ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF MEDICAL BIOCHEMISTRY

ESTIMATION OF TOTAL OXIDATIVE STRESS AND NON-ENZYMATIC ANTIOXIDANT LEVELS IN MALARIA PATIENTS IN LOGIA DUBTI AREA, AFAR, ETHIOPIA: A CASE CONTROL STUDY

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY

JUNE, 2018

ADDIS ABABA, ETHIOPIA

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ESTIMATION OF TOTAL OXIDATIVE STRESS AND NON-ENZYMATIC ANTIOXIDANT LEVELS IN MALARIA PATIENTS IN LOGIA DUBTI AREA, AFAR, ETHIOPIA: A CASE CONTROL STUDY.

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Medical Biochemistry

Addis Ababa University

School of Graduate Studies

This is to certify that the dissertation prepared by Adem Ebrahim, titled: **Estimation of Total Oxidative Stress and Non-Enzymatic Antioxidant levels in Malaria Patients in Logia - Dubti Area, Afar, Ethiopia: A Case-Control study**, 2017/18 and Submitted in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of medical Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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ACKNOWLEDGEMENTS

My greatest and foremost indebtedness goes to the Almighty Allah who gives me the strength throughout my life.

I'm mostly indebted to my supervisors Dr. Solomon Genet and Dr. Natesan Gnanasekaren willingly spending the time and energy for supervision, constructive discussion and correction of this study, patience and encouragement and a word of thanks alone are inadequate.

My special gratitude also goes to Mohammed Mehdi for his support during my laboratory work. I thank Dr. Tamrat for his permission and guidance to use ELISA micro plate reader.

I would like to thank Addis Ababa University College of Health Sciences School of Medicine, Department of Medical Biochemistry for giving me the chance to enroll. I would like also to thank Samara University for sponsoring the study and for the financial support.

It is my pleasure to express my heartfelt thanks to nurses and laboratory technician who helped me during data collection at Logia Health Center, Dubti Hospital and measuring parameters at Zewditu Memorial Hospital: without their support it would have been difficult to find all these participants of the study.

Finally, I owe much to my family and my friends for their patience, support, and for the help I have received from them, which can hardly be expressed in words.

Abbreviations / Acronyms

ABTS:	2,2”- Azino-bis (3-ethylbenzothiazoline-6-Sulphonic acid) diammonium
BCG	Bromocresol Green
BMI	Body Mass Inex
CAT	Catalase
cGMP	cyclic Guanosine MonoPhosphate
DHBS	Dichloro-2-Hydroxy Benzene Sulphonate
DNA	Deoxyribonucleic Acid
DPD	3,5-dichlorophenyl-diazonium tetrafluoro-borate
ELISA	Enzyme-Linked Immunosorbent Assay
FPIX	Ferriprotoporphyrine IX
GPx	Glutathione Peroxidase
GR	Glutathione Redactase
GSH	Reduced glutathione
IL-1	Interlukin-1
IL-6	Interlukin-6
MDA	Manoldialdehyde
NADH	Nicotinamide Adenine Dinucotide Reductase
NADPH	Nicotine Adenine Dinucleotide Phosphate oxidase
NO	Nitric oxide
OS	Oxidative Stress
OSI	Oxidative Stress Index
POD	Peroxidase

RBC	Red blood cells
RNI	Reactive Nitrogen Intermediate
RNS	Reactive Nitrogen species
ROI	Reactive Oxygen Intermediate
ROS	Reactive Oxygen Species
SD	Standard Deviation
SOD	Superoxide dismutase
SSA	Sub-Saharan Africa
TAC	Total Anti-Oxidant Capacity
TNF	Tumor Necrosis Factor
TOS	Total Oxidant Stress
Trx R	thioredoxin Reductase
VCAM-1	Vascular Cell Adhesion Molecule-1

ABSTRACT

Background: Malaria is still a leading cause of morbidity and mortality. Out of the five *Plasmodium* species that cause malaria infection, *P. falciparum* is the deadliest. Total Oxidative Stress might be increased in malaria patients. This may originate from several sources including intracellular parasitized erythrocytes and extra-erythrocytes as a result of haemolysis and host immune response. This might lead to oxidative stress induced oxidation of hemoglobin to methemoglobin (will not have a normal function like the normal hemoglobin) and will cause further complication in the malaria patients.

Aim of the study: To estimate the total oxidative stress and non-enzymatic antioxidant levels in malaria patients at Dubti Referral Hospital, Afar, Ethiopia, from October 2017- June 2018.

Methodology: A case control study was undertaken with 60 malaria patients and 40 healthy controls. The severity of malaria was determined by density of parasitemia. Out of total 60 malaria patients, 32 were low, 16 moderate and remaining 12 were high parasitemia malaria patients. Levels of Total Oxidant Stress, Total Anti-Oxidant Capacity, Oxidative Stress Index, Uric Acid, Albumin, Total Bilirubin and Direct Bilirubin were measured in patients and healthy controls**.**

Result: The ratio of total oxidative stress and total antioxidative capacity, uric acid, total bilirubin and direct bilirubin were significantly increased in serum of malaria patients (30.47, 4.67mg/l, 2.65mg/l, and 3.37mg/l respectively) compared to healthy control groups (8.10, 4.1mg/l, 1.5mg/l, and 2.6mg/l respectively) ($p<0.05$). However, the serum level of albumin was significantly decreased in malaria patients compared to healthy control groups.

Conclusion: Oxidative stress induced complications are important causes of severity as well as morbidity and mortality of malaria infection. Avoiding conditions that maximize oxidative status like alcohol, cigarette smoking etc and taking diets with antioxidant power like fruits and vegetables will help avert oxidative stress induced severity.

Keywords: Malaria parasite, Total Oxidant Stress, Total Antioxidant capacity, Oxidative stress index, Uric Acid, Albumin, Total Bilirubin and Direct Bilirubin.

1. INTRODUCTION

1.1 Background

Malaria is one of the life-threatening infections caused by protozoan parasite and transmitted by female anopheles mosquitoes. It is still a major public health concern of most endemic areas of the world. Five species (*P. falciparum, P. vivax, P. ovale, P. knowlesi, and P. malariae*) cause malarial infection. The major complications are caused by *P. falciparum* and *P. vivax*, with *P. falciparum* being the more virulent (Geleta and Ketema, 2016).

In 2016, an estimated about 216 million cases of malaria and 445,000 malaria related deaths (range 235,000–639,000) occurred worldwide. Most of the disease occurred in Africa (92%) followed by South-East Asia and the Eastern Mediterranean Region(Who, 2017; Gebretsadik *et al.*, 2018).

According to World Health Organization (WHO) report, though malaria is endemic in most tropical and subtropical regions, over 90% of malaria deaths currently occur in sub-Saharan Africa (SSA). The disease is responsible for approximately 15% of all deaths among children younger than five years of age in SSA (Liu *et al.*, 2012; Gebretsadik *et al.*, 2018).

About 75% of the country's landmass is malarious putting 68% of the total population at risk of malaria in Ethiopia (Ghebreyesus *et al.*, 2006; Gebretsadik *et al.*, 2018) . The asexual development of malaria parasite in human being causes disease and modifies the host cell. In the trophozoite-stage, parasite proteins are exported to the surface of the RBC where they play a role in immune recognition and adherence to host endothelium as well as other interactions with host cells. These parasite-host interactions are associated with the pathology of malaria and contribute to the development of severe malaria (Storm and Craig, 2014). It is one of the most widespread human parasitic diseases ranking first in terms of its socioeconomic and public health importance in tropical and subtropical region of the world, especially in sub-Sahara African and Southeast Asian countries (Worku *et al.*, 2014).

Oxidative stress is a general term used to describe a serious imbalance in organisms between the production of reactive oxygen species (ROS) or reactive Nitrogen species (RNS) and the antioxidant capacity of organism (Figure 1). Under these conditions, ROS may damage membrane lipids and DNA, and affect the function of cellular proteins(Buico *et al.*, 2009).

The existence of oxidative stress during malaria infection may be increased through during depletion of antioxidants, increased plasma lipid peroxidation and altered fluidity of erythrocyte membrane. The oxidative stress results from host immune reaction, as an acute phase response, and the intra-erythrocytic parasite's metabolic processes (Ayodele and Oyedele, 2014).

