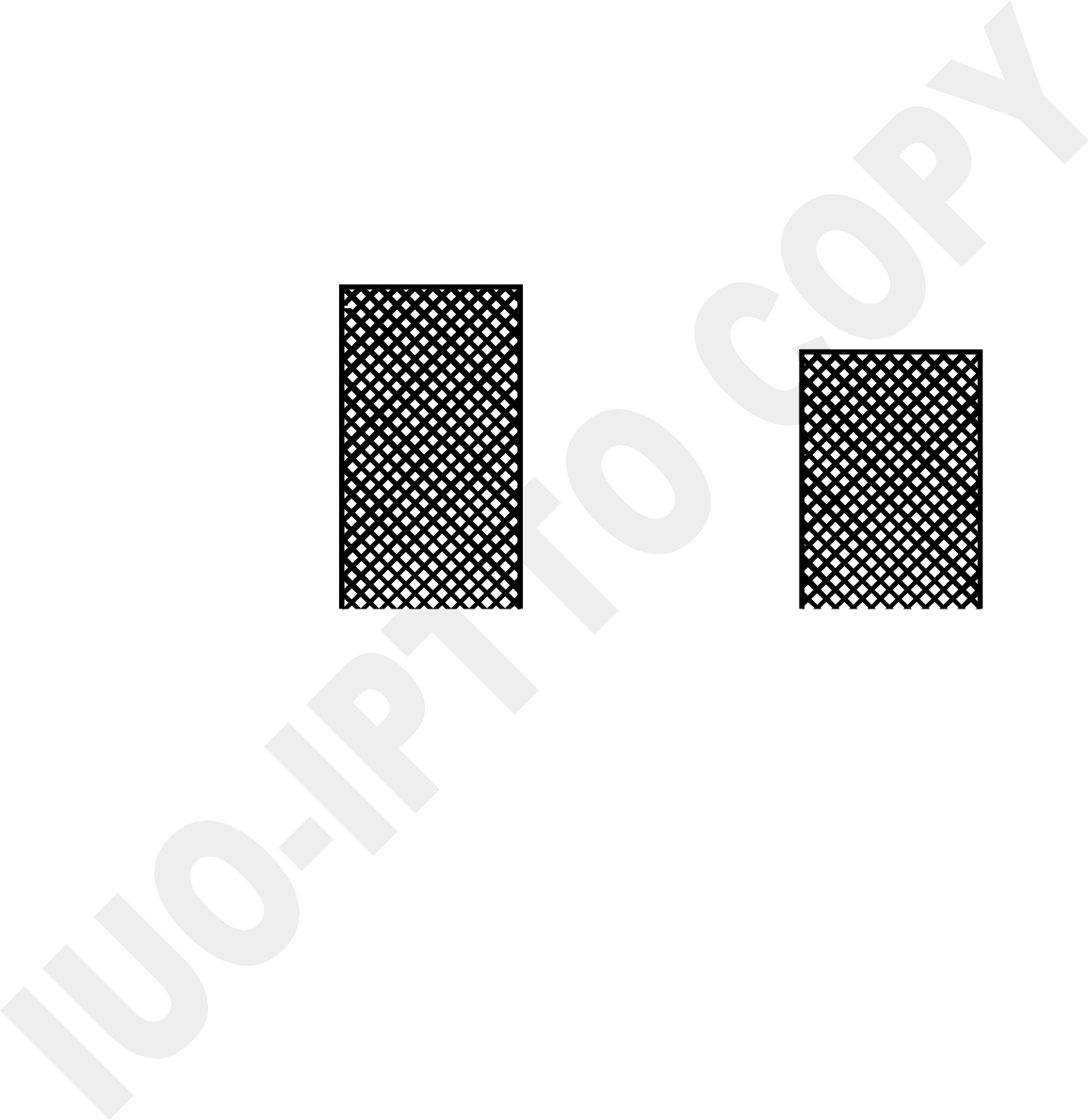


**\***

**\***

**70**

**60**



**Alanine transaminase (IU/L)**

**50**

**40**

**30**

**20**

**10**

**0**

**Male Female**

Figure 4.11: Serum alanine transaminase activity of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

Non-Oil Host Residents

**Male Female**



**50**

Oil Host Residents

**\***

**40**

**\***

**30**

**20**

**10**

**0**

**Aspartate transaminase (IU/L)**

Figure 4.12: Serum aspartate transaminase activity of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

