CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study.

Tuberculosis, (TB) is an infectious disease caused by various strains of *Mycobacteria species*, particularly *Mycobacterium tuberculosis* (Kumar *et al*., 2007). *Mycobacterium tuberculosis* (MTB) is an intracellular pathogen, which grows and replicates in the host macrophages (Yildiz *et al*., 2004). Nigeria is among the fourteen high burden countries for tuberculosis (TB), TB and HIV co- infection and Multi Drug Resistant TB. She is ranked seventh among the 30 high TB burden countries and second in Africa. The problem of TB in Nigeria has been made worse by drug resistant TB and the human immunodeficiency virus or acquired immunodeficiency syndrome (HIV/AIDS) epidemics. It was reported in 2010 by World Health Organization (WHO) that about 210,000 new cases of TB was recorded in Nigeria equivalent to 133/100,000 population. The low TB case finding for both adult and children is a major issue with TB in Nigeria. It was observed that in 2017 only 104,904 TB cases were detected out of an estimated 407,000 for that year (25.8%). This indicates treatment coverage of just 25.8 per cent. This leaves a gap of 302,096 cases which were either undetected or detected but the cases were not reported especially in "non DOTS sites". The huge gap in TB case finding is much higher among children aged zero to fourteen. India, Indonesia and Nigeria account for almost half of the total gap (Delphi and Arun, 2015). The Federal Ministry of Health has made good progress with the drafting of the National Strategic Plan for Tuberculosis Control which aims to provide Universal Access to Prevention, Diagnosis and Treatment by 2020 in line with its commitments to the World Health Organization (WHO, 2015). However, the success of this plan has been marred by several hindrances namely, inadequate budget provisions, access to hard to reach areas, unreported TB cases and inadequate human resources technical capacity. The Federal Ministry of Health declared 2018 a year to accelerate finding and notification of TB cases in Nigeria and by employing active case-finding in key affected populations, over 11,500 TB cases were detected through active house to house case searching in 2017. Furthermore, the unprecedented growth of the tuberculosis epidemic in Africa is due to several factors, the most important being the HIV epidemic. Although HIV is Africa's leading cause of death, tuberculosis is the most common coexisting condition in people who die from AIDS. (Delphi and Arun, 2015). However, it is worthy to note that malaria parasite is also an epidemic disease in Nigeria and could also be

a burden on individuals with tuberculosis and malaria parasite co-infection. Furthermore, about 70% of adults and 88% of children infected with HIV worldwide live in sub-Saharan Africa but almost all of the treatment developed to date has been designed using the research into the North American and European *M. tuberculosis* strains. Analysis of molecular-based data have shown diverse genetic back-grounds among both drug-sensitive and multi drug resistant (MDR) TB isolates in Africa presumably due to underlying genetic and environ-mental differences (Klopper *et al.*, 2013).Moreover, the 2014 WHO report states that, "globally, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360,000 of whom were HIV-positive. The number of people dying from HIV-associated TB has been falling for almost a decade (WHO, 2010). The African Region accounts for about four out of every five HIV-positive TB cases and TB deaths among people who were HIV-positive. Tuberculosis attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have active TB infection cough, sneeze, speak or sing (WHO, 2010). Most infections are asymptomatic and latent, however one tenth of latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. The classic symptoms of active TB infection are chronic cough with blood-tinged sputum, fever, night sweats, and weight loss (Lawn and Zumla, 2011). Diagnosis of active TB relies on chest radiography, demonstration of the presence of *Mycobacterium tuberculosis* in clinical specimens by serial sputum smear acid fast bacillus microscopy, molecular diagnostic technique (gene Xpert) and microbiological culture of sputum (Klopper *et al.*, 2013). On the other hand, diagnosis of latent TB relies on the tuberculin skin test (TST), molecular diagnostic technique or by blood tests such as interferon-gamma release assays (IGRAs). These TB tests work by detecting a cytokine called the interferon gamma cytokine. Two IGRAs that have been approved by the U.S. Food and Drug Administration (FDA), and are commercially available in the U.S., are the QuantiFERON® TB Gold test, and the T-SPOT® TB test (Core Curriculum on Tuberculosis, 2013). Tuberculosis remains unique among the major infectious diseases in lacking accurate and rapid point-of-care tests, largely due to insufficient progress in biomarker discovery. The most pressing priority in TB diagnostics research today is the development of a simple, low-cost, instrument-free rapid test. It has been reported that during *M. tuberculosis* infection, there is increased production of reactive oxygen species (ROS) also known as free radicals as a result of phagocyte respiratory burst (Deveci and Ilhan , 2003: Nnodim *et al*., 2011), and they play a major role in the etiology

of a wide variety of diseases including *M. tuberculosis* by causing significant damage to cell structures. Free radicals are cytotoxic and need to be removed by efficient antioxidant system (Akiibinu *et al*., 2009).In healthy conditions at the cellular level, a critical balance exists between the free radical generation and the antioxidant defense. Any changes in the level of these antioxidants are of key importance for cell viability and great deviations cause cell damage and death (Cooke *et al.*, 2003; Jones, 2008). The generation of lipid peroxides indicates the extent of lipid peroxidation and serves as a marker of cellular damage (Guteridge, 1995; Halliwell and Gutteridge, 2007). Tissue destruction is largely involved during active TB disease due to oxidative stress and *M. tuberculosis* influences the metabolism of affected tissues. It is quite likely that lung containing dormant bacteria during latent infection verse lung associated with progressive disease with excessive tissue damage will produce different metabolites. Similarly, there could be differential expression of host metabolites during TB disease progression (Wallis *et al.*, 2010). A combination of endogenous antioxidants e.g. catalase, glutathione, super-oxide dismutase and exogenous antioxidants e.g. vitamins A, C, E, bioflavonoids, carotenoids are required in adequate amount to remove reactive oxygen species (ROS) from the cell and to protect against oxidative damage (Ihim *et al*., 2013). It has been shown that plasma malondialdehyde (MDA) and copper (Cu) are increased in active tuberculosis prior to treatment (Deveci and Ilhan , 2003). It is therefore expected that a good response to antituberculosis therapy (ATT) on any individual will be able to show a gradual decline in the serum concentration of MDA and Cu .Therefore, studying the levels of serum antioxidants namely non-enzymatic antioxidants (vitamin C(Vit.C) , vitamin E(Vit.E) and selenium(Se),) and enzymatic antioxidants superoxide dismutase (SOD), glutathione reductase (GRX), glutathione peroxidase (GPX) and catalase (CAT)., lipid peroxides; malondialdehye (MDA), lipid profile ; cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol(LDL-C), high density lipoprotein cholesterol(HDL-C), triglyceride (TG), free fatty acids(FFA,) and apolipoproteins (apolipoproteins B (apo B) , apolipoproteins B48 (apo B48) and apolipoproteins B100 (apo B 100) in *Mycobacterium tuberculosis* infected individuals on treatment and those not on treatment may aid in the early detection of tuberculosis infection, disease manifestation and treatment progress.

1.2 Statement of the problem

The prevalence of tuberculosis has been on increase worldwide, with one third of the world population having latent *Mycobacterium tuberculosis* (WHO,2018). HIV/ AIDS epidemics have increased the burden of tuberculosis by enhancing the rate of tuberculosis acquisition and activation of latent *Mycobacterium tuberculosis* to active *Mycobacterium tuberculosis* (WHO, 2008). Screening for latent tuberculosis with Mantoux tests is rarely done in any of the major hospitals in Anambra state. Individuals identified as positive from the rare screening hardly get treated. These individuals and others who may have been missed to non screening have the potentials of progressing to active tuberculosis and infect other people. A vicious cycle of tuberculosis spread is thus set in motion. Sputum smear AFB microscopy, culture and gene Xpert used for the diagnosis of TB currently relies mostly on sputum. However, there are some patients who may not be able to produce sputum and so cannot be screened with it. These problems could result to more deaths. Therefore, it is pertinent to determine biomarkers that could be used for assessing active and latent tuberculosis for patients who can produce sputum and those who cannot. Previous researchers have identified some lipid profile parameters as possible diagnostic biomarkers for tuberculosis (Albanna *et al.,* 2013). The present study was aimed at evaluating the effect of *Mycobacterium tuberculosis* on the levels of serum antioxidants, malondialdehyde, lipid profile and apolipoproteins. Information gathered from this study may suggest the potential of using some of these parameters as prognostic tools for the diagnosis and management of individuals with *Mycobacterium tuberculosis* infection.

1.3 Justification for the study

M. tuberculosis infection is a serious public health challenge especially in developing countries. Tuberculosis infection is curable and preventable. However, treatment of the disease is difficult as a result of long duration of treatment, non compliance to treatment guidelines and emergence of resistant *Mycobacterium tuberculosis.* Treatment requires administration of multiple antibiotics for six months. The most important challenges in the history of tuberculosis has been the impact of the HIV epidemics which increases the rate at which *Mycobacterium tuberculosis* infection is acquired and the likelihood that people who are already infected will develop active tuberculosis (WHO, 2008).The search for appropriate prognostic biomarker may add to knowledge in the management of this condition. This work therefore seeks to determine the influence of *Mycobacterium tuberculosis* infection on antioxidant status and atherogenic markers.

1.4 Significance of the study

This study identified apolipoprotein B 100 to serve as a better marker of cardiovascular risk in individuals with active *Mycobacterium tuberculosis* infection during treatment compared to lipid profile. Furthermore, the measurements of the levels of GRX, Apo B 100 and MDA may serve as screening tests for the prognostic diagnosis of individuals with latent TB.This finding may contribute to efficient and effective management of such individuals.

1.5 Aim

To evaluate the status of some antioxidants, lipid peroxidation, apolipoproteins and atherogenic parameters before, at two months and six months of treatment in individuals with *Mycobacterium tuberculosis* infection.

1.6 Objectives of the study

1. To evaluate the antioxidant and lipid peroxidation status in individuals with active TB infection, latent TB infection and in apparently healthy control by determining the serum levels of lipid peroxidation (MDA), vitamin C (Vit. C) , vitamin E(Vit. E) ,selenium(Se), serum activities of superoxide dismutase (SOD), glutathione reductase (GRX), glutathione peroxidase (GPX) and catalase (CAT).
2. To determine the changes in atherogenic indices in individuals with active TB and latent TB infections and in the control group by measuring serum levels of cholesterol, very low density lipoprotein cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride, free fatty acid and apolipoprotein B (apo B), apolipoprotein B48 (apo B48) and apolipoprotein B100 (apo B100).
3. To evaluate the levels of these parameters in HIV seropositive individuals co-infected with TB and in individuals with active TB infection co- infected with malaria parasite.
4. To determine the levels of these parameters before, after two and six months treatment in individuals with active *Mycobacterium tuberculosis* infection.

1.7 Research questions

1. Are there differences in the serum levels of lipid peroxidation (MDA), vitamin C , vitamin E ,selenium, serum activities of superoxide dismutase, glutathione reductase, glutathione peroxidase and catalase in individuals with active TB infection, latent TB infection and in apparently healthy control?

2. Are there changes in serum levels of cholesterol, very low density lipoprotein, low density lipoprotein, high density lipoprotein, triglyceride, free fatty acid and apolipoproteins B , B48 and B100 in individuals with active TB and latent TB infection and in the control group?.

3. Are there differences in the serum levels of these parameters in HIV seropositive individuals co-infected with TB and in individuals with active TB infection co-infected with malaria parasite?

4 .Are there differences in the levels of these parameters before treatment, after two months and six months treatment?.

1.8 Null hypothesis (H_o)

1. There are no significant differences in the serum levels of lipid peroxidation (MDA), vitamin C , vitamin E ,selenium, serum activities of superoxide dismutase , glutathione reductase , glutathione peroxidase and catalase in individuals with active TB infection, latent TB infection and in apparently healthy control.

2. There are no changes in serum levels of cholesterol, very low density lipoprotein, low density lipoprotein, high density lipoprotein, triglyceride, free fatty acid and apolipoproteins B , B48 and B100 in individuals with active TB and latent TB infection and in the control group.

3. There are no significant differences in the serum levels of these parameters in HIV seropositive individuals co-infected with TB and in individuals with active TB infection co infected with malaria parasite.

4. There are no differences in the levels of these parameters before treatment, after 2months and 6months treatment.

1.9 Alternate hypothesis (H_1)

1. Significant differences exist in the serum levels of lipid peroxidation (MDA), vitamin C , vitamin E ,selenium, serum activities of superoxide dismutase , glutathione reductase , glutathione peroxidase and catalase in individuals with active TB infection, latent TB infection and in apparently healthy control.

2. There are changes in serum levels of cholesterol, very low density lipoprotein, low density lipoprotein, high density lipoprotein, triglyceride, free fatty acid and apolipoproteins B , B48 and B100 in individuals with active TB and latent TB infection and in the control group.

3. Significant differences exist in the serum levels of these parameters in HIV seropositive individuals co-infected with TB and in individuals with active TB infection co infected with malaria parasite.

4. There are significant differences in the levels of these parameters before treatment, after two months and six months treatment.

1.10 Definition of terms

a) Anti tuberculosis therapy (ATT)

They are antibiotics that are used for the treatment of tuberculosis patients.They are limited to Category one, first line drugs with respect to this study.

b) Category one, first line TB positive individuals.

This refers to newly diagnosed TB positive individuals who had not received ATT before and would be treated only with Category one, first line drugs.(they would receive for two months also referred to as Intensive phase (Rifampicin (R), Isoniazid (H), Pyrazinamide (Z), Ethambutol (E)(150/75/400/275mg/kg body weight) tablets and continuation phase RH(150/75 mg/kg body weight) tablets for four months to complete six months treatment duration.

c) Antioxidants

The term antioxidants used in this study represents superoxide dismutase (SOD) , catalase (CAT.), glutathione peroxidase (GPx), glutathione reductase (GRx), vitamin C (vit C),vitamin E (vit E) and selenium (Se). They are molecules that inhibit the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants stop these chain reactions.

d) Lipid peroxidation

Lipid peroxidation is a well defined mechanism of cellular damage in both animals and plants that occurs in vivo during ageing and in certain disease states. The common by-products of lipid peroxidation are malondialdehyde (MDA), 4-hydroxynonel(4-HNE) and 8-iso prostaglandin F2 alpha(8-isoprostane).

CHAPTER TWO

2.0 Literature review

2.1 *Mycobacterium Tuberculosis*

The main cause of TB is *Mycobacterium tuberculosis*, a small, aerobic, rod shaped, non motile bacillus that does not form spores (Gerald *et al.,*2010). The high lipid content of this pathogen accounts for many of its unique clinical characteristics. It divides every 16 to 20 hours, which is an extremely slow rate compared with other bacteria, which usually divide in less than an hour (Jindal, 2011). Mycobacteria have an outer membrane lipid bilayer (Niederweis *et al.*, 2010). Although, they do not stain readily but once stained, they resist decolorization by acid or alcohol and are therefore called 'acid fast bacilli'.If a Gram stain is performed, MTB either stains very weakly "Gram-positive" or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall. MTB can withstand weak disinfectants and survive in a dry state for weeks. In nature, the bacterium can grow only within the cells of a host organism, but *M. tuberculosis* can be cultured in the laboratory.Using histological stains on expectorated samples from phlegm (also called "sputum"), scientists can identify MTB under a microscope. Since MTB retains certain stains even after being treated with acidic solution, it is classified as an acid-fast bacillus (Kumar *et al.,* 2007).The most common acid-fast staining techniques are the Ziehl–Neelsen stain and the Kinyoun stain, which dye acid-fast bacilli a bright red that stands out against a blue background. Auramine-rhodamine staining and fluorescence microscopy, van Lettow, (2008) are also used. The *M. tuberculosis* complex (MTBC) includes four other TB-causing mycobacteria: *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti*. *M. africanum* is not widespread, but it is a significant cause of tuberculosis in parts of Africa. *M. bovis* was once a common cause of tuberculosis, but the introduction of pasteurized milk has almost completely eliminated this as a public health problem in developed countries. *M. canetti* is rare and seems to be limited to the Horn of Africa, although a few cases have been seen in African emigrants. *M. microti* is also rare and is seen almost only in immunodeficient people, although its prevalence may be significantly underestimated (Panteix *et al.,*2010).Other known pathogenic mycobacteria include *M. leprae*, *M. avium*, and *M. kansasii*. The latter two species are classified as "nontuberculous mycobacteria" (NTM). NTM cause neither TB nor leprosy, but they do cause pulmonary diseases that resemble tuberculosis.

The general signs and symptoms of tuberculosis include fever, chills, night sweats, loss of appetite, weight loss, and fatigue (Gerald *et al.*,2010). Significant nail clubbing may also occur. Tuberculosis may infect any part of the body, but most commonly occurs in the lungs (known as pulmonary tuberculosis). Extrapulmonary TB occurs when tuberculosis develops outside of the lungs, although extrapulmonary TB may coexist with pulmonary TB. However, if pulmonary tuberculosis infection does become active, it most commonly involves the lungs in about 90% of cases (Behera, 2010).Symptoms may include chest pain and a prolonged cough producing sputum. About 25% of people may not have any symptoms (i.e. they remain "asymptomatic"). Occasionally, people may cough up blood in small amounts, and in very rare cases, the infection may erode into the pulmonary artery or a Rasmussen's aneurysm, resulting in massive bleeding (Gerald *et al*., 2010; Halezeroğlu and Okur, 2014). Tuberculosis may become a chronic illness and cause extensive scarring in the upper lobes of the lungs. The upper lung lobes are more frequently affected by tuberculosis than the lower ones. The reason for this difference is not clear. It may be due either to better air flow, or to poor lymph drainage within the upper lungs (Gerald *et al*., 2010). Moreover, in 15–20% of active cases, the infection spreads outside the lungs, causing other kinds of TB (Jindal, 2011). These are collectively denoted as "extrapulmonary tuberculosis".Extrapulmonary TB occurs more commonly in immunosuppressed persons and young children. In those with HIV, this occurs in more than 50% of cases. Notable extrapulmonary infection sites include the pleura (in tuberculous pleurisy), the central nervous system (in tuberculous meningitis), the lymphatic system (in scrofula of the neck), the genitourinary system (in urogenital tuberculosis), and the bones and joints (in Pott disease of the spine), among others. When it spreads to the bones, it is also known as "osseous tuberculosis", a form of osteomyelitis. Sometimes, bursting of a tubercular abscess through skin results in tuberculous ulcer. An ulcer originating from nearby infected lymphnodes is painless, slowly enlarging and has an appearance of "wash leather". A potentially more serious, widespread form of TB is called "disseminated tuberculosis", also known as miliary tuberculosis. Miliary TB makes up about 10% of extrapulmonary cases. Miliary TB occurs when tubercle bacilli enter the bloodstream and disseminate to all parts of the body, where they grow and cause disease in multiple sites. This condition is rare but serious. “Miliary” refers to the radiograph appearance of millet seeds scattered throughout the lung. It is most common in infants and children younger than 5 years of age, and in severely immunocompromised persons. Miliary TB

may be detected in an individual organ, including the brain; in several organs; or throughout the whole body. The condition is characterized by a large amount of TB bacilli, although it may easily be missed, and is fatal if untreated. Up to 25% of patients with miliary TB may have meningeal involvement (Core Curriculum on Tuberculosis, 2013).When tuberculosis occurs in the tissue surrounding the brain or spinal cord, it is called tuberculous meningitis. Tuberculous meningitis is often seen at the base of the brain on imaging studies. Symptoms include headache, decreased level of consciousness, and neck stiffness. The duration of illness before diagnosis is variable and relates in part to the presence or absence of other sites of involvement. In many cases, patients with meningitis have abnormalities on a chest radiograph consistent with old or current TB, and often have miliary tuberculosis (Core Curriculum on Tuberculosis, 2013).

2.1.1 Risk factors for tuberculosis

A number of factors make people more susceptible to TB infections. The most important risk factor globally is HIV, 13% of all people with TB are infected by the virus (WHO, 2015). This is a particular problem in sub-Saharan Africa, where rates of HIV are high (Chaisson and Martinson, 2008). Of people without HIV who are infected with tuberculosis, about 5–10% develop active disease during their lifetimes; in contrast, 30% of those coinfected with HIV develop the active disease.

Tuberculosis is closely linked to both overcrowding and malnutrition, making it one of the principal diseases of poverty (Lawn and Zumla, 2011). Those at high risk thus include: people who inject illicit drugs, inhabitants and employees of locales where vulnerable people gather (e.g. prisons and homeless shelters), medically underprivileged and resource-poor communities, high-risk ethnic minorities, children in close contact with high-risk category patients, and health-care providers serving these patients.Chronic lung disease is another significant risk factor. Silicosis increases the risk about 30-fold (AST/CDC ,2000). Those who smoke cigarettes have nearly twice the risk of TB compared to nonsmokers (van Zyl Smit *et al.*,2010).Other disease states can also increase the risk of developing tuberculosis. These include alcoholism Lawn and Zumla, (2011) and diabetes mellitus (three-fold increase)(Restrepo, 2007). Certain medications, such as corticosteroids and infliximab (an anti-αTNF monoclonal antibody), are becoming increasingly important risk factors, especially in the developed world.Genetic susceptibility also exists, Möller and Hoal, (2010), for which the overall importance remains undefined.

2.1.2 Transmission of tuberculosis

When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 μm in diameter. A single sneeze can release up to 40,000 droplets. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very small (the inhalation of fewer than 10 bacteria may cause an infection) (Ahmed and Hasnain ,2011).People with prolonged, frequent, or close contact with people with TB are at particularly high risk of becoming infected, with an estimated 22% infection rate. A person with active but untreated tuberculosis may infect 10–15 (or more) other people per year. Transmission should occur from only people with active TB – those with latent infection are not thought to be contagious. The probability of transmission from one person to another depends upon several factors, including the number of infectious droplets expelled by the carrier, the effectiveness of ventilation, the duration of exposure, the virulence of the *M. tuberculosis* strain, the level of immunity in the uninfected person, and others. The cascade of person-to-person spread can be circumvented by segregating those with active ("overt") TB and putting them on anti-TB drug regimens. After about two weeks of effective treatment, subjects with nonresistant active infections generally do not remain contagious to others. If someone does become infected, it typically takes three to four weeks before the newly infected person becomes infectious enough to transmit the disease to others.

2.1.3 Pathogenesis of tuberculosis

About 90% of those infected with *M. tuberculosis* have asymptomatic, latent TB infections (sometimes called LTBI), with only a 10% lifetime chance that the latent infection will progress to overt, active tuberculous disease. In those with HIV, the risk of developing active TB increases to nearly 10% a year. If effective treatment is not given, the death rate for active TB cases is up to 66%.TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages. Macrophages identify the bacterium as foreign and attempt to eliminate it by phagocytosis. During this process, the bacterium is enveloped by the macrophage and stored temporarily in a membrane-bound vesicle called a phagosome. The phagosome then combines with a lysosome to create a phagolysosome. In the phagolysosome, the cell attempts to use reactive oxygen species and acid to kill the bacterium. However, *M. tuberculosis* has a thick, waxy mycolic acid capsule that

protects it from these toxic substances. *M. tuberculosis* is able to reproduce inside the macrophage and will eventually kill the immune cell.The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe. Tuberculosis of the lungs may also occur through infection from the blood stream. This is known as a Simon focus and is typically found in the top of the lung. This hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and the bones. All parts of the body can be affected by the disease, though for unknown reasons it rarely affects the heart, skeletal muscles, pancreas, or thyroid.Tuberculosis is classified as one of the granulomatous inflammatory diseases. Macrophages, T lymphocytes, B lymphocytes, and fibroblasts aggregate to form granulomas, with lymphocytes surrounding the infected macrophages. When other macrophages attack the infected macrophage, they fuse together to form a giant multinucleated cell in the alveolar lumen. The granuloma may prevent dissemination of the mycobacteria and provide a local environment for interaction of cells of the immune system. However, more recent evidence suggests that the bacteria use the granulomas to avoid destruction by the host's immune system. Macrophages and dendritic cells in the granulomas are unable to present antigen to lymphocytes; thus the immune response is suppressed (Bozzano, 2014). Bacteria inside the granuloma can become dormant, resulting in latent infection. Another feature of the granulomas is the development of abnormal cell death (necrosis) in the center of tubercles. To the naked eye, this has the texture of soft, white cheese and is termed caseous necrosis. If TB bacteria gain entry to the blood stream from an area of damaged tissue, they can spread throughout the body and set up many foci of infection, all appearing as tiny, white tubercles in the tissues (Crowley and Leonard , 2010). This severe form of TB disease, most common in young children and those with HIV, is called miliary tuberculosis. People with this disseminated TB have a high fatality rate even with treatment (about 30%)(Jacob *et al.,* 2009).In many people, the infection waxes and wanes. Tissue destruction and necrosis are often balanced by healing and fibrosis. Affected tissue is replaced by scarring and cavities filled with caseous necrotic material. During active disease, some of these cavities are joined to the air passages bronchi and this material can be coughed up. It contains living bacteria, so can spread the infection. Treatment with appropriate antibiotics kills bacteria and allows healing to take place. Upon cure, affected areas are eventually replaced by scar tissue.

Mycobacteria infect many different animals, including birds, rodents, and reptiles (Shivaprasad and Palmieri, 2012). The subspecies *Mycobacterium tuberculosis*, though, is rarely present in wild animals (Reavill and Schmidt, 2012). An effort to eradicate bovine tuberculosis caused by *Mycobacterium bovis* from the cattle and deer herds of New Zealand has been relatively successful (Mitchell ,2012). Efforts in Great Britain have been less successful. As of 2015, tuberculosis appears to be widespread among captive elephants in the US. It is believed that the animals originally acquired the disease from humans, a process called reverse zoonosis. Because the disease can spread through the air to infect both humans and other animals, it is a public health concern affecting circuses and zoos.(White *et al.*,2008; Ward *et al.,* 2010).

2.1.4 Biochemistry of *Mycobacterium tuberculosis* infection

The *Mycobacterium tuberculosis* cytochrome P450 enzyme CYP142 is encoded in a large gene cluster involved in metabolism of host cholesterol. CYP142 was expressed and purified as a soluble low spin P450 hemoprotein CYP142 binds tightly to cholesterol and its oxidized derivative cholest-4-en -3-one, with extensive shift of the heme iron to the high spin state (Driscoll *et al*., 2010). High affinity for azole antibiotics was demonstrated, highlighting their therapeutic potential. CYP142 catalyzes either 27hydroxylation of cholesterol/ cholest-4-en -3-one or generates 5- cholestenoic acid / cholest-4-en -3-one-27-oic acid from these substrates by successive sterol oxidations, with the catalytic outcome dependent on the redox partner system used (Driscoll *et al*., 2010).The CYP142 crystal structure was solved to1.6A., revealing a similar active site organization to the cholesterol-metabolizing *M. tuberculosis* CYP125, but having a near-identical organization of distal pocket residues to the branched fatty acid oxidizing *M. tuberculosis* CYP124.The cholesterol oxidizing activity of CYP142 provides an explanation for previous findings that CYP125 strains of *Mycobacterium bovis* and *M. bovis* BCG cannot grow on cholesterol, because these strains have a defective CYP142gene. CYP142 is revealed as a cholesterol 27 oxidase with likely roles in host response modulation and cholesterol metabolism. Moreover, the enzyme 3-deoxy-D-arabino-heptulosonate7-phosphate synthase (DAH7PS), catalyzes the first step of the shikimate pathway for the biosynthesis of aromatic compounds. This path way has been shown to be essential in *Mycobacterium tuberculosis,* the pathogen responsible for tuberculosis. DAH7PS catalyzes a condensation reaction between P-enolpyruvate and erythrose 4 –phosphate to give 3-deoxy-D-arabino-heptulosonate-7-phosphate. The enzyme

reaction mechanism is proposed to include a tetrahedral intermediate, which is formed by attack of active site water on the central carbon of P-enol pyruvate during the course of the reaction. Molecular modeling of this intermediate into the active site reported in this study shows a configurational preference consistent with water attack from the re face of P-enolpyruvate (Reichau *et al.,* 2011). Based on this model, an inhibitor of DAH7PS that mimics this reaction intermediate was designed and synthesized. Both enantiomers of this intermediate mimic were potent inhibitors of *Mycobacterium tuberculosis* DAH7PS, with inhibitory constant in the nano molar range. The crystal structure of the DAH7PS- inhibitor complex was solved to 2.35A. Both the position of the inhibitor and the conformational changes of active site residues observed in this structure correspond closely to the predictions from the intermediate modeling. This structure also identifies a water molecule that is located in the appropriate position to attack the re face of P- enolpyruvate during the course of the reaction, allowing the catalytic mechanism for this enzyme to be clearly defined. 3- Deoxy-D- arabino- heptulosonate 7- phosphate synthase (DAH7PS) is an important enzyme in *Mycobacterium tuberculosis* and other pathogens. It catalyzes the first committed step pathway in the shikimate pathway, which is responsible for the biosynthesis of aromatic amino acids and other essential aromatic metabolites in micro organisms, plants, and apicomplexan parasites (Reichau *et al.,* 2011). This pathway is absent in humans and inhibitors of amino acid biosynthesis have been shown to be effective antimicrobial and herbicidal agents . Gene disruption studies have demonstrated that *M. tuberculosis* is not viable if the shikimate pathway is not operational (Reichau *et al.,* 2011). These findings make DAH7PS an attractive target for drug development.

2.1.5 Diagnosis of *Mycobacterium tuberculosis*

Most persons with TB disease have one or more symptoms that lead them to seek medical care while some persons may not have any symptoms of TB disease. Those persons with symptoms of TB disease or either a positive tuberculin skin test (TST) or an interferon-gamma release assay (IGRA) indicative of *M. tuberculosis* infection, should be medically evaluated to exclude TB disease (Gerald *et al*., 2010).A complete medical evaluation for TB disease includes the following five components; Medical history, Physical examination, Test for *M. tuberculosis* infection,Chest radiograph and Bacteriologic examination of clinical specimens.When conducting a medical history, the clinician should ask if any symptoms of TB disease are

present; if so, for how long, and if there has been known exposure to a person with infectious TB disease. Equally important is obtaining information on whether or not the person has been diagnosed in the past with latent tuberculosis infection (LTBI) or TB disease. Clinicians may also contact the local health department for information on whether a patient has a past history of TB infection or disease. If the previous treatment regimen for TB disease was inadequate or if the patient did not adhere to therapy, TB disease may recur and possibly be drug-resistant. It is important to consider demographic factors (e.g., country of origin, age, ethnicity, occupation, or racial group) that may increase the patient's risk for being exposed to TB infection. Clinicians should determine if the patient has underlying medical conditions, especially human immunodeficiency virus (HIV) infection or diabetes, that increase the risk for progression to TB disease in those latently infected with *M. tuberculosis* (Core Curriculum on Tuberculosis, 2013). A physical examination is an essential part of the evaluation of any patient. It cannot be used to confirm or rule out TB disease, but it can provide valuable information about the patient's overall condition, inform the method of diagnosis, and reveal other factors that may affect TB disease treatment, if diagnosed. Selection of the most suitable tests for detection of *M. tuberculosis* infection should be based on the reasons and the context for testing, test availability, and overall cost effectiveness of testing. Currently, there are two methods available for the detection of *M. tuberculosis* infection in the United States. The tests are: Mantoux tuberculin skin test (TST) and Interferon-gamma release assays (IGRAs)*QuantiFERON-TB Gold In-Tube test (QFT-GIT) ;T-SPOT®.*TB* test. Furthermore, with pulmonary TB being the most common form of disease, the chest radiograph is useful for diagnosis of TB disease. Chest abnormalities can suggest pulmonary TB disease (Figure 2.1). A posterior-anterior radiograph of the chest is the standard view used for the detection of TB-related chest abnormalities. In some cases, especially in children, a lateral view may be helpful (Core Curriculum on Tuberculosis, 2013).

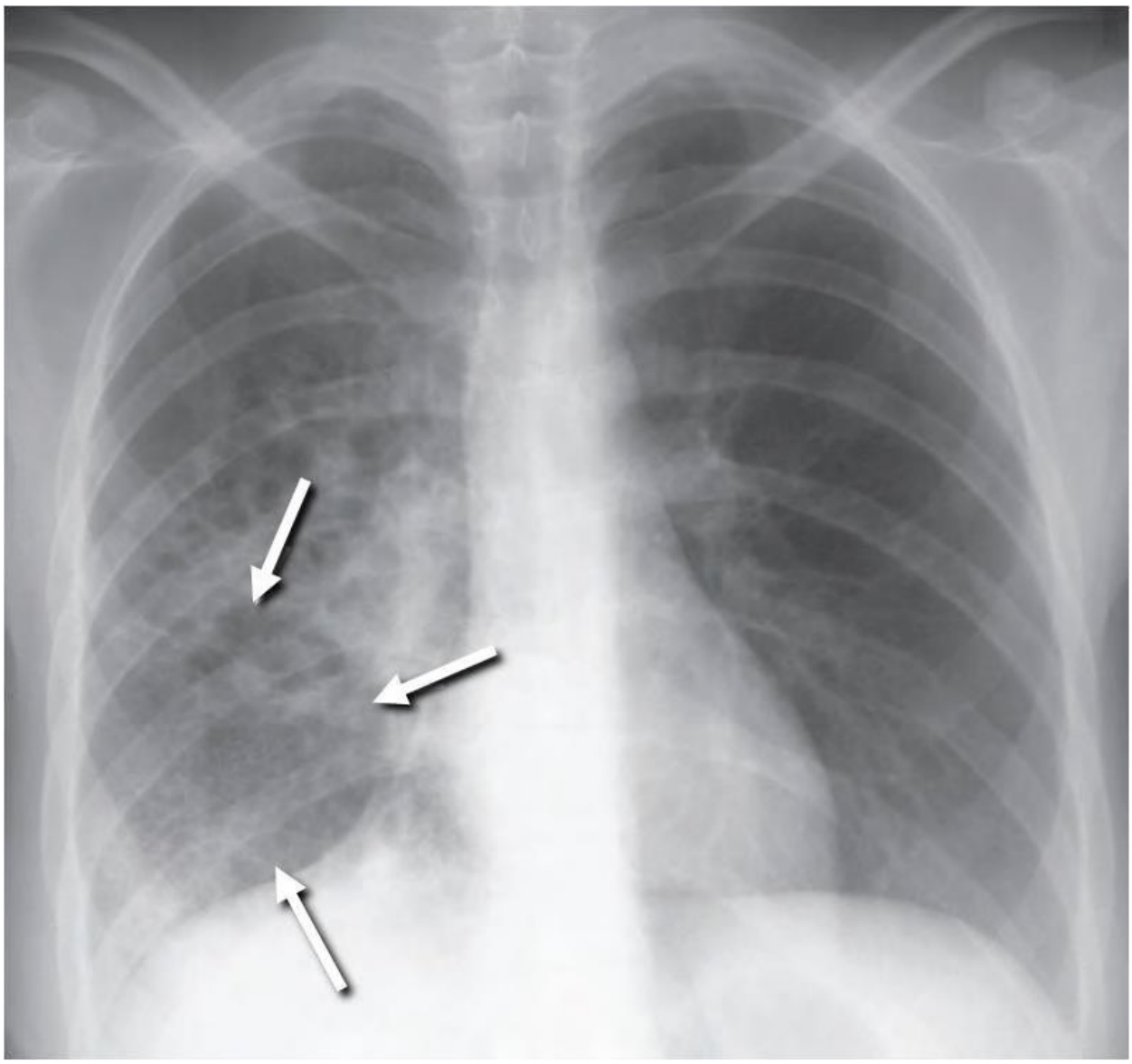

Figure 2.1 Chest radiograph with lower lobe cavity (Core Curriculum on Tuberculosis, 2013).

In some instances, a computerized tomography (CT) scan may provide additional information. A CT scan provides more detailed images of parts of the body that cannot easily be seen on a standard chest radiograph; however, CT scans can be substantially more expensive.In pulmonary TB disease, radiographic abnormalities are often seen in the apical and posterior segments of the upper lobe or in the superior segments of the lower lobe. However, lesions may appear anywhere in the lungs and may differ in size, shape, density, and cavitation, especially in HIV-infected and other immunosuppressed persons. Radiographic abnormalities in children tend to be minimal with a greater likelihood of lymphadenopathy, more easily diagnosed on the lateral film.Mixed nodular and fibrotic lesions may contain slowly multiplying tubercle bacilli and have the potential for progression to TB disease. Persons who have lesions consistent with findings of "old" TB disease on a chest radiograph and have a positive TST reaction or positive IGRA result Bento, (2011) should be considered high-priority candidates for treatment of LTBI (Treatment for Latent Tuberculosis Infection), but only after TB disease is excluded by obtaining three specimens for AFB smear and culture because "old" TB cannot be differentiated from active TB disease based on radiographic appearance alone. Conversely, fully calcified, discrete, nodular lesions without fibrosis likely represent granulomas and pose a lower risk for future progression to TB disease (Core Curriculum on Tuberculosis, 2013). In HIV-infected persons, pulmonary TB disease may present with atypical findings or with no lesions seen on the chest radiograph. The radiographic appearance of pulmonary TB disease in persons infected with HIV might be typical; however, cavitary disease is less common among such patients. More common chest radiograph findings for HIV-infected persons include infiltrates in any lung zone, mediastinal or hilar adenopathy, or, occasionally, a normal chest radiograph. Typical cavitary lesions are usually observed in patients with higher CD4 counts, and more atypical patterns are observed in patients with lower CD4 counts because cavitation is thought to occur as a result of the immune response to TB organisms. In HIV-infected persons, almost any abnormality on a chest radiograph may be indicative of TB disease. In patients with symptoms and signs of TB disease, a negative chest radiograph result does not exclude TB disease. Abnormalities seen on chest radiographs may be suggestive of, but are never diagnostic of TB disease. Chest radiographs may be used to exclude pulmonary TB disease in an HIV-negative person who has a positive TST reaction or IGRA and who has no symptoms or signs of TB disease (Core Curriculum on Tuberculosis, 2013).

2.1.6 Bacteriologic examination of clinical specimens

Examinations of clinical specimens (e.g., sputum, urine, or cerebrospinal fluid) are of critical diagnostic importance (Bento *et al.,* 2011). The specimens should be examined and cultured in a laboratory that specializes in testing for *M. tuberculosis*. The bacteriologic examination has five parts:

i.Specimen collection, processing, and review

ii.AFB smear classification and results

iii.Direct detection of *M. tuberculosis* in clinical specimen using nucleic acid amplification (NAA)

iv.Specimen culturing and identification

v.Drug-susceptibility testing

2.1.7 Specimen collection, processing, and review.

All persons suspected of having TB disease at any site should have sputum specimens collected for an AFB smear and culture, including those with or without respiratory symptoms. At least three consecutive sputum specimens are needed, each collected in 8- to 24-hour intervals, with at least one being an early morning specimen. If possible, specimens should be obtained in an airborne infection isolation (AII) room or other isolated, well-ventilated area (e.g., outdoors) (Lawn and Zumla, 2011). During specimen collection, patients produce an aerosol that may be hazardous to health-care workers or other patients in close proximity. For this reason, precautionary measures for infection control must be followed during sputum induction, bronchoscopy, and other common diagnostic procedures. (Core Curriculum on Tuberculosis, 2013).

2.1.8 Specimen collection methods for pulmonary tuberculosis disease

The four specimen collection methods for pulmonary TB disease are by coughing, induced sputum, bronchoscopy and gastric aspiration.

2.1.9 Coughing

Coughing is the most commonly used method of sputum collection. Coughing should be supervised to ensure that sputum is collected correctly. A health-care worker wearing the

recommended personal protective equipment should coach and directly supervise the patient when sputum is collected. Patients should be informed that sputum is the material brought up from the lungs, and that mucus from the nose or throat and saliva are not good specimens (Lawn and Zumla, 2011). Unsupervised patients are less likely to provide an adequate specimen, especially the first time.

2.1.10 Sputum induction– Sputum is induced for patients unable to cough up sputum, deep sputum-producing coughing may be induced by inhalation of an aerosol of warm, sterile, hypertonic saline (3%– 5%). Induced sputum is very watery and resembles saliva, and should be labeled "induced" to ensure that the laboratory staff workers do not discard it (Core Curriculum on Tuberculosis, 2013).