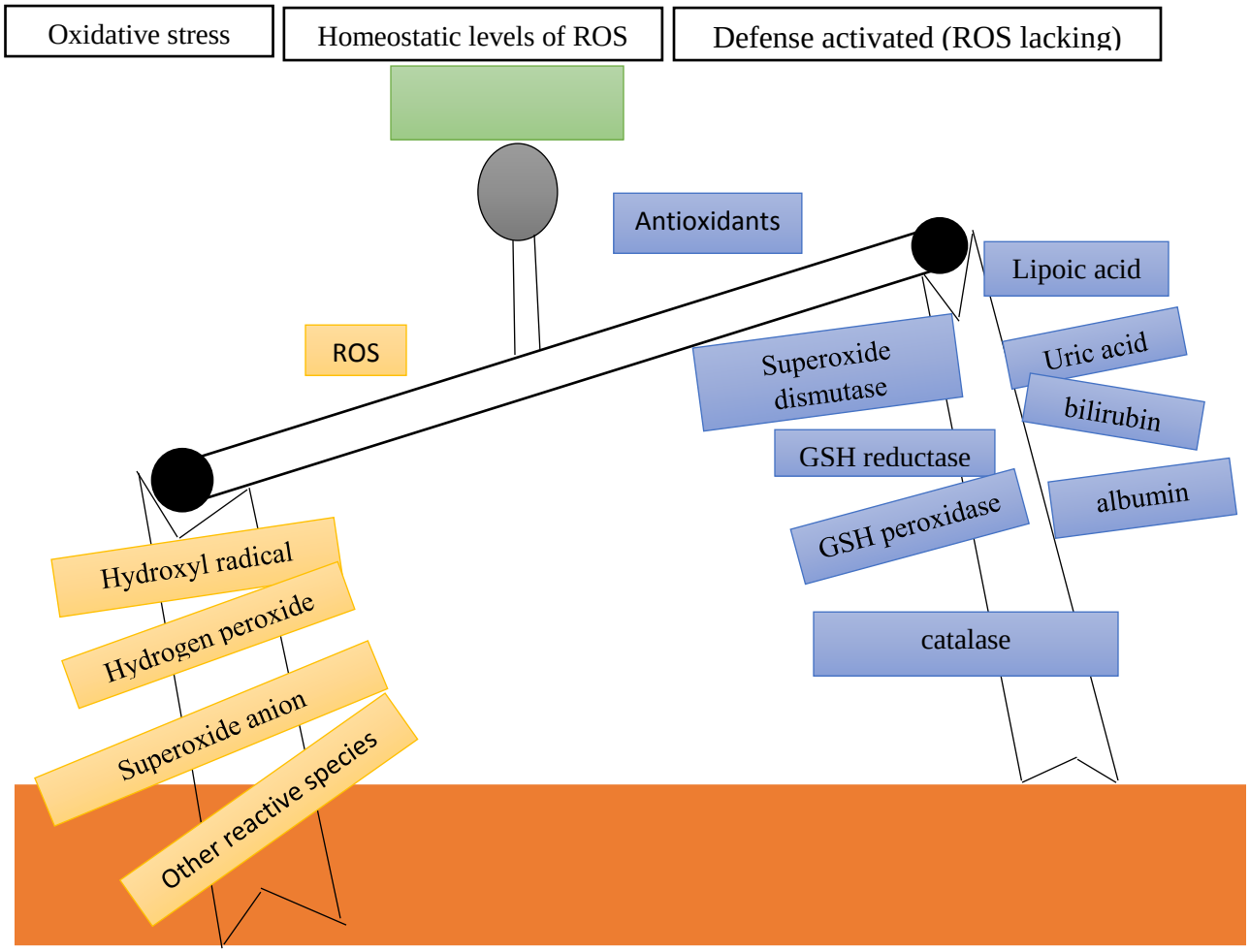

Figure 1. Imbalance between oxidant and antioxidant.

Theses free radicals can be scavenged by the two major groups of antioxidants in living cells: enzymatic antioxidants and non-enzymatic antioxidants (Palmieri and Sblendorio, 2007).

The enzymatic antioxidants are divided into primary and secondary enzymatic defences. The primary defence is composed of three important enzymes that prevent the formation and neutralize free radicals: Glutathione peroxidase, which donates two electrons to reduce peroxides by forming selenols and also eliminates peroxides as potential substrates for the

Fenton reaction; Catalase, which turns hydrogen peroxide into water and molecular oxygen one of the most important and efficient antioxidants in the body and lastly, Superoxide dismutase, converts superoxide anions into hydrogen peroxide as a substrate for subsequent catalase action. The secondary enzymatic defence includes glutathione reductase and glucose-6-phosphate dehydrogenase. Glutathione reductase reduces glutathione (antioxidant) from its oxidized to its reduced form, and by this recycling, continue neutralizing more free radicals. Glucose-6-phosphate dehydrogenase regenerates NADPH, which creates a reducing environment (Palmieri and Sblendorio, 2007; Lü *et al.*, 2010; Shebis *et al.*, 2013).

The non-enzymatic antioxidant defence system (tocopherol, β-carotene, ubiquinol, vitamin C, glutathione, lipoic acid, uric acid, metallothionein, and bilirubin) which quench the damage of oxidative stress (Palmieri and Sblendorio, 2007).

1.2. Literature Review

1.2.1. Malaria

There are a number of researches done about Malaria caused by protozoan parasites belonging to the genus *plasmodium* and transmitted by the infected anopheles' mosquitoes which infects and destroys red blood cells. *P. falciparum* is the most dangerous form of malaria and life threatening in an unprotected, non-immune population causing high morbidity and mortality (Joseph *et al.*, 2011). The life cycle of the *plasmodium* species is completed in two hosts. The primary host being the anopheles mosquito and the secondary host human beings (Prasannachandra *et al.*, 2006).

The major complication of malaria includes severe anemia, cerebral malaria, hypoxia, and placental infection during pregnancy. Whereas severe headache, fever, vomiting, anemia, and loss of appetite are the clinical features of uncomplicated malaria; among these, severe anemia and cerebral malaria constitute the major cause of death (Worku et al., 2014).

In Ethiopia (2015), in a study done in Dilla town and surrounding rural area, Gedeo zone, southern Ethiopia the disease is one of the leading health problem and top ranking in the list of common communicable diseases and one of the leading causes of morbidity and mortality approximately 75% of Ethiopia's landmass is endemic for malaria, with malaria primarily associated with altitude and rainfall. The Carter Centre reported, that about 55.7 million people in Ethiopia faced the risk of malaria. Moreover, malaria transmission peaks bi-annually from September to December and April to May, coinciding with the major harvesting seasons. This seasonality has serious consequences for the subsistence economy of Ethiopia's countryside (Molla and Ayele, 2015).

1.2.2. Risk factor of malaria

The identification of factors influencing malaria risk in households can guide targeted interventions. Important risk factors such as housing type, house proximity to mosquito breeding sites or water drain, toilet facilities, and malaria preventive measures at the household level have been identified in different studies (Ayele *et al.*, 2012). Additional factors such as number of bed nets per household, individual's age and residence altitude, and household wealth, peak monthly rainfall can affect malaria prevalence (Molla and Ayele, 2015).

1.2.3. The Life Cycle of The Malaria Parasite

During blood meal by the infested mosquito, hundreds of sporozoites are introduced into the intradermis. Some of these sporozoites are destroyed by the local macrophages, while others find a blood vessel (Matteelli and Castelli, 2015). Malaria parasites have both asexual (which takes place in the mammalian host), and sexual (which takes place in the anopheles mosquitoes) phases in life cycle (Miller *et al.*, 2002). The parasite goes through various phases during its developmental cycles such as the sporozoites, merozoites, trophozoites, (asexual schizogony stage) and gametocytes (sexual sporogony stage) and all of these stages have been found to have their unique shapes and structures as well as protein complements (Laurence *et al.*, 2002).

When the infected anopheline mosquito takes a blood meal, sporozoites are inoculated into the bloodstream as shown in (Figure 2). Within 2-30 minutes sporozoites enter hepatocytes and begin to divide into exo-erythrocytic merozoites (tissue schizogony). For *P. vivax* and *P. ovale*, dormant forms called hypnozoites may typically remain quiescent in the liver until a later time; *P. falciparum* does not produce hypnozoites (Dickson *et al.*, 2016). Once merozoites leave the liver, they invade erythrocytes and develop into early trophozoites, which are ring shaped, vacuolated and uninucleated. Once the parasite begins to divide, the trophozoites are called schizonts, consisting of many daughter merozoites (blood schizogony). Eventually, the infected erythrocytes are ruptured, releasing merozoites, which subsequently invade other erythrocytes, starting a new cycle of schizogony. The duration of each cycle in *P. falciparum* is about 48 hours(Zhang *et al.*, 2010). At maturity, the merozoites are released into the blood stream and invade red cells by multiple receptor–ligand interactions (Silvie *et al.*, 2008).

The sexual phase results, the erythrocytic cycle generates male and female gametocytes which are picked up by mosquitoes during a blood meal, leading to fertilization and zygote formation within the mosquito stomach (Zhang *et al.*, 2010). Motile zygote (ookinete) penetrates the midgut epithelium to form oocysts within which hundreds of sporozoites are generated. Oocysts rupture to release sporozoites which invade the salivary glands from where they are injected into the mammalian bloods during blood feeding (Ferguson and Read, 2004; Hill, 2006; Chikezie, 2015).

The asexual erythrocyte cycle begins when a single merozoite invades a host erythrocyte and enclosed within a parasitophorous vacuole, which is separate and distinct from the host

erythrocyte cytoplasm. The merozoites eventually released into the blood stream invade more erythrocytes. There are three observable morphologically distinct phases in parasitized erythrocytes (Cholera *et al.*, 2007). Firstly, the ring stage, which last for approximately 24 hours in *P. falciparum* infection, accounts for half of the metabolically non-descript intra-erythrocytic stage. It is followed by the trophozoite stage; a very active period during which most of the erythrocytes cytoplasm is consumed. Finally, the parasite undergoes 4-5 rounds of binary divisions during the schizont stage, producing 8-36 merozoites that burst from the host cell to invade new erythrocytes, and thereby begins another round of infection. This phase of the infection (erythrocytic schizogony) is responsible for malaria pathogenesis (Chikezie, 2015). In erythrocyte, the parasite ingests the haemoglobin in the red cell into a food vacuole and degrades it. It utilizes the amino acids in the haemoglobin for protein biosynthesis and the heme is detoxified by heme polymerase and sequestrated as hemozoin (malaria pigment). The malaria parasite also depends on anaerobic glycolysis for energy, utilizing enzymes such as pLDH, *plasmodium* aldolase (Lew *et al.*, 2003).