Oil Host Residents

Non-Oil Host Residents

**Male Female**



**200**

**175**

**150**

**\***

**\***

**125**

**100**

**75**

**50**

**25**

**0**

**Alkaline phosphate (IU/L)**

Figure 4.13: Serum alkaline phosphatase activity of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Malondialdehyde level

Figure 4.14 shows that the malondialdehyde level was significantly higher (p<0.05) in male (42.92 ± 3.80 uM) and female (39.84 ± 2.81 uM) residents of an oil host community when compared with both male (24.02 ± 2.02 uM) and female (28.51 ± 2.96 uM) of a non-oil host Community.

**50**



Oil Host Residents

\*

\*

Non-Oil Host Residents

**45**

**40**

**Malondiadehyde concentration (uM)**

**35**

**30**

**25**

**20**

**15**

**10**

**5**

**0**

**Male Female**

Figure 4.14: Serum malondialdehyde concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase activities

Figure 4.15 shows that the catalase level was significantly lower (p<0.05) in male (117.7 ± 9.47 uM) and female (196.4 ± 7.25 uM) residents of an Oil Host community when compared with both male (233.0 ± 9.81 uM) and female (237.0 ± 6.24 uM) of a non-oil host Community.

Figure 4.16 shows that the superoxide dismutase level was significantly lower (p<0.05) in male (4.1 ± 0.25 uM) and female (4.2 ± 0.28 uM) residents of an Oil Host community when compared with both male (8.1 ± 0.73 uM) and female (7.5 ± 0.71 uM) of a non-oil host Community.

Figure 4.17 shows that the glutathione reductase level was significantly lower (p<0.05) in male (3.5 ± 0.70 uM) and female (3.5 ± 0.48 uM) residents of an Oil Host community when compared with both male (8.4 ± 1.04 uM) and female (6.4 ± 0.67 uM) of a non-oil host Community.

Figure 4.18 shows that the glutathione peroxidase level was significantly lower (p<0.05) in male (5.1 ± 1.07 uM) and female (4.8 ± 0.47 uM) residents of an Oil Host community when compared with both male (6.8 ± 0.86 uM) and female (6.4 ± 0.82 uM) of a non-oil host Community.

# Oil Host Residents Non-Oil Host Residents

**250**

**\***

**\***

**200**

**150**

**Catalase (uM)**

**100**

**50**

**0**

**Male Female**

Figure 4.15: Serum catalase concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

Oil Host Residents

Non-Oil Host Residents

**Male Female**



**10**

**8**

**6**

**\***

**\***

**4**

**2**

**0**

**Superoxide dismustase (uM)**

Figure 4.16: Serum superoxide dismutase concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**10**



**\***

**\***

Oil Host Residents

Non-Oil Host Residents

**8**

**Glutathione reductase concentration (uM)**

**6**

**4**

**2**

**0**

**Male Female**

Figure 4.17: Serum glutathione reductase concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

**8**



Oil Host Residents

Non-Oil Host Residents

**6**

**Glutathione peroxidase concentration (uM)**

**4**

**2**

**0**

**Male Female**

Figure 4.18: Serum glutathione peroxidase concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Total antioxidant capacity level

Figure 4.19 shows that the total antioxidant capacity level was significantly lower (p<0.05) in male (8.4 ± 1.09 uM) and female (9.2 ± 0.85 uM) residents of an Oil Host community when compared with both male (13.5 ± 1.11 uM) and female (14.3 ± 1.13 uM) of a non-oil host Community.