2.1.11 Bronchoscopy– A bronchoscopy is a medical procedure that allows visualization of the inside of a person's airways. The airways are called the bronchial tubes or bronchi. Bronchoscopy might be needed for specimen collection, especially if previous results have been non diagnostic and doubt exists as to the diagnosis. At other times, bronchoscopy is considered because TB is among several other diagnoses being considered. If possible, examine three spontaneous or induced sputum to exclude a diagnosis of TB disease before bronchoscopy. If possible, avoid bronchoscopy on patients with suspected or confirmed TB disease or postpone the procedure until the patient is determined to be noninfectious, by confirmation of the three negative AFB sputum smear results. Bronchial washings, brushings, and biopsy specimens may be obtained, depending on the bronchoscopy findings. Sputum collected after a bronchoscopy may also be useful for a diagnosis. A bronchoscopy should never be substituted for sputum collection, but rather used as an additional diagnostic procedure.

Whenever feasible, bronchoscopy should be performed in a room that meets the ventilation requirements for an airborne infection isolation (AII) room. Health-care workers should wear N95 respirators while present during a bronchoscopy procedure on a patient with suspected or confirmed infectious TB disease (Core Curriculum on Tuberculosis, 2013)..

2.1.12 Gastric aspiration

Gastric aspiration is a procedure sometimes used to obtain a specimen for culture when a patient cannot cough up adequate sputum. A tube is inserted through the mouth or nose and into the

stomach to recover sputum that was coughed into the throat and then swallowed. This procedure is particularly useful for diagnosis in children, who are often unable to cough up sputum . Gastric aspiration often requires hospitalization and should be done in the morning before the patient gets out of bed or eats, as it is the optimal time to collect swallowed respiratory secretions from the stomach. Specimens obtained by gastric aspiration should be transported to the lab immediately for neutralization or neutralized immediately at the site of collection (Core Curriculum on Tuberculosis, 2013).

2.1.13 Specimen collection methods for extrapulmonary tuberculosis

Tuberculosis disease can occur in almost any anatomical site Thomas *et al.*,(2008), thus, a variety of clinical specimens other than sputum (e.g., urine, cerebrospinal fluid, pleural fluid, pus, or biopsy specimens) may be submitted for examination when extra pulmonary TB disease is suspected. Sample containers that can be used include universal container for urine, pleural fluid, plain tube for pus, cerebrospinal fluid and biopsy container for biopsy. Procedures for the expeditious and recommended handling of the specimen must be in place or assured before the specialist performs an invasive procedure to obtain the specimen. Especially important is rapid transportation to the laboratory according to the laboratory's instructions. It is important to note that the portion of the specimen placed in formalin for histologic examination cannot be used for culture (Core Curriculum on Tuberculosis, 2013).

2.1.14 AFB smears classification and results

Detection of acid-fast bacilli in stained and acid-washed smears examined microscopically may provide the initial bacteriologic evidence of the presence of mycobacteria in a clinical specimen (Figure 2.2). Smear microscopy is the quickest and easiest procedure that can be performed.

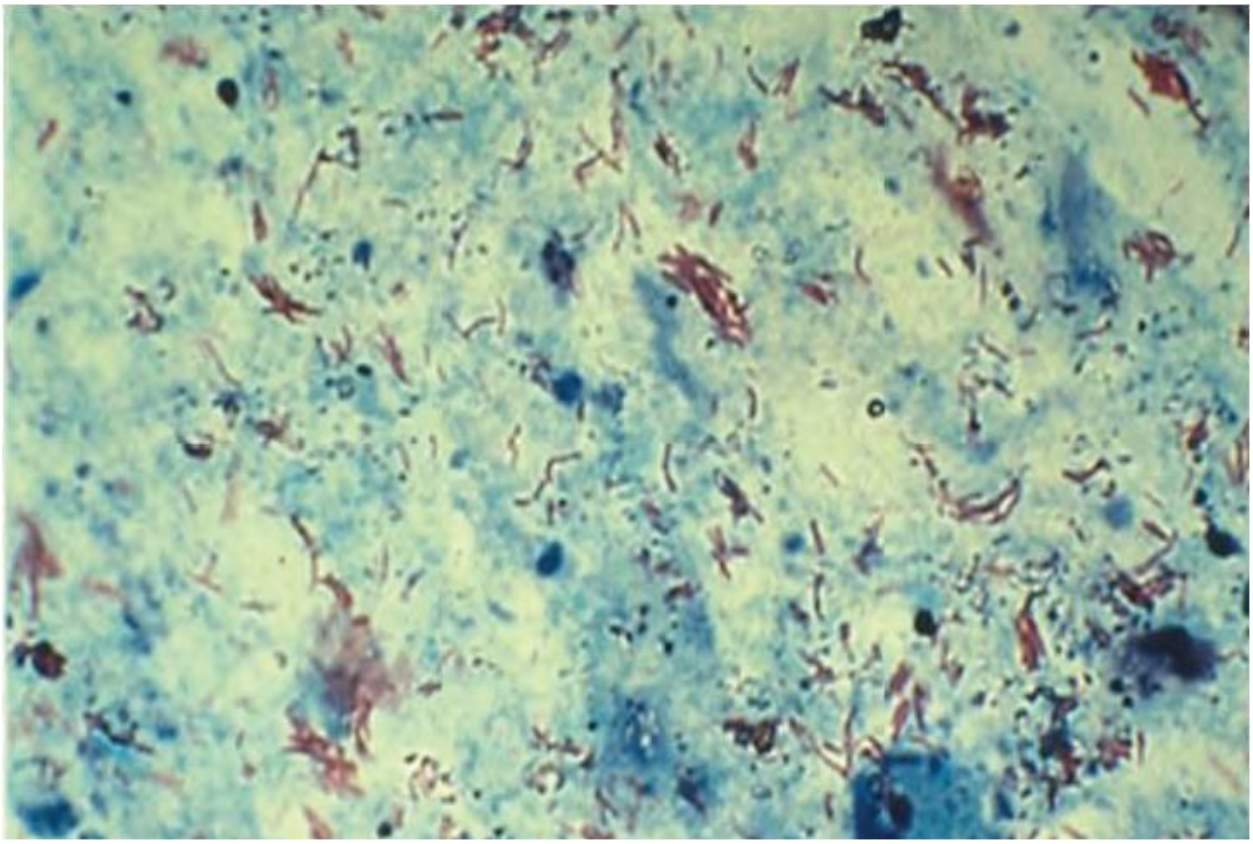

Plate 2.2 Acid-fast bacilli stained in smear tubercle bacilli are shown in red (Lawn and Zumla, 2011)

There are two procedures commonly used for acid-fast staining: Carbolfuchsin methods, Lawn and Zumla, (2011) which include the Ziehl-Neelsen and Kinyoun methods (direct microscopy).

2.1.15 Fluorochrome procedure using auramine-O or auramine-rhodamine dyes (fluorescent microscopy)(Cheesbrough, 2004).

Studies have shown that there must be 5,000 to 10,000 bacilli per milliliter of specimen to allow the detection of bacteria in stained smears. In contrast, 10 to 100 bacilli are needed for a positive culture. Smear examination is a quick procedure; results should be available within 24 hours of specimen collection when specimens are delivered to the laboratory promptly. However, smear examination permits only the presumptive diagnosis of TB disease because the acid-fast bacilli in a smear may be acid-fast organisms other than *M. tuberculosis*. Also, many TB patients have negative AFB smears with a subsequent positive culture. Negative smears do not exclude TB disease (Table 2.1).When acid-fast bacilli are seen in a smear, they are counted. There is a system for reporting the number of acid-fast bacilli that are seen at a certain magnification. According to the number of acid-fast bacilli seen, the smears are classified as 4+, 3+, 2+, or 1+. The greater the number, the more infectious the patient (Core Curriculum on Tuberculosis, 2013). (Table 2.1).

Table 2.1 Smear classification results (Core Curriculum on Tuberculosis, 2013)

Smear Result (Number of AFB observed at 1000X magnification)	Smear Interpretation	Infectiousness of Patient
4+ (>9/field)	Strongly positive	Probably very infectious
3+ (1-9/field)	Strongly positive	Probably very infectious
2+ (1-9/10 fields)	Moderately positive	Probably infectious
1+ (1-9/100 fields)	Moderately positive	Probably infectious
+/- (1-2/300 fields)*	Weakly positive†	Probably infectious
No acid-fast bacilli seen	Negative	Probably not infectious**

* There are variations on labeling for this result, and include listing the number of AFB counted.

† Laboratories may report these smear results as "doubtful" or "inconclusive" based on CDC guidelines.

2.1.16 Direct detection of *M. tuberculosis* in clinical specimen using nucleic acid amplification (NAA) (Core Curriculum on Tuberculosis, 2013)

NAA tests are used to amplify DNA and RNA segments to rapidly identify the microorganisms in a specimen. NAA testing can reliably detect *M. tuberculosis* bacteria in specimens in hours as compared to 1 week or more for culture (Figure 2.3). Possible benefits of using NAA tests include earlier laboratory confirmation of TB disease, earlier treatment initiation, improved patient outcomes, interruption of transmission by early diagnosis, respiratory isolation and appropriate treatment, earlier, more efficient use of respiratory isolation, earlier initiation of contact investigation and more effective public health interventions. CDC recommends that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities, such as contact investigations.

Fig. 2.3 Nucleic Acid Amplification (NAA) Test kit (NAA) **(Core Curriculum on Tuberculosis, 2013)**

Negative NAA test result should not be used as a definitive result to exclude TB disease, especially when the clinical suspicion of TB disease is moderate to high. Rather, the negative NAA test result should be used as additional information in making clinical decisions, to expedite testing for an alternative diagnosis, or to prevent unnecessary TB disease treatment.Culture remains the gold standard for laboratory confirmation of TB disease, and growing bacteria are required to perform drug-susceptibility testing and genotyping. In accordance with current recommendations, sufficient numbers and portions of specimens should always be reserved for culture. Nonetheless, NAA testing should become standard practice for patients suspected of having TB, and all clinicians and public health TB programs should have access to NAA testing for TB to shorten the time to diagnosis. Diagnosing active tuberculosis based only on signs and symptoms is difficult, Bento *et al.*, (2011), as is diagnosing the disease in those who are immunosuppressed (Escalante, 2009). A diagnosis of TB should, however, be considered in those with signs of lung disease or constitutional symptoms lasting longer than two weeks (Escalante, 2009). A chest X-ray and multiple sputum cultures for acid-fast bacilli are typically part of the initial evaluation (Escalante, 2009). Interferon-γ release assays and tuberculin skin tests are of little use in the developing world (Metcalfe *et al.*, 2011; Sester *et al*., 2011) IGRA have similar limitations in those with HIV. Chen *et al*., 2011; Sester *et al*., 2011).

A definitive diagnosis of TB is made by identifying *M. tuberculosis* in a clinical sample (e.g., sputum, pus, or a tissue biopsy). However, the difficult culture process for this slow-growing organism can take two to six weeks for blood or sputum culture. Thus, treatment is often begun before cultures are confirmed. Nucleic acid amplification tests and adenosine deaminase testing may allow rapid diagnosis of TB (Bento, 2011). These tests, however, are not routinely recommended, as they rarely alter how a person is treated. Blood tests to detect antibodies are not specific or sensitive, so they are not recommended (Steingart *et al.*, 2011*).*

2.1.17 Latent tuberculosis

Persons with latent TB infection do not feel sick and do not have any symptoms. They are infected with *M. tuberculosis*, but do not have TB disease. The only sign of TB infection is a positive reaction to the tuberculin skin test or TB blood test. Persons with latent TB infection are not infectious and cannot spread TB infection to others. However, without treatment, about 5 to 10% of infected persons will develop TB disease at some time in their lives.

2.1.18. The Mantoux tuberculin skin test

It is often used to screen people at high risk for TB (Escalante, 2009). Those who have been previously immunized may have a false-positive test result. The test may be falsely negative in those with sarcoidosis, Hodgkin's lymphoma, malnutrition, and most notably, active tuberculosis. Interferon gamma release assays (IGRAs), on a blood sample, are recommended in those who are positive to the Mantoux test. These are not affected by immunization or most environmental mycobacteria, so they generate fewer false-positive results (Pai *et al.*,2008). However, they are affected by *M. szulgai*, *M. marinum*, and *M. kansasii*(Jindal, 2011). IGRAs may increase sensitivity when used in addition to the skin test, but may be less sensitive than the skin test when used alone (Amicosante *et al.*, 2010).

2.1.19 Prevention of tuberculosis

Tuberculosis prevention and control efforts rely primarily on the vaccination of infants and the detection and appropriate treatment of active cases. The World Health Organization has achieved some success with improved treatment regimens, and a small decrease in case numbers (Lawn and Zumla, 2011). The US Preventive Services Task Force recommends screening people who are at high risk for latent tuberculosis with either tuberculin skin tests or interferon-gamma release assays (Bibbins-Domingo *et al*.,2016).

2.1.20.Tuberculosis vaccines and BCG vaccine

The only available vaccine as of 2011 is Bacillus Calmette-Guérin (BCG)(McShane, 2011). It decreases the risk of getting the infection by 20% in children and the risk of infection turning into disease by nearly 60% (Roy *et al*., 2014). It is the most widely used vaccine worldwide, with more than 90% of all children being vaccinated. The immunity it induces decreases after about ten years (Lawn and Zumla, 2011). As tuberculosis is uncommon in most of Canada, the United Kingdom, and the United States, BCG is administered only to those people at high risk. Part of the reasoning against the use of the vaccine is that it makes the tuberculin skin test falsely positive, reducing the test's use in screening (BCG Vaccine Usage in Canada, 2010). A number of new vaccines are currently in development (Lawn and Zumla 2011). The BCG vaccine has limitations, and research to develop new TB vaccines is ongoing. A number of potential

candidates are currently in phase I and II clinical trials. Two main approaches are being used to attempt to improve the efficacy of available vaccines. One approach involves adding a subunit vaccine to BCG, while the other strategy is attempting to create new and better live vaccine (Courtwright and Turner, 2010). MVA85A, an example of a subunit vaccine, currently in trials in South Africa, is based on a genetically modified vaccinia virus (Mason *et al.*, 2015). Vaccines are hoped to play a significant role in treatment of both latent and active disease.To encourage further discovery, researchers and policymakers are promoting new economic models of vaccine development, including prizes, tax incentives, and advance market commitments (Kaufmann, 2010). A number of groups, including the Stop TB Partnership, the South African Tuberculosis Vaccine Initiative, and the Aeras Global TB Vaccine Foundation, are involved with research. Among these, the Aeras Global TB Vaccine Foundation received a gift of more than $280 million (US) from the Bill and Melinda Gates Foundation to develop and license an improved vaccine against tuberculosis for use in high burden countries(Jane *et al.*,2010).

2.1.21.Tuberculosis management

Treatment of TB uses antibiotics to kill the bacteria. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which hinders the entry of drugs and makes many antibiotics ineffective. The two antibiotics most commonly used are isoniazid and rifampicin, and treatments can be prolonged, taking several months. Latent TB treatment usually employs a single antibiotic, Menzies *et al*., (2011) while active TB disease is best treated with combinations of several antibiotics to reduce the risk of the bacteria developing antibiotic resistance (Lawn and Zumla 2011). People with latent infections are also treated to prevent them from progressing to active TB disease later in life (Menzies *et al*., 2011). Unfortunately, treatment of latent TB is either not common in Africa especially Nigeria or not practiced at all more especially in Nnamdi Azikiwe University TB DOT Clinic as at now.

Directly observed Treatment therapy, i.e., having a health care provider watch the person take their medications, is recommended by the WHO in an effort to reduce the number of people not appropriately taking antibiotics (Arch and Mainous, 2010). The evidence to support this practice over people simply taking their medications independently is poor. Methods to remind people of the importance of treatment do, however, appear effective (Liu *et al.*, 2008). A number of medications are being studied for multi drug resistant tuberculosis including: bedaquiline and

delamanid. Bedaquiline received U.S. Food and Drug Administration (FDA) approval in late 2012. The safety and effectiveness of these new agents are still uncertain, because they are based on the results of a relatively small studies (Zumla *et al.*, 2012). However, existing data suggest that patients taking bedaquiline in addition to standard TB therapy are five times more likely to die than those without the new drug, which has resulted in medical journal articles raising health policy questions about why the FDA approved the drug and whether financial ties to the company making bedaquiline influenced physicians' support for its use (Zumla *et al.*, 2012). The recommended treatment of new-onset pulmonary tuberculosis, as of 2010, is six months of a combination of antibiotics containing rifampicin, isoniazid, pyrazinamide, and ethambutol for the first two months, and only rifampicin and isoniazid for the last four months. These drugs are category I first line anti tuberculosis agent. (Lawn and Zumla 2011). If tuberculosis recurs, testing to determine to which antibiotics it is sensitive is important before determining treatment (Lawn and Zumla 2011). If multiple drug-resistant TB is detected, treatment with at least four effective antibiotics for 18 to 24 months is recommended.Primary resistance occurs when a person becomes infected with a resistant strain of TB. A person with fully susceptible MTB may develop secondary (acquired) resistance during therapy because of inadequate treatment, not taking the prescribed regimen appropriately (lack of compliance), or using low-quality medication. Drug-resistant TB is a serious public health issue in many developing countries, as its treatment is longer and requires more expensive drugs. MDR-TB is defined as resistance to the two most effective first-line TB drugs: rifampicin and isoniazid. Extensively drug-resistant TB is also resistant to three or more of the six classes of second-line drugs. Totally drug-resistant TB is resistant to all currently used drugs. It was first observed in 2003 in Italy, but not widely reported until 2012, and has also been found in Iran and India (Velayati *et al.*,2009). Bedaquiline is tentatively supported for use in multiple drug-resistant TB. XDR-TB is a term sometimes used to define extensively resistant TB, and constitutes one in ten cases of MDR-TB. Cases of XDR TB have been identified in more than 90% of countries.

2.1.22 Epidemiology of tuberculosis

In 2007, the number of cases of TB per 100,000 people was highest in sub-Saharan Africa, and was also relatively high in Asia in 2009. Roughly one-third of the world's population has been infected with *M. tuberculosis*, with new infections occurring in about 1% of the population each

year. However, most infections with *M. tuberculosis* do not cause TB disease and 90–95% of infections remain asymptomatic. In 2012, an estimated 8.6 million chronic cases were active. In 2010, 8.8 million new cases of TB were diagnosed, and 1.20–1.45 million deaths occurred, most of these occurring in developing countries (Lozano, 2012). Of these 1.45 million deaths, about 0.35 million occur in those also infected with HIV (Global Tuberculosis Control, 2011).Tuberculosis is the second-most common cause of death from infectious disease (after those due to HIV/AIDS). The total number of tuberculosis cases has been decreasing since 2005, while new cases have decreased since 2002. China has achieved particularly dramatic progress, with about an 80% reduction in its TB mortality rate between 1990 and 2010 (WHO, 2015). The number of new cases has declined by 17% between 2004–2014. Tuberculosis is more common in developing countries; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5–10% of the US population test positive. Hopes of totally controlling the disease have been dramatically dampened because of a number of factors, including the difficulty of developing an effective vaccine, the expensive and time-consuming diagnostic process, the necessity of many months of treatment, the increase in HIV-associated tuberculosis, and the emergence of drug-resistant cases in the 1980s. In 2007, the country with the highest estimated incidence rate of TB was Swaziland, with 1,200 cases per 100,000 people. India had the largest total incidence, with an estimated 2.0 million new cases. In developed countries, tuberculosis is less common and is found mainly in urban areas. Rates per 100,000 people in different areas of the world were: globally 178, Africa 332, the Americas 36, Eastern Mediterranean 173, Europe 63, Southeast Asia 278, and Western Pacific 139 in 2010(WHO, 2010). In Canada and Australia, tuberculosis is many times more common among the aboriginal peoples, especially in remote areas. In the United States Native Americans have a fivefold greater mortality from TB, and racial and ethnic minorities accounted for 84% of all reported TB cases. The rates of TB vary with age. In Africa, it primarily affects adolescents and young adults. However, in countries where incidence rates have declined dramatically (such as the United States), TB is mainly a disease of older people and the immunocompromised. Worldwide, 22 "high-burden" states or countries together experience 80% of cases as well as 83% of deaths.

2.1.23 Biochemical changes in tuberculosis

2.1.24 Tuberculosis and cellular damage:

Role of free radicals and antioxidants in tuberculosis patients Reddy *et al.*, (2004), has observed that Mycobacteria can induce reactive oxygen species production by activating phagocytes and although it could be an important part of the host defense against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation. Consequently, Favier *et al*., (1994), has reported that increased ROS may further contribute to immunosuppression, especially in those with impaired antioxidant capacity, such as HIV infected patients. Severe oxidative stress has been reported in TB patients because of malnutrition and poor immunity. Reddy *et al.,* (2004) investigated the serum lipid peroxidation products and important free radical scavenging enzymes i.e. superoxide dismutase , catalase and antioxidant glutathione levels and total antioxidant status in TB patients. The participants in his work comprised of normal human volunteers (NHV,n=25), TB patients (n=100) – including untreated (TB1, n=55), under treatment (TB2, n=30) and after treatment (TB3, n = 15) with anti-tuberculosis therapy (ATT). The study revealed that levels of lipid peroxidation products malondialdehyde (MDA) were increased significantly in TB1 and TB2 ($p<0.001$) and also in TB3 ($p<0.01$) Similar result was obtained by Lamsal *et al.*,(2007) in his two months follow-up study. These levels gradually decreased with clinical improvement with ATT. Erythrocyte SOD, serum catalase, plasma glutathione levels and serum total antioxidant status were decreased significantly in TB1 and TB2 ($p<0.001$), TB3 ($p<0.001$) patients in comparison with normal healthy volunteers (NHV), these levels gradually increased with clinical improvement with ATT(Reddy *et al.*, 2004). Oxidative stress was observed in all the TB patients (TB1, TB2, TB3), irrespective of treatment status. It was concluded that in TB patients free radical activity was quite high and antioxidant levels were low. However, it was suggested that a suitable antioxidant therapy might prove beneficial and nutritional antioxidant supplementation could represent a novel approach to fast recovery. Moreso, Pugalendhi *et al.*,(2012) in their study lipid peroxidation and antioxidant status in patients of end stage renal disease with and without pulmonary tuberculosis, evaluated oxidative stress and antioxidant status in 102 individuals 63 males and 39 females. These individuals were grouped as follows Group I healthy volunteer (n=16), group II chronic kidney disease (CKD) patients not on dialysis (n=27), Group III CKD patients on hemodialysis (n=21) which further analysed as Group IIIA (before dialysis) and as Group IIIB (after dialysis), Group IV pulmonary

tuberculosis (n=27) and group V CKD with pulmonary TB(n=11) which further analysed as Group V A (before dialysis) and Group V B (after dialysis), plasma LPO was assayed as measure of malondialdehyde (MDA) and enzymatic antioxidants activities, superoxide dismutase (SOD), catalase, Glutathione peroxidase (GPX) in erythrocytes. Vitamin C and Vitamin E were assayed in plasma as a measure of non enzymatic antioxidants. Compared to healthy volunteer the MDA levels were increased significantly in all the patients groups and antioxidants enzymes such as SOD, catalase, GPX, Vitamin E levels in CKD patients groups were significantly higher when compared to healthy volunteers and in patients with TB, however the levels in Vitamin E were significantly lower in patients with TB when compared to healthy volunteers. It was concluded that determination of LPO, Antioxidants status including Vitamin C and Vitamin E is useful to evaluate the oxidative stress in these patients. Further, Shubhangi *et al.*,2012 ,in their study, the roles of glutathione, glutathione peroxidase, glutathione reductase and the carbonyl protein in pulmonary and extra pulmonary tuberculosis which was carried out in different categories of pulmonary and extra pulmonary tuberculosis cases of newly sputum culture positive diagnosed pulmonary categorie I (n=100), extra pulmonary patients categorie (n=35) before and after the DOTS treatment of 6 months, categorie II (n=100), categorie III (n=100) and in normal control subjects (n=100).Result obtained showed that the serum protein carbonyl levels were significantly increased in the pulmonary and extra pulmonary tuberculosis patients. The activities of blood glutathione, glutathione peroxidase, and glutathione reductase were found to be significantly decreased in subjects of all the categories of pulmonary and extra pulmonary tuberculosis. A negative correlation between the carbonyl protein content and glutathione, glutathione peroxidase, and glutathione reductase was seen in pulmonary tuberculosis, $p<0.001$. It was concluded that there was increased antioxidant defense mechanism due to increase oxidative stress in tuberculosis. The changes were reversed after 6 months of antitubercular treatment in patients with a good recovery, but the increase in the oxidative stress was not completely reversed. Nezami *et al*.,2011 in their study, "Atherogenic changes of low-density lipoprotein susceptibility to oxidation, and antioxidant enzymes in pulmonary tuberculosis". Forty-five males with active PTB (case group) and 45 healthy age-matched males (control group) were enrolled in the study. Total antioxidant capacity (TAC), SOD and GPX activities were determined by commercial ELISA kits. MDA levels were measured using the thiobarbituric acid method. LDL susceptibility to oxidation was assessed by measuring lag phase duration.

Result showed that TAC, SOD and GPX activities, and lag phase duration in the case group were significantly lower than the control group (p=.002, p=.004, p=.008, and p=.004, respectively; independent), while the MDA levels was higher in case group (p=.024).Thus it was suggested that a higher susceptibility of LDL to oxidation and higher levels of lipid peroxidation, and therefore, a possible higher risk of atherosclerosis in patients with PTB. Shubhangi *et al*.,2012 in their study, "The Roles of Glutathione, Glutathione Peroxidase, Glutathione Reductase and the Carbonyl Protein in Pulmonary and Extra Pulmonary Tuberculosis" which recruited different categories of pulmonary and extra pulmonary tuberculosis cases of newly sputum culture positive diagnosed pulmonary categorie I (n=100), extra pulmonary patients categorie (n=35) before and after the DOTS treatment of 6 months, categorie II (n=100), categorie III (n=100) and in normal control subjects (n=100), observed that the serum protein carbonyl levels were significantly increased in the pulmonary and extra pulmonary tuberculosis patients. The activities of blood glutathione, glutathione peroxidase, and glutathione reductase were found to be significantly decreased in subjects of all the categories of pulmonary and extra pulmonary tuberculosis. A negative correlation between the carbonyl protein content and glutathione, glutathione peroxidase, and glutathione reductase was seen in pulmonary tuberculosis, $p<0.001$.

Nwanjo and Oze, (2007), also reported higher mean values of plasma lipid peroxide on forty HIV/AIDS seronegative pulmonary tuberculosis patients with the active infection (24 males, 16 females) aged 26-60 ($p<0.05$) when compared with control and reduced levels of non-enzymic antioxidants such as vitamin C, vitamin E and reduced glutathione in plasma of the pulmonary tuberculosis infected subjects ($p<0.05$) when compared with control. It was concluded in the study that pulmonary tuberculosis could probably be associated with excess ROS production(Nwanjo and Oze, 2007). Nnodim *et al*.,2011, in their study the antioxidant status and lipid peroxidation product of newly diagnosed and 6 weeks follow-up patients with pulmonary tuberculosis in Owerri, Imo state, Nigeria obtained result that the levels of vitamin C (0.91±0.42 mg/dL) and vitamin E (0.84±0.31 mg/dL) were significantly decreased in *M. tuberculosis* patients before treatment when compared to the healthy controls [(1.64±0.41 mg/dL) and (1.46±0.38 mg/dL)] respectively at $P<0.05$, while the level of MDA (8.7±1.81 nM/mL) in *M. tuberculosis* patients was significantly higher ($P<0.05$) before ATT as compared with the healthy control (4.91±1.9 nM/mL). Also, there was a significant increase in vitamin C and E levels after 6 weeks of ATT, while MDA levels was decreased when compared with the control

($p<0.05$). It was concluded that the depletion of Vitamins C and E as well as elevation of MDA in tuberculosis patients was suggestive of lipid peroxidation and oxidative stress. The increase in vitamin C and E as well as decrease in MDA after 6 weeks of anti-tuberculosis treatment is suggestive of good response to treatment with standard ATT. Hence, vitamin C and E supplementation improves the quality of life of tuberculosis patients. Mukhtar *et al.*,2012 study on Antioxidant Capacity and Lipid Peroxidation Product in Pulmonary Tuberculosis observed that Trolox Equivalent Antioxidant Capacity (TEAC),and Superoxide Dismutase (SOD) activity were decreased significantly in TB patients than control. The levels of lipid peroxidation products; MDA was significantly high in TB patients (4.04±0.25 μmol/l) than control group; 2.03±0.15 μmol/l, $p<0.05$. Bhimrao *et al*.,2011 study on effect of micronutrients supplementation on oxidative stress and antioxidant status in pulmonary tuberculosis recruited fifty patients with positive sputum for acid fast bacilli (Ziehl Neel-sen (ZN) staining). They were divided into two group and 25 healthy controls without any disease were included for this study. 25 patients with active pulmonary TB were treated with Anti tuberculosis therapy (ATT) and remaining 25 cases were treated with antioxidants as an ad-juvant therapy along with ATT. Serum levels of MDA, Vit.C, Vit.E and total antioxidant status were measured .The results obtained showed significantly decreased level of MDA and in-creased levels of Vit.C, Vit.E and total antioxidant status after six month follow up of anti-oxidants supplementation along with ATT. It was concluded that antioxidants (micro-nutrients) supplementation as an adjuvant therapy helped in reduction of oxidative stress and promoted recovery of patients. Ciftci *et al.*,(2003) in their study, investigated whether the serum levels of Cu, Zn, and Se change during antituberculosis therapy, recruited 22 pulmonary tuberculosis cases that were newly diagnosed with positive sputum and 18 healthy participantss. At the beginning and 2 months after therapy, serum levels of Cu, Zn, and Se were measured by atomic absorption spectrometry. It was shown that Se and Cu levels did not change significantly during the treatment, while there was a significant increase in the levels of Zn and a decrease in the Cu/Zn ratio. It was concluded that serum Zn levels and the Cu/Zn ratio could be used as a valuable laboratory tool for the clinicians to assess response to therapy or effectiveness of the ongoing antituberculosis therapy (Ciftci *et al.*,2003). Further, in the work of Oyedeji *et al*.,(2013) on Oxidative Stress and Lipid Profile Status in Pulmonary Tuberculosis Patients in South Western Nigeria, their literature review cited that malnutrition and increased free radicals generation were common findings with tuberculosis

patients and these can impair their antioxidant capacity.The study was carried out to estimate the levels of non-enzymatic antioxidants:albumin, vitamin C and E, selenium and lipid fractions in sixty–five (M = 29; F = 36) newly diagnosed (untreated) adult active tuberculosis (TB) patients and to compare them with the levels in fifty (M = 25; F = 25) apparently healthy tuberculosis free individuals of the same age group and location. Lipid fractions, MDA, albumin, vitamins C and E were estimated by standard spectrophotometric methods while selenium concentration was determined by atomic absorption spectrophotometric method. There was a significantly increased concentration of MDA ($p < 0.001$) in TB patients when compared with the control participants and significantly reduced concentrations of albumin, vitamin C and E, selenium,total–cholesterol, LDL-cholesterol ($p < 0.001$in each case) and triglyceride ($p < 0.01$) were observed when the TB patients were compared with non TB infected individuals. The study showed that TB patients were predisposed to oxidative stress and reduced concentrations of lipid fractions. Highly nutritious diet fortified with antioxidants supplements was advocated for TB patients alongside chemotherapeutic management and their lipid fractions status should be monitored while managing the patients.Moreover, Akpovi *et al.*,(2013), from Benin Republic in their study, "Tuberculosis treatment raises total cholesterol level and restores high density lipoprotein cholesterol (HDL-C) in patients with pulmonary tuberculosis", recruited 83 patients with pulmonary TB and blood samples were collected from them before and after treatment once and compared to results obtained from 100 control subjects without TB. Before treatment, levels of TC ($p<0.005$), HDL-C ($p<0.005$) and LDL-C ($p<0.005$) were significantly lower in pulmonary TB patients than normal participants. Unlike TC and LDL-C, HDL-C decrease was correlated ($r = 0.96$, $p<0.05$) with smear positivity extent (SPE). At the end of TB treatment, which lasted six months, TC ($p<0.01$) and HDL-C ($p<0.005$) levels were significantly increased than before treatment while LDL-C stayed relatively unchanged. The treatment significantly reduced the atherogenic indices TC/HDL-C ($p<0.001$), LDL-C/HDL-C ($p<0.001$) and log (TG/HDL-C) ($p<0.001$) levels. The results showed that tuberculosis treatment increases TC levels and normalizes HDL-C while reducing atherogenic indices to below levels of controls.Consequently, Taparia *et al.*,(2015)in their research´, "Study of lipid profile in pulmonary tuberculosis patients and relapse cases in relation with disease severity -A pilot study" 32 newly diagnosed and 26 relapsed cases to PTB were recruited for the study. Patients were both male and female with average age 37.16±1.2 years and 39.44±1.5 years respectively.

25 age and gender matched healthy participants that were non family members of patients were taken as controls for comparison. Fasting serum lipid profile (Total Cholesterol (TC),Triglyceride (TG), HDL-cholesterol (HDL-C) , Low density Lipoprotein cholesterol (LDL-C) and Very Low density Lipoprotein cholesterol (VLDL-C) and CRP along with ADA were estimated. Results and discussion: All lipid parameters were significantly ($p<0.05$) low in both newly diagnosed and relapse cases of Pulmonary Tuberculosis (PTB) than controls. TC and LDL-C level were significantly higher in relapsed patients than new PTB cases. Inflammatory markers (ADA and CRP) increased significantly ($p<0.05$) in both new and relapsed group according to control group. Cholesterol and LDL-C were moderately correlated to serum adenosine deaminase deficiency (ADA) as compared to CRP, however no significant correlation was observed between other lipid parameters with ADA or CRP. However, lipid parameters were well correlated with smear positivity extent indicating that SPE is a better measure to assess disease severity which involves progressive decrease in serum lipids. Conclusion: Hypocholesterolemia exists in both newly diagnosed and relapse PTB patients and is one of the many nutritional factors predisposing to TB infection. Serum lipids affect overall strength of immune system with cholesterol being most widely studied in this aspect. SPE (Smear Positivity Extent) shows strong correlation with serum lipids in PTB patients , indicating its reliability in assessing dyslipidemia in PTB patients. Gebremedhin *et al.*(2017) in their study, Lipid Profile in Tuberculosis Patients with and without Human Immunodeficiency Virus Infection, recruited 159 study participants composed of 93 active TB patients [44 HIV coinfected (HIV+TB+) and 49 HIV negative (HIV−TB+)], 41 tuberculin skin test (TST) positive cases [17 HIV coinfected (HIV+TST+) and 24 HIV negative (HIV−TST+)], and 25 healthy controls (HIV−TST−).It was observed that the concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in HIV−TB+ patients were significantly lower compared to HIV−TST+ and to HIV−TST− individuals. Similarly, the concentrations of the TC, LDL-C, and HDL-C in HIV+TB+ were significantly lower compared to HIV−TB+ patients. After the 6 months of anti-TB treatment (ATT), the concentration levels of TC, LDL-C, and HDL-C in HIV−TB+ patients were higher compared to the baseline concentration levels, while they were not significantly different compared to that of HIV−TST+ concentration. It was concluded that the low concentration of lipid profiles in TB patients may be a consequence of the disease and significantly increased in TB patients after

treatment. Amr *et al.*(2017), in their study," Serum Lipid as Biomarker for Therapeutic Monitoring of Latent Tuberculosis Infection" From participants in the ongoing multicentre trial of LTBI treatment (CIHR MCT-94831), 15 randomised to 4 months RIF were selected randomly, and 15 randomised to 9 months INH, matched on age (within 2 years) and sex, were selected. LTBI was defined as a positive tuberculin skin test and/or a positive interferon-γ release assay (positive as defined in Canadian TB standards. To limit the variability of metabolic conditions between individuals, participants were restricted to adults between the age of 18 and 45 years. participants were excluded if they were consuming alcohol daily or taking other drugs that could induce cytochrome P450 (carbamazepine, phenytoin, phenobarbital, felbamate, topiramate, lamotrigine, griseofulvin, nevirapine and oral contraceptives), as these may influence the effect of RIF on lipid blood levels. After signing informed consent, participants provided serum samples before treatment and after 1 month of self-administered LTBI treatment. Participants were not fasting at the time of blood sampling since normal food consumption has no effect on apoA and apoB and a clinically unimportant effect on total cholesterol and HDL-C. Serum samples were labelled with study identity numbers and stored at -80°C until assays of total cholesterol; HDL-C, apoA and apoB were performed. The identity of participants and study drug taken was known only to one investigator (C. Valiquette); all other investigators remained blinded to study drug for data analysis and interpretation. This study was approved by a research ethics board of the McGill University Health Centre (file number 11-046-SDR). Treatment effect on lipids was assessed by comparing the post-treatment serum lipids to the pretreatment levels in each arm, using a paired t-test. The mean change in lipid (treatment effect) was compared between the two intervention groups using linear regression statistics. Residual confounding by age and sex, and potential confounding by other variables (body mass index, alcohol consumption and pretreatment lipid levels) were assessed by comparing different regression models, using stepwise backward regression to select the most parsimonious models. A sample size of 30 subjects was considered adequate for detecting a 10–20% change in serum lipids, considering biological variability of 5–20% . Statistical analyses were conducted using STATA v12.1 (StataCorp LP, College Station, TX, USA).Pretreatment characteristics of the selected subjects receiving INH or RIF were similar. Except for two subjects, all participants had taken more than 80% of their treatment doses during the first month. Result obtained indicated that INH treatment was associated with a significant decrease in both total cholesterol and apoB

levels (mean changes -0.25 $g \cdot L^{-1}$ (95% CI -0.45– -0.06) and -0.07 $g \cdot L^{-1}$ (95% CI -0.12– -0.03), respectively) The change in serum level of apoB, but not total cholesterol, was significantly different between the two treatment groups, mean difference -0.1 $g \cdot L^{-1}$ (95% CI -0.2– -0.004). Change in serum lipids after 1-month treatment with a) rifampicin and b) isoniazid. HDLC: high density lipoprotein cholesterol; apoA: apolipoprotein A-1; apoB: apolipoprotein B.RIF treatment, by contrast, was associated with an increase in mean apoA levels , although this change was not statistically significant (mean change 0.06 $g \cdot L^{-1}$ (95% CI -0.01–0.13).These preliminary observations suggest that lipid metabolism may be altered significantly by LTBI treatment, particularly within one month of starting INH. These observations require confirmation in a larger study, but offer the promise of novel biomarkers in LTBI treatment.This study had several important limitations. First the sample size was small; this limited the ability to identify associations between total cholesterol and INH treatment, or between apoA and RIF treatment. It was unable to evaluate potential drug effects on serum triglycerides and LDL-C as the researchers had to rely on non-fasting serum samples. However, measurement of lipids that require 12 h of fasting before drawing samples are not very practical for therapeutic monitoring in clinical practice. In addition, it compared the effect of two different modalities of treatment, but did not include an untreated control group with LTBI to assess the spontaneous changes in these lipid levels, although the finding that RIF did not have a significant effect on apoB serum level suggests that INH was responsible for the changes seen. Finally, they could not exclude confounding by unknown factors. However, since the initial assignment to treatment arms in this patient population was random, confounding by unknown factors should have been unlikely.Due to lack of clinical symptoms or methods of mycobacterial isolation among patients with LTBI, there is currently no way to evaluate patient response to LTBI treatment. The finding of a significant association between apoB and INH treatment suggests that changes in serum apoB may be a surrogate of adherence to, and possibly also effectiveness of, INH therapy. However, this requires further evaluation with a larger number of subjects to correlate these changes with patient adherence and effectiveness of INH. In addition, this association, which, the researchers acknowledged, has not been investigated before, may explore a novel mechanism for controlling lipid disorders. Although the observed effect of INH treatment, in reducing apoB level by 8.4%, is less than the effect of statins (at least 20%) and other lipid lowering agents (at least 10%), this effect was observed in healthy individuals with normal baseline apoB levels who are different

from those treated with lipid lowering agents.It was concluded that apoB may be a potentially useful biomarker for therapeutic monitoring of LTBI treatment with INH; however, further studies with a larger number of patients, treated for longer periods of time and compared with untreated controls, are required.Osuji *et al*.(2013), in their study which was designed to assess the antioxidant status of HIV infected participants with or without tuberculosis co-infections and in HIV seronegative participants infected with tuberculosis. 193 participants were randomly recruited for the study and grouped into: (i) Symptomatic HIV infected participants with tuberculosis co-infections (n = 67) (ii) symptomatic HIV infected participants without tuberculosis (n = 45) (iii) HIV seronegative participants with Tuberculosis (n = 52) and (IV) HIV seronegative control participants without tuberculosis (n = 29). Blood samples collected from the participants were used for HIV screening; CD4+T cell count, glutathione reductase activity, glutathione peroxidase activity, Total Antioxidant Status and albumin estimations. The results showed that glutathione reductase, glutathione peroxidase and Total Antioxidant Status were significantly lowered in both HIV infected participants with or without tuberculosis and HIV seronegative participants with tuberculosis ($P<0.01$), compared (in each case) with HIV seronegative participants without tuberculosis. The CD4+T cell count were significantly low in HIV infected participants with tuberculosis co-infections and HIV infected group without tuberculosis when compared with HIV seronegative participants with or without tuberculosis. However the CD4+T cell count in HIV infected participants with tuberculosis was not significantly different when compared with HIV infected participants without tuberculosis. Similarly, Audu *et al*.,(2005),remarked that coinfection with tuberculosis or malaria appeared not to have any impact on the degree of depletion of CD4+ T cell counts in HIV infected subjects. The serum albumin were lowered in HIV infected participants with tuberculosis and tuberculosis infected participants ($p<0.01$ in each case). Correlation studies amongst groups showed significant correlation between CD4+T cell count and antioxidants in both HIV and tuberculosis co-infected participants and in HIV infected participants without tuberculosis ($p<0.01$ in each case). Serum albumin correlated positively with the antioxidants in both HIV infected participants and those co-infected with tuberculosis. There was no significant correlation between CD4+T cell count and the antioxidants in HIV seronegative participants with or without tuberculosis. The study observed alterations in the levels of glutathione reductase, glutathione peroxidase, total antioxidant status and albumin in tuberculosis infected participants and in HIV

infected participants with and without tuberculosis. This could be as a result of greater utilization of antioxidants subsequent to increased oxidative stress. These findings also further support a link between oxidative stress, tuberculosis and HIV infection.