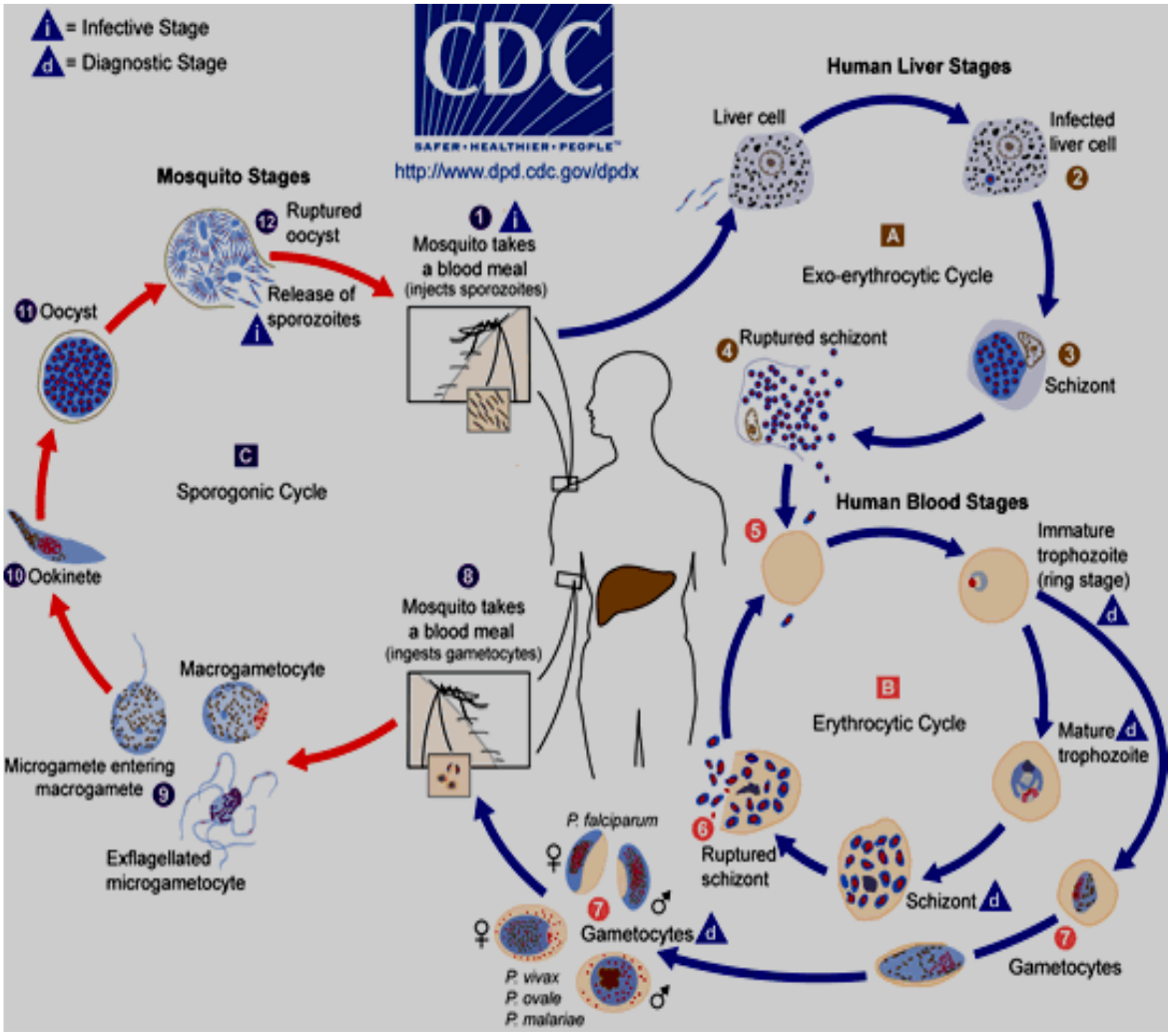

Figure 2: Life Cycle of *Plasmodium falciparum* (Adapted from http//www.dpd.cdc.gov/dpdx)

1.2.4. Reactive oxygen species generation and oxidative stress

A free radical is, by definition, a chemical species containing unpaired electrons and is therefore paramagnetic. Most of the oxygen derived free radicals relevant to cell biology are unstable, short-lived and highly reactive (Palmieri and Sblendorio, 2007). Oxidative stress refers to the imbalance due to excess ROS or oxidants over the capability of the cell to mount an effective antioxidant response (Figure 3). Reactive oxygen species (ROS), such as superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$), consist of radical and non-radical oxygen species formed by the partial reduction of oxygen (Ray *et al.*, 2012).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS, e.g. nitric oxide, $NO^{\bullet)}$ are well recognized for playing a dual role as both deleterious and beneficial species. ROS and RNS are normally generated by tightly regulated enzymes, such as NO synthase (NOS) and NAD(-P-)H oxidase isoforms, respectively. Overproduction of ROS (arising either from mitochondrial electron-transport chain or excessive stimulation of NAD(-P-)H) oxidation results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA (Sen and Chakraborty, 2011).

In contrast, beneficial effects of ROS/RNS (e.g. superoxide radical and nitric oxide) occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as for example in defense against infectious agents, in the function of a number of cellular signalling pathways, and the induction of a mitogenic response (Valko *et al.*, 2007). A study conducted in Poland (2016) reported that low levels of ROS production are required to maintain physiological functions, including proliferation, host defense, signal transduction, and gene expression (Nita and Grzybowski, 2016).

Oxidative stress is the result of an imbalance in pro-oxidant/antioxidant homeostasis that leads to the generation of toxic reactive oxygen species (ROS), such as hydrogen peroxide, organic hydro peroxides, nitric oxide, superoxide and hydroxyl radicals etc. The concept of oxidative stress confined to ROI such as hydroxyl and superoxide radicals, and hydrogen peroxide and singlet oxygen has been extended onto RNI such as nitric oxide (NO), peroxynitrite and, recently, to S-nitrosothiols. Thus, ROI and RNI react with proteins, carbohydrates and lipids, with consequent alteration both in the intracellular and intercellular homeostasis, leading to possible cell death and regeneration (Rahman *et al.*, 2012). The oxidative stress usually results from excessive ROS production, mitochondrial dysfunction, impaired antioxidant system, or a combination of these factors. The pro oxidative/antioxidative cellular imbalance between the ROS production and ability of the biological systems' defense mechanisms to eliminate the cellular stress disturbances leads to the vicious circle, since the oxidative stress reciprocally aggravates ROS production (Nita and Grzybowski, 2016).

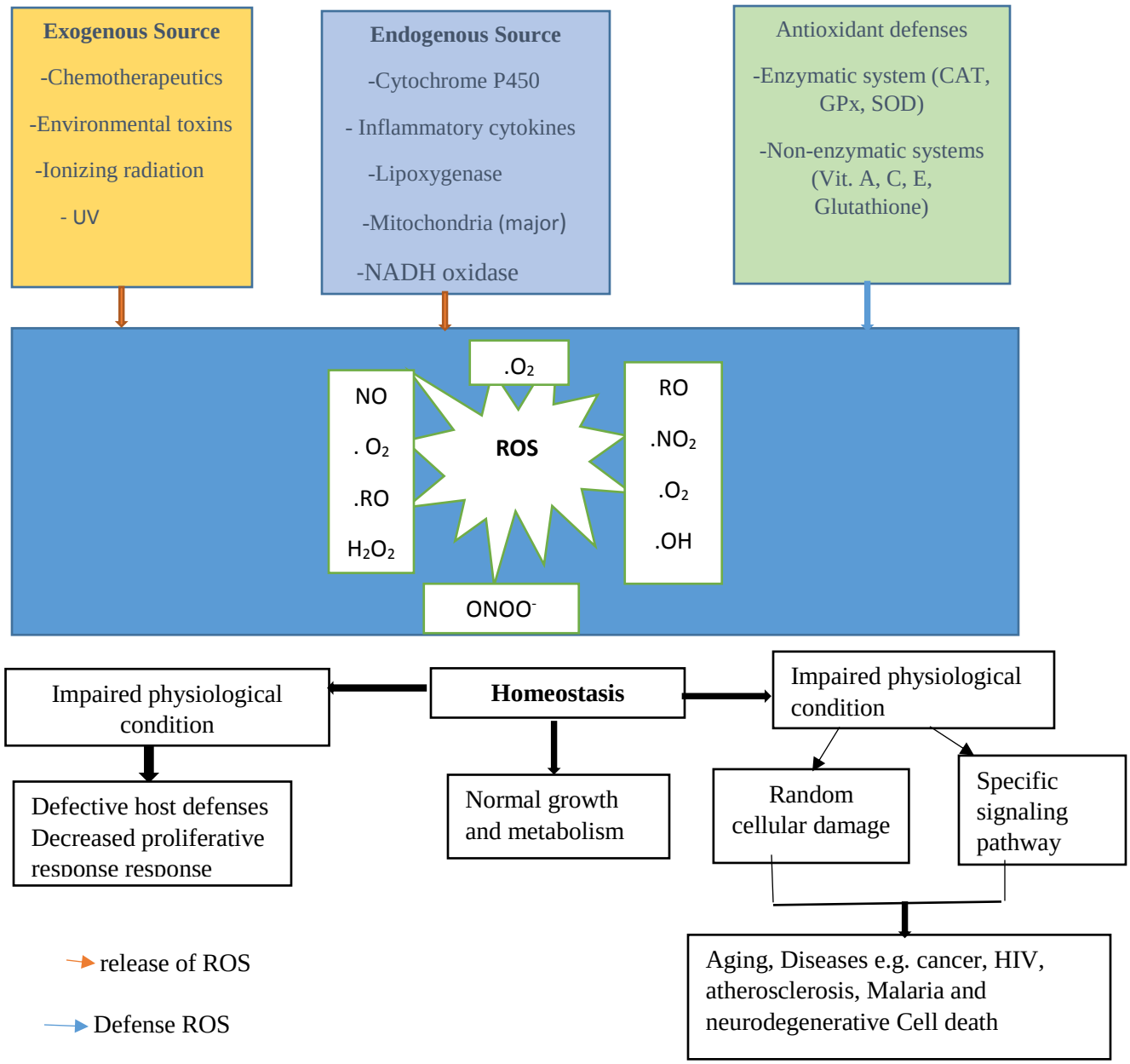

Figure 3: The source and cellular responses to reactive oxygen species ROS.