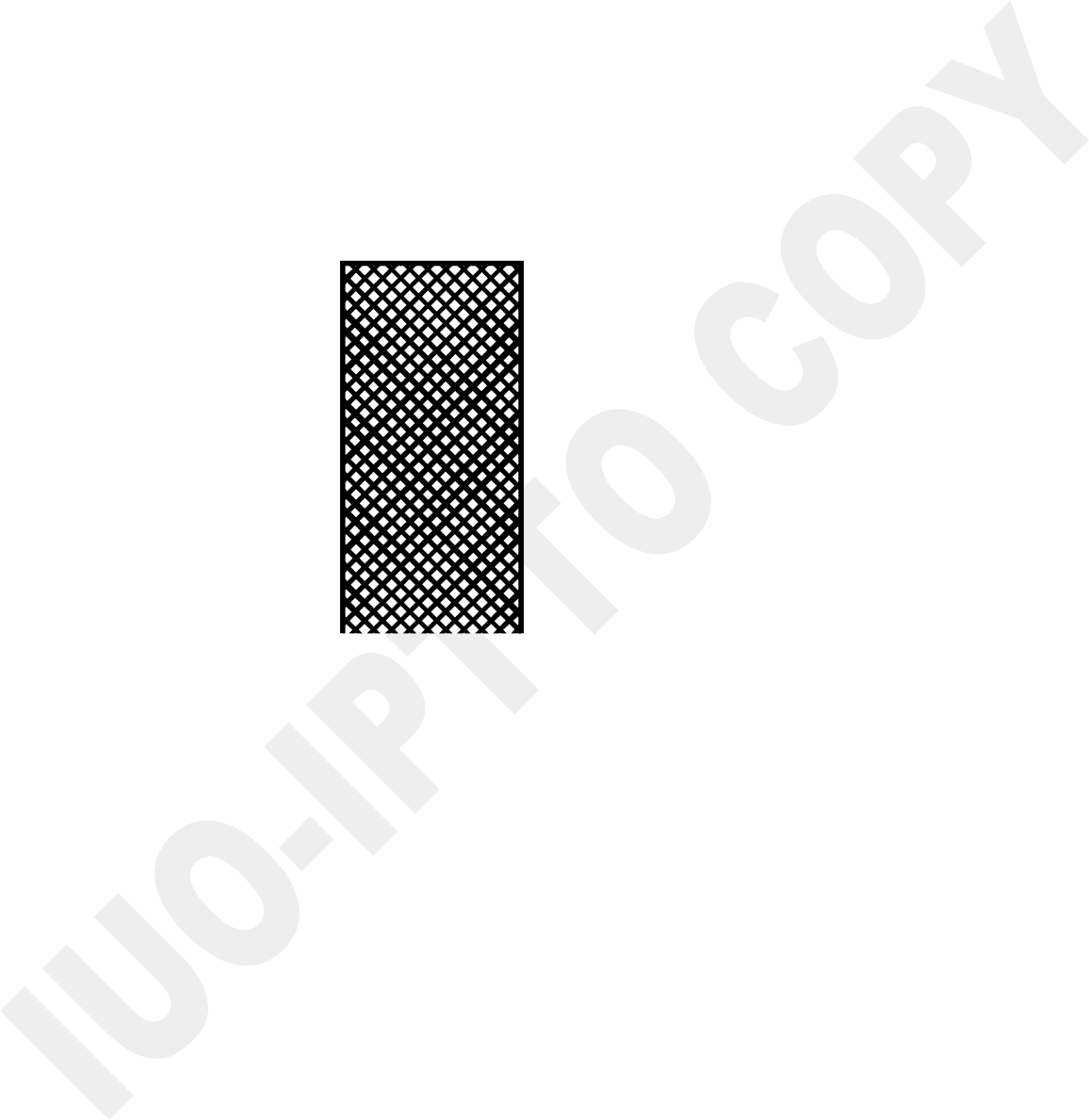
# Oil Host Residents Non-Oil Host Residents



**20**

**18**

**16**



**Total antioxidant capacity (uM)**

**\***

**14**

**12**

**10 \***

**8**

**6**

**4**

**2**

**0**

**Male Female**

Figure 4.16: Serum superoxide dismutase concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### Tumor necrotic factor-alpha

Figure 4.20 shows that the tumor necrotic factor-alpha level was significantly higher (p<0.05) in male (23.8 ± 2.22 pg/mL) and female (35.4 ± 6.98 pg/mL) residents of an Oil Host community when compared with both male (14.4 ± 1.69 pg/mL) and female (16.8 ± 1.22 pg/mL) of a non-oil host Community.