2.2.0 Antioxidants

An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate" (Halliwell and Gutteridge, 1995). The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus.

2.2.1.Free radicals and their chemical reactions

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital (Halliwell and Gutteridge, 1989). The presence of an unpaired electron results in certain common properties that are shared by most radicals. Radicals are weakly attracted to a magnetic field and are said to be paramagnetic. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants or reductants. As a result of this high reactivity, most radicals have a very short half life (10−6 seconds or less) in biological systems, although some species may survive for much longer .The most important free radicals in many disease states are oxygen derivatives, particularly superoxide and the hydroxyl radical. Radical formation in the body occurs by several mechanisms, involving both endogenous and environmental factors

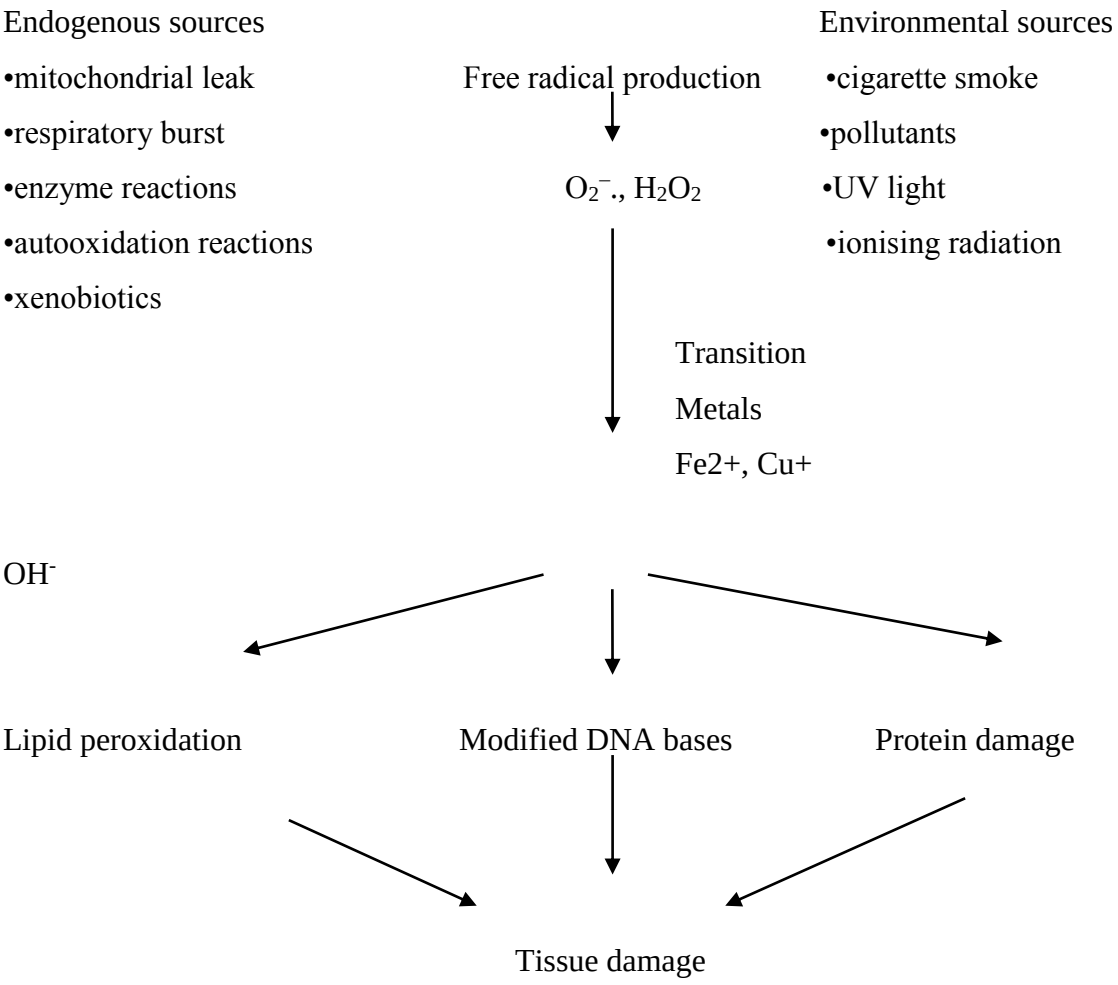

Figure2.4 Major source of free radicals in the body and the consequences of free radical damage (Halliwell and Gutteridge, 1992).

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Superoxide (O2−.) is produced by the addition of a single electron to oxygen, and several mechanisms exist by which superoxide can be produced in vivo (Halliwell and Gutteridge, 1992).Several molecules, including adrenaline, flavine nucleotides, thiol compounds, and glucose, can oxidise in the presence of oxygen to produce superoxide, and these reactions are greatly accelerated by the presence of transition metals such as iron or copper. The electron transport chain in the inner mitochondrial membrane performs the reduction of oxygen to water. During this process free radical intermediates are generated, which are generally tightly bound to the components of the transport chain. However, there is a constant leak of a few electrons into the mitochondrial matrix and this results in the formation of superoxide.(Young and Woodside, 2001). The activity of several other enzymes, such as cytochrome p450 oxidase in the liver and enzymes involved in the synthesis of adrenal hormones, also results in the leakage of a few electrons into the surrounding cytoplasm and hence superoxide formation. There might also be continuous production of superoxide by vascular endothelium to neutralise nitric oxide, production of superoxide by other cells to regulate cell growth and differentiation, and the production of superoxide by phagocytic cells during the respiratory burst. Any biological system generating superoxide will also produce hydrogen peroxide as a result of a spontaneous dismutation reaction. In addition, several enzymatic reactions, including those catalysed by glycolate oxidase and D-amino acid oxidase, might produce hydrogen peroxide directly. Hydrogen peroxide is not a free radical itself, but is usually included under the general heading of reactive oxygen species (ROS). It is a weak oxidising agent that might directly damage proteins and enzymes containing reactive thiol groups. However, its most vital property is the ability to cross cell membranes freely, which superoxide generally cannot do(Young and Woodside, 2001). Therefore, hydrogen peroxide formed in one location might diffuse a considerable distance before decomposing to yield the highly reactive hydroxyl radical, which is likely to mediate most of the toxic effects ascribed to hydrogen peroxide. Therefore, hydrogen peroxide acts as a conduit to transmit free radical induced damage across cell compartments and between cells. In the presence of hydrogen peroxide, myeloperoxidase will generate hypochlorous acid and singlet oxygen, a reaction that plays an important role in the killing of bacteria by phagocytes (Lloyd *et al*., 1997). The hydroxyl radical (OH.), or a closely related species, is probably the final mediator of most free radical induced tissue damage. All of the reactive oxygen species described above exert most of their pathological effects by giving rise to

hydroxyl radical formation. The reason for this is that the hydroxyl radical reacts, with extremely high rate constants, with almost every type of molecule found in living cells including sugars, amino acids, lipids, and nucleotides. Although hydroxyl radical formation can occur in several ways, by far the most important mechanism in vivo is likely to be the transition metal catalysed decomposition of superoxide and hydrogen peroxide .All elements in the first row of the d-block of the periodic table are classified as transition metals. In general, they contain one or more unpaired electrons and are therefore themselves radicals when in the elemental state. However, their key property from the point of view of free radical biology is their variable valency, which allows them to undergo reactions involving the transfer of a single electron. The most important transition metals in human disease are iron and copper. These elements play a key role in the production of hydroxyl radicals in vivo. Hydrogen peroxide can react with iron II (or copper I) to generate the hydroxyl radical, a reaction first described by Fenton in 1894:

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH. + OH^-$

This reaction can occur in vivo, but the situation is complicated by the fact that superoxide (the major source of hydrogen peroxide in vivo) will normally also be present. Superoxide and hydrogen peroxide can react together directly to produce the hydroxyl radical, but the rate constant for this reaction in aqueous solution is virtually zero. However, if transition metal ions are present a reaction sequence is established that can proceed at a rapid rate:

$Fe^{3+} + O_2 \longrightarrow Fe^{2+} + O2$

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH. + OH-$

net result:

$O_2^- + H_2O_2 \longrightarrow OH- + OH. + O2$

The net result of the reaction sequence illustrated above is known as the Haber-Weiss reaction.

Although most iron and copper in the body are sequestered in forms that are not available to catalyse this reaction sequence, it is still of importance as a mechanism for the formation of the hydroxyl radical in vivo. The actual reactions, however, may be somewhat more complex than those described above and it is possible that other reactive intermediates such as the ferryl and perferryl radicals might also be formed. Approximately 4.5 g of iron can be found in the average adult man, most of which is contained in the haemoglobin molecule and other haem containing proteins. Dietary iron is absorbed preferentially from the proximal part of the small intestine in the divalent form and is transferred to the circulation in which it is carried by transferrin. (Young

and Woodside, 2001) Under most circumstances iron remains tightly bound to one of several proteins, including transferrin, lactoferrin, haem proteins, ferritin, or haemosiderin. In addition, however, it seems likely that a small iron pool will be maintained as complexes with a variety of small molecules, such as nucleotides and citrate within the cytoplasm and subcellular organelles. This pool is probably capable of catalysing an iron driven Fenton reaction in vivo. Certainly, these complexes can promote hydroxyl radical formation in vitro. Redox reactive iron can be measured using the bleomycin iron assay, although it remains unclear to what extent iron detected by this assay correlates with any discrete anatomical or physiological pool. In normal circumstances, no bleomycin reactive iron is detectable in plasma from healthy subjects, implying that transferrin or ferritin bound iron is not available to drive hydroxyl radical production. However, transferrin will release its iron at an acidic pH, particularly in the presence of small molecular weight chelating agents such as ADP, ATP, and citrate. Such conditions are found in areas of active inflammation and during ischaemia reperfusion injury, and it is therefore likely that hydroxyl radicals contribute to tissue damage in these settings. Iron is released from ferritin by reducing agents including ascorbate and superoxide itself, and hydrogen peroxide can release iron from a range of haem proteins. Therefore, although the iron binding proteins effectively chelate iron and prevent any appreciable redox effects under normal physiological conditions, this protection can break down in disease states. The role of copper is analogous to that described above for iron. Although free radical production occurs as a consequence of the endogenous reactions described above and plays an important role in normal cellular function, it is important to remember that exogenous environmental factors can also promote radical formation. Ultraviolet light will lead to the formation of singlet oxygen and other reactive oxygen species in the skin. Atmospheric pollutants such as ozone and nitrogen dioxide lead to radical formation and antioxidant depletion in the bronchoalveolar lining fluid, and this may exacerbate respiratory disease. Cigarette smoke contains millimolar amounts of free radicals, along with other toxins.Various xenobiotics also cause tissue damage as a consequence of free radical generation, including paraquat, paracetamol, bleomycin, and anthracyclines.

2.2.2 Antioxidant defence systems

Both endogenous and exogenous, are present to protect cellular components from free radical induced damage because radicals have the capacity to react in an indiscriminate manner leading

to damage to almost any cellular component, an extensive range of antioxidant defences, These can be divided into three main groups: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins (Halliwell and Gutteridge, 1989).

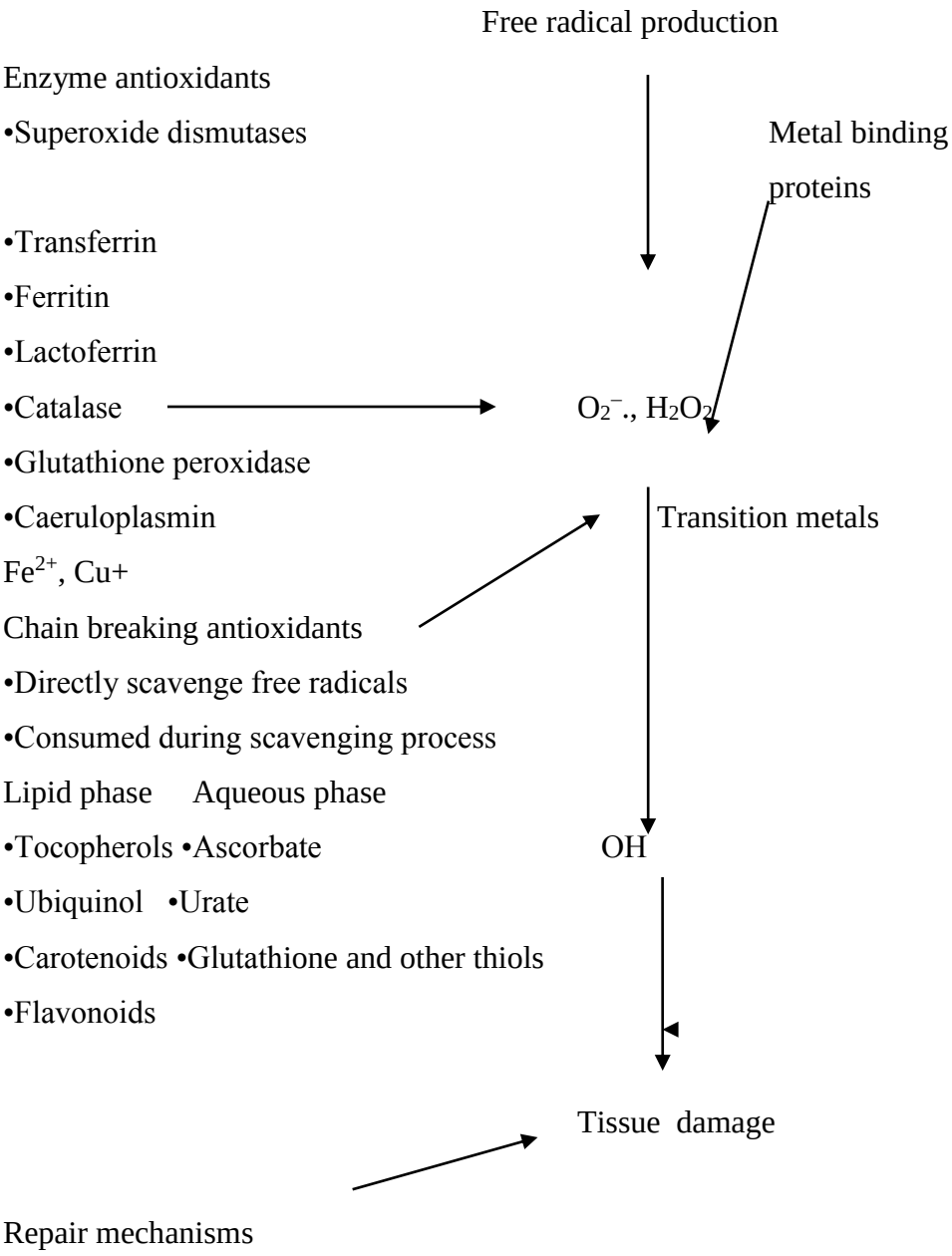

Figure 2.5 Antioxidant defences against free radical attack (Halliwell and Gutteridge, 1992).

Antioxidant enzymes catalyse the breakdown of free radical species, usually in the intracellular environment. Transition metal binding proteins prevent the interaction of transition metals such as iron and copper with hydrogen peroxide and superoxide producing highly reactive hydroxyl radicals. Chain breaking antioxidants are powerful electron donors and react preferentially with free radicals before important target molecules are damaged. In doing so, the antioxidant is oxidised and must be regenerated or replaced. By definition, the antioxidant radical is relatively unreactive and unable to attack further molecules.(Young and Woodside, 2001).

2.2.3. The Antioxidant enzymes

2.2.4. Catalase

Catalase was the first antioxidant enzyme to be characterised and catalyses the two stage conversion of hydrogen peroxide to water and oxygen:

catalase–Fe(III) + H_2O_2 → compound I

compound I + H_2O_2 → catalase–Fe(III) + $2H_2O$ + O_2

Catalase consists of four protein subunits, each containing a haem group and a molecule of NADPH. The rate constant for the reactions described above is extremely high (~107 M/sec), implying that it is virtually impossible to saturate the enzyme in vivo. Catalase is largely located within cells in peroxisomes, which also contain most of the enzymes capable of generating hydrogen peroxide. The amount of catalase in cytoplasm and other subcellular compartments remains unclear, because peroxisomes are easily ruptured during the manipulation of cells. The greatest activity is present in liver and erythrocytes but some catalase is found in all tissues.

2.2.5 Glutathione peroxidases and glutathione reductase

Glutathione peroxidases catalyse the oxidation of glutathione at the expense of a hydroperoxide, which might be hydrogen peroxide or another species such as a lipid hydroperoxide:

ROOH + 2GSH → GSSG + H_2O + ROH .Other peroxides, including lipid hydroperoxides, can also act as substrates for these enzymes, which might therefore play a role in repairing damage resulting from lipid peroxidation. Glutathione peroxidases require selenium at the active site, and deficiency might occur in the presence of severe selenium deficiency. Several glutathione peroxidase enzymes are encoded by discrete genes. The plasma form of glutathione peroxidase is believed to be synthesised mainly in the kidney (Roxborough *et al*.,1999). Within

cells, the highest concentrations are found in liver although glutathione peroxidase is widely distributed in almost all tissues. The predominant subcellular distribution is in the cytosol and mitochondria, suggesting that glutathione peroxidase are the main scavenger of hydrogen peroxide in these subcellular compartments. The activity of the enzyme is dependent on the constant availability of reduced glutathione. The ratio of reduced to oxidised glutathione is usually kept very high as a result of the activity of the enzyme glutathione reductase:GSSG + NADPH + H+ 2GSH + NADP+ .The NADPH required by this enzyme to replenish the supply of reduced glutathione is provided by the pentose phosphate pathway. Any competing pathway that utilises NADPH (such as the aldose reductase pathway) might lead to a deficiency of reduced glutathione and hence impair the action of glutathione peroxidase. Glutathione reductase is a flavine nucleotide dependent enzyme and has a similar tissue distribution to glutathione peroxidase.

2.2.6 Superoxide dismutase

The superoxide dismutases catalyse the dismutation of superoxide to hydrogen peroxide:

$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ The hydrogen peroxide must then be removed by catalase or glutathione peroxidase, as described above. There are three forms of superoxide dismutase in mammalian tissues, each with a specific subcellular location and different tissue distribution.

(1) Copper zinc superoxide dismutase (CuZn- SOD): CuZnSOD is found in the cytoplasm and organelles of virtually all mammalian cells. It has a molecular mass of approximately 32 000 kDa and has two protein subunits, each containing a catalytically active copper and zinc atom.

(2) Manganese superoxide dismutase (MnSOD): MnSOD is found in the mitochondria of almost all cells and has a molecular mass of 40 000 kDa. It consists of four protein subunits, each probably containing a single manganese atom. The amino acid sequence of MnSOD is entirely dissimilar to that of CuZnSOD and it is not inhibited by cyanide, allowing MnSOD activity to be distinguished from that of CuZnSOD in mixtures of the two enzymes.

(3) Extracellular superoxide dismutase (ECSOD): EC-SOD was described by Marklund in 198242 and is a secretory copper and zinc containing SOD distinct from the CuZnSOD described above. EC-SOD is synthesised by only a few cell types, including fibroblasts and endothelial cells, and is expressed on the cell surface where it is bound to heparan sulphates. EC-SOD is the major SOD detectable in extracellular fluids and is released into the circulation from the surface of vascular endothelium following the injection of heparin. EC-SOD might play a role in the

regulation of vascular tone, because endothelial derived relaxing factor (nitric oxide or a closely related compound) is neutralised in the plasma by superoxide (McIntyre *et al.*,1999).

2.2.7 The chain breaking antioxidants

Whenever a free radical interacts with another molecule, secondary radicals may be generated that can then react with other targets to produce yet more radical species. The classic example of such a chain reaction is lipid peroxidation, and the reaction will continue to propagate until two radicals combine to form a stable product or the radicals are neutralised by a chain breaking antioxidant. Chain breaking antioxidants are small molecules that can receive an electron from a radical or donate an electron to a radical with the formation of stable byproducts. In general, the charge associated with the presence of an unpaired electron becomes dissociated over the scavenger and the resulting product will not readily accept an electron from or donate an electron to another molecule, preventing the further propagation of the chain reaction. Such antioxidants can be conveniently divided into aqueous phase and lipid phase antioxidants.

2.2.8 Lipid phase chain breaking antioxidants

These antioxidants scavenge radicals in membranes and lipoprotein particles and are crucial in preventing lipid peroxidation. The most important lipid phase antioxidant is probably vitamin E. Vitamin E occurs in nature in eight different forms, which differ greatly in their degree of biological activity. The tocopherols (α, β, γ, and δ) have a chromanol ring and a phytyl tail, and differ in the number and position of the methyl groups on the ring. The tocotrienols (α, β, γ, and δ) are structurally similar but have unsaturated tails. Both classes of compounds are lipid soluble and have pronounced antioxidant properties. They react more rapidly than polyunsaturated fatty acids with peroxyl radicals and hence act to break the chain reaction of lipid peroxidation. In addition to its antioxidant role, vitamin E might also have a structural role in stabilizing membranes. Frank vitamin E deficiency is rare in humans, although it might cause haemolysis and might contribute to the peripheral neuropathy that occurs in abetalipoproteinaemia. The absorption, transport, and regulation of plasma concentrations of vitamin E in humans has been reviewed by Kayden and Traber, although in general the metabolism of vitamin E is not well described. In cell membranes and lipoproteins the essential antioxidant function of vitamin E is to trap peroxyl radicals and to break the chain reaction of lipid peroxidation.Vitamin E will not

prevent the initial formation of carbon centred radicals in a lipid rich environment, but does minimise the formation of secondary radicals. α-Tocopherol is the most potent antioxidant of the tocopherols and is also the most abundant in humans. It quickly reacts with a peroxyl radical to form a relatively stable tocopheroxyl radical, with the excess charge associated with the extra electron being dispersed across the chromanol ring. This resonance stabilised radical might subsequently react in one of several ways. α-Tocopherol might be regenerated by reaction at the aqueous interface with ascorbate or another aqueous phase chain breaking antioxidant, such as reduced glutathione or urate. Alternatively, two α-tocopheroxyl radicals might combine to form a stable dimer, or the radical may be completely oxidised to form tocopherol quinone. The carotenoids are a group of lipid soluble antioxidants based around an isoprenoid carbon skeleton.(Cooper *et al*.,1999).The most important of these is â-carotene, although at least 20 others may be present in membranes and lipoproteins. They are particularly efficient scavengers of singlet oxygen, but can also trap peroxyl radicals at low oxygen pressure with efficiency at least as great as that of α-tocopherol. Because these conditions prevail in many biological tissues, the carotenoids might play a role in preventing in vivo lipid peroxidation (Chaudiere *et al*., 1999) The other important role of certain carotenoids is as precursors of vitamin A (retinol). Vitamin A also has antioxidant properties, Keys and Zimmerman, (1999) which do not, however, show any dependency on oxygen concentration.

2.2.9 Flavonoids

Flavonoids are a large group of polyphenolic antioxidants found in many fruits, vegetables, and beverages such as tea and wine. Over 4000 flavonoids have been identified and they are divided into several groups according to their chemical structure, including flavonols (quercetin and kaempherol), flavanols (the catechins), flavones (apigenin), and isoflavones (genistein). Epidemiological studies suggest an inverse relation between flavonoid intake and incidence of chronic diseases such as coronary heart disease (CHD). However, little is currently known about the absorption and metabolism of flavonoids and epidemiological associations might be a consequence of confounding by other factors. Available evidence suggests that the bioavailability of many flavonoids is poor, McAnlis *et al*., (1999) and plasma values very low, although there is some evidence that augmenting the intake of flavonoids might improve biochemical indices of oxidative damage (Stein *et al.*,1999) Apart from flavonoids, other dietary phenolic compounds might also make a small contribution to total antioxidant

capacity.Ubiquinol-10, the reduced form of coenzyme Q10, is also an effective lipid soluble chain breaking antioxidant. Although present in lower concentrations than α-tocopherol, it can scavenge lipid peroxyl radicals with higher efficiency than either α-tocopherol or the carotenoids,and can also regenerate membrane bound α-tocopherol from the tocopheryl radical. Indeed, whenever plasma or isolated low density lipoprotein (LDL) cholesterol is exposed to radicals generated in the lipid phase, ubiquinol-10 is the first antioxidant to be consumed, suggesting that it might be of particular importance in preventing the propagation of lipid peroxidation. However, work to clarify further its role has been hampered by the ease with which ubinquinol-10 becomes oxidized during sample handling or analysis.

2.2.10 Aqueous phase chain breaking antioxidants

These antioxidants will directly scavenge radicals present in the aqueous compartment. Qualitatively the most important antioxidant of this type is vitamin C (ascorbate). In humans, ascorbate acts as an essential cofactor for several enzymes catalysing hydroxylation reactions.In most cases, it provides electrons for enzymes that require prosthetic metal ions in a reduced form to achieve full enzymatic activity. Its best known role is as a cofactor for prolyl and lysyl oxidases in the synthesis of collagen. However, in addition to these well defined actions, several other biochemical pathways depend upon the presence of ascorbate (Young and Woodside, 2001). In addition to its role as an enzyme cofactor, the other major function of ascorbate is as a key chain breaking antioxidant in the aqueous phase. Ascorbate has been shown to scavenge superoxide, hydrogen peroxide, the hydroxyl radical, hypochlorous acid, aqueous peroxyl radicals, and singlet oxygen. During its antioxidant action, ascorbate undergoes a two electron reduction, initially to the semidehydroascorbyl radical and subsequently to dehydroascorbate. The semidehydroascorbyl radical is relatively stable owing to dispersion of the charge associated with the presence of a single electron over the three oxygen atoms, and it can be readily detected by electron spin resonance in body fluids in the presence of increased free radical production. Dehydroascorbate is relatively unstable and hydrolyses readily to diketogulonic acid, which is subsequently broken down to oxalic acid. Two mechanisms have been described by which dehydroascorbate can be reduced back to ascorbate; one is mediated by the selenoenzyme thioredoxin reductase and the other is a non-enzyme mediated reaction that uses reduced glutathione. Dehydroascorbate in plasma is probably rapidly taken up by red blood cells before

recycling, so that very little, if any, dehydroascorbate is present in plasma. Apart from ascorbate, other antioxidants are present in plasma in high concentrations. Uric acid efficiently scavenges radicals, being converted in the process to allantoin. Urate might be particularly important in providing protection against certain oxidising agents, such as ozone. Indeed, it has been suggested that the increase in life span that has occurred during human evolution might be partly explained by the protective action provided by uric acid in human plasma. Part of the antioxidant effect of urate might be attributable to the formation of stable non-reactive complexes with iron, but it is also a direct free radical scavenger. Albumin bound bilirubin is also an efficient radical scavenger, and it has been suggested that it might play a particularly crucial role in protecting the neonate from oxidative damage, because deficiency of other chain breaking antioxidants is common in the newborn. The other major chain breaking antioxidants in plasma are the protein bound thiol groups. The sulphydryl groups present on plasma proteins can function as chain breaking antioxidants by donating an electron to neutralise a free radical, with the resultant formation of a protein thiyl radical. Albumin is the predominant plasma protein and makes the major contribution to plasma sulphydryl groups, although it also has several other antioxidant properties. Albumin contains 17 disulphide bridges and has a single remaining cysteine residue, and it is this residue that is responsible for the capacity of albumin to react with and neutralise peroxyl radicals. This property is important in view of the role albumin plays in transporting free fatty acids in the blood. In addition, albumin has the capacity to bind copper ions and will inhibit copper dependent lipid peroxidation and hydroxyl radical formation. It is also a powerful scavenger of the phagocytic product hypochlorous acid, and provides the main plasma defence against this oxidant. Because albumin itself is damaged when it acts as an antioxidant, it has been viewed as a sacrificial molecule that prevents damage occurring to more vital species. The high plasma concentration of albumin and a relatively short half life mean that any damage suffered is unlikely to be of biological importance. However, in vitro work has shown that protein thiyl radicals can themselves act as a potential source of reactive oxidants. The thiyl radical can abstract an electron from a polyunsaturated fatty acid to initiate the process of lipid peroxidation, a reaction that can be inhibited by ascorbate and retinol. The antioxidant effects of albumin and other proteins have been shown to decrease at high concentrations and it has been suggested that this is because thiyl radicals can oxidatively damage other molecules.The importance of these findings to the antioxidant role of albumin in vivo remains unclear.Reduced glutathione (GSH) is

a major source of thiol groups in the intracellular compartment but is of little importance in the extracellular space(Young and Woodside, 2001).GSH might function directly as an antioxidant, scavenging a variety of radical species, as well as acting as an essential factor for glutathione peroxidase . Thioredoxin might also function as a key intracellular antioxidant, particularly in redox induced activation of transcription factors (Arrigo, 1999).

2.2.11 Interactions between chain breaking antioxidants

Although the actions of chain breaking antioxidants have been considered separately above, it is vital to remember that in vivo complex interactions between antioxidants are likely to occur. For instance, it is likely that ascorbate will recycle the tocopheryl radical at the aqueous–lipid interface, so regenerating tocopherol. This might be crucial in ensuring that tocopherol concentrations are maintained in lipoproteins and membranes. In a similar manner, glutathione can regenerate ascorbate from dehydroascorbate. A complex interplay is therefore likely to exist between antioxidants, making it difficult to predict how antioxidants will function in vivo. It therefore becomes meaningless to ask which antioxidant is most important: the answer will depend on the circumstances existing in a particular microenvironment at a specific time, and on the nature of the oxidant injury taking place. A second important property of chain breaking antioxidants is their ability to act as pro-oxidants. In certain circumstances, the presence of an antioxidant might paradoxically lead to increased oxidative damage. For instance, it has been reported that the administration of vitamin C can sometimes lead to an increase in oxidative damage, particularly if iron is also administered. Similarly, it has been clearly shown in vitro that tocopherol might promote LDL oxidation in the absence of an aqueous phase antioxidant such as ascorbate. Whether these reactions are important in vivo is as yet unclear. However, the possibility that antioxidants may have prooxidant effects in vivo must be considered when designing and interpreting the results of clinical trials of antioxidant supplementation.

2.2.12 the transition metal binding proteins

As discussed above, transition metal binding proteins (ferritin, transferrin, lactoferrin, and caeruloplasmin) act as a crucial component of the antioxidant defence system by sequestering iron and copper so that they are not available to drive the formation of the hydroxyl radical.The

main copper binding protein, caeruloplasmin, might also function as an antioxidant enzyme that can catalyse the oxidation of divalent iron.

$4Fe^{2+} + O_2 + 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O$

Fe^{2+} is the form of iron that drives the Fenton reaction and the rapid oxidation of Fe^{2+} to the less reactive Fe^{3+} form is therefore an antioxidant effect.

2.3.0 Consequences of oxidative damage

Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defences, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids. Lipoprotein particles or membranes characteristically undergo the process of lipid peroxidation, giving rise to a variety of products including short chain aldehydes such as malondialdehyde or 4-hydroxynonenal, alkanes, and alkenes, conjugated dienes, and a variety of hydroxides and hydroperoxides. Many of these products can be measured as markers of lipid peroxidation. The measurement of isoprostanes by gas chromatography mass spectroscopy is probably the most specific marker of free radical damage to lipids (Young and Woodside, 2001). Oxidative damage to proteins and nucleic acids similarly gives rise to a variety of specific damage products as a result of modifications of amino acids or nucleotides. Such oxidative damage might also lead to cellular dysfunction, and it is this that might contribute to the pathophysiology of a wide variety of diseases.

2.3.1 Oxidative stress and disease

A role for oxidative stress has been postulated in many conditions, including atherosclerosis, inflammatory conditions, certain cancers, Hecht, (1999) and the process of aging. In many cases, this follows the observation of increased amounts of free radical damage products, particularly markers of lipid peroxidation, in body fluids. It is important to remember, however, that lipid peroxidation is an inevitable accompaniment of cell death from any cause. In most cases peroxidation is a secondary phenomenon, and this does not therefore directly indicate an important role for oxidative stress in the disease concerned. If a primary role for oxidative stress in a particular setting is to be sustained, there should be a plausible mechanism by which increased free radical production or a decrease in antioxidant defences might occur. In addition,

evidence of oxidative stress should be detectable before the onset of tissue damage and augmentation of antioxidant status at an early stage should either prevent or greatly reduce tissue damage. Atherosclerosis can be taken as an example of a process for which there is substantial evidence of a role for oxidative stress. Hypercholesterolaemia is universally accepted as a major risk factor for atherosclerosis. However, at any given concentration of plasma cholesterol, there is still great variability in the occurrence of cardiovascular events. One of the major break throughs in atherogenesis research has been the realisation that oxidative modification of LDL might be a crucially important step in the development of the atherosclerotic plaque. The formation of foam cells from monocyte derived macrophages in early atherosclerotic lesions is not caused by native LDL but only after the modification of LDL by various chemical reactions such as oxidation. Oxidation of LDL is a process initiated and propagated by free radicals or by one of several enzymes, and is believed to occur mainly in the arterial wall in a microenvironment where antioxidants may become depleted. All the cells of the vessel wall—endothelial cells, smooth muscle cells, macrophages, and lymphocytes— can modify LDL in vitro (Young and Woodside, 2001). Several mechanisms are likely to be involved, including transition metal ion mediated generation of hydroxyl radicals, the production of reactive oxygen species by enzymes such as myeloperoxidase and lipoxygenase, and direct modification by reactive nitrogen species. Because oxidation of LDL is primarily a free radical mediated process that is inhibited by antioxidants, antioxidant depletion might be a risk factor for cardiovascular disease (CVD). Evidence for LDL oxidation in vivo is now well established. In immunocytochemical studies, antibodies against oxidised LDL stain atherosclerotic lesions but not normal arterial tissue. LDL extracted from animal and human lesions has been shown to be oxidized and is rapidly taken up by macrophage scavenger receptors. In young survivors of myocardial infarction (MI), an association has been demonstrated between increased susceptibility of LDL to oxidation and the degree of coronary atherosclerosis, whereas the presence of ceroid, a product of lipid peroxidation, has been shown in advanced atherosclerotic plaques. Oxidised LDL has several properties that promote atherogenesis, apart from its rapid uptake into macrophages via the scavenger receptor. Oxidised forms of LDL are chemotactic for circulating macrophages and smooth muscle cells and facilitate monocyte adhesion to the endothelium and entry into the subendothelial space. Oxidised LDL is also cytotoxic towards arterial endothelial cells and inhibits the release of nitric oxide and the resulting endothelium

dependent vasodilation. Therefore, there is a potential role for oxidised LDL in altering vasomotor responses, perhaps contributing to vasospasm in diseased vessels. In addition, oxidised LDL is immunogenic; autoantibodies against various epitopes of oxidised LDL have been found in human serum and immunoglobulin (IgG) specific for epitopes of oxidised LDL can be found in lesions. Oxidised LDL can induce arterial wall cells to produce chemotactic factors, adhesion molecules, cytokines, and growth factors that have a role to play in the development of the plaque (Huang *et al.,* and Kita *et al*.,1999)Apart from the atherogenic consequences of LDL oxidation, it is increasingly recognized that reactive oxygen and nitrogen species directly interact with signalling mechanisms in the arterial wall to regulate vascular function. The activities of oxidant generating enzymes in the arterial wall are regulated by both receptor activation and by non-receptor mediated pathways. The effects of antioxidants on these processes are complex but provide alternative mechanisms by which antioxidant supplementation might ameliorate vascular pathology, for instance by improving endothelial function. Evidence that antioxidant micronutrients potentially reduce the risk of CHD comes from four major sources. First, studies of antioxidant supplementation in animal models of atherosclerosis have generally shown a reduction in disease. Second, many studies have now shown that antioxidant supplementation in healthy subjects or patients with CHD can reduce levels of free radical damage products and protect LDL against oxidation. Vitamin E appears to be the most effective antioxidant; both α-carotene and vitamin C have produced extensions in lag time to oxidation only in a few studies, although it remains possible that they might have a beneficial effect in individuals with poor baseline status. Third, large scale epidemiological studies generally show that low intakes of antioxidants are associated with increased cardiovascular risk after correcting for other risk factors.The epidemiological evidence is strongest in the case of vitamin E. In particular, two large longitudinal studies in the USA examined the association between antioxidant intake and the risk of CHD. In a group of 39 910 male health professionals, men who took vitamin E supplements in doses of at least 100 IU/day for over two years had a 37% lower relative risk of CHD than those who did not take vitamin E supplements, after adjustment for age, coronary risk factors, and intake of vitamin C and β-carotene. In the nurses' health study of 87 245 female nurses, women who took vitamin E supplements for more than two years had a 41% lower relative risk of major coronary disease (Young and Woodside, 2001) This effect persisted after adjustment for age, smoking, obesity,

exercise, blood pressure, cholesterol, and the use of postmenopausal oestrogen replacement, aspirin, vitamin C, and β-carotene. High vitamin E intakes from dietary sources were not associated with a significant decrease in risk, although even the highest dietary vitamin E intakes were far lower than intakes among supplement users. The evidence linking the water soluble vitamin C with CVD is less strong than for vitamin E. In the physicians' follow-up study, a high intake of vitamin C was not associated with a lower risk of CHD in men, whereas in women from the nurses' health survey, an initial effect was attenuated after adjustment for multivitamin use. Only one prospective study involving 11 348 adults demonstrated an inverse relation between vitamin C intake and overall cardiovascular mortality. This effect resulted largely from the use of vitamin C in supplements and might have been caused by other antioxidant vitamins in multivitamin preparations. A prospective population study of 1605 healthy men aged 42, 48, 54, or 60 years in Finland has recently shown that men who had vitamin C deficiency had a relative risk of MI of 2.5 compared with men with higher plasma vitamin C concentrations, after adjustment for other risk factors. There is also some indication that increased dietary intake of β-carotene is associated with reduced risk of CHD, although again the evidence is less convincing than that for vitamin E. In the prospective nurses' health survey, consumption of vitamins A and β-carotene in food and supplements weakly predicted the incidence of CHD; Gaziano and Hennekens calculated a 22% risk reduction for women in the highest quintile of β-carotene compared with those in the lowest. Thus, there is a plausible case supported by experimental studies, animal experiments, and epidemiology linking oxidative stress and atherosclerosis. The key test of such a hypothesis is whether increased antioxidant intake can be shown to prevent the clinical manifestations of atherosclerosis in humans. Several published randomised studies have now considered this issue, and others are currently ongoing. Early results have not been encouraging. The α-tocopherol, β-carotene cancer prevention trial (ATBC), conducted among 29 133 male heavy smokers in Finland, found no reduction in CHD morbidity or mortality during five to eight years of treatment with vitamin E (50 mg daily) or β-carotene (20 mg daily)(Young and Woodside, 2001). Those assigned vitamin E had no significant decrease in deaths from ischaemic heart disease (IHD), but a 50% excess of deaths from cerebral haemorrhage, whereas those assigned to α-carotene experienced an 11% increase in deaths from IHD. In a further analysis, a subgroup of the original subjects who had suffered a previous MI were considered. The endpoint of this substudy was the first major coronary event after randomisation. The