1.2.5. The role of ROS and oxidative stress in the malaria diseases

Malaria caused by *plasmodium* species completes life cycle in host RBC and liver (Malaguamera and Musumeci, 2002) . Oxidative stress is commonly observed to arise from five sources during disease physio-pathogeny(Percário *et al.*, 2012):

1. Inflammatory process initiated in the host in response to infection
2. transition metal catalysis, since in feeding on hemoglobin, the parasite releases significant amounts of free iron
3. the occurrence of ischemia-reperfusion syndrome, resulting from cytoadherence processes and anemia triggered by infection
4. direct reactive species production by the parasite
5. action of antimalarial drugs.

Infection in humans begins when infected mosquito takes a blood meal which results in the release of sporozoites from the salivary glands of the mosquito into the bloodstream. Sporozoite then enters the liver for replication resulting in the release of thousands of merozoite into the blood stream. In the blood, each merozoite invades and engulfs a portion of RBC and appears as thin ring, so called as the "Ring" stage. The ring stage parasite grows to form Trophozoite which produce H_2O_2 and OH^- radicals twice as much as the normal erythrocytes (Shio *et al.*, 2010). Rupture of RBCs release various pro-oxidants such as Hemoglobin (Hb), Methemoglobin (MetHb), Hemin (He) and Haemozoin (Hz) into the circulation (Pamplona *et al.*, 2009; Shio *et al.*, 2010). Then, increased production levels of ROS (reactive oxygen species) are produced by activated neutrophils in the host and during degradation of hemoglobin in the parasite (Idoniji *et al.*, 2011; Tyagi *et al.*, 2017).

Malondialdehyde (MDA) is a known reactive aldehyde formed by the degradation of polyunsaturated lipids by ROS and a widely used biomarker for the oxidative stress response (Ashok *et al.*, 2016). Oxidative stress plays an important role in the development of malarial anemia (Palmieri and Sblendorio, 2007). Oxidative stress is common among malaria patients as a result of the activation of the immune responses by malaria parasite; thereby, causing release of reactive oxygen species (ROS). The increase in lipid peroxidation level in malaria patients

and decrease in ascorbic acid and GSH has been observed to be accountable for the development of oxidative stress in malaria patients (Akanbi *et al.*, 2010).

Oxidative stress is related to the severity of malaria, oxidative stress in malaria may originate from several sources including intracellular parasitized erythrocytes and extra-erythrocytes as a result of haemolysis and host response. Malondialdehyde is an organic compound being generated from reactive oxygen species (ROS) and as such is assayed in vivo as a bio-marker of oxidative stress. Malaria infection has been found to be associated with lipid peroxidation (Malondialdehyde) (MDA) accompanying reduction in antioxidant capacity of the infected patients especially P*lasmodium falciparum* infection. Various studies have established that malaria infection is accompanied by increased production of reactive oxygen species which indicates the environment for oxidative stress. Malaria parasite is sensitive to oxidative stress and the level of oxidative stress is influenced by the severity of malaria infections (Ayodele and Oyedele, 2014).

According to a study done in Brazil (2013), oxidative stress (OS) in malaria can be caused by two main mechanisms. Central to the generation of OS is the degradation of host haemoglobin by the parasite. Secondly, the OS mechanisms involve the host immune response, which initiates a cascade of defense mechanisms culminating with the release of free radicals by activated macrophages, to tackle the parasite (Fabbri *et al.*, 2013). Haemoglobin molecules that are taken up by the parasites' acid food vacuoles lead to the spontaneous oxidation of haem iron from Fe^{2+} to Fe^{3+} (haemin) and the formation of superoxide radicals ($O_2^{\bullet-}$). The combination of $O_2^{\bullet-}$ and haemin inevitably leads to the generation of hydrogen peroxide (H_2O_2) and subsequently, hydroxyl radicals ($^{\bullet-}OH$), which are highly reactive and cytotoxic oxygen intermediates (Figure 4) (Chikezie, 2015).

However, some free ferriprotoporphyrin IX; containing Fe^{3+}) (FPIX) (up to 50% from the food vacuole pass into the parasite compartment. The $O_2^{\bullet-}$ resulting from the oxidation of haem-iron of haemoglobin are either detoxified by superoxide dismutase (SOD) to yield H_2O_2 or in a spontaneous reaction with H_2O_2, lead to the formation of $^{\bullet-}OH$ (Figure 4). In addition, ferric iron (Fe^{3+}) react with molecular oxygen via the Fenton reaction pathways to generate $^{\bullet-}OH$(Figure 4)(Jortzik and Becker, 2012; Chikezie, 2015).

$$O^{\bullet -2} + Fe^{3+} \longrightarrow O^{2} + Fe^{2+}$$

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

Equation 1: The Fenton reaction

These radicals are highly reactive and cause lipid peroxidation and DNA oxidative damage (Jortzik and Becker, 2012).

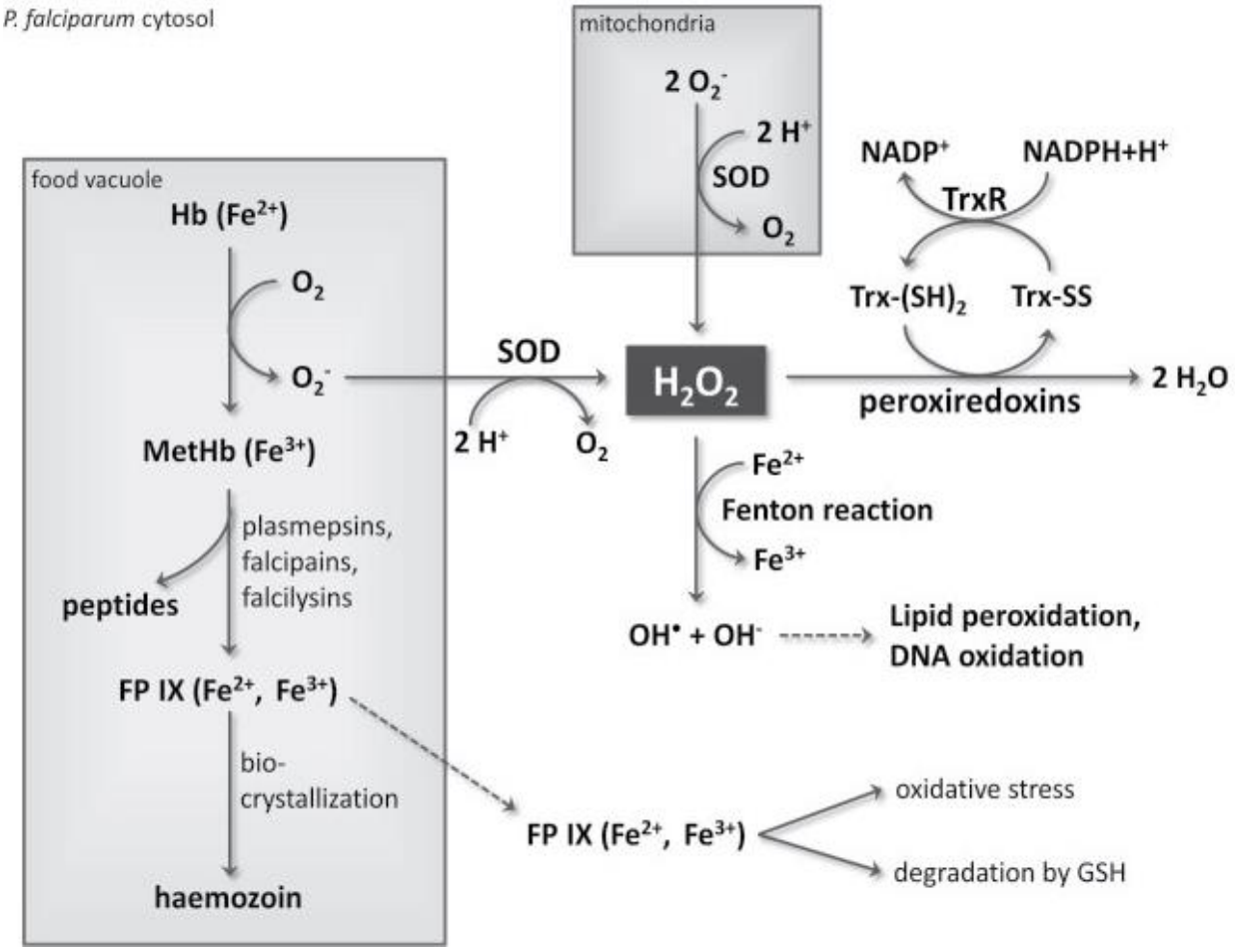

Figure 4: Sources of oxidative stress in *Plasmodium falciparum* (adopted from Chikezie., 2015)

In a study conducted in Australia (2014), it was shown that, in humans, oxidative changes resulting from malaria infection are central to the host protective response against the malaria parasite, and to some of the pathophysiology associated with clinical malaria infection. Hence enhanced oxidative stress reduces erythrocyte deformability, contributing to haemolysis, and the development of anaemia (Zhang *et al.*, 2014). The role of oxidative stress during malaria infection is still unclear. Some authors suggest a protective role, whereas others claim a relation

to the pathophysiology of the disease. However, recent studies suggest that the generation of reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress, plays a crucial role in the development of systemic complications caused by malaria. Malaria infection induces the generation of hydroxyl radicals (OH•) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis (Percário *et al.*, 2012) .