**50**



# Oil Host Residents Non-Oil Host Residents

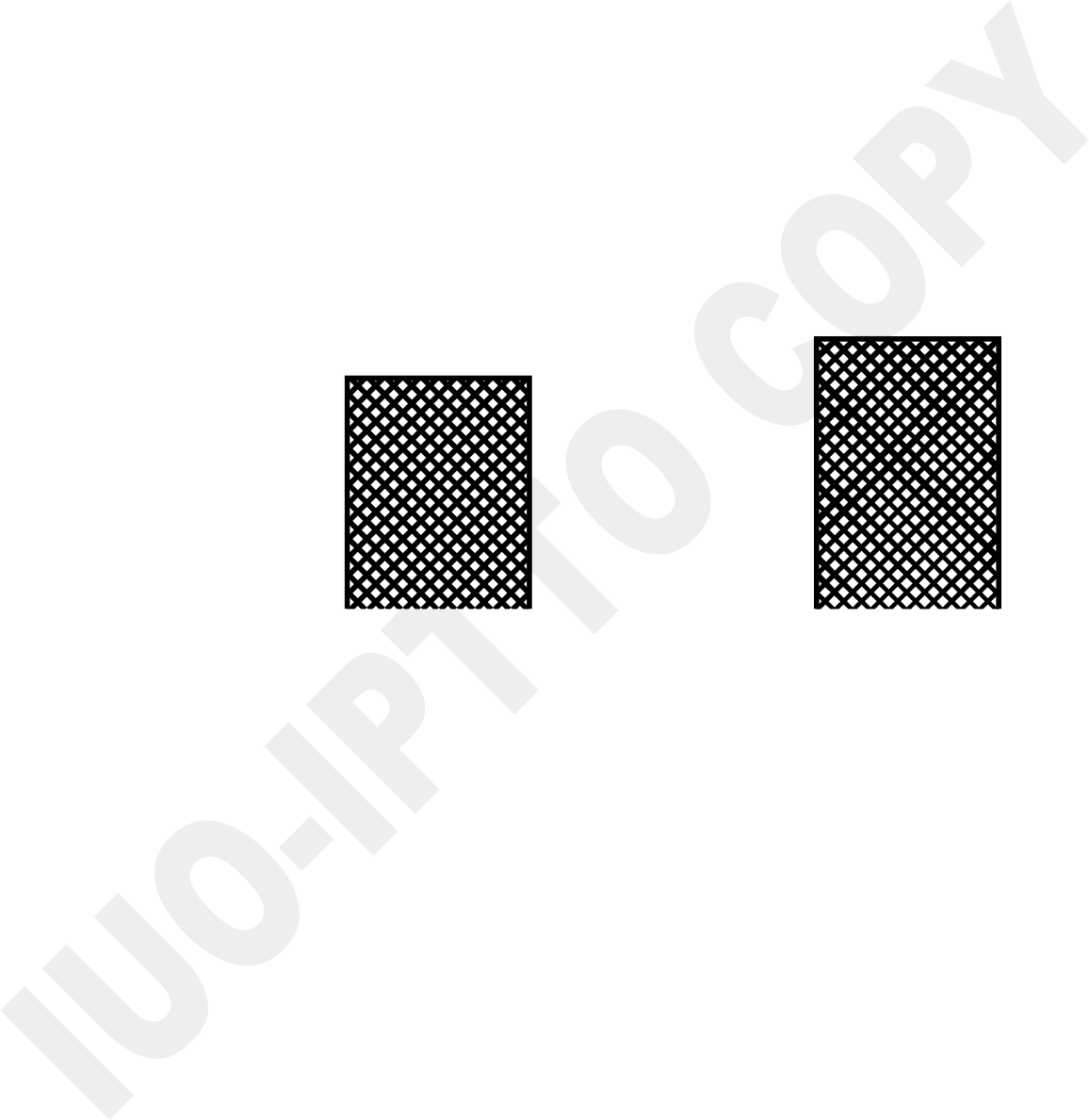
**45**

**\***

**Tumor necrotic factor-alpha (pg/mL)**

**40**

**35**



**30**

**\***

**25**

**20**

**15**

**10**

**5**

**0**

**Male Female**

Figure 4.21: Serum tumor necrotic factor-alpha concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

#### serum 8-hydroxy-2-deoxyguanosine level

Figure 4.21 shows that the 8-hydroxy-2-deoxyguanosine level was significantly higher (p<0.05) in male (23.4 ± 2.37 ng/L) and female (25.9 ± 2.47 ng/L) residents of an Oil Host community when compared with both male (15.2 ± 1.49 ng/L) and female (17.8 ± 1.34 ng/L) of a non-oil host Community.