proportion of major coronary events did not decrease with either α-tocopherol or β-carotene supplements. In fact, β-carotene conferred an excess of fatal IHD (75% increase in risk). There was a beneficial effect of vitamin E on non-fatal MI with a risk reduction of 38%. By contrast, in the Chinese cancer prevention study conducted among 29 584 poorly nourished residents of Linxian, China, those randomised for 5.25 years to a combined regimen of 15 mg/day β-carotene, 30 mg/day vitamin E, and 50 μg/day selenium had a significant 9% reduction in total mortality, a significant 21% decrease in stomach cancer deaths, and a non-significant 10% decrease in cerebrovascular mortality (Young and Woodside, 2001) However, the wisdom of generalising these findings to well nourished populations remains uncertain. The β-carotene and retinol efficacy trial (CARET), designed to test the effects of a combined supplement of 30 mg β-carotene and 25 000 IU retinol daily among 18 314 cigarette smokers and individuals with occupational asbestos exposure, was ended early when researchers recognised a raised risk of death from lung cancer in those receiving β-carotene and, again, no beneficial effect on CVD was found.134 For CVD mortality, there was a non significant 26% increase in the treated group ($p = 0.06$). The physicians' health study followed more than 22 000 US male doctors treated with 50 mg β-carotene or placebo every other day for an average of 12 years. The trial appears to have been conducted meticulously and its results seriously question any beneficial effect with such supplementation on CVD in well nourished populations. There were no significant effects on individual outcomes, or on a combined endpoint of non-fatal MI, non-fatal stroke, and cardiovascular death, for which the relative risk was 1.0 (95% confidence interval, 0.91 to 1.09).There was also no evidence of harm (or benefit) among the 11% of participants who were current smokers at baseline, although small effects could not be ruled out. The effect of β-carotene supplementation (50 mg/day) in 1720 male and female subjects for a median period of 4.3 years with a median follow up of 8.2 years was also studied.136 Subjects whose plasma values of β-carotene were in the highest quartile at the beginning of the study had the lowest risk of death from all causes compared with those in the lowest quartile. However, supplementation had no effect on either all cause or cardiovascular mortality. Thus for α-carotene supplementation, it would appear that there are no overall benefits among those individuals with a good nutritional status who are at low or average risk of developing CHD. The situation might be different, however, for those with a previous history of such disease. Hodis *et al* have shown a reduction in CAD progression (as measured angiographically) in men given 100 IU vitamin E

daily, although no benefit was found for vitamin C. Singh *et al* found that a combination of vitamins A, C, E, and α -carotene administered within a few hours after acute MI and continued for 28 days led to significantly fewer cardiac events and a lower incidence of angina pectoris in the supplemented group. The Cambridge heart antioxidant study (CHAOS), a trial of vitamin E supplementation on 2002 patients with angiographic evidence of coronary disease, was carried out with mean treatment duration of 1.4 years. It was found that this short term supplementation with α-tocopherol (268 or 537 mg/day) reduced CHD morbidity in patients, in that patients had a significantly (77%) decreased risk of subsequent non-fatal MI. However, no benefit was found in terms of cardiovascular mortality, with a non-significant excess among vitamin E allocated participants.The GISSI-P study randomised 11 324 men surviving a myocardial infarction to 300 mg vitamin E, 1 g n-3 polyunsaturated fatty acids (PUFAs), both, or neither in a randomised, placebo controlled trial (Valagussa *et al*.,1999). Results suggested a beneficial effect of n-3 PUFAs but no benefit with vitamin E ($p = 0.07$). However, further analysis of secondary endpoints suggested some beneficial effects of vitamin E. In addition, the effect of vitamin E might have been ameliorated by the Mediterranean diet of the subjects. Neither of these qualifications holds true for the HOPE study,(Yusuf,2000)which recruited over 9000 subjects likely to be eating a typical northern European diet, who were at high risk for cardiovascular events because they had CVD or diabetes in addition to one other risk factor. Subjects were randomly assigned according to a two by two factorial design to receive either 400 IU of vitamin E daily from natural sources or matching placebo, and either an angiotensin converting enzyme inhibitor (ramipril) or matching placebo for a mean of 4.5 years. Vitamin E supplementation had no effect on primary or secondary cardiovascular endpoints. Thus, for vitamin E in Western populations, the only available trial data in primary prevention are from the ATBC trial, which showed no effect. In secondary prevention, the accumulating trial data for vitamin E are less consistent, although not particularly encouraging. The CHAOS study was positive, although it suffers from design limitations. The GISSI-P study gave a borderline result, whereas the HOPE study was unequivocally negative. How should we interpret the discordance between data from cohort studies and the results so far available from clinical trials? In general, it might be that the duration of clinical trials is too short to show a benefit, and that antioxidant intake over many years is required to prevent atherosclerosis. Thought needs to be given to trial design, with dose, duration of treatment and follow up period, initial antioxidant values and dietary intake, and

extent and distribution of existing atherosclerosis being taken into consideration. Animal models have nearly always tested the effects of antioxidants on the early atherosclerotic lesions. Whether or not antioxidants have inhibitory effects on the later stages remains to be seen. In addition, the complex mixture of antioxidant micronutrients found in a diet high in fruit and vegetable intake might be more effective than large doses of a small number of antioxidant vitamins. It could be that several of these compounds work together but have no effect individually, or that other dietary components (such as trace elements) might be effectors of antioxidant action. The trial evidence available so far relates only to α-tocopherol and β-carotene. Although effective at protecting against lipid peroxidation, these antioxidants have little effect on arterial endothelial function. Ascorbate, in contrast, seems more effective in improving endothelial function, although there is less epidemiological support for a protective effect of ascorbate. Alternatively, the significant results linking antioxidant intake with CHD risk observed in cohort studies might be the result of confounding with other lifestyle behaviours. Young and Woodside, (2001), examined dietary antioxidants and plasma lipids in the coronary artery risk development in young adults (CARDIA) study and found that a higher intake of antioxidants was associated with other lifestyle factors such as physical activity and non-smoking. Plasma concentrations of antioxidants are linked with social class, being higher in more affluent groups. Although these variables can be individually controlled for in analyses, it might be that a complex lifelong behaviour pattern needs to be studied before conclusions regarding antioxidants and CHD can be made. For example, passive smoking has recently been shown to have an atherogenic effect on LDL, yet exposure to smoke is a difficult lifestyle variable to control for in cohort analyses. There is overwhelming evidence that oxidative stress occurs in cells as a consequence of normal physiological processes and environmental interactions, and that the complex web of antioxidant defence systems plays a key role in protecting against oxidative damage. These processes appear to be disordered in many conditions, and a plausible hypothesis may be constructed implicating oxidative stress as a cause of tissue damage. However, as illustrated by the example of CHD, attempts to intervene therapeutically by using antioxidant supplements have so far been largely unsuccessful. A more complete understanding of the biochemical events occurring at a cellular level to influence oxidative damage is required to guide future therapeutic advances.

2.4.0 Lipids

Lipids have important roles in virtually all aspects of life:(1) serving as hormones,(2) serving as an energy source,(3) aiding in digestion and (4) acting as structural components in cell membranes in addition, lipids and lipoproteins are intimately involved in the development of atherosclerosis, a pathogenic process that is the underlying cause of the common cardiovascular disorders of (1) myocardial infarction,(2) cerebrovascular disease, and (3) peripheral vascular disease (Burtis, *et al*.,2008).

2.4.1Basic lipids

The term Lipid applies to a class of compounds that are soluble in organic solvents but nearly insoluble in water. Chemically, lipids contain primarily non polar carbon-hydrogen (C-H) bonds and typically yield fatty acids and or complex alcohols after hydrolysis. Some lipids also contain charged or polar groups such as (1) sialic,(2)phosphoryl,(3)amino,(4) sulphuryl,or(5)hydroxyl groups. The presence of these chemical groups gives lipid molecules an affinity for both water and organic solvents (amphipathic) (Burtis *et al.,* 2008). This allows them to exist at the aqueous interface of biological membranes. Overall, lipids are broadly subdivided into six groups based on their chemical structure, namely (1) cholesterol, (2) fatty acids (3) acylglycerols,(4) sphingolipids,(5)prostaglandins ,and (6) terpenes.

2.4.2 Cholesterol

Cholesterol is found almost exclusively in animals and is a key membrane component of all cells. It Is a steroid alcohol with 27 carbon atoms that are arranged in a tetracyclical sterane ring system, with a C-H side chain. Knowledge of the numbering system for the carbon atoms in cholesterol is important because it is the basis of the nomenclature system of numerous enzymes involved in various biochemical pathways related to cholesterol, such as (1) vitamin D,(2) steroid hormones, and (3) bile acid biosynthetic pathways. Cholesterol is primarily composed of C-H bonds, and hence it is fairly water insoluble. It does, however, contain a polar hydroxyl (OH) group on its A-ring .Thus, it is both a polar and non polar molecule (amphipathic) (Burtis *et al*.,2008).

2.4.3 Cholesterol absorption

The average American diet is estimated to contain approximately 300 to 450mg of cholesterol per day, which mostly comes from the consumption of animal products. A similar amount of cholesterol enters the gut from biliary secretions and the turnover and release of intestinal mucosal cell. Practically all cholesterol in the intestine is present in the unesterified (free) form. Esterrified cholesterol, which contains a fatty acid attached to the hydroxyl groups on the A-ring is rapidly hydrolyzed in the intestine to free cholesterol and fatty acids by cholesterol esterases secreted from the pancreas and small intestine. Before being absorbed, cholesterol is first solubilized through a process called emulsification. Emulsification occurs by the formation of mixed micelles that contain (1) unesterified cholesterol, (2)fatty acids,(3) monoglycerides,(4) phospholipids,and (5) conjugated bile acids(Carl *et al.*,2008). Bile acids, by acting as detergents, are the most critical factor in micelle formation. In their absence, digestion and absorption of both cholesterol and triglyceride are severely impaired. The ability of cholesterol to form micelles is also influenced by quantity of dietary fat but not in its degree of saturation. Increased amounts of fats in the diet results in the increase of mixed micelles, which in turn allows for more cholesterol absorption. Most cholesterol absorption occurs in the middle jejunum and terminal ileum parts of the small intestine and is mediated by the enterocyte surface protein. This protein is the target for the drug ezetimibe that blocks cholesterol absorption. Typically, between 30% to 60% of dietary cholesterol is absorbed per day, which represents as much as 1g/day when one is on a high fat diet. Once cholesterol enters the intestinal mucosal cell, it is packaged with triglycerides, phospholipids, and a large protein called apolipoprotein (apo) B-48 into large lipoprotein particles called chylomicons. Chylomicrons are secreted into the lymph and eventually enter the circulation where they deliver the absorbed dietary lipid to the liver and peripheral tissues (Burtis *et al*.,2008).

2.4.4 Cholesterol synthesis

Cholesterol also is endogenously synthesized with almost 90% of its synthesis occurring in the liver intestine. Most peripheral cells instead depend on the exogenous delivery of cholesterol by lipoproteins. Cholesterol biosynthesis occurs in three stages. In the first stage,acetyl-CoA,a key metabolic intermediate derived from carbohydrates, amino acids, and fatty acids,forms the six carbon thioester HMG-CoA (3-Hydroxy-3-methylglutaryl-CoA). In the second stage, HMG-CoA

is reduced to mevalonate and then is decarbxylated to a series of five- carbon isoprene units. These isoprene units are then condensed to form first a 10-carbon (geranyl pyrophosphate)and then a 15-carbon intermediate (farnesyl pyrophosphate). Two of these C_{15} molecules then combine to produce the final product of the second stage, squalene,a 30-carbon acyclic hydrocarbon. The second stage is important because it contains the steps involving the microsomal enzyme HMG-CoA reductase, which is the rate-limiting enzymes in cholesterol biosynthesis and is inhibited by the statin-type drugs. The enzyme that forms farnesyl pyrophosphate, geranyltransferase, is an important second site of regulation because inhibition here permits the formation of physiologically important intermediate isoprenoidsin the absence of cholesterol synthesis. The third stage occurs in the endoplasmic reticulum with many of the intermediate products being bound to a specific carrier protein. Squalene is initially oxidized and then undergoes cyclization to form the 4-ring, 30-carbon intermediate, lanosterol. In a series of oxidation-decarboxylation reactions,a number of side chains are removed from tetracyclic sterane ring structure to form the 27 –carbon molecule of cholesterol.

2.4.5. Cholesterol esterification

Cholesterol is esterified to a fatty acid to form a cholesteryl ester by two different enzymes. In the cell, excess cholesterol is esterified by acylcholesterol acyltransferase (ACAT), which helps reduce the cytotoxicity of excess free cholesterol. Once esterified, cholesteryl esters are stored in intracellular lipid drops. The esterification of cholesterol by ACAT involves the energy dependent activation of a fatty acid with thio coenzymes A (CoASH) to form an acyl-CoA, which in turn reacts with hydroxyl group on cholesterol to form an ester .

Cholesteryl esters also are formed in the circulation by the action of lecithin cholesterol acyltransferase (LCAT) on cholesterol in lipoproteins, particularly on high density lipoproteins (HDL). The LCAT reaction does not require CoASH. It results from fatty acid transfer from the second carbon position of lecithin (phosphatidylcholine) to cholesterol . Cholesteryl ester account for about 70% of the total cholesterol in plasma,and LCAT is responsible for the formation of most of the cholesteryl esters in plasma. LCAT is secreted by the liver into the circulation and is activated by apoliproprotein A-I,the main protein on HDL . Once cholesterol is esterified, it loses its free hydroxyl group and becomes much more hydrophobic and goes from surface of lipoprotein particles to the hydrophobic core (Burtis *et al*.,2008).

2.4.6.Cholesterol catabolism

Except for specialized endocrine cells that uses cholesterol for the synthesis of steroid hormones, most peripheral cells have limited ability to further catabolize cholesterol . Cholesteryl esters are hydrolyzed to free cholesterol by various lipases in all cells, but thereafter, cholesterol has to be returned to the liver to undergo any further catabolism. Approximately one third of the daily production of cholesterol,or about 400 mg/day,is converted in the liver into bile acids. About 90% of bile acids are reabsorbed in the lower third of the ileum and eventually returned to the liver by the enterohepatic circulation. Bile acids that enter the large intestine are partially deconjugated by bacterial enzymes to secondary bile acids. Cholic acid is converted, for example to deoxycholic acid,and chenodeoxycholic acid is converted to lithocholic acid .Not all cholesterol delivered to the liver is converted to bile salts. Much of it is resecreted into the circulation on lipoproteins and the remainder is directly excreted into the bile unchanged,where it is solubilized into mixed micelles by bile acids and phospholipids. When the amount of cholesterol in bile exceeds the capacity of these solubilizing agents,it is possible for cholesterol to precipitate and form cholesterol gallstones.

2.4.7 Fatty acids

RCOOH is the general chemical formula for a fatty acid, where "R" is an alkyl chain. Fatty acid chain lengths vary and are commonly classified as short-chain (2 to 4 carbon atoms), medium-chain (6 to 10 carbon atoms),or long-chain (12 to 26 carbon atoms) fatty acids. Those of importance in human nutrition and metabolism are the long-chain class that contains an even number of carbon atoms.Fatty acids are further classified according to their degree of saturation. Saturated fatty acids have no double bond (C=C)between their carbon atoms; monounsaturated fatty acids contain one double bond and polyunsaturated fatty acids contain multiple double bonds . The double bonds in polyunsaturated fatty acids are usually three carbon atoms apart. Fatty acids from marine fish living in deep, cold water, such as salmon, possess up to six unsaturated double bonds and are usually more than 20 carbon atoms long. Unsaturated fatty acids are prone to oxidation by non enzymatic reaction of oxygen with their double bonds. The labeling of the carbon atoms in fatty acids is either from the carboxyl terminal end (numbering system) or from the methyl terminal end (η-or ω – numbering system;). In addition, the carbon atoms may be labeled with Greek symbols, with α being adjacent to the carboxyl group and ω

being farthest away. In the Δ -system fatty acids are abbreviated according to the (1) number of carbon atoms,(2) number of double bonds, and (3) position(s) of double bond(s). for example, linoleic acid would be written as $C_{18}:2^{9,12}$ and contains 18 carbons and two unsaturated bonds between 9 and 10 and carbons 12 and 13. Using the η-or ω –system, linoleic acid would be abbreviated as C_{18}:2n-6, where only the first carbon forming the unsaturated pair is written. The Geneva or systematic classification, which is based on their chemical names, is a third common nomenclature system for fatty acids In saturated fatty acids, the chain is extended and flexible; the carbon atoms rotates freely around their longitudinal axis. Unsaturated fatty acids, however, have fixed 30^0 bends in their chains at each double bond. Depending on the plane in which this bend occurs, either the cis or trans isomer is produced. In mammals, all naturally occurring unsaturated fatty acids are of the cis variety. Trans fatty acids results from catalytic hydrogenation in which the unsaturated double bonds are chemically reduced to raise their melting point. This process is used to "harden" or solidify fats in the manufacture of certain foods such as margarine. Most fats in the human body are derived from the diet, which on average contains up to 40% fat, 90% of which is triglyceride. In addition, humans are able to synthesize most fatty acids. They are unable, however, to synthesize some fatty acids, such as linoleic acid ($C_{18}:2^{9,12}$), which is found only in plants. Because it is vital for health, growth, and development , it is termed an essential fatty acid. Linoleic acid is converted to arachidonic acid, which is a precursor for prostaglandin synthesis and is also important in the myelinization of the central nervous system. Fatty acids exist in the circulation in either an unesterified or free state, the latter primarily bound to albumin,or in various esterified forms, such as triglycerides, phospholipids,or cholesteryl esters. The free fatty acid carboxyl group has a pK_a of approximately 4.8; thus free fatty acid molecules primarily exist in their ionized forms. The normal concentration of free fatty acids in human plasma is 0.3 to 1.1 mmol/l (8 to 31 mg/dl). The flux of free fatty acids through the plasma is considerable and sensitive to physiological energy demands and the availability of alternative forms of metabolic fuel,such as glucose.

2.4.8.Fatty acid catabolism

Fatty acids are catabolized by enzymatic oxidation in the mitochondria and produce energy by a series of reactions known as β-oxidation. The process works repetitively and shortens the fatty acid chain by two carbon atoms at a time from the carboxy terminal end of the molecule. For

example, one of palmitic acid (C_{16}) is converted to eight moles of acetyl-CoA. Acety-CoA does not normally accumulate in the cell, but is condensed enzymatically with oxaloacetate, derived largely from carbohydrate metabolism,to yield citrate, a major component of the tricarboxylic acid cycle (Krebs cycle). The Krebs cycle is a common pathway for final oxidation of nearly all metabolic fuels, whether derived from carbohydrate, fat or protein, and ultimately results in the production of adenosine triphosphate (ATP), the main energy storage molecule in the body. The complete catabolism of palmitic acid, for example, yields 16 moles of CO_2,16 moles of H_2O and 129 moles of ATP(2340 calories). The amount of energy produced by the catabolism of 1 mol of palmitic acid (16 carbon atoms) is approximately twice that produced by the catabolism of an equivalent amount (2.5mol) of glucose (6carbon atoms per molecule). Triglycerides contain three fatty acid molecules and are therefore a relatively efficient storage form of metabolic energy. Furthermore, energy storage by triglycerides is also efficient in terms of space because it does not require any water for hydration unlike carbohydrate.

2.4.9 Ketone formation

During prolonged starvation or when carbohydrate metabolism is impaired, such as in uncontrolled diabetes mellitus, the formation of acetyl-CoA exceeds the supply of oxaloacetate. The abundance of acetyl-CoA results from excessive degradation of the fatty acids by β – oxidation in the liver. The resulting acetyl-CoA excess is diverted to an alternative pathway in the mitochondria for the formation of (1) acetoacetic acid (2) β-hydroxybutyric acid and (3) acetone, the three compounds known collectively as ketone bodies. Ketosis, therefore, develops from excessive production of acetyl-CoA , as the body attempts to obtain necessary energy from stored fat in the absence of an adequate supply of carbohydrate metabolites. The entire process of ketosis is reversed through restoration of an adequate concentration of carbohydrates. In cases of starvation, restoration consists of adequate carbohydrate ingestion. In diabetes mellitus, ketosis is reversed by insulin administration, which permits circulating blood glucose to be taken up by the cells. Once a normal metabolic state is restored, the release of fatty acids from adipose tissue is suppressed and the resumed production of oxaloacetate enables it to be conjugated with acetyl-CoA,which inhibits further ketone formation .

2.4.10 Acylglycerols (glycerol esters)

Glycerol is a three-carbon alcohol that contains a hydroxyl group on each of its carbon atoms. Chemically, it is possible to esterify each hydroxyl group with a fatty acid. The two terminal carbon atoms in the glycerol molecule are chemically equivalent and designated α and α'. The center carbon is labeled β. A common alternative labeling system uses the numeral 1 for the α'-carbon,2 for the β-carbon,and 3 for the α'-carbon. The class of acylglycerol is determined by the number of fatty acyl groups present: (1) one fatty acid, monoacylglycerols (monoglycerides);(2) two fatty acids diacylglcerols (diglycerides);and (3)three fatty acids triacylglycerols (triglycerides). In a monoacylglycerol, the fatty acid may be linked to any of the three carbon atoms. For example,1-monoglyceride indicates a fatty acid is attached to the α-carbon. This numbering system applies to all acylglycerols, including the phosphoglycerides . Triglycerides constitute 95% of tissue storage fat and are the predominant form of glyceryl esters found in plasma. The fatty acid residues found in (1) monoglycerides,(2) diglycerides,or (3) triglycerides vary considerably and usually include different combinations of long-chain fatty acids. In general, triglycerides from plant sources, such as corn,sunflower and safflower,tend to be enriched in unsaturated fatty acids such as C_{18}:2 or linoleic acid and are liquid oils at room temperature. Triglycerides from animals, especially ruminants, tend to have saturated acids ranging from C_{12}:0 through C_{18} :0 and are solids at room temperature. Dietary triglycerides are digested in the duodenum and absorbed in the proximal ileum. Through the action of pancreatic and intestinal lipases and in the presence of bile acids, they are first hydrolyzed to glycerol, monoglycerides, and fatty acids. After absorption, these components of triglycerides are reassembled as triglycerides in the intestinal epithelial cells and then packaged with cholesterol and apo B-48 to form chylomicrons. Chylomicrons are secreted into the lymphatic system and eventually reach the circulation. Triglycerides are the main metabolic fuel carried by chylomicrons and are delivered to the liver and peripheral cells after they are hydrolyzed to fatty acids by lipases. Another major class of acylglycerols are those containing phosphoric acid at the third (α') carbon atom, which are referred to as phosphoglycerides . In their simplest form the A group is a hydrogen atom and the molecule is called a diacylphoglyceride. Usually,the A group is some sort of alcohol,such as (1) choline,(2)serine,(3)inositol,or (4) ethanolamine. If the A group is choline, for example,the molecule is referred to as phosphatidylcholine (lecithin). If ethanolamine, the molecule is referred to as phosphatidylethanolamine. As the types of fatty acid

residues R_1 and R_2 are varied, numerous types of phospholipids are formed. These phosphoglycerides are named according to the fatty acid acyl esters attached at C-1 and C-2 of the glycerol. Saturated fatty acids are typically esterified to the C-1 position, whereas polyunsaturated fatty acids are often attached to the C-2 position. In inner mitochondrial membranes more complex phosphoglycerides, known as cardiolipins,are found. They are derived from two phosphogylceride molecules joined by Glycerol Bridge.

2.4.11 Sphingolipids

Sphingolipids are a fourth class of lipids found in humans and are derived from the amino alcohol sphingosine . This dihydric 18-carbon alcohol contains an amino group at C-17. A fatty acid containing 18 or more carbon atoms is attached to the amino group through an amide linkage to form ceramide. This is an intermediate structure in the formation of (1) sphingomyelin,(2) galactosylceramide,and (3) glucosylceramide. In addition, the sugar containing ceramides also have a sulfate group attached usually to the 2-position of the galactose residue to form the sulfatides. The glycosyl ceramides also have additional monosaccharide moieties,such as (1)galactose,(2) N-acetylgalactosamine,and (3)N-acetylneuraminic acid,to form complex globosides and gangliosides.Gangliosides are especially abundant in the membrane of the gray matter of the brain. Whereas glycosphingolipids have a more general role in cellular interactions and are also a source of blood group and tumor antigens.

2.4.12 Prostaglandins

Prostaglandins and related compounds are derivatives of fatty acids, primarily arachidonate. Thromboxanes, some hydroperoxy and hydroxyl-fatty acid derivatives, and leukotrienes are all chemically related to prostaglandins. These bioactive lipids exert diverse physiological actions at concentrations as low as 1μg/L. These prostaglandins are a series of C_{20} unsaturated fatty acids containing a cyclopentane ring; the parent fatty acid has been given the trivial name prostanoic acid. By convention,prostaglandins are abbreviated PG, with the class designated by a capital letter (A,B,E,F,G,H,and I),followed by a number and in some cases a Greek letter namely PGA, PGB,PGE, PGF, PGG or PGH and PGI (Burtis, *et al*.,2008). With the exception of PGG and PGH,which have the same ring structure (cyclopentane endoperoxide) and are intermediates in the formation of other PGs, the letters refer to different ring structures. The number after the

capital letter is usually written as subscript and is used to designate the number of unsaturated bonds in the PG side chains and not within the ring structure itself. The use of the Greek letter (α or β) applies only to the F series and refers to the hydroxyl group found at C-9. In the α-series, the hydroxyl group projects below the ring plane in the same direction as the C-11 hydroxyl group, whereas the β-series denotes that the hydroxyl at C-9 is above the plane of the ring. Sixteen naturally occurring prostaglandins have been described, but only seven, along with two thromboxanes are commonly found throughout the body. These are termed the primary prostaglandins. Although prostaglandins appear hormones-like in action, they are different from conventional hormones in that they are synthesized at the site of action and are made in almost all tissues. Linoleic acid ($C_{18:}2^{9,12}$) is the precursor of two of the three 20-carbon fatty acids that form prostaglandins; linoleic acid ($C_{18}:2^{9,12,15}$) is the other precursor. Both of these fatty acids are considered essential because they are not synthesized in the body and therefore must be present in the diet. The three C20 fatty acids subsequently found are (1) $C_{20}:3^{5,8,11}$ (eicosatrienoic acid), (2) $C_{20}:4^{5,8,11,14}$ (eicosatetraenoic or arachidonic acid),and $C_{20}:5^{8,11,14,17}$ (eicosatetraenoic acid).These three fatty acids form the PG_1,PG_2,and PG_3 series, respectively. Once formed, prostaglandins have short-lived effects and are catabolized within seconds. Inactivation of prostaglandin appears to be mediated by two enzymes, 15α-hydroxy-prostaglandin dehydrogenase and Δ^{13} prostaglandin reductase. Prostaglandins are not stored. However, the precursor C_{20} fatty acids are present in the tissue attached to the C-2 position of phosphoglycerides. When prostaglandin synthesis is stimulated, the C_{20} precursor is hydrolyzed from phospholipids by phospholipase A_2. The release of the C_{20} fatty acid appears to be the rate –limiting step in prostaglandin synthesis and is stimulated by various mediators, such as bradykinin, thrombins,or angiotensin11. Although it is probable that all prostaglandins follow a similar synthetic pathway, C_{20}:4 (arachidonic acid) has been the most intensively studied and is used to illustrate the general pathway. Once released, arachidonic acid follows one of two pathways. The lipoxygenase route produces 12-$_L$-hydroperoxy-5,8,10,14 eicosatetraenoic acid (HPETE); HPETE spontaneously decompose 12-$_L$-hydroperoxy-5,8,10,14 eicosatetraenoic acid (HETE). The alternative pathway is mediated by cyclooxygenase (COX) to produce the endoperoxides PGG_2 and PGH_2 . what controls the entry into a specific pathway remains speculative: however it is known that nonsteroidal anti-inflammatory drugs ([NSAIDs]):asprin,ibuprofen and indomethacin)inhibit the COX enzymes, thereby decreasing

prostaglandin synthesis. COX-1 and COX-2 are two isoforms of COX. COX-1 is constitutively expressed in cells, whereas COX-2 is synthesized in response to inflammation. Drugs that are specific for COX-2 have been developed to reduce the nephrogenic and ulcerogenic side effects from the inhibition of COX-1,but long-term use of these drugs has recently been associated with increased incidence of myocardial infarction, which may limit their clinical utility.$PG1_2$,or prostacyclin,is derived from arachidonic acid in the vascular endothelium. It has a powerful vasodilatory action, especially on the coronary arteries, and is also responsible for inhibiting platelet aggregation. Thromboxane A_2 is synthesized from arachidonic acid,but is also produced by platelets. It has the opposite effect of prostacyclin because it stimulates the contraction of arterial smooth muscle and enhances platelet aggregation. It has half-life of about seconds and is rapidly converted to its inactive metabolite,thromboxane B_2. The thromboxanes are slightly different in structure from the other prostaglandins in that they contain six-sided rings of five carbon atoms and one oxygen atom.

2.4.13 Terpenes

Tarpenes are polymers of the five-carbon isoprene unit and include vitamins A,E,and K and the dolichols, which play an important role in protein glycation.

2.4.14 Lipoproteins

Lipids synthesized in the liver and intestine are transported in the plasma in macromolecular complexes known as lipoproteins. They are typically spherical particles with non polar neutral lipids (triglycerides and cholesterol esters) in their core and more polar amphipathic lipids (phospholipids and free cholesterol) at their surfaces. The association of the core lipids with phospholipid and apolipoproteins is noncovalent occurring primarily through hydrogen bonding and van der Waals forces. The binding of lipids to apolipoproteins is weak and allows the exchange of lipids and apolipoproteins among the plasma lipoproteins and between cell membranes and lipoproteins. The binding is sufficiently strong, however, to allow the various classes of lipoprotein to be isolated by a variety of analytical techniques.

2.4.15 Classification of lipoproteins

Lipoproteins have different physical and chemical properties because they contain different proportions of lipids and proteins. Traditionally, lipoproteins have been categorized based on

differences in their densities, as determined by ultracentrifugation (Burtis *et al.*,2008). These categories include (1) chylomicrons (2) very-low density lipoprotein cholesterol (VLDL-C), (3) intermediate-density lipoprotein cholesterol (IDL-C) (4) low density lipoprotein cholesterol (LDL-C), (5) high density lipoprotein cholesterol (HDL-C) and lipoprotein(a) [Lp(a)]. In general the larger lipoproteins contain more core lipids, triglyceride and cholesteryl ester, and are lighter in density and contain a smaller percent of protein. In the fasting state, most plasma triglycerides are present in VLDL-C. In the post prandial state, chylomicrons appear transiently and contribute significantly to the total plasma triglyceride concentration. LDL-C carries about 70% of total plasma cholesterol, but very little triglyceride. HDL-C typically contains about 20% to 30% of plasma cholesterol. Lp(a) is a distinct class of lipoprotein which is structurally related to LDL-C because both lipoproteins possess one molecule of apo B-100 per particle and have similar lipid compositions. Unlike LDL-C, Lp(a) also contains a carbohydrate-rich protein apo(a)] that is covalently bound to the apo B-100 through a disulfide linkage. Apo(a) exhibits a significant sequence homology with plasminogen and a high degree of variation in polypeptide chain length. Apo(a) contains a tandem array of a protein motif called a kringle domain. The different size polymorphisms of Apo(a) are due to a variable number of kringle 4type 2 domains. Lipoproteins are also separated by various electrophoretic techniques. At pH 8.6, HDL-C migrates with the α-globulins, LDL-C with the β-globulins, and VLDL-C and Lp(a) between the α- and β-globulins in the pre-β—globulins region. IDL forms a broad band of between β- and pre-β—globulins. Chylomicrons remain at the application point. The major lipoprotein classes have been referred to by their electrophoretic locations: pre-β-lipoproteins, VLDL; β-lipoprotein, LDL; and α-lipoproteins, HDL-C. The electrophorectic separation of lipoproteins was the foundation for the older phenotypic classification (type 1-5) of familial dyslipidaemias (Burtis *et al.*,2008).

2.4.16 Apolipoproteins

Apolipoproteins are the protein component of lipoproteins i.e protein that bind lipids (oil-soluble substances such as fat and cholesterol) to form lipoproteins. They transport the lipids through the lymphatic and circulatory systems.The lipid components of lipoproteins are insoluble in water. However, because of their detergent-like (amphipathic) properties, apolipoproteins and other amphipathic molecules (such as phospholipids) can surround the lipids, creating the lipoprotein

particle that is itself water-soluble, and can thus be carried through water-based circulation (i.e., blood, lymph). Apolipoproteins also serve as enzyme cofactors, receptor ligands, and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in tissues (Burtis *et al*.,2008).

2.4.17 Functions

Apolipoproteins function as structural components of lipoprotein particles by maintaining the structural integrity, cofactors for enzymes and facilitating the uptake of lipoproteins by acting as ligands for specific cell-surface receptors in lipid transport,.ApoA1 in particular is the major protein component of high-density lipoproteins; apoA4 is thought to act primarily in intestinal lipid absorption. Further, apoE is a blood plasma protein that mediates the transport and uptake of cholesterol and lipid by way of its high affinity interaction with different cellular receptors, including the low-density lipoprotein (LDL) receptor. Recent findings with apoA-I and apoE suggest that the tertiary structures of these two members of the human exchangeable apolipoprotein gene family are related (Saito *et al*.,2004). The three-dimensional structure of the LDL receptor-binding domain of apoE indicates that the protein forms an unusually elongated four-helix bundle that may be stabilised by a tightly packed hydrophobic core that includes leucine zipper-type interactions and by numerous salt bridges on the mostly charged surface. Basic amino acids important for LDL receptor binding are clustered into a surface patch on one long helix (Wilson *et al.*,1991).They are enzyme coenzymes (C-II for lipoprotein lipase and A-I for lecithin-cholesterol acyltransferase).Lipid transport proteins and ligands for interaction with lipoprotein receptors in tissues (apoB100 and apoE for LDL-receptors, apoA-I for HDL receptors).

2.4.18 Classes of apolipoproteins

There are two major types of apolipoproteins. Apolipoproteins B form low-density lipoprotein (sometimes referred to as "bad cholesterol") particles. These proteins have mostly beta-sheet structure and associate with lipid droplets irreversibly. Most of the other apolipoproteins form high-density lipoprotein ("good cholesterol") particles. These proteins consist of alpha-helices and associate with lipid droplets reversibly. During binding to the lipid particles these proteins

change their three-dimensional structure. There are also intermediate-density lipoproteins formed by Apolipoprotein E. There are six classes of apolipoproteins and several sub-classes:
A (apo A-I, apo A-II, apo A-IV, and apo A-V), B (apo B48 and apo B100), C (apo C-I, apo C-II, apo C-III, and apo C-IV), D, E, H. Apo A-I is the major protein in HDL, Apo CI,II, III, and E are present in various proportions in all lipoproteins. Apo B -100 and apo B -48 are firmly bound to lipoproteins and do not exchange between the different lipoproteins like the other apolipoproteins.Exchangeable apolipoproteins (apoA, apoC and apoE) have the same genomic structure and are members of a multi-gene family that probably evolved from a common ancestral gene. ApoA1 and ApoA4 are part of the APOA1/C3/A4/A5 gene cluster on chromosome 11(Fullerton *et al*.,2004). Hundreds of genetic polymorphisms of the apolipoproteins have been described, and many of them alter their structure and function.

2.4.19 Synthesis and regulation

Apolipoprotein synthesis in the intestine is regulated principally by the fat content of the diet.
Apolipoprotein synthesis in the liver is controlled by a host of factors, including dietary composition, hormones (insulin, glucagon, thyroxin, estrogens, androgens), alcohol intake, and various drugs (statins, niacin, and fibric acids). Apo B is an integral apoprotein whereas the others are peripheral apoproteins.

2.4.20 Lipid peroxidation and its products

Lipid peroxidation is a well defined mechanism of cellular damage in both animals and plants that occurs in vivo during ageing and in certain disease states. Lipid peroxides are unstable markers of oxidative stress which decompose to form complex reactive by-products. Most common by-products of lipid peroxidation are malondialdehyde (MDA),4-hydroxynonel(4-HNE) and 8-iso prostaglandin F2 alpha(8-isoprostane). Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism.

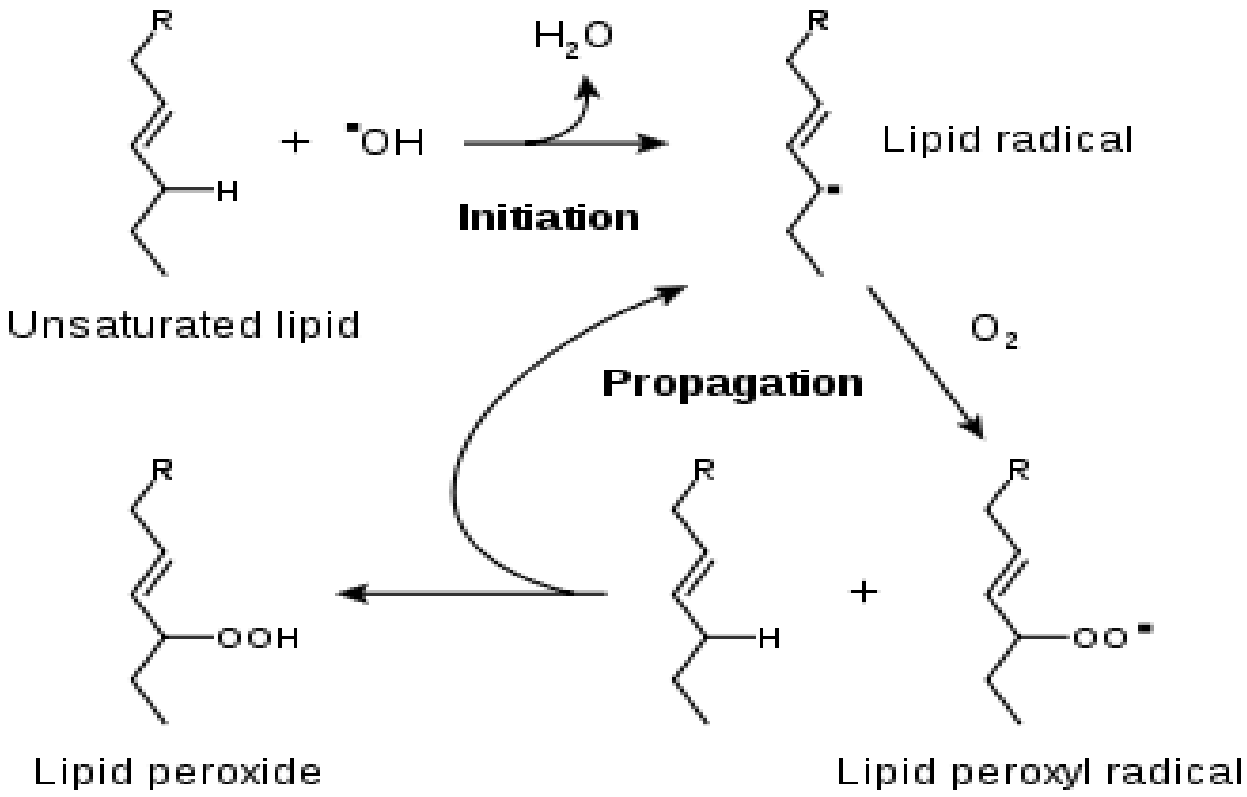

Figure 2.6 Mechanism of lipid peroxidation (Catala, 2007).

It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene -CH2- groups that possess especially reactive hydrogens. As with any radical reaction, the reaction consists of three major steps: initiation, propagation and termination.

2.4.21Initiation

Initiation is the step whereby a fatty acid radical is produced. The initiators in living cells are most notably reactive oxygen species (ROS), such as OH· or HO2, which combines with a hydrogen atom to make water and a fatty acid radical.

2.4.22 Propagation

The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This too is an unstable species that reacts with another free fatty acid producing a different fatty acid radical and a lipid peroxide or a cyclic peroxide if it had reacted with itself. This cycle continues as the new fatty acid radical reacts in the same way.