1.2.6. Role of Antioxidants in malaria patients

Antioxidants are the components produced by the body to neutralize the effect of free radicals, but the effect will be limited to specific antioxidants. In human body oxidants and antioxidate ratio will be maintained, any alteration in these oxidants and antioxidats will causes accumulation of ROS within the body, this process is called as oxidative stress (Kattappagari *et al.*, 2015). Excessive formation of free radicals and concomitant damage at the cellular and tissue level are controlled by antioxidant defenese systems (Dokuzeylul *et al.*, 2015). The antioxidants are also associated with reduction of free radical generation and improves antioxidant status in patients, thus it may be beneficial to recover normal function and treatment of such diseases (Sen and Chakraborty, 2011). A study conducted in Nigeria (2014) indicated that, malaria infection is associated with lipid peroxidation (Malondialdehyde) (MDA) accompanying reduction in antioxidant capacity of the infected patients especially *Plasmodium falciparum* infection (Ayodele and Oyedele, 2014).

1.2.7. Non-Enzymatic Antioxidants

1.2.7.1. Uric Acid

Uric acid, the final product of purine metabolism in humans, is an abundant aqueous, potent antioxidant and scavenger of reactive oxygen species (Richard *et al.*, 2013); contributes as much as two-thirds of all free radical scavenging capacity in human serum/plasma (Gallego-Delgado *et al.*, 2014). It is particularly effective in quenching or scavenging hydroxyl radicals hydrogen peroxide, superoxide and peroxynitrite radicals (Sergio *et al.*, 2010) and may serve a protective physiological role by suppression of Fenton reaction (iron catalysed oxidation) and prevention of lipid peroxidation (Sergio *et al.*, 2010; Nilgün, 2012); to prevent damage to cellular components arising from the activity of chemical reactions (Olisekodiaka *et al.*, 2017).

1.2.7.2. Bilirubin

Anti-oxidants are reactive agents which detoxify both intracellular and extracellular ROS and RNS, providing protection against them. Glutathione, albumin, uric acid and bilirubin are common anti-oxidants, important in maintaining the balance between pro-oxidants and antioxidants in healthy states (Narmada *et al.*, 2016). Heme oxygenase (rate limiting enzyme in heme degeradative pathway) inducible by high heme levels, enhanced production of ROS, elevated oxidized lipids and by several agents that causes oxidative damage in stressed condition (Nitin *et al.*, 2013). Biliverdin is converted to bilirubin, which function as a potent antioxidant. CO mediates vasodilation by inducing cyclic guanosine monophosphate (cGMP) and ferritin, over-induced due to accumulation of iron, sequesters the pro-oxidant free iron (Sumanta *et al.*, 2014).

In oxidative stress, free heme is increased with the breakdown of hemoproteins such as hemoglobin, myoglobin, or cytochrome P450 and induces the production of reactive oxygen species and low-density lipoprotein oxidation, which injures endothelial cells (Thomas and Daiber, 2013). Bilirubin, an endogenous product of heme degradation, stronger antioxidant potential than α-tocopherol, attenuate H_2O_2-induced endothelial leukocyte rolling, inhibit NADPH oxidase activity *in vitro* and possesses multiple biological activities (Douwe *et al.*, 2011; Valášková and Muchová, 2016).

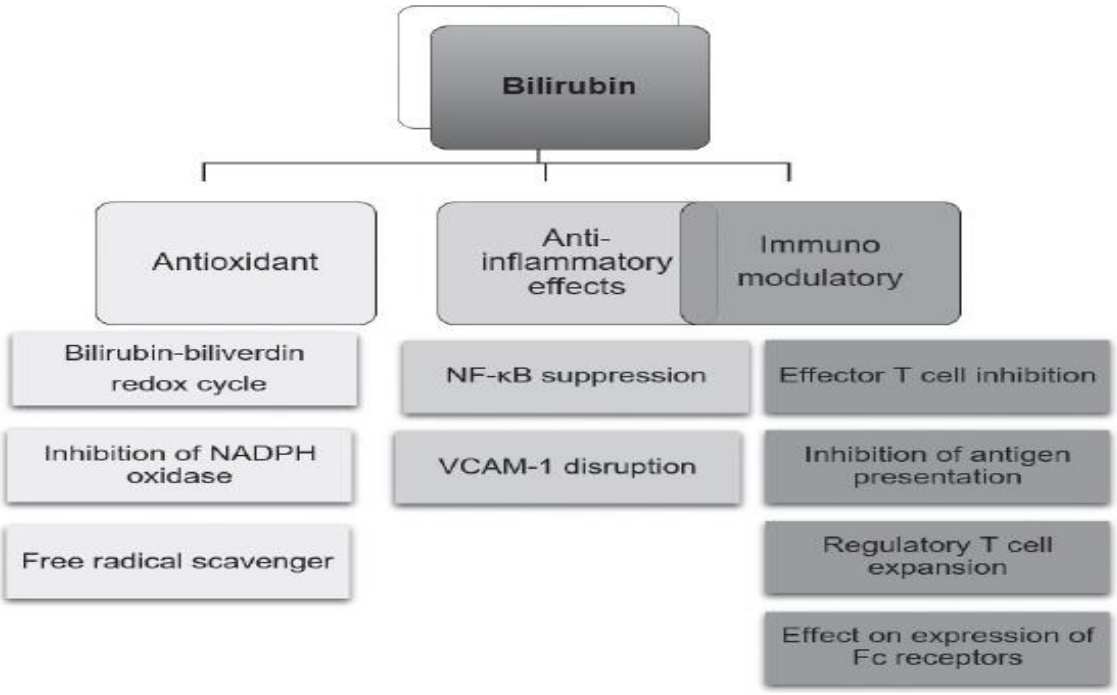

Figure 5: The overview of protective effects of bilirubin (adopted from Valášková and Muchová, 2016).

1.2.7.3. Albumin

Albumin is the most abundant plasma protein synthesized exclusively in the liver. Albumin constituents over half of total plasma protein, a concentration of 35-50g/l, in healthy individual (Prakash, 2017). Albumin is the most important circulating visceral protein in the body; functions in many physiologic processes including: vasodilation, endothelial cell apoptosis, antioxidant reactions and has a role as a transport protein for a number of organic and inorganic ligands such as lipid soluble hormones, bile salts, unconjugated bilirubin, free fatty acids (apoprotein), calcium, ions (transferrin), and some drugs like warfarin, phenobutazone, clofibrate & phenytoin. For this reason, it's sometimes referred as a molecular "taxi" (Ballmer, 2001; Burl and George, 2010).

Albumin is known for its regulation of colloid osmotic pressure, buffer acid-base changes, transportation of a wide range of endogenous ligands and drugs, and potent antioxidant and free radical scavenging activities (Mustafa *et al.*, 2013). Its concentration has been used by physicians and dieticians to help detect and monitor protein nutritional status (Burl and George, 2010).

1.3. Statement of the problem

Malaria is a major health problem in the world. In 2016, an estimated 216 million cases of malaria occurred worldwide. According to WHO, most malaria cases in 2016 were in African region (90%), South-East Asia Region (7%) and Mediterranean Region (2%). 85- 90% of malaria fatalities is occurred in sub-Saharan Africa (Who, 2017; Lake *et al.*, 2016).

In Ethiopia, Malaria remain the leading cause of morbidity and mortality. *Plasmodium falciparum* and *Plasmodium vivax* are the two species commonly known in which accounting for 60% and 40% proportion, respectively (Gebretsadik *et al.*, 2018). The distribution and transmission of malaria in Ethiopia varies from place to place and season to season. Altitude affects the pattern of malaria distribution in Ethiopia through its effect on temperature. Risk of malaria is highest in the western lowland of Oromia, Amhara, Tigray, entire regions of Gambella and Benishangul Gumuze region and eastern low lands of Ethiopia, primarily Afar and Somali(Alelign and Dejene, 2016) .

The Ethiopian Federal Ministry of Health put malaria diseases as serious health problem in the country where it has been the major cause of illness and death for many years. According to Ethiopian Federal Ministry of Health report, up to 75% of the country is malarious with about 68% of the total population living in areas at risk of malaria. That is, more than 50 million people are at risk from malaria, and four to five million people are affected by malaria annually(Gebretsadik *et al.*, 2018). These cases depend on the environmental, seasonal, climatic and others different socioeconomic factors (Bedane *et al.*, 2016).