# Oil Host Residents Non-Oil Host Residents

**30**

**\***

**\***

**25**

**8-Hydroxy-2-deoxyguanosine (ng/L)**

**20**

**15**

**10**

**5**

**0**

**Male Female**

Figure 4.20: Serum 8-hydroxy-2-deoxyguanosine concentration of the residents of an oil host community in Delta State and non-oil host community in Edo State, Nigeria.

Bars represent Mean ± SEM. \*p<0.05 was considered significant when compared with the residents of a non-oil host community.

### CHAPTER FIVE

### DISCUSSION

Body mass index (BMI) was developed as a risk indicator of metabolic diseases. Some common conditions related to overweight and obesity include cardiovascular diseases, high blood pressure, osteoarthritis, some cancers, and diabetes (WHO, 2018). The BMI was no different in both the residents of an oil host and non-oil host communities.

Zinc is an essential antioxidant in the human body (Shazia *et al.,* 2012) and one of the most important trace mineral in the body (Markiewicz *et al.,* 2015). Zinc deficiency is usually associated with reduced immunity (Prasad *et al.,* 2013), DNA damage, and induced oxidative toxicity (Jomova *et al.,* 2011). In this study, the serum zinc level in both female and male residents of the oil host and non-oil host communities was similar. The serum zinc level in the study is within the normal range. Abdel *et al.* (2019) reported deficiency of zinc and an increase in oxidative stress in individuals exposed to petroleum. Also, studies by Olawoyin *et al.* (2018) and Berglund *et al.* (2000) showed low serum zinc levels in individuals exposed to petroleum pollution

Lead exposure may increase the susceptibility of membranes by altering their integrity via causing deterioration of their components (Gurer *et al.,* 2000). While cadmium has been reported to be unable to generate free radicals directly, indirect generation of various radicals has been reported (Abdel *et al.,* 2019). Such a mechanism involves displacement of other redox-active metals from their binding sites thus increasing their free form and enhancing their capability of producing free radicals (Galan *et al.,* 2001). As an environmental contaminant, Pb is often associated with Cd. Since both elements have similar properties and their health effect are similar and additive (Xie *et al.,* 2011; Tella *et al.,* 2016). In this study, serum Pb and Cd concentrations were significantly higher in the residents of the oil host community. The present results follow other studies' trends (Berglund *et al.,* 2000; Gurer *et al.,* 2000 and Olawoyin *et al.,* 2018). This increase in serum Pb and Cd could be due to the increased activities of crude oil factories in the environment thus affecting the residents through inhalation of the gases emitted by the crude oil factories and fish farming which is a major sourse of food and occupation.

Biologically important elements have a significant role in the maintenance of homeostasis and participate in various physiological activities such as neuromuscular irritability, nerve conduction, and prevention of the development of an age-related complication. Alteration of levels in of these elements may induce a series of events such as slow movement, postural abnormality, impaired balance, extensive membrane damage, and peripheral vascular resistance (Dutta *et al.,* 2015). Serum Na+ and HCO3- concentration were significantly lower among residents of an oil host community than in the control group (non-oil host community) and also in another study reported by Prabhunath *et al.,* (2016). Potassium was significantly higher in residents of oil host community. Potassium and sodium are known to play integral roles in the electrophysiology of cellular functions (Kianifard and Chopra, 2018) and neuronal transmission (Eipe *et al.,* 2016). Due to the observed significant reduction in sodium and bicarbonate and a significant increase in potassium, there is a possible reduction in urinary output and cardiac output which can possibly lead to hypertension among the residents of oil host community.

Urea and creatinine were significantly higher in the residents of oil host community in this study than in non oil host community and in another study reported by Ibrahim *et al.,* (2012) among individuals exposed to crude oil. This significant decrease in electolytes (Na+ and HCO3-), and increase in potassium, urea and creatinine may due to the increase in Pb and Cd level in the residents of an oil host community. Relationship between electrolytes and residents of an oil host community has not been well documented.

Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphate (ALP) are indicators of hepatic damage and dysfunction (Khan *et al.,* 2001), these enzymes play important roles in biological processes (specifically in detoxification, metabolism, and biosynthesis of energetic macromolecules) which are important for different essential functions. ALP is often used as a marker to measure hepatic and biliary tract function (Mark, 2013). The serum ALT, AST, and ALP were significantly increased among residents in oil host community in this study. Other studies also reported significant increase in ALP, AST and ALT (Mark, 2014). The observed increase in the levels of these hepatic enzymes in the serum could be due to crude oil toxicants exposure among the residents of the community, thereby allowing liberation of these enzymes into the circulation. An increase in the serum level of ALP observed in this study proposes a possibility of biliary tract dysfunction among the residents of the oil host community.

This study indicates that increase in serum Pb and Cd level seems to play a role in hepatic toxicity.

Serum total antioxidant capacity is an integrative index used to reflect the antioxidant capacity of the body (Obida *et al.,* 2018). Measurement of total antioxidant capacity (TAC) is appropriate for the evaluation of the total antioxidant defenses of blood, cells, different types of tissues, and organs. TAC has been reported to reduce by exposure to radiation, herbicides, carbon monoxide, carbon tetrachloride, lead arsenic, mercury, cadmium, aluminum, and other toxic elements (Goraca *et al.,* 2006). A significant decrease in TAC among the residents of an oil host community was observed in this study. Individuals who have been exposed to petroleum showed highly significant reductions in TAC (Hegacy *et al.,*2014).

The superoxide dismutase (SOD) catalyzes the destruction of the superoxide radical, with potential toxicity arising from dismutation and hydrogen peroxide formation, while the glutathione reductase (GR) and glutathione peroxidase (GPx) catalyzes the conversion of hydrogen peroxide to water and directly reduce tissue injury from lipoperoxidation (Obida *et al.,* 2018). This study results show that SOD, CAT, GR, and GPx activities in the serum of residents in oil host community were decreased. With the decrease in TAC, SOD, CAT, GR, and GPx, there is an increase in free radicals in the body system. Therefore, it can be seen that heavy metals contamination interfered with the normal functions of the body, bringing harm to the human health of individuals living in the vicinity of an oil factory (Shen *et al.,* 2019).

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells, an increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress (Gaweł *et al.,* 2004). Malondialdehyde (MDA) is one of the end-products of the peroxidation of membrane lipids caused by ROS formation, especially by the superoxide ion. This is in agreement with other studies which illustrated that petroleum exposure has been associated with an increase in the overall formation of MDA (Georgieva *et al.,* 2002). In this study, MDA was significantly increased in the residents of an oil host community. A significant increase of serum MDA levels was seen in another study reported by Hegacy *et al.,* (2014). This result indicates that increase in serum lead level has an effect on kidney and hepatic toxicity which induces oxidative damage in the residents of an oil host community.

TNF-ɑ is a well-known inflammatory. There was a significant increase in the level of serum TNF-ɑ in the residents of an oil host community in this study. Elevated TNF-ɑ levels have been associated with the pathophysiology of reoxygenation injury, myocarditis, cardiac allograft vasculopathy, heart failure progression (Han *et al.,* 2019), arthritis, diabetes, Crohn’s disease, and also cachexia which correlated with terminal malignancy (Cameron *et al.,* 2017).

An experimental study showed that oxidative damage permanently occurs to lipids of cellular membranes, proteins, and DNA. In nuclear and mitochondrial DNA, 8-hydroxy-2- deoxyguanosine (8-OHdG) is among the predominant forms of the free radical-induced oxidative lesion and has therefore been widely used as a biomarker for oxidative stress and carcinogenesis (Athanasios *et al.,* 2009). This study shows that serum 8-hydroxy-2-deoxyguanosine level was significantly increased in the residents of the oil host community than in the control group. This study shows that there is a high possibility of DNA damage due to long-time exposure to crude oil activities in the environment. Other studies on serum 8-OHdG in crude oil host community are limited.

### CONCLUSION

In conclusion, the residents of an oil host community in Nigeria may be at high risk of kidney and liver toxicity through oxidative stress induction associated probably with heavy metal accumulation in the system.

### RELEVANCE TO SCIENCE

* + 1. This study proves possible pathological conditions such as kidney and hepatic damage, oxidative stress, TNF-α and DNA damage among residents to oil host community, however this scientific study cannot be used for diagnostic purpose.
    2. This study helps to determine the level of damage done by crude oil factories on the residents of oil host community

I recommend for further scientific research in oil host communities to sort out the damage and possible solution to curb up this problem.

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