2.4.23 Termination

When a radical reacts with a non-radical it always produces another radical, which is why the process is called a "chain reaction mechanism." The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of two radicals actually colliding. Living organisms have evolved different molecules that speed up termination by catching free radicals and therefore protect the cell membrane. One important such antioxidant is vitamin E. Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase.

2.4.24 Hazards

Hazard occurs if is not terminated fast enough, there will be damage to the cell membrane, which consists mainly of lipids. Phototherapy may cause hemolysis by rupturing red blood cell membranes in this way.In addition, end products of lipid peroxidation may be mutagenic and carcinogenic . For instance, the end product malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts to them, primarily M1G (Marnett,1999).The toxicity of lipid hydroperoxides to animals is best illustrated by the lethal phenotype of

glutathione peroxidase ,Trevisan *et.al.*,(2000) ,(GPX4) knockout mice. These animals do not survive past embryonic day 8, indicating that the removal of lipid hydroperoxides is absolutely essential for mammalian life (Muller *et.al.*,2007).

2.4.25 Tests for the quantification of the end products of lipid peroxidation

Certain diagnostic tests are available for the quantification of the end products of lipid peroxidation, specifically malondialdehyde (MDA) (Marnett,1999).The most commonly used test is called a TBARS Assay (thiobarbituric acid reactive substances assay). Thiobarbituric acid reacts with malondialdehyde to yield a fluorescent product. However, there are other sources of malondialdehyde, so this test is not completely specific for lipid peroxidation (Trevisan *et.al.*,2000). In recent years, development of immunochemical detection of HNE-histidine adducts opened more advanced methodological possibilities for qualitative and quantitative detection of lipid peroxidation in various human and animal tissues as well as in body fluids, including human serum and plasma samples. In biochemistry and metabolism, beta-oxidation is the catabolic process by which fatty acid molecules are broken down in the cytosol in prokaryotes and in the mitochondria in eukaryotes to generate acetyl-CoA, which enters the citric acid cycle, and NADH and $FADH_2$, which are co-enzymes used in the electron transport.

Oxidative stress moreover, is an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (Ihim *et al*, 2012).

2.5.0 The body mass index (BMI)

The body mass index or Quetelet index is a value derived from the mass (weight) and height of an individual. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in metres.

The BMI may also be determined using a table or chart which displays BMI as a function of mass and height using contour lines or colours for different BMI categories, and which may use other units of measurement (converted to metric units for the calculation).The BMI is an attempt to quantify the amount of tissue mass (muscle, fat, and bone) in an individual, and then categorize that person as *underweight*, *normal weight*, *overweight*, or *obese* based on that value. However, there is some debate about where on the BMI scale the dividing lines between categories should be placed(Malcolm, 2015). Commonly accepted BMI ranges are underweight: under 18.5 kg/m^2, normal weight: 18.5 to 25, overweight: 25 to 30, obese: over 30.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out at TB DOT Clinics of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Immaculate Heart Hospital Nkpor and St. Charles Boromeo Hospital Nkpor in Anambra State.

3.2 Study design

The study was a prospective follow up study in which blood sample was collected from each individual before initiation of antituberculosis therapy, two months into ATT, six months into ATT, grouped accordingly and involved, laboratory investigations and use of questionnaires to obtain personal data and relevant medical history. See appendix 1

3.3 Study population

The study population consisted of five thousand five hundred and eighteen (5518) suspected individuals with cardinal symptoms of tuberculosis who presented at TBDOT clinics of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi between May, 2015 and January, 2018, five hundred and thirteen (513) of such individuals who presented at TBDOT clinics of Immaculate Heart Hospital Nkpor (IHHN) between May, 2015 and January, 2018 and eight hundred and thirty four (834) of such individuals who presented at TBDOT clinics of St. Charles Boromeo Hospital Nkpor (SCBHN) between June, 2017 and January, 2018. The clinics are located at Anambra State. They were chosen because they were the three major clinics for tuberculosis treatment in Anambra State. They were individuals on category 1 first line TB treatment. Therefore, they received for two months also referred to as Intensive phase (Rifampicin (R), Isoniazid (H), Pyrazinamide (Z), Ethambutol (E)(150/75/400/275mg/kg body weight) tablets and for four months also referred to as continuation phase RH (150/75 mg/kg body weight) tablets to complete six months treatment duration.

3.4 Sampling Technique

A convenience and consecutive sampling techniques were used to select individuals from the TB DOT centres before the initiation of therapy.Participants were individuals who met the inclusion criteria and consented after the purpose was explained to them and were recruited as they became available until the sample size was attained.

3.5 Sample size determination

Prevalence rate of tuberculosis in NAUTH TB DOTS Clinic for 2013 was calculated using the figures below. Number of suspected TB patients of NAUTH TB DOTS Clinic for 2013= 2112

Number of TB positive patients of NAUTH TB DOTS Clinic for 2013 = 169

TB prevalence rate for suspected TB positive patients = 169/2112 X 100% = 8% (NAUTH, 2013).Sample size was determined using the formula of Araoye, (2004).

$N=Z^2Pq\ (1-P)/D^2$

Where;

N=minimum sample size

P= expected prevalence rate (%) of NAUTH TB DOT Clinic for 2013=169/2112 X 100% = 8%

D= desired level of significance = 0.05 (5%)

Z= standard normal deviation set at 1.96 which corresponds to the 95% confidence level;

q= alternate proportion (1-p) (1 - 0.08 =0.92)

Substituting in the above formula

$N= (1.96)^2 \times 0.08\ X\ 0.92)/\ (0.05)^2$

= 3.842 X 0.08X 0.92/0.0025

=0.2827712/0.0025

=113. A total sample size of 113 was calculated. In order to take care of possible attrition, a total of 159 individuals infected with active TB were recruited for the study.

3.6 Recruitment of individuals with tuberculosis infection

One hundred and fifty nine (159) individuals who presented at TB DOTS clinics between May, 2015 and January, 2018, comprising of ninety six(96)(F=32,M=64) from NAUTH, forty six (46;F=17,M=29)from IHHN and seventeen (17;F=5,M=12)from SCBHN with symptoms such as chronic cough with blood-tinged sputum, fever, night sweats, chest pain and weight loss were confirmed by serial sputum smear acid fast bacillus(AFB) microscopy, molecular diagnostic technique (gene Xpert) and microbiological culture of sputum to be infected with *Mycobacterium tuberculosis* were recruited for this study.Their weights and heights were measured, recorded and used to compute their BMI. They were screened for malaria parasite (MP) and human immuno-deficiency virus (HIV) upon recruitment. They were classified into three groups namely;individuals with active TB (TB+), individuals with TB and HIV co-infection (TBHIV+), then individuals with TB and MP co-infection (TBMP+), based on their screening test results. Upon recruitment TB+, TBHIV+ and TBMP+ were (107;F=37,M=70), (35;F=11,M=25) and 17(F=6,M=11) respectively in number. One hundred and seven (107) TB+ comprising of sixty three (63;F=21,M=42) from NAUTH, thirty two(32;F=12,M=20) from IHHN and twelve (12;F=4,M=8) from SCBHN. Thirty five (35) TBHIV+ comprising of twenty one (21)(F=7,M=14) from NAUTH, ten(10)(F=3,M=7) from IHHN and four(4)(F=1,M=3) from SCBHN while TBMP+ comprises of twelve (12)(F=4,M=8) from NAUTH, four(4)(F=2,M=2) from IHHN and one (1)(F=0,M=1) from SCBHN. They were followed up for six months on antituberculosis therapy (ATT). They were individuals on category 1 first line TB treatment. Therefore, they received for two months also referred to as Intensive phase (Rifampicin (R), Isoniazid (H), Pyrazinamide (Z), Ethambutol (E)(150/75/400/275mg/kg body weight) tablets. Sputum smear microscopy and drug susceptibility test (DST) by rapid molecular based method (gene Xpert) for monitoring treatment outcome, predicting treatment progress, relapse, failure and pre-treatment Isoniazid resistance were carried out on completion of two months treatment. At this point few were already negative to sputum smear microscopy test only. Based on the results of gene xpert, individuals who were found to harbor multi drug resistance tuberculosis (MDR-TB) strains were excluded from the research because they would be registered as having failed treatment and would be treated with different regimen while those without MDR-TB strains proceeded with continuation phase RH (150/75 mg/kg body weight) tablets for four months to complete six months treatment duration.

Upon completion of two months ATT. Twenty six (26)(F=11,M=15) were lost due to attrition. Out of this twenty six (26) individuals, Nine (9)(F=4,M=5) were lost due to transfer of treatment location upon recruitment , thirteen (13)(F=4,M=9) to death and four (4)(F=3,M=1) due to treatment failure.The remaining one hundred and thirty three (133) individuals infected with TB consisting of eighty nine (89;F=30,M=59:TB+=54,F=18,M=36, TBHIV+=26,F=9,M=17,TBMP+=9, F=3,M=6) from NAUTH, thirty four (34) (F=11,M=23:TB+=24,F=8,M=16,TBHIV+=7,F=2,M=5,TBMP+=3,F=1,M=2)from IHHN and ten(10)(F=2,M=8:TB+=7,F=2,M=5, TBHIV+=2,F=0,M=2, TBMP+=1,F=0,M=1)from SCBHN proceeded with continuation phase RH(150/75 mg/kg body weight) tablets for four months to complete six months treatment duration. Upon completion of six months ATT. Thirteen individuals (13) were lost due to attrition. Out of this thirteen (13) individuals, seven (7) (F=3,M=4) were lost due to transfer of treatment location , six(6)(F=2,M=4) to death.

The remaining one hundred and twenty (120) individuals comprising of seventy five(75) (F=24,M=51:TB+=50,F=16,M=34,TBHIV+=16,F=5,M=11,TBMP+=9,F=3,M=6)from NAUTH, thirty five(35) (F=12,M=23:TB+=25,F=8,M=17,TBHIV+=8,F=2,M=6,TBMP+=2,F=2,M=0)from IHHN and ten(10)(F=2,M=8:TB+=7,F=2,M=5,TBHIV+=2,F=0,M=2, TBMP+=1,F=0,M=1)from SCBHN successfully completed six months follow up. They consisted of eighty two(n= 82,F=26,M=56) individuals with active TB, twenty six (n=26,F=7,M=19) individuals with TB and HIV co-infection, and twelve (n=12,F=5,M=7) individuals with TB and MP co-infection.

In addition, one hundred and thirty one individuals were also recruited for this study from Nnewi and Nkpor. These were household contacts of patients suffering from tuberculosis or others who were in close contact with tuberculosis patients. They comprised mostly of relatives and caregivers. Others were apparently healthy volunteers who had no known contacts with patient with tuberculosis or suspected tuberculosis cases. They were administered with purified protein derivative (PPD) for the mantoux test which detects latent tuberculosis and screened for malaria and HIV. The weights and heights of all the individuals were measured, recorded and used to calculate their BMI. Questionnaire was used to obtain relevant data and personal medical history of all the individuals. They were classified into two groups of individuals namely; apparently healthy individuals referred to as normal healthy control (NHC) and individuals with latent

tuberculosis infection (LTBI), based on their screening test results. Out of these individuals, one hundred and five individuals (n=105,F=29,M=76) were apparently healthy control and twenty six (n=26,F=9,M=17) individuals with latent tuberculosis infection (LTBI).

3.7 Inclusion criteria

Newly diagnosed TB positive individuals with or without mp, and or HIV co -infections were recruited. The individuals above were Category one, first line TB positive individuals between 15-66years, attending the TB DOTS Clinic, NAUTH, Nnewi, St. Charles Boromeo Hospital and Immaculate Heart Hospital Nkpor and were followed up on six months course anti tuberculosis therapy.

3.8 Exclusion criteria.

Individuals infected with TB on antiretroviral therapy were excluded. Patients diagnosed with pulmonary tuberculosis but having diabetes mellitus were excluded from the study.Tobacco smokers, alcohol drinkers and participants who had other clinical problems such as diabetics and cardiovascular diseases were excluded from the study.

3.9.0 Ethical consideration

Ethical approval for the study was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTHEC) NAUTH/CS/66/VOL.7/79 Nnewi, Immaculate Heart Hospital Ethics Committee (IHHEC)(14/04/16) and St.Charles Boromeo Hospital Nkpor(24/7/17). See appendices 3,4 and 5.

3.9.1 Informed consent

The aim, benefits and purpose of the study was explained to the individuals. Participation was voluntary and informed consent was obtained from all the individuals. The individuals were allowed at any time they so desired to discontinue and that would not in any way affect their care. The information obtained from the individuals was kept highly confidential in observance of the privacy act. See appendix 2

3.9.2. Research Questionnaire

Socio-demographic information such as age, sex, educational status, marital status, occupation etc., including clinical details such as symptoms, history of infection, smoking, alcohol use etc were obtained using a questionnaire.

3.9.3 Sample collection

3.9.3.1 Sputum collection

Sputum samples were collected using the Directly Observed treatment short Course (DOTs) strategy specification. Sputum sample was collected twice (consisting of on the spot sample and early morning sample next day) into a wide mouth container from each of the individuals.

3.9.3.2 Sputum processing

Sputum samples were processed using the Ziehl Neelsen Staining Method and confirmed using the Gene Xpert by Cepheid, especially for HIV positive cases with cough.

The individuals with positive TB results were referred to the DOTs clinic where they commenced treatment with the intensive phase using Rifampicin, Isoniazid, pyrazinamide and ethambutol for two months. After the intensive phase of treatment, a repeat sputum microscopy was done to determine if the individuals still had open TB, before continuation phase was started with Rifampicin and Isoniazid for four months. Individuals who had open TB, were suspected of resistance and their samples were sent for Gene Xpert confirmation. (Category 1 failure). (Gene Xpert confirms TB infection but most importantly, it confirms resistance to Rifampicin).

3.9.3.3 Blood sample collection

Blood samples were collected three times respectively from individuals with active *Mycobacterium tuberculosis* infection. Firstly, immediately the individual(s) was confirmed to be positive for pulmonary tuberculosis by Ziehl Neelsen's staining technique and GeneXpert MTB/RIF assay, before the initiation of anti tuberculosis treatment (ATT).

Secondly on successful completion of two months course ATT. At this point, some individuals were AFB negative (-) while some were AFB +, both continued with ATT.Thirdly on successful completion of six months course ATT. Blood samples were collected from individuals with latent TB and apparently healthy individuals (control) once. Eight milliliters (8mls) of blood was collected from each individuals at each period of blood collection, thick and thin blood films were made for microscopic detection of *P. falciparium on* recruitment and malaria *plasmodium*

falciparum/pan rapid test device (Startcare ™ Accessio USA) which is a chromatographic immunoassay for the qualitative detection of circulating *P. falciparum* antigen in whole blood was also used. Two milliliters (2ml) of blood was dispensed in ethylene diamine tetracetic acid (EDTA) bottle and six milliliters (6ml) of blood was dispensed in plain tube to separate serum for various biochemical assays (Nwanjo *et al.*, 2007). The blood in the plain tube was allowed to stand for 30 minutes to clot and further centrifuged at 3500 rpm for five minutes using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with micropipette into sterile serum sample bottle for the measurement of biochemical parameters. Each individual's blood sample was stored frozen at -20^0C in aliquot, in three vials to avoid repeated thawing and storing that would affect the result of the analysis.

3.9.4 Anthropometric measurements

The following anthropometric indices were measured for each individual using standardized procedure (Frisancho, 1990; Gibson, 1990):

a. Weight was measured to the nearest 0.5 kilogram using a standard weighing scale with the individuals wearing light clothes and on bare foot. The scale was on a hard and flat surface, and calibrated frequently using known standard 10kg weight, while the pointer of the scale was adjusted to zero before each measurement.
b. Height was measured to the nearest 0.1 cm using a stadiometer in an erect position against the wall without foot wears, head scarf or caps.
c. Body Mass Index (BMI) was then calculated by dividing the weight (W) in kg by the square of the participant's height (H^2) in meters i.e. BMI = W/H^2 in (kg/m^2) and all values were taken to the nearest one decimal place.

3.9.5 Laboratory methods and procedures

All the reagents were commercially purchased and the manufacturers' standard operating procedure (SOP) was strictly followed.

Ziehl-Neelsen (ZN) sputum smears to detect AFB (as described by Cheesbrough, 2004)

Principle of Ziehl-Neelsen

The sputum smears were stained with carbol fuchsin combined with phenol. The stain was bound to the mycolic acid in the mycobacterial cell wall which resists decolorization by 3% v/v acid

alcohol decolorizing solution and upon counterstaining with malachite green or methylene blue provided a background contrast colour against the red AFB.

Test procedure

Smears were spread evenly on slides covering a diameter of about 20mm using wooden spatula. The smear was air dried completely and then fixed with 2 drops of 70% v/v methanol. The methanol was left on the smear for 3 minutes. The slide was rapidly passed; smeared uppermost, three times through the flame of a bunsen burner. The smear was allowed to cool before staining. The smear was covered with carbol-fuchsin stain and the stain was heated until vapor begins to rise. The heated stain was left on the slide for 5 minutes and stain was washed off with clean water. The smear was covered with 3% v/v acid alcohol (decolorizing solution) for 3 minutes and was washed with clean water. The smear was then covered in methylene blue for 60 seconds, and washed with water. The back of the slide was wiped and placed on a draining rack to air dry. The smear was examined microscopically, using ×100 oil immersion objectives.

Result

AFB: Red, straight or slightly curved rods, occurring singly or in a group and background material appeared blue. The numbers of bacteria present was reported per high power field as follows;

more than 10 AFB/FIELD:+++

1-10 AFB/FIELD:++

10-1000 AFB/100 FIELDS:+

1-9 AFB/FIELDS:exact number was reported

3.9.6 GeneXpert methods for detection of mycobacterium tuberculosis and rifampin resistance (GeneXpert MTB/RIF) (As reported by Blakemore *et al,* 2010).

Principle

GeneXpert MTB/RIF assay is a catridge based nucleic acid amplification (NAA) test which identifies DNA sequences specific for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF) (i.e. mutation of the rpoB gene) in less than 2 hours by polymerase chain

creaction. The Xpert® MTB/RIF purifies and concentrates *Mycobacterium tuberculosis bacilli* from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR (Danica *et al*., 2010).

Procedure

The sputum samples were batched to a maximum of four per run. The patient(s) details were entered on theXpert MTB/Rif worksheet.

Lid of leak proof sputum collection container were unscrewed. The sputum volume was measured using a graduated plastic disposable pipette.The volume was recorded on the Xpert/Rif worksheet. (Note: the maximum volume on the graduated disposable pipette is 3ml). Carefully the pipette was discarded. The sample reagent was added at 2:1 (v/v) ratio to the sputum sample by using separate plastic disposable pipette. The lid of sputum cup was replaced carefully and leakages on the cup were avoided. The sputum cup was shaken vigorously 10-20 times using back and forth movements in a single shake and incubated for 15 minutes at room temperature. It was also shaken at least once, as described above during incubation. After incubation, the sputum sample should be liquefied with no visible clumps of sputum. Particulate matter may exist that is not part of the sample. Each Xpert MTB/RIF cartridge was labelled with the Laboratory number on the front side bottom of the cartridge as the same given on the sputum cup with marker pen.

Preparing the cartridge

The liquefied sample was drawn into the transfer pipette until the meniscus of pipette was above the minimum mark by using the sterile transfer pipette provided in theXpert/Rif kit. (Sample (s) with insufficient volume(less than 2ml) was not processed further) . Sample was transferred into the open port of the Xpert MTB/RIF cartridge and dispensed slowly to minimize the risk of aerosol formation. The transfer pipette was discarded into bio-hazard waste bin. The cartridge lid was closed by making sure the lid snaps firmly into place.The remaining liquefied sample was kept for up to 12 hours at 2-8°C (for repeat testing). The cartridge was loaded into the GeneXpert Dx instrument. The system was confirmed attached to a working uninterrupted power source, then the computer and GeneXpert Dx instrument were turned on. The Sample ID and Laboratory number were recorded in the gene Xpert window. The Laboratory number must match the

number on the cartridge and on sputum cup.The test was started within 30 minutes of adding the sample to the cartridge by clicking start test. The instrument module door would open and displayed the blinking green light, and the cartridge was loaded. The door of the module was closed firmly (an audible click sound was heard). The test started and the green light stopped blinking. When the test was finished, the light turned off. Next cartridge was loaded following the steps described above. Result was printed automatically once the run was completed. It took around 1 hour 55 minutes to complete run. The cartridge was removed when the system released the door lock at the end of run. The used cartridge was disposed in the biohazard waste container.

Quality controls

Each Xpert MTB/RIF cartridge includes a Sample processing control (SPC) and Probe Check control (PCC). Print out of the test result indicates the validation of controls.

Interpretation of results

The results were interpreted by the GeneXpert Dx system from measured fluorescent signals and embedded calculation algorithms. Result was displayed in the "View Results" window. Lower Ct values represented a higher starting concentration of DNA template; higher Ct values represented a lower concentration of DNA template.

MTB detected

If MTB target DNA was detected- the MTB result displayed High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sputum sample. Below table lists the Ct value ranges for the displayed MTB results (Boehme *et al*.,2011).

MTB result	Ct range
High	<16
Medium	16-22
Low	22-28
Very Low	>28

Rifampicin resistance result types, when MTB is detected:

Rifampicin resistance DETECTED: a mutation in the rpoB gene has been detected that falls within the valid delta Ct setting.

Rifampicin resistance NOT DETECTED: no mutation in the rpoB has been detected.

Rifampicin resistance INDETERMINATE: the MTB concentration was very low and resistance could not be detected.

MTB not detected

MTB target DNA is not detected. Sample Processing Control (SPC) meets acceptance criteria.

MTB NOT DETECTED-MTB target DNA is not detected

SPC- Pass; SPC has a Ct value range and endpoint above the endpoint minimum setting.

Probe Check- PASS: all probe check results pass.

RIF not detected

Rifampicin target DNA is not detected, SPC meets acceptance criteria.

RIF NOT DETECTED- Rifampicin target DNA is not detected.

SPC- Pass; SPC has a Ct value range and endpoint above the endpoint minimum setting.

Probe Check- PASS: all probe check results pass.

INVALID

Presence or absence of MTB cannot be determined, repeat test with extra specimen. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

MTB INVALID- Presence or absence of MTB DNA cannot be determined

SPC-FAIL; MTB target results is negative and the SPC Ct is not within valid range.

Probe Check- PASS; all probe check results pass.

3.9.7 Mantoux test (as described by Cheesbrough, 2004)

The Mantoux Tuberculin skin test is the standard method of determining whether a person is infected with *Mycobacterium tuberculosis* or have exposure to tubercle bacilli . It was developed by Kock in 1890 but the intradermal technique currently in use was described in 1912 by a French physician , Charles Mantoux after whom the test is named. This test is known as the

tuberculin skin test. The PPD used was obtained from BB – NCIPD Ltd, sofia, Bulgaria. Each vial contained 1ml (10 doses) containing 50TU of PPD = 5TU/0.1ml per dose.

Principle

Tuberculin skin test is the classic clinical demonstration of the function of the delayed type hypersensitivity response. When an antigen i.e PPD of tubercle bacilli is injected intradermally in an individual, immune response of person who has been exposed to the bacteria is expected to mount within 48-72hours leading to the formation of an induration, a raised bumb in the volar surface of the forearm (area of the injection) which is due to the influx and activation of macrophages.

Injection Technique (According to the manufacturer's instruction)

The dorsum of the middle third of the forearm was the site of injection and was cleaned with alcohol and allowed to dry. The rubber cap of the vial was wiped clean with a sterile piece of cotton wool moistened with alcohol and allowed to dry. One milliliter (1.0ml) graduated tuberculin syringe was inserted gently through the cap and filled to slightly above 0.1ml with the tuberculin PPD. Any air bubbles were removed and the volume was adjusted to exactly 0.1ml (test dose 0.1 ml of PPD containing 5 tuberculin units). The needle was inserted into skin with the bevel up, entering just the superficial layer of the skin. The solution was injected slowly and a small papule (bleb) about 6mm to10mm in diameter appeared. This disappeared within minutes. Using a marker, a circle of about 3cm was drawn around the site of injection for easy site identification. The subject was instructed to return for the test reading after 48 hours, taking care during the period not to scratch, or irritate the area.

Evaluation of the reaction

A positive reaction to tuberculin purified protein derivative was shown by a flat, uneven, slightly raised palpable induration with a diameter of at least 5 millimeters. Reactions with a diameter larger than 15mm was defined as strongly positive and that gave a strong indication of infection with mycobacterium tuberculosis complex.

3.9.8 Detection of Antibodies to HIV – 1 and HIV – 2

The HIV status of study participants was determined using the Determine HIV-1/2 (Abbott

laboratories, Japan) as the screening test, the Capillus HIV-1/2 (Trinity Biotech, Ireland) as the confirmatory test and Uni-Gold HIV-1/2 recombinant (Trinity Biotech, Ireland) as a tie breaker test. Tests were carried out according to the manufacturer's instruction.

Principle of DETERMINE^{TM}

Determine HIV – 1 and 2 is an in vitro, usually read, qualitative immunoassay for the detection of antibodies to HIV – 1 and HIV – 2 in human serum, plasma and whole blood. Determine is an immunochromatograghic test which relies on the migration of liquid across the surface of a nitrocellulose membrane. Sample is added to the sample pad followed by addition of running buffer. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid – antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV – 1 and /or HIV – 2 are present in the samples, the antibodies bind to the antigen selenium colloid at the patient window forming a red line at the patient window site. If antibodies to HIV – 1 and/or HIV – 2 are absent, the antigen – selenium colloid flows past the patient sample window and no red line is formed at the site. There should always be a red line at the control window. This is the incorporated procedural control in assay device to ensure assay validity.

Procedure

As described by the manufacturer, 50µl of subject serum was separated from clotted whole blood sample and was delivered onto appropriately labelled pads. After 15 minutes of sample application, the result was read. This, method has in built quality control which validates the result. Two visible red lines in the region labeled control and test represented a HIV seropositive reaction while a single red line in the region labeled control only represented a HIV seronegative reaction.

Principle of STAT – PAK

The chembio HIV – 1 and 2 STAT – PAK which is an immunochromatographic assay , relies on the migration of liquid across the surface of a nitrocellulose membrane. It employs a unique combination of a specific antibody binding protein which is conjugated colloidal gold dye. HIV – 1 and 2 antigens are bound to the membrane solid phase. The sample is delivered onto the

sample well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigen; if present, the antibodies bind to the gold conjugative antibody binding protein. In a reactive sample, the dye conjugate – immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the control area containing immunoglobulin G antigens

Procedure

The procedure was as described by the manufacturer. 5µl of each subject's serum was collected using sample loop from the kit by touching it on the specimen and allowing the opening in the loop to fill. The samples were then dispensed on the sample wells in appropriately labeled sample pad. Three drops of buffer supplied with the test kit were added to the sample wells. The result was read after 5 minutes. This method also has inbuilt quality control. The presence of two pink/purple lines in the control and test regions indicated a HIV seropositive reaction while a single pink/purple line at the control region indicated a HIV seronegative reaction.

3.9.9 Diagnosis of Malaria

Whole blood was used for the diagnosis of *P. falciparum* malaria using thick and thin blood smears for microscope detection and malaria plasmodium falciparum rapid test device (CARESTART^{TM} Malaria HRP2 (Pf) by ACCESS BIO,INC. USA) which is a chromatographic immunoassay for the qualitative detection of circulating *p. falciparum* antigen in whole blood. It contains a membrane strip, which is pre-coated with a monoclonal antibody as a single line across a test strip. The monoclonal antibody is specific to the HRP2 (histidine-rich protein 2) of the P. *falciparium.* The conjugate pad is dispensed with antibody absorbed on gold particles, which are specific to HRP2 of P. *falciparium.*

The thick blood film concentrated red blood cells (RBCs) on a small surface and was more sensitive than the thin film method in detecting low levels of parasitaemia. Giemsa stain an alcohol based Romanowsky stain was used. It is a mixture of eosin which stains the parasite chromatin red or pink and methylene blue which stains the parasite cytoplasm blue. The RBCs were lysed during the staining process leaving white blood cells, platelets and any parasites.A drop of blood was placed on the centre of a clean grease free slide and spread in a circular

manner on the slide with the corner of another slide till it covered an area four times its original area. The film was allowed to dry properly in the incubator at 37^0 C for 30 minutes before immersing in a staining jar containing Giemsa stain (freshly diluted with buffered water (10%) for 30 minutes before washing off. The slides were stood upright to air dry. Slides were viewed using X100 objective (oil immersion) for detection of malaria parasite. In the thin blood films, RBCs were fixed and a parasites inclusion in RBCs was demonstrated. The morphological identification of the parasite to the species level was much easier and provided greater specificity than the thick film examination. For the procedure, a small drop of blood was placed at the bottom of the slide and using a spreader, a thin film was made. This was air dried and stained using Leishman stain first for 2 minutes before double diluting in buffered water and staining further for 8 minutes. Subsequently, the slides were washed and air dried before viewing using X100 objective. For the rapid malaria parasites test, whole blood was used for the diagnosis using malaria plasmodium falciparum/pan rapid test device (CARESTART^{TM} Accessio USA).

Principle

The principle is based on a rapid chromatographic immunoassay for the qualitative detection of circulating P. falciparum antigen in whole blood. Immunochromatography relies on the migration of liquid across the surface of a nitrocellulose membrane. The immunochromatographic tests are based on the capture of parasite antigens from peripheral blood using monoclonal antibodies prepared against a malaria target and conjugated to gold particles in a mobile phase. A second capture monoclonal antibody applied to a strip of nitrocellulose acts as the immobile phase. The migration of the antigen – antibody complex in the mobile phase along the strip enables the labeled antigen to be captured by the monoclonal antibody of the immobile phase, thus producing a visible coloured line. The test cassette (CARESTART^{TM}) contains a membrane strip, which is pre-coated with a monoclonal antibody as a single line across a test strip. The monoclonal antibody is specific to the HRP2 (histidine-rich protein 2) of the P. *falciparium.* The conjugate pad is dispensed with antibody absorbed on gold particles, which are specific to HRP2 of P. *falciparium.* The assay buffer borax buffered SDS and saponin solution. The mouse monoclonal antibodies react with the malaria antigen if present in the sample they move along the membrane chromatographically to the test region and form a visible line as the antibody – antigen – antibody gold particle complex with high degree

of sensitivity and specificity. Both the test line and control line in the result window are not used for procedural control and should always appear if the test has been performed correctly.

Procedure (as described by the manufacturer)

Briefly, 5µl of the whole blood specimen from the subjects was delivered unto labeled round specimen wells in the cassettes, using the specimen loops provided. 2 drops of assay buffer (supplied with the test device) was added into square assay diluents wells. After 20 minutes, the results were read.

For positive result:

P. falciparum positive: the presence of two colour bands ("P.F" Test line and "C" Control line) or three colour bands ("P.F", "Pan" Test lines and "C"Control) within the result window, no matter which bands appears first, P. *falciparum* positive result would be indicated.

Procedure for parasite count (as described by Cheesbrough, 2005)

The parasite count was done using thick blood films. The films were stained using Giemsa stain as previously described. The density of malaria parasites were estimated by counting the number of parasites against 200 white blood cells (WBCs) in the thick film. The parasite density (per µl) was obtained using the formula:

$$\frac{\text{No of parasite counted}}{\text{WBC counted (200)}} \times \frac{\text{WBC count}}{1}$$

3.19. Estimation of serum lipids.

(a)Total Cholesterol

(a)Total Cholesterol was estimated by Cholesterol enzymatic End-point Method (Roeschlau *et.al.,* 1974). The kit was purchased from Randox Diagnostic LTD,Cat. No. CH 200.

Principle

Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4- amino antipyrine in the presence of phenol and peroxidase (Trinder, 1969).

Cholesterol ester + $H_2O \xrightarrow{\text{cholesterolesterase}}$ Cholesterol + fatty acids

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

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

Procedure

10(ul) of serum, distilled water, control and standard cholesterol was collected and transferred into four test tubes labelled sample ,reagent blank,control and standard respectively.1000(ul) of reagent(R1) was added into all the test tubes, mixed and incubated for 5 minutes at 37^{O}C. The absorbance of the sample (A_{sample}) against the reagent blank was read spectrophotometrically within 60 minutes at 500nm.

(b) Triglycerides

Triglycerides was estimated by enzymatic method (Tietz, 1990 and Trinder, 1969).

The kit was purchased from Randox Diagnostic LTD,Cat. No. TR 210.

Principle

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide,4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.(Tietz,1990 and Trinder,1969).

Triglycerides + $H_20 \xrightarrow{lipase}$ glycerol + fatty acids

Glycerol +ATP $\xrightarrow{GK}$ glycerol-3-phosphate +ADP

Glycerol-3-phosphate + $O_2 \xrightarrow{GPO}$ dihydroxyacetone + phosphate + H_2O_2

$2H_2O_2$ +4-aminophenazone +4 chlorophenol $\xrightarrow{POD}$ quinoneimine +HCl +$4H_2O$

Procedure

10(ul) of serum, distilled water, control and standard cholesterol was collected and transferred into four test tubes labelled sample ,reagent blank, control and standard respectively.1000(ul) of reagent(R1=reagent mixture R1a +R1b) was added into all the test tubes, mixed and incubated for 5 min at 37^{O}C. The absorbance of the sample (A_{sample}) against the reagent blank was read spectophotometrically within 60 minutes at 500nm.

(c) High Density Lipoproteins-cholesterol (HDL-C).

The kit was purchased from Randox Diagnostic LTD,Cat. No. CH 203.

Principle

Low Density Lipoprotein (LDL AND VLDL) and chylomicron fractions are precipitated quantitatively from serum by the addition of phosphotungstic acid in the presence of

magnessium ion. After centrifugation, the cholesterol concentration in the HDL (High Density Lipoprotein) fraction which remains in the supernantant is determined.

Procedure

(i) Precipitation reaction, 500(ul) of sample and standard was transferred into two test tubes, each containing 1000(ul) of precipitant (R1),and labelled, sample and standard respectively. The tubes were mixed and allowed sitting for 10minutes at room temperature. Then centrifuged for 10 minutes at 4000 rpm, or 2 minutes at 12000rpm. The cleared supernatant was separated off within two hours and was used for determining the cholesterol content as follows.

(ii) 100(ul) of supernatant, distilled water and standard supernatant was collected and transferred into three test tubes labelled sample ,reagent blank and standard respectively.1000(ul) of reagent (R1) was added into all the test tubes, mixed and incubated for 5 min at 37^{O}C. The absorbance of the sample (A_{sample}) against the reagent blank was read spectophotometrically within 60 minutes at 500nm.

(d) Low Density Lipoprotein Cholesterol (LDL-C) in mmol/l was estimated by a clearance method for the direct measurement of LDL cholesterol using Randox Diagnostic LTD kit.

Principle

The assay consists of two distinct reaction steps

Elimination of chylomicrons, VLDL-Cholesterol and HDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase.

Specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by Cholesterol enzymatic End-point Method (Roeschlau *et.al.,* 1974).

(e) Atherogenic index (AI)

Atherogenic index was calculated by dividing the values of low density lipoprotein cholesterol with that of high density lipoprotein cholesterol.

(f) Coronary risk index (CRI)

Coronary risk index was calculated by dividing the values of total cholesterol with that of high density lipoprotein cholesterol.

3.9.10 Estimation of Apolipoprotein B (APOB)

APOB was estimated by sandwich enzyme immunoassay technique as described by (Brodsky, 2008).

Principle of the Assay

The AssayMax Human Apolipoprotein B ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of human ApoB in plasma, serum, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human ApoB in less than 4 hours. A polyclonal antibody specific for human ApoB has been pre-coated onto a 96-well microplate with removable strips. ApoB in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for ApoB, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Assay Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. The assay was performed at room temperature (27-30°C).Excess microplate strips were removed from the plate frame and returned immediately to the foil pouch with desiccants inside. The pouch was resealed securely to minimize exposure to water vapor and stored in a vacuum desiccator.50 µl of Human ApoB Standard or sample was added per well. Wells were covered with a sealing tape and incubated for 2 hours. The timer was started after the last addition to time for 2 hours.Wells were washed five times with 200 µl of Wash Buffer manually. The plate was inverted each time and the contents decanted; hit 4-5 times on absorbent material to completely remove the liquid.50 µl of Biotinylated Human ApoB Antibody was added to each well and incubated for 1 hour. The microplate was washed again as described above. 50 µl of Streptavidin-Peroxidase Conjugate was added to each well and incubated for 30 minutes. The Microplate reader was turned on and set up for the program in advance.The microplate was washed again as described above.

50 µl of Chromogen Substrate was added per well and incubated for 10 minutes or till the optimal blue color density developed. The Microplate was gently tapped to ensure thorough mixing and bubbles in the well were broken with pipette tip. 50 µl of Stop Solution was added to

each well. The color changed from blue to yellow.Absorbance was read on a microplate reader at a wavelength of 450 nm immediately and results obtained were recorded.

Estimation of apolipoprotein B 48 (APOB 48)

APOB 48 was estimated by sandwich enzyme immunoassay technique as described by (Brodsky, 2008).

Test principle The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to ApoB48. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for ApoB48 and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain ApoB48, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of ApoB48.Concentrations of ApoB48 in the samples were calculated by comparing the OD of the samples to the standard curve.

Assay Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. The assay was performed at room temperature (27-30°C). The samples were centrifuged again after thawing before the assay. All the reagents were mixed thoroughly by gently swirling before pipetting and foaming was avoided.

Add Sample: 100µL of Standard, Blank, or Sample was added per well. The blank well was added with Reference Standard and Sample diluent. Solutions were added to the bottom of micro ELISA plate well, foaming and inside wall touching were avoided. The solution was mixed gently. The plates were covered with sealer provided with the kit and incubated for 90 minutes at 37°C.

Biotinylated Detection Ab: The liquid of each well were removed by aspiration without washing. Immediately, 100µL of Biotinylated Detection Ab working solution was added to each well. Covered with the Plate sealer. The plate was gently tapped to ensure thorough mixing and incubated for 1 hour at 37°C.

Wash: Each well was aspirated and washed; the process was repeated three times. Washing was done by filling each well with Wash Buffer (approximately 350µL). Complete removal of liquid at each step was essential. After the last wash, the remaining Wash Buffer was removed by decanting. The plate was inverted and patted against thick clean absorbent paper.

HRP Conjugate: 100µL of HRP Conjugate working solution was added to each well and the plate was covered with the sealer and incubated for 30 minutes at 37°C. The wash process was repeated for five times as in above..

Substrate: 90µL of Substrate Solution was added to each well. Covered with a new Plate sealer. Incubated for 15 minutes at 37°C and was protected from light. (The reaction time could be shortened or extended according to the actual color change, but not more than 30minutes). When apparent gradient appeared in standard wells, user should terminate the reaction.

Stop:50µL of Stop Solution was added to each well. Then, the color turned to yellow immediately.

OD Measurement: The micro-plate reader was opened in advance, preheated and the testing parameters were set. The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450 nm. The concentrations of the samples were calculated from the standard curve.

Estimation of Apolipoprotein B 100 (APOB 100)

APOB 100 was estimated by sandwich enzyme immunoassay technique as described by

(Brodsky, 2008).

Principle

This assay employs an antibody specific for human ApoB100 coated on a 96-well plate. Standards and samples are pipetted into the wells and ApoB100 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human ApoB100 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of ApoB100 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. Removable 8-well strips were labeled appropriately for the experiment.100 µl of each standard and sample wash added into appropriate wells. The plates were covered well and incubated for 2.5 hours at room temperature. The solution was discarded by decanting and washed 4 times with Wash Buffer. The plate was inverted and blotted against clean paper towels.100 µl of 1X prepared biotinylated antibody was added to each well and incubated for 1 hour at room temperature with gentle shaking. The solution was discarded and washing was repeated as in above.100 µl of prepared Streptavidin solution was added to each well. The plate was incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded by decanting and washed 4 times with Wash Buffer. 100 µl of TMB One-Step Substrate Reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50µl of Stop Solution was added to each well. Absorbance was read at 450 nm immediately.

3.9.11 Estimation of free fatty acid quantification colorimetric/fluorometric Kit

Principle

Fatty Acids are converted to their CoA derivatives, which are subsequently oxidized with the concomitant generation of color or fluorescence. C-8 (octanoate) and longer fatty acids can then be easily quantified by either colorimetric (spectrophotometry at 570 nm wavelenght) or fluorometric (at Ex/Em = 535/587 nm) methods with detection limit 2 µM free fatty acid in variety samples.

Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use.

Standard Curve Preparation:

For the colorimetric assay, 0, 2, 4, 6, 8, 10 µl Palmitic Acid Standard was added into 96-well plate individually and volume was adjusted to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Fatty Acid Standard.

Sample Preparation: different volume of samples was added to each well in a 96-well plate and the volume was brought up to 50 µl/well with Assay Buffer.

Acyl-CoA Synthesis: 2 µl ACS Reagent was added into all the standard and sample wells. Mixed well; the reaction was incubated at 37°C for 30 min. 50 µl of the Reaction Mix was added to each well containing the Standard or test samples. The reaction was incubated for 30 min at 37°C, avoiding exposure to light. O.D. was measured at 570 nm for colorimetric assay in a micro-plate reader. The Fatty Acid amount in the sample wells were calculated from the standard curve. Fatty Acid Concentration = Fa/Sv (nmol/µl or mM) Fa is the Fatty Acid amount (nmol) in the well obtained from standard curve. Sv is the sample volume (µl) added to the sample well.

3.9.12 Determination of Lipid Peroxidation:

Lipid peroxidation was estimated by the method of (Gutteridge and Wilkins, 1982).

Principle:

Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 540nm.

Procedure

Three test tubes were set up and labeled test, blank and serum control. 50µl of serum, distilled water (d/w), and control were added to the test tubes respectively. 1ml of 1% TBA in 20% NaOH was added to all the test tubes. And 1ml of glacial acetic acid was added to all the test tubes respectively. The reagents were mixed and incubated in boiling water for 15 minutes. Allowed to Cool and absorbance of the tests were read at 532 nm against the reagent blank. Results were calculated using MDA (nmol/ml) = (OD X 1000000)/156000

Where E_{532} = Molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$)

3.9.13 Determination of enzymic antioxidants

i. Determination of superoxide dismutase activity

Method: Misra and Fredovich (1972) as described by Akinduko *et al*., (2014) with little modifications.

Principle

The ability of superoxide dismutase (SOD) to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O_2) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH (Valerino and McCormack, 1971) and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

Procedure

Four test tubes were set up and labeled test, standard, blank and control respectively. 20 microlitre (µl) of serum, standard, distill water and control were added into the test tubes respectively.250µl of 0.05M carbonate buffer (pH 10.2) was added to all the test tubes and allowed to equilibrate at 37 ^{0}C for five minutes. 30µl of 0.3mM freshly prepared epinephrine was added to all the test tubes. Absorbance of the tests were read after 30s at 30s interval for 150s at 480 nm against the reagent blank.

Calculation:

Actual OD reading (R) = $OD_{150} - OD_{30}/2$

% inhibition = $(R_{blank} - R_{test} / R_{blank})$ X 100

Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor

ii. Determination of Catalase Activity

Catalase activity was estimated by the method of (Sinha, 1972).

Principle

This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured colorimetrically at 570 to 610nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Procedure

Four test tubes were set up and labeled test, standard, blank and control respectively.1.2ml of 0.2M hydrogen peroxide and 3ml of 0.01M phosphate buffer (pH 7.0) were added into the test tubes respectively. Then 0.3ml of serum, standard, distill water and control were added to the test tubes respectively. The reagents were gently mixed and incubated at RT, 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at one minute interval. Mixed and incubated in a boiling water for 10 minutes, cooled and tests absorbance were read at 570 nm.

Calculation:

Change in OD (R) = $OD_1 – OD_{2or3}/t$

The amount of H_2O_2 degraded in (umol) is extrapolated from the already prepared H_2O_2 standard curve using the value of R..

Procedure for the Preparation of H_2O_2 Standard Curve

Six test tubes were set up and labeled 1-6 respectively. 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 millilitres (mls) of 0.019 M H_2O_2 were added respectively to the test tubes while 0.9, 0.8, 0.7,0.6,0.5 and 0.4 mls of water (H_2O) were added respectively to the test tubes, 2mls of potassium dichromate ($K_2Cr_2O_7$) was added to all the test tubes and H_2O_2 concentrations of 20,40,60,80,100,and 120 in the test tubes were obtained respectively. The absorbances of the tests were read at 570nm against the reagent blank and recorded. The H_2O_2 Standard Curve was obtained by plotting a graph of the absorbances against the concentrations.

iii. **Determination of Glutathione peroxidase (GPX, E.C. 1.11.1.9)**.

The activity of glutathione peroxidase was determined by the method of Rotruck *et al.,* (1973) with slight modifications.

Principle

A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specific time period. The GSH content remaining after the reaction was measured by the methods of Beutler *et al*., (1963). $2GSH + H2O2 = GSSG + 2 H2O$

Procedure

The reaction mixture in a total volume of 1 ml contained 0.2 ml of phosphate buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.5 ml of the enzyme preparation (tissue homogenate/ plasma/ hemolysate). 0.2 ml of glutathione and 0.1 ml of H_2O_2 were added to this mixture and incubated

at 37°C for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH by the method of Beutler and Kelley. A blank was treated similarly to which 0.2 ml of enzyme was added after incubation.

The activity of glutathione peroxidase was expressed as U/l of plasma (U- mmoles of GSH utilised/ minute) and recorded.

iv. Evaluation of Glutathione Reductase

Glutathione Reductase activity is evaluated by colorimetric method as described by Anderson and Davis, (2004).

Principle

Glutathione Reductase catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), which plays an important role in the GSH redox cycle that maintains adequate levels of reduced GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress.

Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. Samples were treated to destroy GSH before the assay. 100 µl sample and 5 µl 3% H_2O_2 were added, mixed and incubated at 25°C for 5 min. Then 5 µl of catalase was added, mixed and incubated at 25°C for another 5 min. 2 -50 µl of the pretreated samples were added into a 96-well plate, the volume was brought to 50 µl with Assay Buffer. Several doses of the sample was added to make sure the readings were within the standard curve range. 10 µl /well Positive Control was used (optional) and adjusted to 50 µl with Assay Buffer.

TNB standard curve: 0, 2, 4, 6, 8, 10 µl of the TNB Standard was added into 96-well plate in duplicate to generate 0, 10, 20, 30, 40, 50 nmol/well standard. The final volume was brought to 100 µl with Assay Buffer. 50 µl of the Reaction Mix was added to each test samples. Mixed well. OD was measured at 405 nm at T1 (reading A1). The reaction was incubated at 25°C for 10 min (or incubated longer time if the GR activity was low), protected from light, OD was measured at 405 nm again at T2 (reading A2). ΔA405 nm = A2 – A1. Note: A1 and A2 were essentially read in the reaction linear range. Calculation: TNB standard Curve was plotted. ΔA405nm was applied to the TNB standard curve to get ΔB nmol of TNB (TNB amount generated between T1 and T2 in the reaction wells).

GR Activity = ( ) sample dilution factor = nmol/min/ml = mU/mL

Where: ΔB is the TNB amount from TNB standard Curve (in nmol).

T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

0.9 is the sample volume change factor during sample pre-treatment procedure.

Unit definition: One unit is defined as the amount of enzyme that generates 1.0 µmol of TNB per minute at 25°C. The oxidation of 1 mole of NADPH to NADP+ generated 2 mole TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.

3.9.14 Estimation of non-enzymic antioxidants.

i. Methodology for vitamin C (ascorbic acid) Estimation

Vitamin C was estimated by Omaye *et. al.*,(1979) method

Principle

The ascorbic acid is converted to hydroascorbic acid by cupric sulphate solution and this couples with 2,4 dinitrophenyl hydrazine in the presence of thiourea as a mild reducing agent, sulphuric acid then converts the DNPH into a red coloured compound which is assayed spectrophotometrically.

Procedure

Serum vitamin C (ascorbic acid) concentration was measured by Omaye *et. al.*,(1979) method. To 0.5 ml of serum, 1.5 ml of 6% TCA was added and centrifuged at 3500g for 20 minutes using Wisperfuge model 1384 centrifuge (Samson Holland).To 5ml of supernatant, 0.5 ml of DNPH reagent (2% DNPH) and 4% thiourea in 9 N sulphuric acid) was added and incubated for 3 h at room temperature. After incubation 2.5 ml of 85% sulphuric acid was added and colour developed was read at 530 nm after 30 min.

iii. Methodology for vitamin E (_α-tocopherol) estimation

Serum vitamin E (_α-tocopherol) was estimated by the method of Desai (1984).

Principle

The principle is based on the reduction of ferric to ferrous ion by vitamin E which then form a red complex with α-α-dipyridyl. Vitamin E and carotenes were first extracted into xylene and the extinction read at 460nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520nm

Procedure

Vitamin E was extracted from serum by addition of 1.6 ml ethanol and 2.0 ml petroleum ether to 0.5 ml serum and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2 ml of 0.2% 2,2-dipyridyl, 0.2 ml of 0.5% ferric chloride was added and kept in dark for 5 min, an intense red colour layer obtained on addition of 4 ml butanol was read at 520 nm.

iii. Selenium

Selenium analysis was conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of APHA 1995 (American Public Health Association)

Working principle: Atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since selenium has its own characteristic absorption wavelength, a source lamp composed of selenium is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of selenium in the sample.

Preparation of reference solutions

A series of standard selenium solutions in the optimum concentration range are prepared, the reference solutions were prepared daily by diluting the single stock selenium solutions with water containing 1.5 mL concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the selenium stock solutions.

Calibration curve forselenium was prepared by plotting the absorbance of standards versus their concentrations.

Sample Digestion (Adrian, 1973)

1. 1ml of serum was measured and added 4 ml of distilled water
2. Mixed properly, boiled at 37^0c for 20mins
3. Measured using FS240AA agilent atomic absorption spectroscopy

Treatment

The treatment was based on a combination of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) for a period of 6 months. It comprises an initial phase with a combination of ERHZ for two months, followed by a four month treatment of HR.

3.9.15 Quality control measures

Quality control sera were run along test in each batch of analysis this was compared with the reference range of values of the control material. Then the standard deviation (SD) and coeffient of variation CV) of between batch (inter) was calculated using the formula: $CV=\delta/\dot{x}$ where $\delta=$ standard deviation, $\dot{x}=$ mean. The intra CV of the control results calculated are as follows:

TEST ANALYSE	MEAN±SD	INTER CV	(% CV)
FFA	0.31±0.01	0.03	3
TG	1.54±0.04	0.03	3
TC	4.20±0.07	0.02	2
LDL-C	2.76±0.09	0.03	3
HDL-C	1.20±0.08	0.07	7
VLDL-C	0.32±0.02	0.06	6
MDA	1.73±0.02	0.01	1
APOB	1346.75±31.9	0.02	2
APOB48	102.14±2.04	0.02	2
APOB 100	2044.92±15.63	0.01	1
SOD	1.56±0.02	0.01	1
CAT	50.7±1.7	0.03	3
GPX	48.38±0.46	0.01	1
GRX	151595.58±4485.38	0.03	3
VITE	59.11±4.59	0.08	8
VITC	2.17±0.02	0.01	1
SELENIUM	20.79±0.51	0.02	2

CV= coefficient of variation

3.9.16 Statistical analysis

The IBM Statistical Package for Social Sciences (SPSS) VERSION 21 was used for the statistical analysis. In order to decide whether to use parametric or non – parametric tests to calculate significance and correlation, the distribution of each group was tested for normality. For normally distributed data, ANOVA was used to determine if there were any statistical differences between the means of apparently healthy control, individuals with active *Mycobacterium tuberculosis* infection, individuals with latent *Mycobacterium tuberculosis* infection, individuals seropositive to HIV co-infected with *Mycobacterium tuberculosis* and individuals with *Mycobacterium tuberculosis* infection co-infected with malaria parasite. LSD's post hoc multiple comparisons were run to confirm where the differences occurred in the groups mentioned above. The paired sample t-test was used to determine the mean difference between individuals with *Mycobacterium tuberculosis* infection before treatment, two months and six months following treatment with antituberculosis therapy. The results were presented as mean ± standard deviation. Significant levels were considered at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 Sociodemographic parameters of individuals with LTBI, active TB infections and apparently healthy control (mean±SD).

There was a significant difference in the mean level of body weight (kg) and body mass index (kg/m^2) in individuals with latent tuberculosis infection (LTBI), active tuberculosis infection (TB+) and apparently healthy control (AHC) ($p<0.05$) while there was no significant difference in their mean ages ($p>0.05$). The mean level of body weight in individuals with LTBI (66.20±10.08) and active TB infection (61.61±12.03) were significantly lower compared with AHC (73.31±14.88)($p<0.05$) while there was no significant difference in the mean body weight (kg) of individuals with LTBI (66.20±10.08) and TB+ infection (61.61±12.03) compared with AHC (73.31±14.88) ($p>0.05$). Further, there was a significantly lower mean level of BMI in individuals with LTBI (25.57±6.53) and active TB (24.11±3.84) compared with AHC (29.19±7.22) ($p<0.05$) while there was no significant difference in the mean BMI level of individuals with LTBI (25.57±6.53) and TB+ infection (24.11±3.84) compared with AHC (29.19±7.22) ($p>0.05$).

Table 4.1 Sociodemographic parameters of individuals with LTBI, active TB infections and apparently healthy control (mean±SD).

Group	Body weight(kg)	BMI(kg/m^2)	Age (years)
1. AHC(n=105)	73.31±14.88	29.19±7.22	35.61±9.60
2.LTBI(n=26)	66.20±10.08	25.57 ±6.53	38.31±10.83
3.TB+ (n=82)	61.61±12.03	24.11±3.84	36..44±14.20
f-value	29.17	51.40	3.57
p-value	<0.001	<0.001	0.331
1 vs 2	0.004	0.002	0.623
1 vs 3	<0.001	<0.001	0.567
2 vs 3	0.061	0.624	0.434

AHC: apparently healthy control, **LTBI:** latent tuberculosis infection,:**TB+** active tuberculosis infection.

Table 4.2 Sociodemographic parameters of individuals with human immunodeficiency virus and tuberculosis co-infection (HIV&TB) , individuals with tuberculosis and malaria parasite co-infection (TB&MP) and apparently healthy control (mean±SD).

There was a significant difference in the mean level of body weight (kg) and body mass index (kg/m^2) in individuals with human immunodeficiency and tuberculosis co-infection (HIV&TB) , individuals with tuberculosis and malaria parasite co-infection (TB&MP)and apparently healthy control (AHC) ($p<0.05$) while there was no significant difference in their mean ages ($p>0.05$). The mean level of body weight of individuals with human immunodeficiency and tuberculosis co-infection (53.61±8.74) was significantly lower compared with the control (73.31±14.88)($p<0.05$) while there was no significant difference in the mean body weight (kg) of individuals with tuberculosis and malaria parasite co-infection (69.70±12.95) compared with the control group (73.31±14.88) ($p>0.05$). However, a significantly lower mean level of BMI was observed in individuals with human immunodeficiency and tuberculosis co-infection (22.48±2.38) compared with the control (29.19±7.22) ($p<0.05$) while no significant difference existed in the mean BMI level of individuals with tuberculosis and malaria parasite co-infection (26.69±4.01) compared with individuals with human immunodeficiency and tuberculosis co-infection (22.48 ±2.38) and the control group (29.19±7.22) ($p>0.05$).

Table 4.2 Sociodemographic parameters of individuals with human immunodeficiency virus and tuberculosis co-infection (HIV&TB) , individuals with tuberculosis and malaria parasite co-infection (TB&MP) and apparently healthy control (mean±SD).

Group	Body weight(kg)	BMI(kg/m^2)	Age (years)
1. AHC(n=105)	73.31±14.88	29.19±7.22	35.61±9.60
2.TBHIV+n=26	53.61±8.74	22.48 ±2.38	34.61±11.12
3. TB&MP (n=12)	69.70±12.95	26.69±4.01	39.33±7.43
f-value	21.17	28.39	13.17
p-value	<0.001	<0.001	0.315
1 vs 2	<0.001	<0.001	0.736
1 vs 3	0.205	0.113	0.567
2 vs 3	<0.001	0.041	0.541

AHC: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite co-infection.

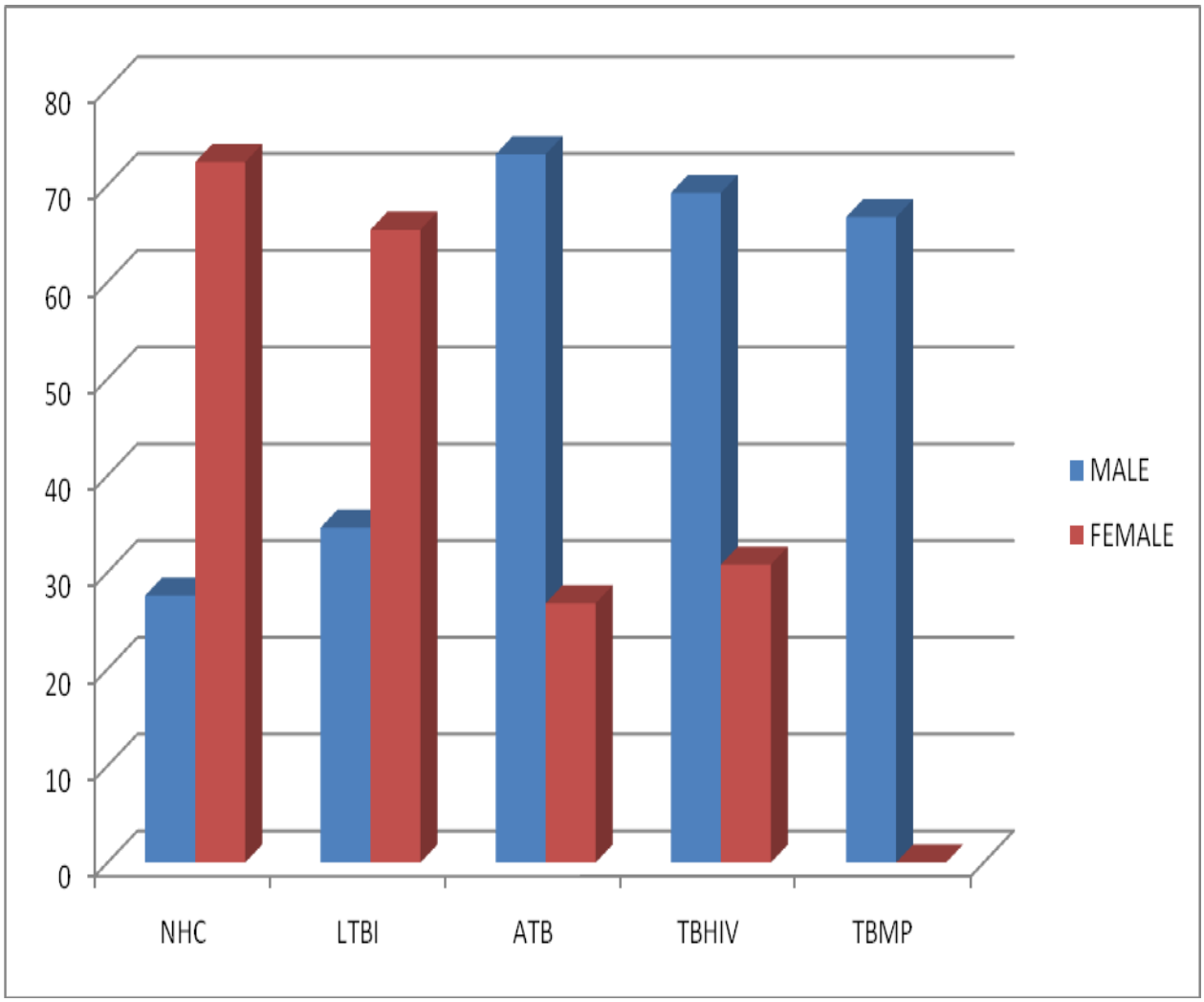

Figure 4.1 Barchart showing gender distribution of the study group

AHC: apparently healthy control, **LTBI:** latent tuberculosis infection,:**ATB** active tuberculosis infection,**TBHIV:** tuberculosis and HIVco- infection,:**TBMP:** tuberculosis and malaria pararsite infection

Table 4.3 Serum baseline levels of non enzyme antioxidants and malondialdehyde in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

Significant difference exists in the mean serum levels of vitamin E(mg/dl), vitamin C(mg/dl),selenium (mg/l) and malondialdehyde (MDA)(nmol/ml) in individuals with latent tuberculosis infection (LTBI), active tuberculosis infection (ATB) and apparent healthy control(AHC) ($p<0.05$) respectively. There was no significant difference in the mean serum level of Vitamin E in individuals with LTBI (13.75±4.55) compared with AHC (14.21±3.96)($p>0.05$) while the mean serum level of Vitamin E was significantly lower in TB+ compared with AHC (14.21±3.96)($p<0.05$). Likewise, there was significantly lower mean serum level of Vitamin E in individuals with active TB (7.01±3.4) compared with LTBI (13.75±4.55) ($p<0.05$) . Moreover, there was significantly lower mean serum level of Vitamin C in individuals with active TB (0.37±0.20) and LTBI (1.95±0.75) compared with AHC (2.18±0.69)($p<0.05$) and the mean serum level of vitamin C was also significantly lower in individuals with active TB (0.37±0.20) compared with LTBI(1.95±0.75)($p<0.05$). There was significantly lower mean serum level of selenium in individuals with active TB (0.42±0.22) compared with LTBI (18.21±24.17) and AHC (20.57±12.53) ($p<0.05$). However, no significant difference existed in the mean serum level of selenium in individuals with LTBI (18.21±24.17) compared with AHC (20.57±12.53) ($p>0.05$).Further, the mean serum level of MDA was significantly higher in individuals with LTBI (1.25±0.39) and active TB (2.55±1.49) compared with AHC (0.55±.30) ($p<0.05$).

Table 4.3 Serum baseline levels of non enzyme antioxidants and malondialdehyde in apparently healthy control, individuals with LTBI and active TB infections (mean±SD).

Group	Vit E(mg/dl)	Vit C(mg/dl)	Se(mg/l)	MDA(nmol/ml)
AHC 1n=105	14.21±3.96	2.18±0.69	20.57±12.53	0.55±0.30
LTBI2 n=26	13.75±4.55	1.95±0.75	18.21±24.17	1.25±0.39
TB+ 3 n=82	7.01±3.40	0.37±0.20	0.42±0.22	2.55±1.49
f-value	83.55	172.11	27.79	47.52
p-value	<0.001	<0.001	<0.001	<0.001
1 vs 2	0.477	0.043	0.063	0.004
1 vs 3	<0.001	<0.001	<0.001	<0.001
2 vs 3	<0.001	<0.001	<0.001	<0.001

AHC: apparently healthy control, **LTBI:** latent tuberculosis infection,: **TB+** active tuberculosis infection.

Table 4.4 Serum baseline activities of enzyme antioxidants in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

The mean serum activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in individuals with latent tuberculosis, active tuberculosis and apparently healthy control differed significantly ($p<0.05$). There was no significant difference in the mean serum activity of superoxide dismutase in LTBI (1.61±0.23) compared with AHC (1.58±0.32)($p>0.05$) while the mean serum activity of superoxide dismutase was significantly lower in active TB (1.13±0.46) compared with individuals with latent tuberculosis (1.61±0.23) and AHC (1.58±0.32)($p<0.05$).Further, there was no significant difference in the mean serum activity of catalase in LTBI (52.46±34.46) compared with AHC (50.80±24.55)($p>0.05$) while serum activity of catalase was significantly lower in active TB (31.96±13.69) compared with individuals with latent tuberculosis (52.46±34.46) and AHC (50.80±24.55) ($p>0.05$).Furthermore, the mean serum activity of glutathione peroxidase was significantly higher in LTBI (0.58±0.25) and active TB (0.54±0.22) compared with AHC (0.46±0.20) ($p<0.05$). Moreover, the mean serum activity of glutathione peroxidase in individuals with latent tuberculosis (0.58±0.25) was significantly higher compared with AHC (0.46±0.20) ($p<0.05$). However, the mean serum activity of glutathione reductase (mu/ml) in LTBI (87388±58.17) was significantly higher compared with AHC (49320.11±10.30)($p<0.05$), while serum activity of glutathione reductase (mu/ml) was significantly lower in individuals with active TB (26452.31±16.25) compared with individuals with LTBI (87388.42±58.17) and AHC (49320.11±10.30) ($p<0.05$).

Table 4.4 Serum baseline activities of enzyme antioxidants in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

Group	SOD (U/ml)	CAT (U/l)	GPX (µmol/l)	GRX (mu/ml)
AHC1. n=105	1.58±0.32	50.80±24.55	0.46±0.20	49320.11±10.30
LTBI.2 n=26	1.61±0.23	52.46±34.46	0.58±0.25	87388.42±58.17
TB.+3 n=82	1.13±0.46	31.96±13.69	0.54±0.22	26452.31±16.25
f-value	96.85	18.12	4.83	8.67
p-value	<0.001	<0.001	0.030	<0.001
1 vs 2	0.746	0.425	0.040	0.028
1 vs 3	<0.001	<0.001	<0.001	<0.001
2 vs 3	<0.001	<0.001	<0.001	<0.001

AHC: apparently healthy control, **LTBI:** latent tuberculosis infection,: **TB+** active tuberculosis infection.

Table 4.5 Serum baseline levels of lipid profile in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

The mean serum levels of free fatty acids (nmol/µl), triglyceride, total cholesterol(mmol/l), LDL-C(mmol/l),VLDL-C (mmol/l)and HDL-C(mmol/l) in individuals with latent tuberculosis, active tuberculosis and apparently healthy control differed significantly($p<0.05$) respectively. There was no significant difference in the mean serum level of FFA (nmol/µl) in individuals with LTBI (0.29±0.11) compared with AHC(0.32±0.15)($p>0.05$)while the mean serum level of FFA (nmol/µl) was significantly lower in individuals with active TB(0.19±0.08) compared with individuals with LTBI (0.29±0.11) and AHC (0.32±0.15)($p>0.05$). The mean serum level of TG(mmol/l) was significantly lower in individuals with LTBI(1.12±0.49) and active TB (0.56±0.27)compared with AHC (1.55±0.87)($p<0.05$).Further, the mean serum level of TC(mmol/l) was significantly lower in individuals with active TB (2.44±1.50) compared with individuals with LTBI(4.98±1.80) and AHC (5.12±1.11)($p<0.05$) while there was no significant difference in the mean serum level of TC(mmol/l) in individuals with LTBI compared with NHC (5.12±1.11)($p>0.05$).Likewise, the mean serum level of LDL-C(mmol/l) was significantly lower in individuals with active TB (0.51±0.48) compared with individuals with LTBI(2.47±1.53) and AHC (2.71±1.30)($p<0.05$) while there was no significant difference in the mean serum level of LDL-C(mmol/l) in individuals with LTBI (2.47±1.53) compared with AHC (2.71±1.30)($p>0.05$). Furthermore, the mean serum level of VLDL-C(mmol/l) was significantly lower in individuals with LTBI(0.51±0.22) and active TB (0.25±0.12) compared with AHC (0.71 ±0.39)($p<0.05$). Similarly, the mean serum level of HDL-C(mmol/l) was significantly lower in individuals with active TB(1.68±1.32) compared with individuals with LTBI(2.00±1.20) and AHC (1.71±1.00)($p<0.05$) while there was no significant difference in the mean serum level of HDL-C(mmol/l) in individuals with LTBI (2.00±1.20) compared with AHC (1.71±1.00) ($p>0.05$).

Table 4.5 Serum baseline levels of lipid profile in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

GROUP	FFA (nmol/µl)	TG (mmol/l)	TC (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	HDL-C (mmol/l)
1.AHC n=105	0.32±0.15	1.55±0.87	5.12±1.11	2.71±1.30	0.71 ±0.39	1.71±1.00
2.LTBIn=26	0.29±0.11	1.12±0.49	4.98±1.80	2.47±1.53	0.51±0.22	2.00±1.20
3.TBn=82	0.19±0.08	0.56±0.27	2.44±1.50	0.51±0.48	0.25±0.12	1.68±1.32
f-value	15.35	39.95	24.12	67.51	5.52	2.36
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.045
1 vs 2	0.19	0.04	0.54	0.13	0.03	0.30
1 vs 3	<0.01	<0.01	<0.01	<0.01	<0.01	0.03
2 vs 3	<0.01	<0.01	<0.01	<0.01	<0.01	0.04

AHC: apparently healthy control, **LTBI:** latent tuberculosis infection,: **TB+** active tuberculosis infection: AI atherogenic index :CRI coronary risk index

Table 4.6 Serum baseline levels of apo lipoprotein B, apo lipoprotein B48 , B100, atherogenic and coronary risk indices in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

The mean serum levels of apolipoprotein B48 (ng/ml), B100 (ng/ml),atherogenic and coronary risk indices in individuals with latent infection, active tuberculosis infection and apparently healthy control differed significantly ($p<0.05$) while there was no significant difference in the mean serum level of apolipoprotein B(µg/ml)($p>0.05$). However, the mean serum level of apolipoprotein B 48(ng/ml) was significantly higher in individuals with active TB infection (364.4±8.5) compared with individuals with LTBI(106.3±1.1) and apparently healthy control (100.2±5.5)($p<0.05$), similarly the mean serum level of apo lipoprotein B 48(ng/ml) was significantly higher in individuals with LTBI (106.3±1.1) compared with AHC (100.2±5.5) ($p<0.05$).Likewise, there was a significantly higher mean serum level of apolipoprotein B 48(ng/ml) in individuals with active TB infection(364.4±85) compared with individuals with LTBI (106.3±1.1) ($p<0.05$). More so, the mean serum level of apolipoprotein B 100(ng/ml) was significantly higher in individuals with LTBI (2489.4±0.84) and active TB infection (2879.5±1.03) compared with AHC (2032.3±1.11) ($p<0.05$), likewise, there was a significantly higher mean serum level of apolipoprotein B 100(ng/ml) in individuals with active TB infection(2879.5±1.03) compared with individuals with LTBI (2489.4±0.84) ($p<0.05$). The mean serum level of AI was significantly lower in individuals with LTBI(1.24±0.96) and active TB(0.30±0.04) compared with AHC (1.58±1.08)($p<0.05$), while there was no significant difference in the mean serum level of AI in individuals with active TB(0.30±0.04) compared with individuals with LTB infection(1.24±0.96)($p>0.05$). The mean serum level of CRI was significantly lower in individuals with LTBI (2.49±1.97) and active TB (1.45±0.45) compared with AHC (1.58±1.08)($p<0.05$) while there was no significant difference in the mean serum level of CRI in individuals with active TB(1.45±0.45) compared with individuals with LTB infection(2.49±1.97)($p>0.05$) ($p<0.05$).

Table 4.6 Serum baseline levels of apo lipoprotein B, apo lipoprotein B48 , B100, atherogenic and coronary risk indices in individuals with LTBI, active TB infection and apparently healthy control (mean±SD).

GROUP	APOB (µg/ml)	APOB48 (ng/ml)	APOB100 (ng/ml)	AI	CRI
1.AHC n=105	2033.2± 1.10	100.2 ± 5.50	2032.3± 1.11	1.58±1.08	2.99±1.93
2.LTBI n=26	2493.7± 0.83	106.3± 1.10	2489.4± 0.84	1.24±0.96	2.49±1.97
3.TB n=82	2879.3± 1.03	364.4±8.5	2879.5± 1.03	0.30±0.04	1.45±0.45
f-value	0.686	0.402	15.30	11.09	26.28
p-value	0.287	<0.001	<0.001	<0.001	<0.001
1 vs 2	0.995	0.036	0.019	0.04	0.002
1 vs 3	0.177	<0.001	<0.001	<0.001	<0.001
2 vs 3	0.172	<0.001	<0.001	0.074	0.624

AHC: apparently healthy control, **LTBI:** latent tuberculosis infection,: **TB+** active tuberculosis infection.

Table 4.7 Serum baseline levels of non enzyme antioxidants and malondialdehyde in individuals with TBHIV co-infection, individuals with TBMP co-infection and apparently healthy control (mean±SD).

The mean serum levels of vitamin E(mg/dl), vitamin C(mg/dl),selenium (mg/l) and malondialdehyde (MDA)(nmol/ml) in individuals with TBHIV co-infection, individuals with TBMP co-infection and apparently healthy control(AHC) differed significantly($p<0.05$). There was significantly lower mean serum level of Vitamin E in individuals with TBHIV (1.57 ±1.30) and TBMP co-infections (2.23±1.70) compared with AHC (14.21±3.96) ($p<0.05$) but there was no significant difference in the mean serum level of Vitamin E in individuals with TBHIV(1.57 ±1.30) compared with individuals with TBMP co-infection (2.23±1.70) ($p>0.05$).Moreover, the mean serum level of vitamin C(mg/dl) was significantly lower in individuals with TBHIV (0.51±0.22) and TBMP co-infections (0.32±0.17) compared with AHC (2.18±0.69) ($p<0.05$) but there was no significant difference in the mean serum level of Vitamin E in individuals with TBHIV(0.51±0.22) compared with individuals with TBMP co-infections (0.32±0.17) ($p>0.05$).Further, the mean serum level of selenium (mg/l) was significantly lower in individuals with TBHIV (0.62±0.18) and TBMP co-infections (0.57±0.16) compared with AHC (20.57±12.53)($p<0.05$) but there was no significant difference in the mean serum level of selenium in individuals with TBHIV(0.62±0.18) compared with individuals with TBMP co-infections (0.57±0.16) ($p>0.05$). Furthermore, the mean serum level of malondialdehyde (nmol/ml) was significantly higher in individuals with TBHIV (2.35±1.64) and TBMP co-infections (3.00±1.84) compared with AHC (0.55±0.30)($p<0.05$) but there was no significant difference in the mean serum level of malondialdehyde (nmol/ml) in individuals with TBHIV(2.35±1.64) compared with individuals with TBMP co-infections (3.00±1.84) ($p>0.05$).

Table 4.7 Serum baseline levels of non enzyme antioxidants and malondialdehyde in individuals with TBHIV co-infection, individuals with TBMP co-infection and apparently healthy control (mean±SD).

Group	Vit E(mg/dl)	Vit C(mg/dl)	Se(mg/l)	MDA(nmol/ml)
1.AHC n=105	14.21±3.96	2.18±0.69	20.57±12.53	0.55±0.30
2.TBHIV n=26	1.57 ±1.30	0.51±0.22	0.62±0.18	2.35±1.64
3.TBMP n=12	2.23±1.70	0.32±0.17	0.57±0.16	3.00±1.84
f-value	83.55	172.11	27.79	47.52
p-value	<0.001	<0.001	<0.001	<0.001
1 vs2	<0.001	<0.001	<0.001	<0.001
1 vs3	<0.001	<0.001	<0.001	<0.001
2 vs3	0.873	0.297	0.993	0.095

AHC: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite co-infection.

Table 4.8 Serum baseline activities of enzyme antioxidants in individuals with TBHIV co-infection,TBMP co-infection and apparently healthy control (mean±SD)

A significant difference exists in the mean serum activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in individuals with latent tuberculosis, active tuberculosis and apparently healthy control ($p<0.05$). The mean serum activity of superoxide dismutase was significantly lower in individuals with TBHIV (0.48±0.40) and TBMP co-infections (0.65±0.63) compared with AHC (1.58±0.32)($p<0.05$) while there was no significant difference in the mean serum activity of superoxide dismutase in individuals with TBHIV (0.48±0.40) compared with individuals with TBMP co-infection (0.65±0.63) ($p>0.05$).However, the mean serum activity of catalase was significantly lower in individuals with TBHIV (11.53±1.14) and TBMP co-infections (11.27±0.60) compared with AHC (50.80±24.55)($p<0.05$) while there was no significant difference in the mean serum activity of catalase in individuals with TBHIV (11.53±1.14) compared with individuals with TBMP co-infections (11.27±0.60) ($p>0.05$).Furthermore, the mean serum activity of glutathione peroxidase (μmol/l) was significantly higher in individuals with TBHIV (1.36±1.12) and TBMP co-infections (2.00±1.32) compared with AHC (0.46±0.20)($p<0.05$) but there was no significant difference in the mean serum activity of glutathione peroxidase in individuals with TBHIV(1.36±1.12) compared with individuals with TBMP co-infections(2.00±1.32) ($p>0.05$).Moreover, the mean serum activity of glutathione reductase was significantly lower in individuals with TBHIV (1212.70±1.33) and TBMP co-infections (1111.40±1.30) compared with AHC (49320.11±10.30)($p<0.05$) while there was no significant difference in the mean serum activity of glutathione reductase in individuals with TBHIV (1212.70±1.33) compared with individuals with TBMP co-infection(1111.40±1.30) ($p>0.05$).

Table 4.8 Serum baseline activities of enzyme antioxidants in individuals with TBHIV co-infection,TBMP co-infection and apparently healthy control (mean±SD)

Group	SOD (U/ml)	CAT (U/l)	GPX (μmol/l)	GRX (mu/ml)
1.AHC n=105	1.58±0.32	50.80±24.55	0.46±0.20	49320.11±10.30
2.TBHIV n=26	0.48±0.40	11.53±1.14	1.36±1.12	1212.70±1.33
3.TBMP n=12	0.65±0.63	11.27±0.60	2.00±1.32	1111.40±1.30
f-value	96.85	18.12	4.83	8.67
p-value	<0.001	<0.001	0.003	<0.001
1 vs2	<0.001	<0.001	<0.001	<0.001
1 vs3	<0.001	<0.001	<0.001	0.009
2 vs3	0.248	0.936	0.059	1.000

AHC: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite co-infection.

Table 4.9 baseline serum levels of lipid profile and FFA in individuals with TBHIV co-infection,TBMP co-infection and apparently healthy control (mean±SD).

The mean serum levels of free fatty acids (nmol/µl), triglyceride(mmol/l), total cholesterol(mmol/l), LDL-C(mmol/l),VLDL-C (mmol/l)and HDL-C(mmol/l) in normal healthy control, individuals with TBHIV and TBMP co-infections were significantly difference.($p<0.05$) respectively.The mean serum level of FFA (nmol/µl) in individuals with TBHIV (0.23±0.07) and TBMP co-infections (0.20±0.07) was significantly lower compared with AHC(0.32±0.15)($p<0.05$) while there was no significant difference in the mean serum level of FFA (nmol/µl) in individuals with TBHIV(0.23±0.07) compared with individuals with TBMP co-infection (0.20±0.07) ($p>0.05$).Moreover, the mean serum level of triglyceride (mmol/l) in individuals with TBHIV (0.44±0.26) and TBMP co-infections (0.41±0.21) was significantly lower compared with AHC(1.55±0.87)($p<0.05$) while there was no significant difference in the mean serum level of triglyceride (mmol/l) in individuals with TBHIV(0.44±0.26) compared with individuals with TBMP co-infection (0.41±0.21) ($p>0.05$). Likewise, the mean serum level of total cholesterol (mmol/l) in individuals with TBHIV (2.35±1.64) and TBMP co-infections (2.46±1.84) was significantly lower compared with AHC (5.12±1.11) ($p<0.05$) while there was no significant difference in the mean serum level of triglyceride (mmol/l) in individuals with TBHIV co-infection (2.35±1.64) compared with individuals with TBMP co-infection (2.46±1.84) ($p>0.05$). Further, the mean serum level of LDL-C (mmol/l) in individuals with TBHIV (0.48±0.53) and TBMP co-infections (0.67±0.46) was significantly lower compared with AHC(2.71±1.30)($p<0.05$) while there was no significant difference in the mean serum level of LDL-L (mmol/l) in individuals with TBHIV co-infection (0.48±0.53) compared with individuals with TBMP co-infection (0.67±0.462.71±1.30) ($p>0.05$).Furthermore, the mean serum level of VLDL-C (mmol/l) in individuals with TBHIV (0.20±0.12) and TBMP co-infections (0.19±0.10) were significantly lower compared with the control group (0.71 ±0.39)($p<0.05$) while there was no significant difference in the mean serum level of VLDL-C (mmol/l) in individuals with TBHIV (0.20±0.12) compared with individuals with TBMP co-infection (0.19±0.10) ($p>0.05$).Also, the mean serum level of HDL-C (mmol/l) in individuals with TBHIV (1.36±1.10) and TBMP co-infections (1.60±1.48) was significantly lower compared with NHC (1.71±1.00)($p<0.05$).

Table 4.9 baseline serum levels of lipid profile and FFA in individuals with TBHIV co-infection,TBMP co-infection and apparently healthy control (mean±SD).