Earlier studies have demonstrated that cells infected with parasites exhibit an increased generation of reactive oxygen species (ROS) with decreased antioxidant production, leading to the activation of redox-dependent transcription factors and the production of various cytokine (Ashok *et al.*, 2016).

As far as the knowledge of investigator, no previous study exists in Ethiopia about the status of oxidative stress among malaria patients. Therefore, this study designed to estimate of Total Oxidative Stress and Non-enzymatic Antioxidant levels among newly diagnosed malaria patients in Logia and Dubti area in Afar region. So, the present study on estimation of total oxidative stress could be helpful for health professionals to predict a treatment plan for malaria patients.

1.4. Significance of the study

Malaria is one of the major diseases of poor people in developing countries, particularly in sub-Saharan Africa. Oxidative stress is believed to be a key factor in the pathogenesis of malaria however, its role in the pathogenesis of the disease still remain to be elucidated. As a result, it is necessary to identify early markers for diagnosis, prognosis and a potential therapeutic target for malaria diseases. Therefore, this study is intended to identify early metabolic markers for diagnosis, prognosis and therapeutic target for malaria patients through evaluation of total oxidative stress, total antioxidant capacity, uric acid, bilirubin and albumin as compared to control groups. The estimation of total oxidative stress could provide early indication of disease progression and potential target for treatment. Besides, it can also serve as baseline information to undertake further studies on similar settings in the future.

1.5. Hypothesis

- There will be an imbalance between free radical production and antioxidant capacity in malaria patients that leads to severity of disease and complications.

2. OBJECTIVE

2.1. General objective

- To assess serum levels of Total Oxidative Stress among Malaria Patients and to identify correlation of TOS, TAC and OSI with severity of malaria in Logia Dubti area, Afar, Ethiopia.

2.2. Specific objectives

- To measure serum levels of Total Oxidative Status in malaria patients and compare with apparently healthy control groups.
- To evaluate total antioxidant capacity and non-enzymatic antioxidants in malaria patients and compare with apparently healthy control groups.
- To compare oxidative stress and antioxidant levels in low, moderate and highly infected malaria patients.
- To investigate the correlation between total oxidant and antioxidant capacity and oxidative stress index in malaria patients.
- To examine the correlation between total Oxidant - antioxidant capacity and oxidative stress index with severity of malaria patients.

3. MATERIALS AND M ETHODS

3.1. Study area

The study was conducted in Logia Health Center and Dubti Referral Hospital, Afar, Ethiopia. Dubti hospital Logia Health center are found in Zone one, which are located 620 km to East of Addis Ababa, the capital city of Ethiopia and 12km away from Samara, the administrative city of Afar regional state. Both health institutions have high flow rate of malaria patients.

3.2. Study design and period

A case control study design was applied to estimate total oxidative stress and non-enzymatic antioxidant levels in malaria patients between the period October 2017 to June 2018.

3.3. Source population

Peoples attending at Dubti Hospital

3.4. Study population

The study population was all malaria patients and healthy control who visit the Referral Hospitals and Health Center of Dubti, Afar, Ethiopia during the study period.

3.5. Eligibility criteria

3.5.1. Inclusion criteria

All malaria patients who were volunteer and willingness to participate in the study were included.

3.5.2. Exclusion Criteria

Malaria patients who were pregnant women, HIV patients, Diabetic, liver disease, smoking cigarettes, drinking alcohol, TB patients and other chronic diseases were excluded.

3.6. Sample size determination

The size of study participants that were recruited into the research was calculated using the G* power version 3.1 software by selecting t-test of means. Sample size was calculated by considering alpha =0.05, power (1-Beta) =0.8 (80%) with malaria case and apparently health controls based on unmatched ratio and effect sized (d) =0.5. The total sample size became 128.

The number of participants enrolled into the study was 100 (60 for malaria case and 40 for apparently health controls).

3.7. Sampling procedure and techniques

A convenience sampling technique was applied, all malaria cases and health controls individuals fulfilling the inclusion criteria and attending logia health center and Dubti Hospital during the study period were included until the required sample size was achieved.

3.8. Study variables

3.8.1. Dependent variables

- Total Oxidant Stress (TOS)
- Total antioxidant capacity (TAC)
- Oxidative Stress Index
- Uric Acid
- Total Bilirubin (TB)
- Direct Bilirubin (DB)
- Albumin

3.8.2. Independent variables

- Socio demographic characteristics
- Age
- Sex
- Marital status
- Severity of malaria
- BMI
- Diet

3.9. Data and Specimen collection handling and storage

The data was collected by experienced laboratory technologists and nurses working at Dubti Hospital and Logia Health Center. Interviewer administered structured questionnaire data collection tool was used. The data collection was mainly focused on the objective of the study. All necessary information was collected from patients using clinical data and patient's medical records(charts) using structured formats (see Annex) consisting of collection of data on socio-

demographic characteristics, medical history and laboratory investigations and recorded using data abstract format (see Annex-3).

Under preceding instructions malaria patients and healthy controls were checked regarding fasting by interview on the morning of the examination by Nurse. Malaria Patients were selected who confirmed to be infected with the *falciparum* malarial parasite by microscopic examination of Giemsa stained thin blood smears. Appropriate information on demography and health status was obtained from standardized clinical files designed for the program and from a predesigned. Five milli liters of fasting venous blood samples were obtained by vein puncture from vein of the arm of each participant using sterile techniques then transferred to serum separator container and allowed to stand for 30 min to coagulate and centrifuge at 3000 rpm for 10 minutes in order to separate the serum. Serum was separated by sterile pipette and transferred to 3ml Eppendorf test tubes and incubated at -80^{0}C until analyzed.

3.9.1. Malaria Parasite Density Determination

P. falciparum parasitaemia was determined in various blood smears stained by Giemsa stain. Parasitaemia was calculated based on previous methodology (Mohamed *et al.*, 2016): low (+) 1–10/100 fields, mild (++) 11–100/100 fields, moderate (+++) 1–10/one field, and high parasitaemia (++++) >10/one field.

3.10. Biochemical assays and Laboratory analysis

Measurement of total oxidative status and total antioxidant capacity were assessed using ELISA microplate reader LT 4000 in Microbiology lab of Addis Ababa University. Measurement of uric acid, bilirubin (total and direct) and albumin were using calibrated fully automated Mind ray BS-200E, clinical analyzer (china) according to the reagent manufacture's instruction in central laboratory of Zewditu Memorial Hospital.

3.10.1. Determination of Total Oxidant Stress (TOS)

Principle: Absolute oxidant status of plasma was measured by robotized colorimetric estimation way for Total oxidant stress (TOS). In this process, oxidants present in the plasma were determined by oxidation of ferrous particle by o-dianisidine complex to ferric complexes. The oxidation reaction was upgraded by glycerol molecules, which were richly present in the reaction medium. A colored compound was formed when the ferric ion reacted with xylenol

orange in an acidic medium. The color strength, which can be measured by ELISA microplate reader LT 4000, was correlated to the total quantity of oxidant molecules present in the plasma. The assay was calibrated with hydrogen peroxide, and the results were expressed as mmol H_2O_2 Equiv./l. Standard curve was used to calculate the concentration of the study sample (figure 6) **(**Erel, 2005).

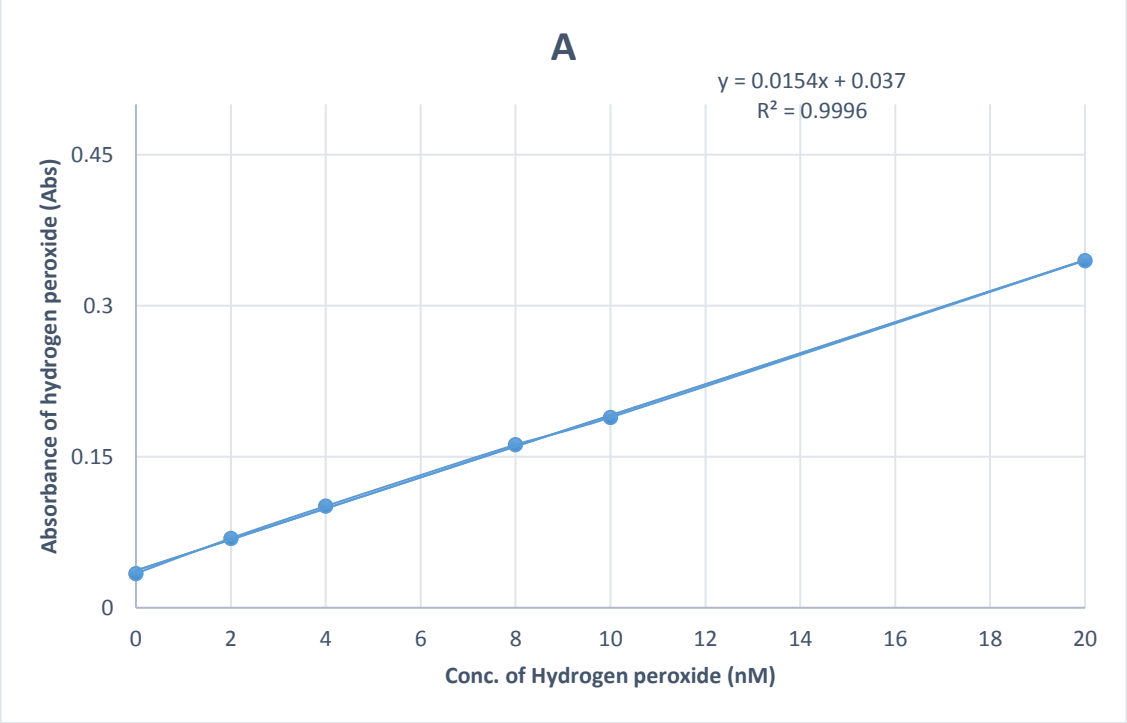

Figure 6: Standard curve for estimation of Total Oxidant Stress (TOS)

Procedure**:** Reagent **1** and **2** were prepared.