GROUP	FFA (nmol/µl)	TG (mmol/l)	TC (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	HDL-C (mmol/l)
1.AHC n=105	0.32±0.15	1.55±0.87	5.12±1.11	2.71±1.30	0.71 ±0.39	1.71±1.00
2.TBHIV n=26	0.23±0.07	0.44±0.26	2.35±1.64	0.48±0.53	0.20±0.12	1.36±1.10
3.TBMP n=12	0.20±0.07	0.41±0.21	2.46±1.84	0.67±0.46	0.19±0.10	1.60±1.48
f-value	15.35	39.95	24.12	67.51	5.52	2.36
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.045
1 vs 2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1 vs 3	<0.01	<0.01	<0.01	<0.01	<0.01	0.08
2 vs 3	0.43	0.88	0.19	0.59	0.06	0.22

AHC: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite co-infection.

Table 4.10 Serum baseline levels of apo lipoprotein B, apo lipoprotein B48, B100, atherogenic and coronary risk indices in individuals with TBHIV and TBMP co-infections and apparently healthy control (mean±SD).

A significant difference was observed in the mean serum levels of apolipoprotein B(μg/ml), apolipoprotein B48(ng/ml), B100(ng/ml),atherogenic and coronary risk indices in individuals with TBHIV and TBMP co-infections and apparently healthy control($p<0.05$) .The mean serum level of apolipoprotein B (μg/ml) was significantly higher in individuals with TBMP (2656±0.462) and TBHIV co- infection (2759±0.375) compared with normal healthy control (2033±1.095)($p<0.05$), while there was no significant difference in the mean serum level of apolipoprotein B (μg/ml) in individuals with TBMP co-infection (2656.3±0.46) compared with individuals with TBHIV co-infection (2759.6±0.37) ($p>0.05$). However, the mean serum level of apolipoprotein B 48(ng/ml) was significantly higher in individuals with TBMP (386.4±2.36) and TBHIV co- infection (358.7±1.98) compared with control group (100.2±5.5) ($p<0.05$), while no significant difference was observed in the mean serum level of apolipoprotein B 48(ng/ml) in individuals with TBMP co-infection (386±2.36) compared with individuals with TBHIV co-infection (358±1.98) ($p>0.05$). Furthermore, the mean serum level of apolipoprotein B 100(ng/ml) was significantly higher in individuals with TBMP (2880.8±0.46) and TBHIV co-infection (3448.3±0.22) compared with the control group (2032.3±1.11) ($p<0.05$), while no significant difference was observed in the mean serum level of apolipoprotein B 100(ng/ml) in individuals with TBMP co-infection (2880±0.462) compared with individuals with TBHIV co-infection (3448.3±0.22) ($p>0.05$).However, the mean serum level of atherogenic index was significantly lower in individuals with TBMP (0.42±0.19) and TBHIV co- infections (0.35±0.03) compared with apparently healthy control (1.58±1.08) ($p<0.05$), while no significant difference was observed in the mean serum level of antherogenic index in individuals with TBMP co-infection (0.42±0.19) compared with individuals with TBHIV co- infection (0.35±0.03) ($p>0.05$).The mean serum level of coronary risk index was significantly lower in individuals with TBMP (1.54±0.03) and TBHIV co- infection (1.73±0.64) compared with apparently healthy control (2.99±1.93) ($p<0.05$), likewise the mean serum level of CRI in individuals with TBMP co-infection (1.54±0.03) was significantly lower compared with individuals with TBHIV co-infection (1.73±0.64) ($p< 0.05$).

Table 4.10 serum baseline levels of apo lipoprotein B, apo lipoprotein B48 , B100, atherogenic index and coronary risk index in individuals with TBHIV andTBMP co-infections and apparently healthy control(mean±SD).

GROUP	APOB (µg/ml)	APOB48 (ng/ml)	APOB100 (ng/ml)	AI	CRI
1.AHC n=105	2033.2± 1.10	100.2 ± 5.50	2032.3± 1.11	1.58±1.08	2.99±1.93
2.TBHIV n=26	2759.6±0.37	358.7± 1.98	3448.3±0.22	0.35±0.03	1.73±0.64
3.TBMP n=12	2656.3± 0.46	386.4±2.36	2880.8±0.46	0.42±0.19	1.54±0.03
f-value	125.83	117.70	123.86	11.09	26.28
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
1 vs 2	<0.001	<0.001	<0.001	<0.001	<0.001
1 vs 3	<0.001	<0.001	<0.001	0.001	0.113
2 vs 3	0.999	0.990	0.993	0.615	0.041

AHC: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite co-infection.

Table 4.11 Serum levels of lipid profile and FFA at baseline, two months and six months following treatment(mean±SD).

A significantly higher difference was observed in the mean serum levels of LDL-C (mmol/l) at 6months (0.73±0.30) compared with 2months (0.56±0.52) and baseline (0.52±0.49) on ATT ($p<0.05$) while no significant difference was observed in the mean serum level of LDL-C(mmol/l) at 2months(0.56±0.52) compared with baseline(0.52±0.49) on ATT($p>0.05$). Further the mean serum level of VLDL-C (mmol/l) at 2months (0.48±0.22) and 6months (0.49±0.22) on ATT were significantly higher compared with baseline (0.40±0.21) ($p<0.05$) while no significant difference existed in the mean serum level of VLDL-C(mmol/l) at 2months (0.48±0.22) compared with 6months(0.49±0.22) on ATT($p>0.05$). Furthermore, the mean serum levels of TG (mmol/l) following treatment for 6months (1.742±0.30) was significantly higher compared with 2months(1.01±0.48) and baseline (0.52±0.27) ($p<0.05$) on ATT while the mean serum levels of FFA (nmol/µl) following treatment for 6months(0.11±0.07) was significantly lower compared with 2months(0.15±0.09) and baseline(0.20±0.08) ($p<0.05$).

Table 4.11 Serum levels of lipid profile and FFA at baseline, two months and six months following treatment(mean±SD).

Group	TC (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	FFA (nmol/µl)
Baseline1 n=120	2.55±1.55	0.52±0.49	0.40±0.21	1.65±1.38	0.52±0.27	0.20±0.08
2months2 n=120	2.75±1.39	0.56±0.52	0.48±0.22	1.67±1.37	1.01±0.48	0.15±0.09
6months3 n=120	2.90±1.89	0.73±0.30	0.49±0.22	1.70±1.35	1.742±0.30	0.11±0.07
1 vs 2	0.271	0.342	<0.001	0.854	<0.001	<0.001
1 vs 3	0.549	<0.001	<0.001	0.417	<0.001	<0.001
2 vs 3	0.602	<0.001	0.574	0.900	<0.001	<0.001

Table 4.12 Serum levels of apolipoprotein B, apolipoprotein B 48, apolipoprotein B 100 and MDA at baseline, 2months and 6months during treatment(mean±SD).

There were significant lower mean serum levels of Apo B(µg/ml), Apo B48(ng/ml) and MDA (nmol/ml) following 6months treatment. (1179.2±85.5; 158.6±60.3; 1.39±0.65) compared with 2months (1263.4±72.3; 162.8±67.4; 1.49±0.86) and baseline levels (1366.1±39.3;359.7±81.2; 1.74±0.77) ($p<0.05$) . A significant higher mean serum level was observed in Apo B100 (ng/ml) levels in individuals with active TB at 2months (3439.5±9.16) and 6months(3442±9.04) levels following treatment compared with baseline (3003.4±9.35) ($p<0.05$). Furthermore, the mean serum levels of Apo B (µg/ml), Apo B48 (ng/ml) and MDA (nmol/ml) were significantly higher at 2months (1263.4±72.3; 162.8±67.4; 1.49±0.86)following treatment compared with baseline(1366.1±39.3;359.7±81.2; 1.74±0.77) ($p<0.05$) respectively, while the mean serum levels of MDA (nmol/ml) was significantly lower at 2months (1.49±0.86)following treatment compared with baseline (1.74±0.77) ($p<0.05$).

Table 4.12 Serum levels of apolipoprotein B, apolipoprotein B48,apolipoprotein B100and MDA at baseline, two months and six months during treatment (mean±SD).

Group	APOB (µg/ml)	APOB48 (ng/ml)	APOB100 (ng/ml)	MDA (nmol/ml)
Baseline1 n=120	1366.1± 39.3	359.7±81.2	3003.4±9.35	1.74±0.77
2months2 n=120	1263.4±72.3	162.8±67.4	3439.5±9.16	1.49±0.86
6months3 n=120	1179.2±85.5	158.6±60.3	3442.9±9.04	1.39±0.65
1 vs 2	<0.01	<0.01	<0.01	<0.01
1 vs 3	<0.01	<0.01	<0.01	<0.01
2 vs 3	<0.01	<0.01	<0.01	<0.01

Table 4.13 Serum levels of non enzyme antioxidants at baseline, two months and six months during treatment (mean±SD).

The mean serum levels of vitamin E(mg/dl) and selenium (mg/l) were significantly higher at 6months (51.84±18.16and 42.69±17.94) compared with 2months (47.86±23.17 and 33.96±27.77) and baseline(27.75±13.46 and 17.75±18.24) following treatment ($p<0.05$).

Table 4.13 Serum levels of non enzyme antioxidants at baseline, two months and six months during treatment(mean±SD).

Group	VITE (mg/dl)	VITC (mg/dl)	Se (mg/l)
Baseline1 n=120	27.75±13.46	1.63±0.62	17.75±18.24
2months2 n=120	47.86±23.17	1.75±0.53	33.96±27.77
6months3 n=120	51.84±18.16	1.78±0.49	42.69±17.94
1 vs 2	<0.001	0.386	<0.001
1 vs 3	<0.001	0.174	<0.001
2 vs 3	0.186	0.836	0.029

Table 4.14 Serum activities of enzyme antioxidants at baseline, two months and six months during treatment(mean±SD).

The mean serum activities of SOD (U/ml), CAT (U/L), GPX (umol/l) and GRX (mu/ml) at 6months(1.61±0.43; 65.22±17.42; 0.76±22and 50857.50±27.82) were significantly higher compared with 2months(1.31±0.55; 48.57±13.33; 0.65±0.31 and 26846.31±14.61) and baseline(1.19±0.42; 33.58 ±13.24; 0.58±0.20 and 25715.70±17.31) while no significant difference was observed in the mean serum activities of SOD (U/ml)(1.31±0.55), GPX (umol/l)(0.65±0.31) and GRX (mu/ml)(26846.31±14.61) at 2months compared with baseline(1.19±0.42; 0.58±0.20 and 5715.7±17.31) following treatment($p>0.05$) .

Table 4.14 Serum activities of enzyme antioxidants at baseline, two months and six months during treatment (mean±sd).

Group	SOD (U/ml)	CAT (U/L)	GPX (umol/l)	GRX (mu/ml)
Baseline1 n=120	1.19±0.42	33.58 ±13.24	0.58±0.20	25715.70±17.31
2months2 n=120	1.31±0.55	48.57±13.33	0.65±0.31	26846.31±14.61
6months3 n=120	1.61±0.43	65.22±17.42	0.76±22	50857.50±27.82
1 vs 2	0.029	<0.001	0.020	0.773
1 vs 3	<0.001	<0.001	<0.001	<0.001
2 vs 3	<0.001	<0.001	<0.001	<0.001

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CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Many factors contribute to tuberculosis burden especially in sub-Saharan Africa. These include the drawback of developing an effective vaccine, diagnostic challenges, length of treatment, the increase in HIV burden and emergence of *Mycobacterium tuberculosis* drug resistant strains. In this work, the status of lipid peroxidation, apolipoproteins , antioxidants and some atherogenic parameters were evaluated in individuals with *Mycobacterium tuberculosis.* The study individuals were both male and female age matched, but predominantly male. There was a significantly lower mean BMI value observed in test groups compared with the control.This could be a consequence of *Mycobacterium tuberculosis* infection. However, malaria parasite co-infection did not affect the body weight of the patients. BMI has been used as an indicator of malnutrition, Gebremedhin *et al.,* (2017) or total adiposity, Pusapati *et al.*,(2017); although with many limitations. Malnutrition and tuberculosis are positively associated. However, the mean BMI values of the entire study group were within the normal reference range.The lower mean levels of BMI observed in the test group might be as a result of depletion in body lipids and free fatty acids associated with *Mycobacterium tuberculosis infection.* This lower mean levels of BMI observed in the test group is in agreement with that of (Yung-Feng , 2016 :Aaron and Marco, 2017). The mean levels of antioxidants were significantly reduced in the test groups compared with the control while there was a significant increase in the mean values of malondialdehyde in the test groups compared with the control.Vitamin-E has been described as the major chain breaking antioxidant in humans because of its lipid solubility. Vitamin-E is located in cell membranes, where it interrupts lipid peroxidation and probably plays a major role in modulating intracellular signaling pathway that relay on reactive oxygen species. Vitamin E can also directly quench ROS, including superoxide radical, OH, and O2.Vitamin–E plays a primary defense against lipid peroxidation and oxidation (Cooper *et al*.,1999). Vitamin-C is the most abundant water soluble antioxidant in the body. It directly scavenges oxygen radical, hydroxyl radical and hydrogen peroxide. Vitamin-C neutralizes oxidants from stimulated neutrophils. Vitamin-C contributes to the regeneration of vitamin-E to its active form. Vitamin-C is the most important compound implicated in recycling of alpha- tocopherol radicals(Young and Woodside, 2001). Individuals with LTBI are immune competent, which explains the no significant differences

observed in Vitamin E levels, SOD, and CAT activities in individuals with LTBI compared with the control group. The significantly lower mean serum levels of vitamins C and E observed in this study have been previously reported by some authors (Reddy *et al.*, 2004: Nwanjo and Oze, 2007, Pugalendhi *et al.*,2012 and Oyedeji *et al*.,2013). The reported no significant difference in the mean serum level of selenium in individuals with LTBI compared with AHC and significant difference observed in individuals with active tuberculosis corroborated the work of Oyedeji *et al*.,(2013). Further, the significant higher difference observed in the mean serum level of MDA (nmol/ml) in LTBI and in individuals with active tuberculosis compared with AHC, and a significantly lower difference in the mean serum level of MDA in LTBI compared with active TB in this study was consistent with the findings of Reddy *et al.*, (2004), Nwanjo and Oze, (2007) and Oyedeji *et al*.,(2013), who reported higher mean values of plasma lipid peroxide (MDA) in pulmonary tuberculosis patients with individuals with active tuberculosis infection compared with control and may be due to high oxidative stress in TB infected individuals. Further, the mean serum activity of superoxide dismutase was significantly lower in active TB individuals compared with LTBI and AHC while the mean serum activity of catalase was significantly lower in active TB individuals compared with individuals with LTBI and AHC.There was also significantly higher mean serum activity of catalase in individuals with LTBI compared with the control group. Similar result was also obtained by (Reddy *et al.*, 2004).Severe oxidative stress has been reported in TB patients because of malnutrition and poor immunity. Reddy *et al.*, (2004) investigated the serum lipid peroxidation products and important free radical scavenging enzymes i.e. superoxide dismutase (SOD), catalase and antioxidant glutathione levels and total antioxidant status in TB patients. The individuals in his study comprised of normal human volunteers (NHV,n=25), TB patients (n=100) – including untreated (TB1, n=55), under treatment (TB2, n=30) and after treatment (TB3, n = 15) with anti-tuberculosis therapy. The study revealed that levels of lipid peroxidation products malondialdehyde (MDA) were increased significantly in TB1, TB2 and TB3. Similar results were also obtained by Pugalendhi *et al.*,(2012) and Lamsal *et al.*,(2007) in their two months follow-up study. MDA levels gradually decreased with clinical improvement following treatment. Erythrocyte SOD, serum catalase, plasma glutathione levels and serum total antioxidant status were decreased significantly in TB1 and TB2 , TB3 patients in comparison with the control, these levels gradually increased with clinical improvement with ATT(Reddy *et*

al., 2004). Oxidative stress was observed in all the TB patients (TB1, TB2, TB3), irrespective of treatment status, however, none of the researchers recruited individuals with latent tuberculosis. Further, there was a significantly higher mean serum activity of GPX (mu/ml) in individuals with LTBI and active TB compared with AHC.This observation supported the work of Pugalendhi *et al.*,(2012). Besides, there was a significantly lower mean serum activity of GRX in active TB compared with LTBI and AHC in this research, these findings substantiated the study by Shubhangi *et al.*,2012 ,in their work.This study confirmed the role of oxidative stress in the pathogenesis of TB , reduced antioxidant levels and free radical generation in the TB patients than in control group. Significantly increased MDA in TB patients than in control group has been previously reported by some authors (Reddy *et al*., 2004: Nwanjo and Oze, 2007: Reddy *et al*.,2009: Pugalendhi *et al.*, 2012 and Oyedeji *et al*.,2013).The finding in this study correlated with these previous findings. Increased production of reactive oxygen species secondary to phagocyte respiratory burst occur in pulmonary TB. Evidence suggest that increased circulating levels of free radical activity are found in pathogenesis of active pulmonary TB and hence may play a role in resultant fibrosis (Ramesh *et al*.,2011). Free radicals have been implicated in the development of lung fibrosis which may be a long term sequel of pulmonary tuberculosis. Free radicals attack the cell membrane causing tissue damage and wasting disease in pulmonary TB patients. *Mycobacterium* can induce reactive oxygen species production by activating phagocytes (Reddy *et al*.,2004). Moreover, a significantly lower mean serum level of FFA and lipid profile in individuals with active TB compared with individuals with LTBI and AHC was observed .Also no significant difference was observed in the mean serum level of TC,FFA, LDL-C and HDL-C in individuals with LTBI compared with AHC. The reduced FFA and cholesterol in tuberculosis infected groups could be explained by the finding that *M. tuberculosis* maintains viability by extracting and utilizing essential nutrients from the host, and is a requirement for all of the pathogenic activities that are exploited by the bacterium. Specifically, *M. tuberculosis* preferentially acquires, stores in the bacterial cytoplasm and metabolizes host-derived lipids (fatty acids and cholesterol), and the bacterium consumes these substrates to effect and maintain disease (Kaley *et al*.,2018 and Rahul *et al*.,2019). The reduced lipid profile findings in this study is consistent with the study by (Gebremedhin *et al.*2017).Similar result was obtained by (Gebremedhin *et al.*,2017) from Ethiopia, which observed that the concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-

density lipoprotein cholesterol (HDL-C) in active TB patients were significantly lower compared with LTBI and control individuals. Likewise the findings corroborated with the results reported from Southern Nigeria by Oyedeji *et al*.,(2013) and from Benin Republic by Akpovi *et al.*,(2013), in their study. "Also, no significant difference existed in the mean serum level of HDL-C in individuals with LTBI compared with AHC . This report is consistent with the study by Taparia *et al*.,(2015). Furthermore, the observed no significant difference in the mean serum levels of FFA and TC in individuals with LTBI compared with AHC agreed with the work of Gebremedhin *et al.*, (2017) and could be probably because LTBI individuals are immune competent, do not have TB disease or active infection ,are indisposed to malnutrition and severe oxidative stress.Furthermore, no significant difference existed in the mean serum level of Apo B across the groups (AHC, LTBI, and TB+) . Although, ApoB is an important component of most atherogenic lipoprotein particles (Benn *et al*., 2007). ApoB occurs in two main forms, apoB 48 and apoB 100. ApoB 48 is synthesized mainly by the small intestine (Benn *et al*., 2007).It was also found that there was significantly higher mean serum level of Apo B48(ng/ml) in individuals with LTBI and active TB compared with AHC. Also, a significant higher mean difference was observed in the mean serum level of apolipoprotein B 48(ng/ml) in individuals with LTBI compared with AHC . ApoB 48 is primarily found in chylomicrons (Chenn, 1986, Benn *et al*., 2007 and Burtis *et al*.,2008). Consequently, there was a significantly higher mean serum level of Apo B100 in individuals with LTBI and active TB compared with AHC. Likewise, there was significantly higher mean serum level of Apo B100 in individuals with LTBI compared with AHC . ApoB-100 is the largest of the apoB group of proteins, consisting of 4563 amino acids. ApoB 100 is the apolipoprotein found in lipoproteins synthesized by the liver and it is found in chylomicrons, VLDL, IDL, LDL and LP (a) particles(Burtis *et al*.,2008). All these particles are atherogenic. Each of these particles contains a single apoB molecule. Several studies have shown that apoB may be a better predictor of cardiovascular disease risk than LDL-C (Chan and Watts, 2006:Benn *et al*., 2007 and Paramjit *et al*., 2016). Furthermore, it has been shown that apoB may be elevated despite normal or low concentrations of LDL-C. ApoB also appears to predict on-treatment risk, when LDL-C has been lowered by statin therapy. The measurements of apoB represent the total burden of the main lipoprotein particles involved in the atherosclerotic process(Benn *et al*., 2007) Therefore, from the viewpoint of atherosclerosis and cardiovascular risk, apoB100 is the important

one(Sniderman *et al*., 2012 and Hem *et al*., 2014).In this study the mean serum level of Apo B48 and B100 in individuals with active TB+ and LTBI were significantly higher compared with AHC and were more raised in individuals with active TB while there was no significant difference in the mean serum level of Apo B across the groups. It could be recalled that in this study too total Cholesterol, LDL-C, TG, AI and CRI were significantly low in active TB+ individuals compared with AHC individuals. This finding cannot jettison their predisposition to cardiovascular instability since it has been shown that apoB may be elevated despite normal or low concentrations of LDL-C and apoB may be a better predictor of cardiovascular disease risk than LDL-C(Chan and Watts ,2006:Benn *et al*., 2007), as in this study apo B48 and B100 were raised in individuals with active TB while LDL-C was low. Moreover, increasing evidence indicates a link between low blood cholesterol levels and a number of human diseases including tuberculosis (TB) (Akpovi *et al*., 2013). Apo B containing lipoproteins are the ones that are most likely to enter the wall of the arteries. They are capable of trafficking cholesterol into the artery wall, and if present in increased numbers they may be the main initiating factor in atherosclerosis. Retention of ApoB containing lipoprotein particles within the arterial wall is an essential part of the process. In this study, from the data available, individuals with active TB are most likely predisposed to cardiovascular disease risk.The mean serum levels of baseline non enzyme antioxidants (vitamin E, vitamin C and selenium were significantly lower in individuals with TBHIV and TBMP co-infections compared with the control group. This could be as a result of oxidative damage that occurs during tuberculosis disease. Hence, malondialdehyde a product of lipid peroxidation was significantly raised in *Mycobacterium tuberculosis* co morbidity in this study. Vitamin E is a lipid phase chain breaking antioxidants. Vitamins E and C scavenge radicals in membranes and are crucial in preventing lipid peroxidation. However there is paucity of information on the effect of *Mycobacterium tuberculosis* - *Plasmodium falciparium* (Pf)(TBMP) and *Mycobacterium tuberculosis*- *Human Immunodeficiency virus* (HIV)co - infection (TBHIV) on non enzyme antioxidants(vitamins E and C, selenium)and MDA.Previous studies, mostly compared active TB individuals and control only (Nwanjo and Oze, 2007:Nnodim *et al*.,2011, and Oyedeji *et al*.,2013). All the studies observed reduced levels of non enzyme antioxidants and raised levels of MDA in individuals with TB compared with the control. Unlike, in this study, individuals with tuberculosis with either HIV or MP co- infection were compared with the control group. They suffered enhanced lipid peroxidation due to

tuberculosis and this probably impaired their antioxidant capacity One of the manifestations of these free radical mediated processes is lipid peroxidation(Bhimrao *et al*.,2011).This finding corroborates with the study of Oyedeji *et al*.,(2013).The observed no statistically significant difference in individuals with TBHIV and TBMP comorbidity, could be because they had in common active TB infection. Similar result was obtained by Osuji *et al*., (2013), in their study. Likewise, Audu *et al*.,(2005),remarked that co-infection with tuberculosis or malaria appeared not to have any impact on the degree of depletion of CD4+ T cell counts in individuals living with HIV .Furthermore, the observed significantly reduced mean serum activities of baseline enzyme antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in individuals with TBHIV and TBMP co-infection could be attributed to tuberculosis disease. This is consistent with the work of Osuji *et al.,* (2013), which reported lower serum activities of glutathione peroxidase, glutathione reductase and total antioxidant capacity in both HIV infected participants with or without tuberculosis and HIV seronegative participants with tuberculosis, (in each case) compared with apparently healthy control and no significant difference was also observed compared with comobidity or TB participants as in this study. Moreover, FFA, TG, TC, LDL-C, VLDL-C and HDL-C were also significantly reduced in individuals with tuberculosis co- infected with HIV or malaria parasite than in the control group. This probably indicated that TB diseases might be a risk factor for low lipid profiles at diagnosis. Similar result was obtained by Gebremedhin *et al.*,(2017) from Ethiopia, which observed that the concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in active TB patients were significantly lower compared with the control group. Prior epidemiologic studies have also shown that increased body mass index is associated with higher total cholesterol and low-density lipoprotein cholesterol (Pusapati *et al.*, 2017).Hence, it could be recalled that the control group had higher BMI value than the test group in this study. Subsequently, the mean serum levels of apo lipoprotein B, apo lipoprotein B48 and B100 were significantly raised in individuals with TBHIV and TBMP co- infection than in the control group while the mean serum level of AI and CRI were significantly lower in individuals with TBMP and TBHIV co- infection compared with the control group. The observed significant increase in the mean levels of serum apolipoproteins at baseline in the individuals with tuberculosis disease could probably be as a result of tuberculosis disease. Hence, they might be at a higher risk of predisposition to cardiovascular

disease despite having low lipid profile values. Apolipoprotein B 100 because of its low molecular size can traffic cholesterol into the artery wall, and if present in increased numbers they may be the main promoting factor in atherosclerosis. Sniderman *et al*.,(2012), in their study , suggested that high levels of apo B are indicative of a higher risk to cardiovascular disease even when LDL-C or non-HDL-C levels stay low and concluded that concentrations of apo B are superior indicators of vascular/heart disease and CVD risk prediction than standard lipid profile. Free fatty acid concentration was significantly low at 6months and 2months following treatment than at baseline. The reason for FFAs reduction in this study following treatment is poorly understood. However it has been stated that an increase in FFAs is followed by production of ROS and activation of endogenous radical oxygen scavengers (Binienda *et a.l.*,1998). The reduction in FFAs levels at 6months and 2months than at baseline probably suggest enhanced tubercle bacilli clearance with treatment duration which is an indication of good therapeutic response. Likewise, high concentrations of FFAs are associated with insulin resistance, fatty liver disease, atherosclerosis and myocardial dysfunction. Hence reduction in the FFAs level could be likened to good therapeutic response. Furthermore, the observed significantly higher mean serum levels of TC, LDL-C, VLDL -C and TG, following treatment at 2months and 6months compared with baseline suggest improvement due to response to TB treatment. Active TB and TB-HIV co-infection pose an additional metabolic, physical, and nutritional burden, resulting in further increase in energy expenditure, mal-absorption, and micronutrient deficiency .*Mycobacterium tuberculosis* causes pulmonary and extra pulmonary tuberculosis .It is an infectious disease which could lead to inflammation of any affected organ especially the lungs. It triggers the production of free radicals and reactive oxygen species which negatively affects host lipid by causing Enhanced lipid peroxidation.Malnutrition and tuberculosis are synergistically associated to each other.In fact, the combination of malnutrition leading to decreased “supplementation” of lipid and reduction of immune parameters might be indicating the pathogenesis of tuberculosis. *Mycobacterium tuberculosis* activates invaded macrophages resulting in free radical burst. High serum levels of the free radicals and high concentration of lipid peroxidation products are characteristics of patients with advanced tuberculosis (Gebremedhin *et al.,2017*). Lipid peroxidation could cause reduced concentration of serum lipids and tissue inflammation. Similarly, Prabakaran *et al.,* (2017) has stated that low serum cholesterol in the new cases may

be due to high oxidative stress. However, the low cholesterol level in this study normalized with treatment and it was treatment duration dependent. The rise in lipid profile concentration after taking ATT treatment in TB patients might be due to the nutritional status and immune function improving and the cleaning of circulating bacilli in the blood. The finding that TB patients on ATT had significantly raised lipid profile concentration compared to baseline is consistent with the previous findings (Taparia *et al*., 2015 and Gebremedhin, 2017).Likewise, significantly higher mean serum levels of Apo B and Apo B48 in individuals with TB at baseline compared with individuals with TB at 2months and 6months was observed following treatment. The progressive reduction in ApoB and ApoB48 with ATT observed in this study corroborated the work of Albanna *et al*.,(2017), whose research indicated that isoniazid (H) was associated with the significant decrease in total cholesterol and apoB levels. In line with their study, isoniazid (H) was used with rifampicin (R) throughout the six months treatment period for the TB individuals recruited in this study. Further, there was a significantly lower mean serum level of Apo B100 in TB individuals at baseline compared with individuals with TB at 2months and TB at 6months on ATT. Previous study has shown that ApoB 100 is the apolipoprotein found in lipoproteins synthesized by the liver and it is found in chylomicrons, VLDL, IDL, LDL and LP (a) particles(Burtis *et al*.,2008). All these particles are atherogenic (Sniderman *et al*., 2012). The observed increase in ApoB 100 at 2 months and 6months on ATT could be the predisposing factor for the risk of cardiovascular disease in tuberculosis patients even on treatment. Sniderman *et al*., (2012), have shown in their work that from the viewpoint of atherosclerosis and cardiovascular risk, apoB100 is the important one. Further, there was a significantly higher mean serum level of MDA in individuals with TB at baseline compared with individuals with TB at 2months and 6months on ATT.This finding is in agreement with the work of Reddy *et al.*, (2004), Lamsal *et al.*,(2007)and Pugalendhi *et al.*,(2012)which reported that levels of lipid peroxidation products malondialdehyde (MDA) were increased significantly in individuals with TB before treatment and decreases with treatment, hence, oxidative stress was observed in all the TB patients irrespective of treatment status. Further, the observed significantly lower mean serum level of selenium in TB individuals at baseline compared with individuals with TB at 2months and 6months in this work agreed with the work of Oyedeji *et al*.,(2013),whose case control study demonstrated a significantly reduced concentrations of selenium in individuals with TB than in control. In this study, the progressive increase in the serum level of selenium in

individuals with TB following treatment was an indication of good therapeutic response and could be used for therapeutic monitoring but differ from the study of Ciftci *et al.*,(2003), who investigated whether the serum levels of Cu, Zn, and Se change during antituberculosis therapy, they recruited 22 pulmonary tuberculosis cases that were newly diagnosed with positive sputum and 18 healthy individuals. At the beginning and 2 months after therapy, serum levels of Cu, Zn, and Se were measured by atomic absorption spectrometry. It was shown that Se and Cu levels did not change significantly during the treatment but Zn did. This observation by Ciftci *et al.*,(2003),could be attributed to poor therapeutic response on the TB individuals because Copper (Cu), zinc (Zn), and selenium (Se) are essential elements that play a crucial role in the immune system and tuberculosis is associated with malnutrition and immuno-deficiency. However, there was no significant difference in the mean serum level of Vitamin C (mg/dl) in individuals with TB at baseline compared with individuals with TB at 2months and 6months on ATT. This finding agreed with the case control work of Pugalendhi *et al.*,(2012), but differed with those of (Nwanjo and Oze, 2007:, Bhimrao *et al*.,2011:, Nnodim *et al*.,2011 and Oyedeji *et al*.,2013).
Moreover, a significantly lower mean serum activities of SOD and CAT in individuals with TB at baseline compared with 2months and 6months following treatment were observed. Similar results on SOD and CAT activities in individuals with TB were obtained by Reddy *et al.*, (2004), Lamsal *et al.*,(2007) and Mukhtar *et al.*,2012 in their follow-up study in TB individuals and these enzyme activities gradually increased with clinical improvement on ATT, Reddy *et al.*, (2004), as in this study. Further, there was a significantly lower mean serum activity of GPX in TB individuals at baseline compared with 2months and 6months following treatment.This finding is consistent with the work of (Pugalendhi *et al.*,2012). However, there was no significant difference in the mean serum activity of GRX (mu/ml) in individuals with TB at baseline compared with 2months following treatment but there was a significantly lower mean serum activity of GRX(mu/ml) in individuals with TB at baseline and 2months compared with individuals with TB at 6months following treatment. This is also consistent with the research conducted by (Shubhangi *et al*.,2012).Similar result was also shown by(Nezami *et al*.,2011).The significant difference in the mean serum activity of GRX in individuals with TB at baseline and 2months compared with individuals with TB at 6months following treatment could be associated to better therapeutic response considering that the gap between initiation of treatment , 2months and 6months on ATT is twice longer. It is now not surprising why a repeat sputum

microscopy and or Gene Xpert confirmation after the intensive phase (2 months) of treatment might not clear the tubercle bacilli until the 6th month in some patients.

5.2 Conclusion

The findings of this study suggest reduced levels of antioxidants in individuals with active *Mycobacterium tuberculosis* infection with increased levels of malondialdehyde. However, no significant differences were observed in the levels of most of these parameters in individuals with latent *Mycobacterium tuberculosis* infection compared with the control group while the cardiac indices were lower in individuals with active *Mycobacterium tuberculosis* infection. The antioxidants status, atherogenic indices and lipid peroxidation responded to antituberculosis therapy after six months duration of treatment in individuals with active *Mycobacterium tuberculosis* infection. However, the levels of apo B100 increased with increased duration of treatment, suggesting persistent risk of cardiovascular events in individuals with active *Mycobacterium tuberculosis* infection even on treatment.

5.3 Contributions to Knowledge

Oxidative stress and lipid peroxidation took place in individuals infected with *Mycobacterium tuberculosis* infection and that after treatment, malondialdehyde level a marker of oxidative stress reduced. Some of the biochemical parameters measured in individuals with latent *Mycobacterium tuberculosis* infection was not significantly different from apparently healthy control. Apolipoprotein B 100 may serve as a better marker than routine lipid profile for the screening of cardiovascular risk in individuals with *Mycobacterium tuberculosis* following treatment.

4. Apolipoproteins B48 and B 100 were higher in individuals with *Mycobacterium tuberculosis* infection.
5. The levels of antioxidants and lipid profiles were significantly elevated after treatment.
6. Individuals treated for tuberculosis may be predisposed to cardiovascular disease.

5.4 Limitations of study

Patients'follow up was very challenging as a sizeable number of participants were lost in this process.

5.5 Recommendations

Measurement of the levels of apo B 100 should be considered as routine in the prognostic cardiac evaluation of individuals with active *Mycobacterium tuberculosis* infection. Determination of activities of catalase and GRX, levels of Apo B 100 and MDA may be used as adjuct indicators in the prognostic management of individuals with latent *Mycobacterium tuberculosis* infection. Routine evaluation of antioxidants status and levels of free fatty acids is recommended in the management of individuals with active *Mycobacterium tuberculosis* infection.Further research in this area with larger sample size is additionally recommended.

References

Aaron, R.C., Marco, S. (2017). The link between tuberculosis and body mass index. *Journal of Thoracic Disease,* 9(3): E301–E303.

Adrian, W.J. (1973). A Comparison of a wet pressure digestion method with other commonly used wet and dry ashing methods. *Analyst*, 98:213-215.

Ahmed, N., Hasnain, S. (2011). Molecular epidemiology of tuberculosis in India. Moving forward with a systems biology approach. *Tuberculosis,* 91 (5): 407–413.

Akiibinu, M.O., Arinola, O. G., Ogunyemi E.O. (2009). Plasma neopterin and peroxide levels in pulmonary tuberculosis patients on chemotherapy with or without micronutrient supplementation. *Pakistan Journal of Medical Sciences,* 25(3):380-385.

Akinduko, D.S.,Temitope, M.O.,Chukwuemeka,Z.U.(2014). Antioxidant capacity of *Moringa Oleifera* seed oil against CCL_4 induced hepatocellular lipid peroxidation in wistar albino rats. *European Journal of Experimental Biology,* 4(1):514-518.

Akpovi, D.C., Gbaguidi, L.H.S., Amago, E.,Affolabi, D.,Dougnon,T.V.,Faihun, F.,Anagonou,S. (2013). Tuberculosis treatment raises total cholesterol and restores HDL-C in patients with pulmonary tuberculosis. *African Journal of Biotechnology,* 12(41):6019-6024.

Albro, P.W., Corbelt, J. L., Schroeder, J. L. (1986). Application of the thiobarbiturate assay to the measurement of lipid products in microsomes. *Chemico- Biological Interactions,* 86:185-194.

Amicosante, M., Ciccozzi, M., Markova, R (2010). Rational use of immunodiagnostic tools for tuberculosis infection, guidelines and cost effectiveness studies. *The new microbiologica.* 33 (2): 93–107.

Albanna, K. B., Amr, S., Donald, W., Chantal, V., Dick, M. (2013). Serum lipids as biomarkers for therapeutic monitoring of latent tuberculosis infection. *European Respiratory Journal,* 42: 547-550.

Anderson, J.V., Davis, D.G. (2004). Abiotic stress alters transcript profiles and activity of glutathione *S*-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula. International Journal for plant Biology,* 120 (3):421-433.

APHA, (1995). Standard methods for the examination of water and wastewater. 19th edition, American Public Health Association Inclination, New York, pp.213-223.

Araoye, M.O. (2004) Sample size determination in research methodology with statistics for health and social sciences. 1st edition, Nathadex Publishers, Ilorin, pp.115-121.

Arch ,G., Mainous, III. (2010). Management of antimicrobials in infectious diseases, impact of antibiotic resistance. Totowa, N.J. Humana Press, pp. 69-71.

Arrigo, A.P. (1999). Gene expression and the thiol redox state. *Free Radical Biolology and Medicine* ,27:936–944.

ATS/CDC Statement committee on latent tuberculosis infection (2000). Targeted tuberculin testing and treatment of latent tuberculosis infection. *American Thoracic Society*, MMWR. Recommendations and reports, 49 (RR–6): 1–51.

Audu, D.C., Onwujekwe, J.,Onubuogu, N.,Adedoyin, A.,Oyeyejepu,J., Aje, C.,Onyewuche, C.,Oparango, M., Ewuru, A.,Anichabe,Musa,C. (2005). Possible impact of co-infections of tuberculosis and malaria on the CD4+ cell counts in HIV infected subjects. *Annal of African Medicine,* 4:10-13.

Babar, S. (2015). Evaluating the performance of 4 Indices in determining adiposity. Clinical *Journal of Sports Medicine, Lippincott Williams and Wilkins,* 2: 183-184.

Behera, D. (2010). Textbook of Pulmonary Medicine 2nd edition. New Delhi: Jaypee Brothers Medical Publishers, pp. 457.

Benn, M., Nordestgaard, B.G., Jensen, G.B., Tybjaerg-Hansen. A (2007). Improving prediction of ischemic cardiovascular disease in the general population using apolipoprotein B, the Copenhagen City Heart Study. *Arteriosclerosis, Thrombosis and Vascular Biology*, 27(3): 661– 670.

Bento, J., Silva, A.S., Rodrigues, F., Duarte, R (2011). Diagnostic tools in tuberculosis. *Acta Médica Portuguesa*. 24 (1): 145–154.

Bhaskaran, K. I., Douglas, Forbes, H., dos-Santos-Silva, H., Leon, D.A., Smeeth, L (2014). Body-mass index and risk of 22 specific cancers, a population-based cohort study of 5·24 million UK adults. *Lancet*, 384 (9945): 755–765.

Beutler, E., Duron, O.,Kelly, B.M.,(1963).Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine,* 61:882-888.

Bibbins-Domingo, Kirsten., Grossman, D. C., Curry, S. J., Bauman, L., Davidson, K. W., Epling, J. W., García, F. A.R.., Herzstein, J., Kemper, A. R., Krist, A. H., Kurth, A. E., Landefeld, C., Seth,M., Carol, M., Phillips, W. R.., Phipps, M. G., Pignone, M. P.
(2016). Screening for Latent Tuberculosis Infection in Adults . *Journal of the American Medical Association* 316 (9):962-969.

Blakemore, R., Story, E., Helb, D., Kop, J., Banada, P., Owens, M., Chakravorty, S., Jones, M., Alland, D.(2010). Evaluation of the analytical performance of the Xpert MTB/RIF Assay. *Journal of Clinical Microbiology*, 48 (7): 2495–2501.

Bozzano, F. (2014). Immunology of tuberculosis. *Mediterranian Journal of Hematology and Infectious Diease,* 6 (1): e2014027.