Reagent 1: [114mg of xylenol orange and 8.18gm of NaCl] were dissolved in 900mL of H_2SO_4 solution 25mM. The final reagent was composed of 150mM xylenol orange, 140mM NaCl, and 1.35M glycerol, pH 1.75.

Reagent 2: [1.96 gm of ferrous ammonium sulfate and 3.17 gm of o-dianisidine dihydrochloride] were dissolved in 1000mL of H_2SO_4 solution 25mM. TOC of serum was measured by adding 225µL reagent 1 into 35µL serum sample and then 11µL of reagent 2 was added. Finally, absorbance was measured by **ELISA** microplate reader LT 4000 at wavelength 560 nm.

3.10.2. Measurement of total antioxidant capacity (TAC)

Principle: Total antioxidant capacity of plasma was measured by robotized colorimetric estimation system for Total antioxidant capacity (TAC). In this technique, the hydroxyl radical, the most powerful natural radical, was generated by the Fenton reaction and it responds with the colorless substrate O-dianisidine to create the dianisyl radical, which was splendid yellowish-brown in colour. Upon the addition of a plasma sample, the oxidative responses started by the hydroxyl radicals present in the reaction are scavenged by the antioxidant agents of the plasma, keeping the color change and consequently giving a viable estimation of TAC. The test results were expressed as micro mol ascorbic acid Eq/l, and the accuracy of this measure is fabulous, being lower than 3% (Koracevic *et al.*, 2001).

A direct measurement method for total antioxidant capacity using a new generation, more stable 2,2‟-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical cation was used to determine the total antioxidant capacity (Erel, 2005**)**.

Assay Principle of the Novel Measurement Method

The reduced ABTS molecule was oxidized to ABTSS+ using hydrogen peroxide alone in acidic medium (the acetate buffer 30 Mm, pH 3.6). In the acetate buffer solution, the concentrate (deep green) ABTS+ molecules stay more stable for a long time. While it is diluted with a more concentrated acetate buffer solution at high pH values (the acetate buffer 0.4 M, pH 5.8), the color spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations. This reaction was monitored spectrophotometrically and the bleaching rate was inversely related with the TAC of the sample. The reaction rate was calibrated with Ascorbic acid standard for TAC measurement assays, and the assay results were expressed in µmol Ascorbic acid equivalent/L (figure 7).

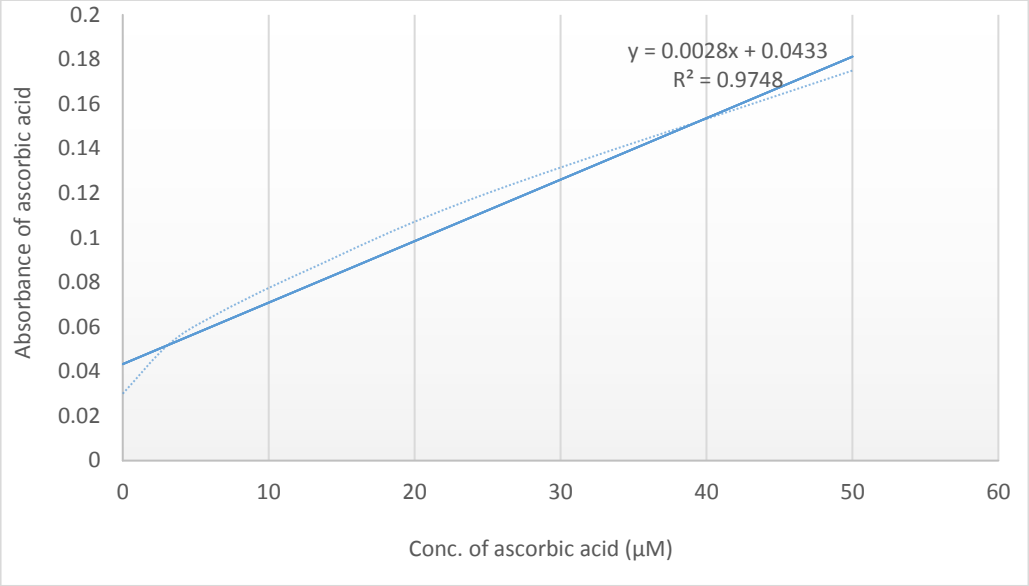

Figure 7: Standard curve for estimation of Total antioxidant capacity (TAC)

Reagent Preparation

Reagent 1

1. The 0.4 M acetate buffer solution (pH 5.8) was obtained as follows: 32.8 g of CH3COONa was dissolved in 1000 ml of deionized water (final concentration: 0.4 M).

2. Reagent-grade glacial acetic acid (22.8 ml) was diluted to 1000 ml with deionized water (final concentration: 0.4 M).

3. The sodium acetate solution (940 ml) was mixed with 60 ml of the acetic acid solution under a pH meter; the pH of the acetic acid–sodium acetate buffer was 5.8.

4. The buffer solution was stable for at least 6 months at 4 ^{0}C.

Reagent 2

1. The 30 mM acetate buffer solution, pH 3.6, was prepared as follows: 2.46 g of CH3COONa was dissolved in 1000 ml of deionized water (final concentration: 30 mM).

2. Reagent-grade glacial acetic acid (1.705 ml) was diluted to 1000 ml with deionized water (final concentration: 30 mM).

3. The sodium acetate solution (75 ml) was mixed with 925 ml of the acetic acid solution under a pH meter; the pH of the acetic acid–sodium acetate buffer was 3.6.

4. Then 200 micro liters of commercial H_2O_2 solution (30%, Sigma) was diluted to 1000 ml of the buffer solution (final concentration, 2 mM).

5. Then 50 mg ABTS (Sigma lot-030M8213V) was dissolved in 9.11 ml of prepared solution (final concentration: 10 mM).

6. After 1 hour of incubation at room temperature the characteristic deep green color of ABTS+ was appeared. The colored reagent was stable for at least 6 months at 4 ^{0}C.

7. The standard Ascorbic acid was prepared in acetate buffer (30 mM) solution and mixed just like the sample and run on micro plate with respect to the sample in duplicate.

Procedure of the Assay

Prepared reagents and solutions for the experiment were brought to room temperature before the experiment had begun. This procedure was done according to the modified micro plate assay (improved method of total antioxidant assay) for total antioxidant capacity (Gupta *et al.*, 2009). First 10μL of reagent 1 was added to the micro plate reader followed by 5 μL of serum sample using the multi-Chanel dispenser and mix carefully. Thereafter, the first absorbance had been read on automated micro plate reader (Labtek LT-4000, India) and immediately reagent 2 was added and mixed. Thereafter, it was incubated for 5 minutes and the last absorbance was read at 600 nm. The calibration type was linear and ascorbic acid was used as a standard between 10-50 μM concentration ranges.

The total antioxidant capacity of the sample was calculated from the standard curve drawn for ascorbic acid and the assay results were expressed in mmol of Ascorbic acid equivalent/L of sample.

3.10.3. Measurement of oxidative stress index (OSI)

The TOC/TAC ratio provides the OSI, which is an indicator of the degree of oxidative stress. OSI value can be calculated using the following formula: OSI (arbitrary unit) = TOC (mmol H_2O_2 equivalent/l)/TAC (mmol Trolox equivalent/l00) (Erel, 2005**)**.

3.10.4. Determination of serum uric acid

Principle: Uric acid was measured colorimetrically by **(**uricase/ PAP) (Piero *et al.*, 2010). Uricase catalyzes the oxidation of uric acid to allantoin and hydrogen peroxide (H_2O_2). In the presence of peroxidase (POD), H_2O_2 react with 4- aminoantipyrine (4-AA) and 3, 5, dichloro-2-hydroxybenzenesulphonate (DHBS) to form a quinoneimine dye. The reaction sequences are as follows:

Uric acid + O_2+ H_2O **URICASE** Allantoin + CO_2 + H_2O_2

H_2O_2 + 4-AA +DHBS **POD** Quinoneimine + $4H_2O$.

The intensity of the red color produced is directly proportional to the concentration of uric acid in the sample when read at 545 nm.

3.10.5. Determination of serum bilirubin

Principle: The total bilirubin and direct bilirubin were measured by Jendrassik and Grof, method (Cheesbrough, 2006). Bilirubin reacts with the diazotized sulphanilic acid (diazo reagent) to form azobilirubin. Caffeine is an accelerator and gives a rapid and complete conversion to azobilirubin. The pink colour of acidic azobilirubin is converted to blue azobilirubin by alkaline tartrate, which can be measured colourimetrically.

Total bilirubin is coupled with diazonium compound, 3, 5-dichlorophenyl – diazonium – tetraflouro - borat (DPD) to yield the corresponding azobilirubin. The absorbance of this dye at 546 nm is directly proportional to the total bilirubin concentration in the sample.