Boehme, C.C., Nicol, M.P., Nabeta. P., Michael, J.S., Gotuzzo, E., Tahirli, R., Gler, M.T., Blakemore, R., Worodria, W., Gray, C., Huang, L., Caceres, T., Mehdiyev, R., Raymond, L., Whitelaw, A., Sagadevan, K., Alexander, H., Albert, H., Cobelens, F., Cox, H., Alland, D., Perkins, M.D.,(2011). Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance, a multicentre implementation study. *Lancet,* 30:377(9776):1495-1505.

Brodsky, J.L (2008). *Trends Endocrinology Metabolism.* 19(7):254-259.

Burtis, A.C., Ashwood, E.R., Bruns, D.E.(2008). Lipids, Lipoproteins, Apolipoproteins and other Cardiovascular Risk Factors in: Tietz Fundamentals of Clinical Chemistry, 6th edition, published by Elsevier, Sri Pratap Udyog, 274, Capt. Gaur Marg, Sriniwaspuri, New Delhi India-110065, chapter 23,pp.402-411.

Butov, D.A., Pashkov, Y.N., Stepanenko, A.L.,(2011). Phase IIb randomized trial of adjunct immunotherapy in patients with first-diagnosed tuberculosis, relapsed and multi-drug-resistant (MDR) TB. *Journal of Immune Based Therapeutic Vaccines*,9:3-4.

Catala, A.(2007) The ability of melatonin to counteract lipid peroxidation in biological membranes: *Current Molecular,* 7(7)1- 7.

Chaisson, R.E., Martinson, N.A. (2008). Tuberculosis in Africa combating an HIV-driven crisis. *The New England Journal of Medicine*. 358 (11): 1089–1092.

Chan, D.C., Watts, G.F.(2006) Apolipoproteins as markers and managers of coronary risk *An International Journal of Medicine, 99:277–287.*

Chaudiere, J., Ferrari-Iliou, R. *(*1999). Intracellular antioxidants: from chemical to biochemical mechanisms. *Food and Chemical Toxicology Journal,* 37:949–962.

Cheesbrough, M. (2004). Staining malaria parasite. In: District laboratory practice in tropical countries. United Kingdom: Cambridge University Press, pp. 247-251.

Cheesbrough, M. (2004). Protein. In: District Laboratory Practice in Tropical Countries Part 1 United Kingdom: Cambridge University Press, pp. 434-436.

Chen, S.H.,, Yang, C.Y., Chen, P.F., Setzer, D., Tanimura, M., Li, W.H., Gotto, AM Jr, Chan L (2011). The complete cDNA and amino acid sequence of human apolipoprotein B-100. *Journal of Biological Chemistry*, 261 (28): 12918–12921.

Ciftci, U. T., Ciftci , B. , Yis, Ö. , Guney ,Y., Bilgihan, A., Ogretensoy, M. (2003). Changes in serum selenium, copper, zinc levels and Cu/Zn ratio in patients with pulmonary tuberculosis during therapy. *Biological Trace Element Research*, 95 (1): 65–71.

Cooke, M.S., Evans, D.M., Dizdaroglu, M., Lunec, J.(2003). Oxidative DNA damage, mechanisms, mutation, and disease. *The Federation of American Societies for Experimental Biology Journal,*17:1195–1214.

Cooper, D.A., Eldridge, A.L., Peters, J.C. (1999). Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration. A review of recent research. *Nutrition Reviews,* 57:201–214.

Core Curriculum on Tuberculosis (2013). What the clinician should know 6th edition. Centers for Disease Control and Prevention. Division of Tuberculosis Elimination, 2011, pp. 24.

Courtwright, A., Turner, A .N. (2010). Tuberculosis and stigmatization, pathways and interventions. *Public Health Reports,* 125 *Supplementary* 4: 34–42.

Crowley, Leonard, V. (2010). An introduction to human disease, pathology and pathophysiology correlations 8th edition, Sudbury, Mass. Jones and Bartlett. pp. 274-275.

Danica, H., Martin, J., Elizabeth, S., Catharina, B., Ellen,W., Ken, H., JoAnn, K., Michelle, R. O., Richard, R., Padmapriya, B., Hassan, S., Robert, B., Ngoc, N. T. L., Edward, C., Jones-López, M. L., Michele, B., Irene, A., Roy, D., Mugerwa, B. M., Emily, W., Lee, C., Peter, D., Mark, D. P., David, H. P., David, A.,(2010).Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology,48:229-237.*

Debes, R., Pradeep, D.,Kalyan, G., Neelam, G. (2007). Antioxidant vitamin level in sickle cell disorders. *National Medical Journal of India,* 20(1): 11-13.

Delph, C., Arun, K. P. (2015). Tuberculosis in the African continent. A comprehensive review. *Pathophysiology,* 22:73-83.

Desai,(1984). Vitamin E analysis methods for animal tissues. *Methods in Enzymology*, 105:138-47.

Deveci ,F., Ilhan ,N.(2003). Plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis. *Biological Trace Element Research,* 95(1): 29-38.

Driscoll, M. D., Kirsty, J.M., Colin, L., Natalia, M., Irina, A. P., Pierre, L., Stephen, E.J. R., David, L., Andrew, W. M. (2010). Structural and biochemical characterization of *Mycobacterium tuberculosis* CYP142 evidence for multiple cholesterol 27- hydroxylase activities in a human pathogen. *Journal of Biological Chemistry* 285(49): 38270- 38282.

Escalante, P. (2009). In the clinic, tuberculosis. *Annals of Internal Medicine,* 150 (11): ITC61–614.

Favier, A., Sappey, C., Laclerc, P., Faure, P., Micoud, M.(1994). Antioxidant status and lipid

peroxidation in patients infected with HIV. *Journal of Chemical Biology Interaction*, 91 : 165- 180.

Flegal, K.M., Kruszon-Moran, D., Carroll, M.D., Fryar, C.D., Ogden, C.L. (2016). Trends in Obesity Among Adults in the United States, 2005 to 2014. *Journal of American Medical Association.*. 315 (21): 2284–2291.

Frisancho, A. R..(1990). Anthropometric standards for the assessment of growth and nutritional status. Ann Arbour. University of Michigan Press, pp. 1-2.

Fullerton, S.M., Buchanan, A.V., Sonpar, V.A., Taylor, S.L., Smith, J.D., Carlson C.S., Salomaa, V., Stengård, J.H., Boerwinkle, E., Clark, A.G., Nickerson, D.A., Weiss, K.M. (2004). The effects of scale, variation in the APOA1/C3/A4/A5 gene cluster. *Human Genetics*, 115 (1): 36–56.

Gebremedhin, G.,Yemane, A., Feyissa, C., Atsbeha, G., Girmay, M. Mistire, W., Desta, K (2017). Lipid profile in tuberculosis patients with and without human immunodeficiency virus infection. *International Journal of Chronic Diseases.* (3843291):1- 7.

Gerald, L., Mandell, J. E., Bennett, R. (2010). Mandell, D., and Bennett's principles and practice of infectious diseases 7th edition. Philadelphia, PA, Churchill Livingstone/Elsevier, Chapter 250.

Gibson, R.S.(1990). Principles of nutritional assessment. Oxford university press, new York, pp.1-2.

Guteridge, J.M.C.(1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry*. 41:18199-18208.

Gutteridge, J.M., Wilkins, S. (1982). Copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. *FEBS Letters.*, 137:327-330.

Halezeroğlu, S., Okur, E. (2014). Thoracic surgery for haemoptysis in the context of tuberculosis: what is the best management approach?. *Journal of Thoracic Disease*, 6 (3): 182–185.

Halliwell, B., Gutteridge, J.M. (1989).*Free radicals in biology and medicine*,2nd edition. Oxford: Clarendon Press, pp.112-114.

Halliwell, B., Gutteridge, J.M.C.(2007). Cellular responses to oxidative stress, Adaptation, damage, repair, senescence and death, third edition, New York Oxford University press. pp.187-267.

Halliwell, B., Gutteridge, J.C. (1992). Biologically relevant metal ion-dependent hydroxyl radical generation an update. *FEBS Letters.* 307:108–112.

Halliwell, B., Gutteridge, J.C. (1995).The definition and measurement of antioxidants in biological systems. *Free Radical Biology and Medicine,* 18:125–126.

Hecht, S.S. (1999). Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute,* 91:1194–2100.

Hem, K. T. Uddhav, T., Khelanand, P. S., Sanjit, S., Ramendra, K.R., Pujan, P., Preeti, K., Laxmi, K., Chandika, D.(2014). Apo B/Apo A-I ratio is statistically a better predictor of cardiovascular disease than conventional lipid Profile: A Study from Kathmandu Valley, *Nepal. Journal of Clinical and Diagnostic Research,*8 (2): 34–36..

Huang, Y.H., Schafer-Elinder, L, Wu, R.(1999). Lysophosphatidylcholine induces proinflammatory cytokines by a platelet-activating factor receptor-dependent mechanism. *Clinical and Experimental Immunology,* 116:326–31.

Ihim, A. C., Nwosu, D.C., Nwanjo, H.U., Onyenekwe, C.C., Nnodim, J., Manafa, P.O., Okeke A. C., Oluboyo, A.O., Nwobodo E.I., Nwadike, C., Oze, G.(2013). Anti lipid peroxidative and hypolipidaemic potentials of *Nauclea latifolia* leaves extract on ciprofloxacin induced oxidative stress in rats. *Asian Journal of Pharmaceutical and Biological Research,* 3(1):18-23.

Ihim, A. C., Obi, P.C., Meludu, C. S., Onyenekwe, C.C., Anyabolu, E. A., Njoku ,C. M. (2018). Free fatty acid and lipid profile in active TB, Latent TB, Active TB and HIV, active TB and malaria parasite in subjects in Anambra. *European Journal of Biomedical and Pharmaceutical sciences,* 5(11): 506-511.

Jacob, J.T., Mehta, A.K., Leonard, M.K. (2009). Acute forms of tuberculosis in adults. The *American Journal of Medicine,* 122 (1): 12–17.

Jane, N., Zuckerman, Elaine, C. (2010). Travelers' vaccines 2nd edition. Shelton C.T. people's medical publishing house. pp.319-320.

Janiszewska-Drobinska,B., Kowalski, J., Blaszczyk, J., Kedziora, J., Kaczmarek, P., Ciecwierz,J.,(2001). Estimation of plasma malonyldialdehyde concentration in patients with pulmonary tuberculosis. *Pol Merkur Lekarski.* 11: 310-313.

Jindal, S.K. (2011). Textbook of pulmonary and critical care medicine. 1st edition, New Delhi: Jaypee Brothers Medical Publishers, pp.549-550.

Jones, D.P.(2008). Radical-free biology of oxidative stress. *American Journal of Physiology Cell Physiology,*295:C849–C868.

Kaufmann, S.H. (2010). Future vaccination strategies against tuberculosis. Thinking outside the box. *Immunity*. 33 (4): 567–577.

Keys, S.A., Zimmerman, W.F.(1999). Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. *Experimental Eye Research*,68:693–702.

Kinoshita,M., Kojima, M., Matsushima,T., Teramoto,T. (2005). Determination of apolipoprotein *Clinica Chimica Acta,* 351:115-120.

Kita, T., Kume, N., Ishii, K.,(1999).Oxidized LDL and expression of monocyte adhesion molecules. *Diabetes Research and Clinical Practice.* 45:123–126.

Klopper, M., Warren, R.M., Trolip, P.A. (2013).Emergence and spread of extensively and totally drug-resistant tuberculosis, South Africa. *Emerging Infectious Disease.*19: 449–455.

Kumar, V., Abbas, A.K,, Fausto, N., Mitchell, R.N (2007). *Robbins Basic Pathology* 8th edition. Saunders Elsevier pp.516–522.

Kwiatkowska, S., Piasecka, G., Zieba, M., Piotrowski, W., Nowak., D.(1999). Increased serum concentrations of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. *Respiratory Medicine,* 93:272-276.

Lamsal, M.,Gautam, N.,Bhatta, N.,Toora, B.D.,Bhattacharya, S. K.,Baral, N. (2007) Evaluation of lipid peroxidation product, nitrite and antioxidant levels in newly diagnosed and two months follow-up patients with pulmonary tuberculosis, *Southeast Asian Journal of Tropical Medicine and Public Health*, 22(8):25-28.

Lawn, S.D., Zumla, A.I. (2011). Tuberculosis. *Lancet*, 378 (9785): 57–72.

Lawn, S.D., Nicol, M.P. (2011). Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future microbiology*, 6 (9): 1067–1082.

Liu, Q., Abba, K., Alejandria, M.M., Balanag, V.M., Berba, R. P. Lansang, M.A. (2008). Liu, Qin, edition. Reminder systems and late patient tracers in the diagnosis and management of tuberculosis. *Cochrane database of systematic reviews , (4): CD006594- CD006595.*

Lloyd, R.V., Hanna, P.M., Mason, R.P.(1997). The origin of the hydroxyl radical oxygen in the Fenton reaction. *Free Radical Biology Medicine,* 22:885–888.

Lozano, R (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010, a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380 (9859): 2095–128.

MacKay, N.J. (2010). Scaling of human body mass with height, the body mass index revisited. *Journal of Biomechanics,43 (4): 764–66.*

Malcolm K. (2015).Why being overweight means you live longer. https://www.independent.co.uk. Retrieved 4th December, 2017.

Marnett, L.J.(1999) Lipid peroxidation-DNA damage by malondialdehyde. *Mutation research, 424(1-2):83-95*.

Mason, P.H., Roy, A. Spillane, J. Singh, P. (2015). Social, historical and cultural dimensions of tuberculosis. *Journal of Biosocial Science,* 48: 1–27.

Menzies, D., Al Jahdali, H., Al Otaibi, B (2011). Recent developments in treatment of latent tuberculosis infection. *The Indian journal of medical research*,133 (3): 257–266.

McAnlis, G.T., McEneny, J., Pearce, J. (1999).Absorption and antioxidant effects of quercetin from onions in man. *European Journal of Clinical Nutrition*, 53: 92–96.

McIntyre, M., Bohr, D.F., Dominiczak, A.F.(1999). Endothelial function hypertension the role of superoxide anion. *Hypertension*, 34:539–545.

Metcalfe, J.Z., Everett, C.K., Steingart , K.R., Cattamanchi, A., Huang, L.,Hopewell P.C., Pai, M.(2011). Interferon-γ release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries, systematic review and meta-analysis. *The Journal of Infectious Diseases,* 204 supplementary 4: S1120–129.

Misra, H.P. and Fridovich, I. (1972). The Role of Superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chem*istry,247: 3170.

Mitchell, M.A. (2012). Mycobacterial infections in reptiles. The Veterinary Clinics of North America. *Exotic Animal Practice,* 15 (1): 101–111.

Möller, M., Hoal, E.G. (2010). Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. *Tuberculosis*, 90 (2): 71–83.

Mukhtar A. H., Bilal, A., Syed I. A. S., Muhammad, A.U.K.(2012). Antioxidant capacity and lipid peroxidation product in pulmonary tuberculosis. Al Ameen *Journal of Medical Sciences*, 5 (3) :3 1 3 -3 1 9.

Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A.,Van Remmen, H. (2007) Trends in oxidative aging theories. *Free Radical Biology Medicine,* 43: 477-503.

Nagamani, M. , Prahaladu, P. , Vijayababu, P.V.S.S., Ashalata , K., Kusuma, P., kumara,(2015). Lipid peroxidation product as a marker of oxidative stress in Psoriasis -A case control study in North Coastal Andhra Pradesh. IOSR *Journal of Dental and Medical Sciences,* 14(5):18-20.

Nezami, N., Ghorbanihaghjo, A., Rashtchizadeh, N., Argani, H., Tafrishinejad, A., Ghorashi, S., Hajhosseini, B. (2011).Atherogenic changes of low-density lipoprotein susceptibility to oxidation, and antioxidant enzymes in pulmonary tuberculosis. *Atherosclerosis,* 217(1):268-273.

Niederweis, M., Danilchanka, O., Huff, J., Hoffmann, C., Engelhardt, H. (2010). Mycobacterial outer membranes: in search of proteins. *Trends in Microbiology,* 18 (3): 109–116.

Nnamdi Azikiwe University Teaching Hospital, (2013) Tuberculosis directly observed treatment short course clinic-microbiology laboratory record.

Nnodim, J., Anyadoh, S., NwosuNjoku, E.(2011) The antioxidant status and lipid peroxidation product of newly diagnosed and six weeks follow up patients with pulmonary tuberculosis in Owerri, Imo State, Nigeria. *Asian Pacific Journal of Tropical Disease,* 1: 292-294.

Nwanjo, H. U., Oze, G. O. (2007). Oxidative imbalance and non-enzymic antioxidant status in pulmonary tuberculosis infected subjects. Carcinogenic potential. *Pakistan Journal of Nutrition*, 590-592.

Nwanjo,H.U., Okafor ,M.C., Oze,G.(2007).Protective role of A- tocopherol and ascorbic acid supplementation on halofantrine induced hepatotoxicity in rats . *The Internet Journal of Nutrition and Wellness,* 3(2)1-2.

Omaye, S.T., Turbull, T.P., Sauberchich, H.C. (1979). Selected Methods for determination of ascorbic acid in cells, tissues and fluids. *Methods Enzymology*, 6: 3-11

Oyedeji,S.O., Oguntuase, N. R., Esan, A., Adesina,A. A., Oke, O. T.(2013).Oxidative stress and lipid profile status in pulmonary tuberculosis patients in South Western Nigeria. *Greener Journal of Medical Sciences,* 3(6): 228–232.

Osuji, F.N., Onyenekwe, C.C., Ifeanyichukwu, M. O., Ahaneku, J.E., Ezeani, M. Ezeugwunne, I. P.(2013). Impact of HIV and *Mycobacterium tuberculosis* co-infections on antioxidant status in Nigeria. *Pakistan Journal of Nutrition.* 12(5):496-504.

Paglia, D. E., Valentine, W.N.(1967). *Journal of Laboratory Clinical Medicine*70:158.

Pai, M., Zwerling, A., Menzies, D. (2008). Systematic Review: T-Cell–based Assays for the Diagnosis of Latent Tuberculosis Infection. An Update *Annals of Internal Medicine*, 149 (3): 1–9.

Panteix, G., Gutierrez, M.C., Boschiroli, M.L., Rouviere, M., Plaidy, A., Pressac, D., Porcheret, H., Chyderiotis, G., Ponsada, M., Van Oortegem, K., Salloum, S., Cabuzel, S., Bañuls, A.L., Van de Perre, P., Godreuil, S. (2010). Pulmonary tuberculosis due to *Mycobacterium microti*, a study of six recent cases in France. *Journal of Medical Microbiology,*59 (8): 984–989.

Paramjit, K. S., Salma, M.A. Musaad, A. T. R., Stephanie, S. B., Sonya, S., James, H. D., Hubert, W. V., Anne, R., Colleen, S. S., Robert, H. C. (2016).Lipoprotein biomarkers and risk of cardiovascular disease, a laboratory medicine best practices systematic review. *Journal of Applied Laboratory Medicine, 1(2): 214–229.*

Pepys, M.B., Hirschfield, G.M. (2003). C-reactive protein, a critical update. *Journal of Clinical Invesion.* 111 (12): 1805–1812.

Prabakaran, J. J., Biniam, E., Matiwos, A., Mussie , M., Henok, Z., Michael, Y., Adiam, S., Awet, G.(2017). Association of Dietary and Serum Cholesterol with Active Pulmonary Tuberculosis: A Hospital Based Study. *International Journal of Current Microbiology and Applied Sciences,* 6 (12):4056-4073.

Pugalendhi1,V., Prema Gurumurthy, Panicker, T. M. R., Paul Korath, M., Jagadeesan1, K., Tracy Tina Angelina, J. (2012). Lipid peroxidation and antioxidant status in patients of end stage renal disease with and without pulmonary tuberculosis. *Journal of International Medical Sciences Academy,* 25(4):225-227.

Pusapati, M., R., Girijasankar, G., Ramesh, B. P. (2017). Association of lipid profile, atherogenic indices, and LPL Hind-III gene polymorphism with coronary artery disease positive subjects. *International Journal of Pharmaceutical and Clinical Research*. 9(1): 6-15.

Ramesh, S.K. , Amareshwara, M., Sameer, R..M., (2011).Study of protein oxidation and antioxidants status in pulmonary tuberculosis patients. *International Journal of Pharma and Bio Sciences,*2(3):104-109.

Reavill, D.R., Schmidt, R.E. (2012). Mycobacterial lesions in fish, amphibians, reptiles, rodents, lagomorphs, and ferrets with reference to animal models. The Veterinary clinics of North America. *Exotic Animal Practice,*15 (1): 25–40.

Reddy, Y. N., Murthy, S.V.,Krishna, D. R., Prabhakar, M.C.(2004) Role of free radicals and antioxidants in tuberculosis patients. *Indian Journal of Tuberculosis,*213-218.

Reddy Y.N., Murthy S.V., Krishna D.R., (2009). Oxidative metabolic changes in pleural fluid of tuberculosis patients. Bangladesh. *Pharmacology.* 4:69-72.

Reichau, S., Wanting, J., Scott, R. W., Richard, D. H., Edward, N. B., Emily, J.P.(2011). Potent inhibitors of a shikimate pathway enzyme from *Mycobacterium tuberculosis* combining mechanism and modeling based design. *Journal of Biological Chemistry* 286(18): 16197-16207.

Restrepo, B.I. (2007). Convergence of the tuberculosis and diabetes epidemics. Renewal of old acquaintances .*Clinical Infectious Diseases,* 45 (4): 436–438.

Rifadin, (2000). Hoechst Marion Roussel in: PDR Physicians desk reference. 54th edition . Oradell, N J. Medical Economics Data, pp: 1382-1383.

Roeschlau, P.,Bernt, E.and Gruber,J.W.,(1974) Cholesterol Estimation Clinical Biochemistry 1st edition .*Journal of the American Medical Association publication*,12:403-404.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G.(1973).Selenium. Biochemical role as a component of glutathione peroxidase.179 (4073):588-590.

Roxborough, H.E., Mercer, C., McMaster, D.,(1999). Plasma glutathione peroxidase activity is reduced in haemodialysis patients. *Nephron.* 81:278–283.

Roy, A., Eisenhut, M., Harris, R.J., Rodrigues, L.C., Sridhar, S., Habermann, S., Snell, L., Mangtani, P., Adetifa, I., Lalvani, A., Abubakar, I. (2014). Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: systematic review and meta-analysis. *British Medical Journal* .Clinical Research edition, 349: g4643- 4648.

Saito, H., Lund-Katz, S., Phillips, M.C. (2004). Contributions of domain structure and lipid interaction to the functionality of exchangeable human apolipoproteins. *Progress in Lipid Research, 43 (4): 350–380.*

Schneider, H. J., Friedrich, N., Klotsche, J. Pieper, L., Nauck, M., John, U. (2010). The predictive value of different measures of obesity for incident cardiovascular events and Mortality. *Journal of Clinical Endocrinology and Metabolism, 95 (4): 1777–1785.*

Sester M., Sotgiu G. Lange C., Giehl C., Girardi E., Migliori G.B., Bossink A., Dheda K., Diel R., Dominguez J., Lipman M., Nemeth J., Ravn P., Winkler S., Huitric E., Sandgren A., Manissero D. (2011). Interferon-γ release assays for the diagnosis of active tuberculosis, a systematic review and meta-analysis. *The European Respiratory Journal.* 37 (1): 100–111.

Shivaprasad, H.L., Palmieri, C. (2012). Pathology of mycobacteriosis in birds. The veterinary clinics of North America. *Exotic Animal Practice*, 15 (1): 41–55.

Si, G., Li, M., Meng, K., Qiao, D.,(2013).Phase III clinical study of efficacy and safety of vaccae to prevent tuberculosis. https://clinicaltrials.gov/ct2/show/NCT01979900 (retrieved 27 March, 2019).

Sinha, A.K.(1972) .Colorimetric assay of catalase. *Analytical Biochemistry*. 47:389-394.

Shubhangi, M.D.,Vinayak,W.P.,Nagsen,N.R.(2012). The Roles of glutathione, glutathione peroxidase, glutathione reductase and the carbonyl protein in pulmonary and extra pulmonary tuberculosis. *Journal of Clinical Diagnostic Research,* 6(9):1462-1465.

Sniderman, A.D., Cianflone K, Arner P, Summers LK, Frayn KN(1998). The adipocyte, fatty acid trapping, and atherogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*,18:147-151.

Sniderman, A.D., Maslowska M, Cianflone K.(2000). Of mice and men and women and the acylation-stimulating protein pathway *Current Opinion in Lipidiology*.11:291-296.

Stein, J.H., Keevil, J.G., Wiebe, D.A.(1999). Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation.* 100:1050–1055.

Steingart, K.R., Flores, L.L., Dendukuri, N., Schiller, I., Laal, S., Ramsay, A., Hopewell, P.C., Pai, M. (2011). Evans Carlton edition. Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis, an updated systematic review and meta-analysis *PLOS Medicine,*8 (8): e1001062.

Taparia, P., Yadav, D., Koolwal, S.,Mishra,S.(2015).Study of lipid profile in pulmonary tuberculosis patients and relapse cases in relation with disease severity.A pilot study . *International Journal of Sciences and Applied Research.* 2(1): 41-50.

Tapel, A. L ., Zalkin, H.(1959). Inhibition of lipid peroxidation in mitochondria by Vitamin E. *Archives of Biochemistry and Biophysis,* 80: 333-336.

Tasduq, S.A., Peerzada, K., Koul, S.,(2005) . Biochemical manifestations of anti-tuberculosis drugs induced hepatotoxicity and the effect of silymarin. *Hepatology Research*, 31:132–135.

The United states pharmacopeia, (2000). The national formulary.USP 24th revision NF 19th Edition, Rock ville, MD The United States Pharmacopeial Convention inc,1999 pp: 689-690. Thomas, M., Habermann and Amit, K. (2008). Mayo Clinic internal medicine: concise textbook. Rochester, MN: Mayo Clinic Scientific Press, pp. 789-790.

Tietz, N.W.(1990).Cholesterol Estimation in: Clinical guide to laboratory tests, 2nd edition W .B.Saunders Company, Philadelphia,USA, 554-556.

Trevisan, M., Browne, R., Ram, M., Freundheim, J., Mariecarosella, A., Armstrong, D.(2000) Correlates of Markers of Oxidative Status in the general population. *Americant Journal of Epidemiology,* 154(4)1-4.

Trinder, P. (1969). Serum Cholesterol estimation enzymatic method, *Annal of Clinical Biochemistry*. 6 :(24)1-2.

Ukibe, N.R. , Onyenekwe,C.C. , Ahaneku, J.E. , Meludu,S.C. , Ukibe,S.N., Ilika,A. , Ifeanyichukwu,M.O., Ezeani,M.C., Igwegbe, A.O., Ofiaeli,N., Onochie, A. Abor, N.,(2010).CD4+ T-cells count in HIV-malaria co-infection in adult population in Nnewi,South Eastern Nigeria. *International Journal of Biological and Chemical Sciences,* 4(5): 1593-1601.

Valagussa, F., Franzosi, M.G., Geraci, E.(1999). Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction. Results of the GISSI Prevenzione trial. *Lancet*,354:447–455.

Valerino D.M. (1971). Xanthine Oxidase-Mediated Oxidation of Epinephrine Biochemical Pharmacology,20(1);47-55.

van Lettow, M., Whalen, C. (2008). Nutrition and health in developing countries 2nd edition. Totowa, N.J. Richard, D. Semba and Martin W. Bloem, editions. Humana Press, pp. 291-293.

van Zyl Smit, R.N., Pai, M., Yew, W.W., Leung, C.C., Zumla, A., Bateman, E.D., Dheda, K., (2010). Global lung health: the colliding epidemics of tuberculosis, tobacco smoking, HIV and COPD. *European Respiratory Journal*,35 (1): 27–33.

Velayati, A.A., Masjedi, M.R., Farnia, P., Tabarsi, P., Ghanavi, J., Ziazarifi, A.H., Hoffner, S.E. (2009).Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug resistant strains in Iran. *Chest,* 136 (2): 420–425.

Wallis, R.S. , Doherty, T. M. , Onyegbujo, P., Vahedi, M., Laang, H., Olesen, O., Parida, S.(2010). Biomarkers for tuberculosis disease activity, cure, and relapse. *Lancet Infectious Diseases*, 10: 68–69.

Wang, Y. (2012). Use of Percentiles and Z-Scores in Anthropometry in: Handbook of t Anthropometry. New York: Springer. Chapter 2. pp. 29.

Ward, A.I., Judge, J., Delahay, R.J. (2010). Farm husbandry and badger behaviour: opportunities to manage badger to cattle transmission of Mycobacterium bovis. *Preventive Veterinary Medicine*. 93 (1): 2–10.

White, P.C., Böhm, M., Marion, G., Hutchings, M.R. (2008). Control of bovine tuberculosis in British livestock: there is no 'silver bullet. *Trends in Microbiology.* 16 (9): 420–427.

Wiid, I., Seaman, T., Hoal, E.G., Benade, A.J., Van Helden, P.D.(2004) Total antioxidant levels are low during active TB and rise with anti-tuberculosis therapy. *International Union of Biochemistry and Molecular Biology Life.* 56:101-106.

Wilson, C., Wardell, M.R., Weisgraber, K.H., Mahley, R.W., Agard, D.A (1991).Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. *Science. 252 (5014): 1817–1822.*

Wooliams, J.A.,Wiener,G.,Anderson, P.H., McMurray, C.H.(1983). *Superoxide Dismutase Research in Veterinary Science.* 35:47-52.

World Health Organisation, (2000). Standard Regimens for New TB patients in, Treatment of tuberculosis guidelines. WHO Library, 4th Edition, cataloguing in publication Data.

World Health Organization (2008).https://www.who.int>tb>publications>tb control. Geneva: World Health Organization.(retrieved 2/11/19):179.

World Health Organization. (2013). Nigeria Tuberculosis Fact sheet N°104. Retrieved 28 May,2013.

World Health Organisation, (2015).National Strategic Plan for Tuberculosis Control Towards Universal Access to Prevention, Diagnosis and Treatment, presentation on the new post-2015 targets, accessed at: http://www.who.int/tb/post_2015_tb_presentation.pdf?ua=1, (Retrieved September 6, 2019).

World Health Organisation, (2018). Latent TB Infection, Updated and consolidated guidelines for programmatic management.https://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/(retrieved October 10, 2019):1-78.

Yıldız, G., Ayse, B., Tansu, U.C., Filiz, Ç., Ozgür, C.(2004). Serum malondialdehyde levels and superoxide dismutase activities in pulmonary tuberculosis and lung cancers. *Ankara Üniversitesi Dikimevi Saglık Hizmetleri Meslek Yüksekokulu Dergisi, Cilt* (6) 2.

Young, I. S., Woodside, J. V (2001).Antioxidants in health and disease Antioxidants in health and disease. *Journal of Clinical Pathology, 176–186.*

Yung-Feng, Y., Pei-Hung, C., Muh-Yong, Yen, S.L., Peing ,C., Mei-Jen, Y., Bo-Lung, H., Pesus, C., Chung-Yeh, D. Association of body mass index with tuberculosis mortality a population-based follow-up study. Medicine (Baltimore)v.95(1):e2300.

Zumla, A., Hafner, R., Lienhardt, C., Hoelscher, M., Nunn, A (2012). Advancing the development of tuberculosis therapy. Nature Reviews., *Drug Discovery*. 11 (3): 171–172.

APPENDIX 1

QUESTIONAIRE

The purpose of the study is to assess **Serum Antioxidants, Lipid Peroxidation, Lipid Profile, And Apolipoproteins In *Mycobacterium tuberculosis* Infected Individuals And during Treatment In Anambra State.** It is hoped that findings from the study would help to improve management of the individuals.

The following questions require voluntary and truthful response. The information given is considered confidential.

SECTION A; BIODATA

INSTRUCTION: Please answer the following questions by ticking [√] in the appropriate space provided and supply answer where necessary.

Code

Age 15-20[ ] 21-30 [ ] 31-40[ ] 41-50 [ ] 51-60 [ ] 61-65 [ ] 66 and above [ ]

Occupation: student [ ] Trader[ ] farmer [ ] civil servant[ ] unemployed[ ] others(specify)[ ]

Religion

Level of Education: Non literate [ ] Primary [ ] Post primary [ ] Tertiary [ ]
Marital Status single[ ] married [ ] divorced [ ] widowed [ ]

Married, what is your type of marital union? Monogamous [ ] Polygamous [ ]

Sex: Male [ ] Female [ ]

SECTION B CLINICAL FEATURES (SYMPTOMS)

1.Which of the following symptom(s) do you experience?

A. weakness[ ]B.weight loss[ ]C.fever[ ].D. rashes [ ]E .diarrhoea[ ]

F. cough[ ] G. itching[ ]

2 When did you test positive to Mycobacterium tuberculosis?

A. ≤ 1 month [ ]; B. one month now [ ] C. 6months ago [ ] ; D.12 months ago [ ] E.≥12months [ ]

3. What remedies have you sought?

A. Traditional/herbal [ ] B. religious [ ] C. orthodox [ ]D. None of the above [ ]

4.Have you been on anti tuberculosis therapy before?

A. yes[ ] B.No [ ]

5.Do you suffer from any of the following? Diabetes [ ] cancer [ ] liver problems [ ] kidney problems [ ] opportunistic infections [ ]

SECTION C

6.What is your HIV Status? HIV Negative [ ] HIV Positive [ ]

If HIV positive when were you diagnosed positive?

A. ≤ 1 month [ ]; B. one month now [ ] C. 6 months ago [ ] ; D.12 months ago [ ] E.≥12months [ ]

7.What remedies have you sought?

A. Traditional/herbal [ ] B. religious [ ] C. orthodox [ ]D. None of the above [ ]

8..Have you been on antiretroviral therapy before?

`A. yes [ ] B .No [ ]

9. Do you have injection from patent medicine dealers ?

A. yes [ ] B .No [ ]

10. When last did you have unprotected sex?

A. 0-3 months B. 4-6 months C. 7-12 months D. 13-18 months E. > 8months.

11.Do you have more than one sexual partner? A. Yes [ ] B .No [ ]

12. Have you been treated for STD? A. Yes [ ] B .No [ ]

SECTION D: MALARIA

13. Do you sleep under the net? A. Yes [ ] B .No [ ]

14. How do you know you have malaria?

A. Fever B. headache C. joint pains D. weakness

15. When last were you treated of malaria?

A. a month ago B. 3 months ago C. 6 months ago D.> 6 months ago

16. When last did you have malaria?

A. a month ago B. 3 months ago C. 6 months ago D.> 6 months ago

SECTION E Tuberculin Purified Protein Derivative (PPD)

17. Have you had BCG vaccine before? A. Yes [ ]B No [ ] if Yes when A. < a month ago B. a month ago C. 2 months ago D.>3 months ago E.in childhood.

18. Have you had chicken pox, small pox, or oral polio vaccine before? A. Yes [ ] B .No [ ]

if Yes when A.< a month ago B. a month ago C. 2 months ago D.>3 months ago E.in childhood.

19. Have you had varicella, BCG or oral typhoid (TY21a) vaccine before? A. Yes [ ] B .No [ ] if Yes when A. < a month ago B. a month ago C. 2 months ago D.>3 months ago E.in childhood.

SECTION F

20. Do you smoke cigarette? A. Yes [ ]B No [ ] if Yes when last did you smoke ? A. < a month ago B. a month ago C. 2 months ago D.>3 months ago E. .>6 months ago.

21. Are you a diabetic patient? A. Yes [ ]B No [ ].

22. Are suffering of any heart disease? A. Yes [ ]B No [ ]

23. Do you take alcohol? A. Yes [ ]B No [ ]

APPENDIX 2

INFORMED CONSENT FORM

Dear Participant,

I , IHIM AUGUSTINE C. a postgraduate student of Nnamdi Azikiwe University, is to evaluate, **Serum Antioxidants, Lipid Peroxidation, Lipid Profile And Apolipoproteins In *Mycobacterium tuberculosis* Infected Individuals And During Treatment In Anambra State.**Antioxidants and Lipid Peroxidation status of Mycobacterium tuberculosis infected subjects and their roles in predicting prognosis predisposing to disease states. *M. tuberculosis* infection lowers the body defense system (antioxidants) and exposes the patient to various ailments (opportunistic diseases). This study wants to find out ways of preserving this natural defense system in patience with this infection. It is expected that the outcome of the study will help in better understanding of how the infection and drug interaction affect the antioxidants and lipid status of the subjects. This will be used as biomarker for predicting prognosis of active and latent TB infection and improve the medical care of those affected by the disease.This work will involve administration of self structured questionnaire which will be given to consented patient .Patient's sputum and blood samples will be collected three times and processed at no cost to the patient.Participation is voluntary and you are free to withdraw at any time without any repercussion whatsoever. You are also assured of strict confidentiality and your health will not be adversely affected in any way by participating in this study.The result of the study will be communicated to the patient's physician for better management of the patient. Ethical issues arising from the study will be referred to ethics committee for appropriate action. Please do indicate your interest to participate.

A) Yes I want to participate ☐
B) No I do not want to participate ☐

……..	…..……	……
Signature/thumb print of participant	**Date**	**phone number**
……..		…..……
Signature of principal investigator		**Date**

For enquiries, complaints or any information regarding this study, please contact the principal investigator, Ihim Augustine Chinedu at Medical Laboratory Science Department, Nnamdi Azikiwe University, Nnewi Campus or can be reached on phone at 08035985883

I appreciate your co-operation and interest. Thank you.

NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL

P.M.B. 5025, NNEWI, ANAMBRA STATE, NIGERIA

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Secretary to the Board

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Our Ref: NAUTH/CS/66/VOL.7/79

Your Ref: _____

Date: 17th August, 2015

Ihim Augustine Chinedu
Department of Medical Laboratory Science
Faculty of Health Sciences and Technology,
Nnamdi Azikiwe University,
Nnewi Campus

ETHICS COMMITTEE APPROVAL

RE: ANTIOXIDANTS AND LIPID PEROXIDATION AS INDEX OF TUBERCULOSIS DIAGNOSIS AND MANAGEMENT IN ANAMBRA

We write to inform you that after due consideration of your research proposal, approval is hereby conveyed for you to commence the study.

The principal investigator is required to send a progress report to the Ethics Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out her oversight function.

Please note that this approval is subject to revocation if you fail to obtain proper authorization from your study site/unit.

.....
Prof. P.U Ele
Chairman, NAUTH Ethics Committee

.....
Udemezue N.O (Mrs)
Sec., NAUTH Ethics Committee

MOTTO: Caring With The Compassion of Jesus & Mary

IMMACULATE HEART HOSPITAL AND MATERNITY

P.O. Box 29, Nkpor, Anambra State
Nigeria, West Africa.
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April 14, 2016

Ihim Augustine Chinedu
Medical Laboratory Science Department,
Faculty of Health Sciences and Technology,
Nnamdi Azikiwe University,
Nnewi Campus.

APPROVAL TO CONDUCT STUDY

ANTIOXIDANTS AND LIPID PEROXIDATION AS INDEX OF TUBERCULOSIS DIAGNOSIS AND MANAGEMENT IN NNEWI, ANAMBRA STATE.

This is to inform you the committee assent to your demand to conduct part of your research work in this hospital.

You are expected to be guided by the rules of good practice and uphold the ethical standard while doing your study.

In case of any violation from recognized standard practice, an immediate withdrawal of this approval will be effected.

Goodluck.

MR. EZENWA UCHE H.
Chairman

REV.SR. OSUJI MARY FAUSTINA
Secretary

ST. CHARLES BORROMEO HOSPITAL

P. O. Box 379, Onitsha, Anambra State, Nigeria. Phone: 08138955442.

Our Ref. _____ Your Ref. _____ Date: 24/7/2017

Ihim Augustine Chinedu
Medical Laboratory Science Department
Faculty of Health Sciences and Technology
Nnamdi Azikiwe University,
Nnewi Campus

APPROVAL TO CONDUCT STUDY

ANTIOXIDANTS AND LIPID PEROXIDANT AS INDEX OF

TUBERCULOSIS DIAGNOSIS AND MANAGEMENT IN ANAMBRA STATE

This is to convey the committee, assent to your demand to conduct part of your research work in this hospital.

You are bound by rules of good practice, and we believe you will uphold the ethical standard while doing your work.

Be informed that in case of infraction and deviation from recognized standard practice an immediate withdrawal of this facility will be effected.

Good luck.

MADUEKWE FUR
Chairman

24/7/2017
REV. SR MARIA MODESTA OKAFOR
Secretary