Reaction sequences is as follows:

Bilirubin + DPD + caffeine ⟶ Total azobilirubin

Conjugated (direct) bilirubin concentration is liberated by the detergent. The stabilized diazonium salt 3,5-diclorophenyl – diazonium –tetraflouro - borat (DPD) couples directly with direct (conjugated) bilirubin in an acid medium to yield the corresponding azobilirubin. The absorbance of this dye at 546 nm is directly proportional to the direct bilirubin concentration in the sample.

Reaction sequences is as follows:

Bilirubin + DPD⟶ Direct azobilirubin

Bilirubin and the diazo reagent form an azobilirubin complex, which can be measured colorimetrically.

The color of the azobilirubin varies with pH. It is based on the following principle that

(1) conjugated (directed) bilirubin is water soluble and therefore will react with diazo reagent in a water solution,

(2) Un-conjugated (indirect) bilirubin is not water soluble, therefore, alcohol is necessary to put the un-conjugated bilirubin in solution. So that it can react in the diazo reaction.

3.10.6. Estimation of albumin concentration

Principle: Albumin in the sample reacted with bromocresol green in acid medium forming a coloured complex that can be measured by colorimeter(Young, 1995)..

Albumin +BCG (yellow) $\xrightarrow{\text{pH 4.2}}$ Albumin-BCG complex (blue)

The absorbance of this dye at 545 nm is directly proportional to the albumin concentration in the sample.

3.10.7. Data processing and analysis

All data were checked, cleared and feed into Epi-data (version 3.5.1, 2008) and then exported to SPSS (version 22.0, 2012, America) software for statistical analysis. Descriptive statistics like mean, standard deviation, standard error and percentage were carried out. Student independent sample T-test was performed to detect differences between groups on continuous variables. One-way ANOVA followed by post hoc analysis was used to compare parasitemia case of malaria patients. Bivariate Pearson correlation test was carried out to determine the relationship (association) of TOS, TAC with OSI. $P < 0.05$ was considered as a statistically significant.

3.11. Data Quality Assurance and management

- ✓ There was a well-prepared data collection questionnaire to assess participant's demographic information.
- ✓ The blood samples for biochemical assay was collected by adherence with standard operation procedures and measurement of analysis were carried out after running quality control samples.
- ✓ All the laboratory procedures were handled by professional laboratory technologists.
- ✓ All the tests were standardized and automated.

3.12. Ethical Consideration

The ethical clearance was obtained from Biochemistry Department college of Health Sciences Addis Ababa University with protocol number 02/17 and meeting number DRERC 01/17 with reference no. Furthermore, Collaboration letter for data collection was also obtained from Afar Regional Health Bureau. The objective of the study was briefly clarified and explained for each participant, before enrolling any of the eligible study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability and academic honesty was maintained throughout the study by code (see Annex). The findings of the study will be disseminated for health care professionals and other concerned bodies for better care of the malaria patients than ever.

4. RESULTS

4.1. Socio-demographic characteristics

One-hundred (60 malaria cases and 40 health controls) participated in this study. The mean age for malaria case and health controls was 23.5±8 and 23.8±7.4 years old respectively. Therefore, malaria patients and health controls were age-matched (p= 0.86). The number of males were 38/60 (63.3 %) in the malaria patients and 24/40 (60 %) in the healthy controls. So, the sex of malaria patients was well matched with the healthy controls (p = 0.9). Out of 60 malaria patients, 34(56.7%) were married. Most of malaria patients in the study were living in rural areas 44/60 (73.3%). Out of sixty, 49 (81%) were illiterate. Most of the malaria patients did not use bed nets 39/60 (65%). 48/60 (80%) malaria patients have lower income.

The clinical severity was determined according to intensity of infections (Parasitemia). Accordingly, 32/60 (53.3%) patients were low parasitemia, 16/60 (26.7%) patients were moderate parasitemia and 12/60 (20%) high parasitemia.

Table 1. General characteristics of study participants of cases and controls at Dubti Hospital and Logia Health center, Afar, Ethiopia, 2018

Variables		**Malaria patient N=60**	**Control group N=40**	**p-value**
Age mean (SD)		23.5±8	23.8±7	0.86
Sex, male [n (%)]		38 (63.3)	24 (60)	0.9
Residence [n (%)]	Urban	16 (26.7)	35 (87.5)	0.01*
	Rural	44 (73.3)	5 (12.5)	
Educational level [n (%)]	Illiterate	49 (81.7)	5 (12.5)	0.01*
	High school or less	7 (11.7)	13 (32.5)	
	College and above	4 (6.7)	22 (55)	
Marital status [n (%)]	Single	23 (38.3)	27 (67.5)	0.06
	Married	34 (56.7)	13 (32.5)	
	Widowed	3 (5)	0 (0.0)	
Income [n (%)]	Low (<500)	48 (80)	24 (60)	0.01*
	Middle (500-1000)	12 (20)	13 (32.5)	
	High (>1000)	0 (0.0)	3 (7.5)	
Use of bed nets [n (%)]	Yes	21 (35)	29 (72.5)	0.01*
	No	39 (65)	11 (27.5)	
Severity of malaria [n (%)]	Low (<1000)	32 (53.3)		**NA**
	Moderate (1000-10000)	16 (26.7)		
	High (>10000)	12 (20)		
BMI [n (%)]	<18.5	4 (14.8)	2 (7.4)	
	18.5-24.9	14 (51.9)	18 (66.7)	
	25-29.9	6 (22.2)	4 (14.8)	0.45
	≥30	3 (11.1)	3 (11.1)	

SD= Standard Deviation, %= percentage, n= number, BMI=Body Mass Index, NA= Non-Applicable. Categorical variables are presented in frequency and percentage while continuous variables are presented as mean (SD), Income Level: low= ≤ 500, medium = 500 -1000 and high = ≥ 1000 Ethiopian Birr. * statically significant

4.2. Estimated levels of biochemical parameters

4.2.1. Serum levels of TOS and OSI in malaria patients and control groups

The concentration of total oxidative status (TOS) and oxidative stress index (OSI) of malaria patients and control groups were examined (table 2). Total oxidative status in serum of malaria patients (3.1±0.5 µmol H_2O_2 Equiv/l) were significantly (p = 0.03) higher than control groups (1.8±0.5 µmol H_2O_2 Equiv/l) and oxidative stress index in serum were found to be significantly (p = 0.04) higher in malaria patients (30.5±8.6) compared to control group (8.1±1.4).

Table 2. Serum parameters of oxidative status biomarkers in the study participants.

Serum parameter	Control group (n=40, mean ±SE)	Malaria case (n=60, mean ±SE)	p-value
TOS (µmole H_2O_2 eqv. /l])	1.8±0.5	3.1±0.5	**0.03***
TAC (nmole ascorbic acid eqv /l)	28.9±1.9	19.4±1.3	**0.01***
OSI (ratio of TOS/TAC) *100	8.1±1.4	30.5±8.6	**0.04***
Uric Acid (mg/dL)	4.1±0.1	4.7±0.1	**0.02***
DB (mg/dL)	2.6±0.1	3.4±0.2	**0.03***
TB (mg/dL)	1.5±0.1	2.7±0.2	**0.01***
Albumin (mg/dL)	4.9±0.1	3.4±0.1	**0.01***

Values are expressed as Means± SE. **TOS**= total oxidative stress, **TAC**= total antioxidant capacity, **OSI**=oxidative stress index, **TB**= total bilirubin, **DB**= direct bilirubin. *= statistically significant

4.2.2. Total Antioxidant Capacity and Non-enzymatic Antioxidants in malaria patients and control groups

The mean serum total antioxidant status was higher in controls than patients (28.9±1.9 and 19.4±1.3 nmole ascorbic acid eqv/l) respectively and the difference was significant (p = 0.01) (table 3).

Uric acid, total and direct bilirubin level were significantly ($P < 0.05$) increased (4.7±0.1mg/dl, 2.7±0.2mg/dl and 3.4±0.2mg/dl respectively) in malaria patients compared to control group (4.1±0.1mg/dl, 1.5±0.1mg/dl and 2.6±0.1mg/dl respectively).

Serum albumin level of malaria patients (3.4±0.1mg/dl) was significantly (p = 0.01) lower compared to control group (4.9±0.1mg/dl).

Table 3. **Total Antioxidant Capacity and Non-enzymatic Antioxidants in test and control groups.**

Serum Parameter	Control group (n = 40)	Malaria case (n = 60)	p-value
TOS (µmole H_2O_2 eqv. /l])	1.8±0.3	3.15±0.5	**0.03***
TAC (nmole ascorbic acid eqv /l)	28.9±1.9	19.4±1.3	**0.01***
OSI (ratio of TOS/TAC) *100	8.1±1.4	30.5±8.6	**0.04***
Uric Acid (mg/dL)	4.1±0.1	4.7±0.1	**0.02***
DB (mg/dL)	2.6±0.1	3.4±0.2	**0.03***
TB (mg/dL)	1.5±0.1	2.7±0.2	**0.01***
Albumin (mg/dL)	4.9±0.1	3.4±0.1	**0.01***

Values are expressed as mean±SE. **TOS**= total oxidative stress, **TAC**= total antioxidant capacity, **OSI**=oxidative stress index, **TB**= total bilirubin, **DB**= direct bilirubin. *= statistically significant.

4.2.3. To Compare the Oxidative stress and antioxidant levels in low, moderate and highly infected malaria parasite.

Total oxidative stress (TOS), oxidative stress index, total antioxidant status, uric acid, direct bilirubin, total bilirubin and albumin in the blood sample were compared in relation to the stage of malaria (table 4).

The total oxidative stress level of high parasitemic patients (6.2±1.8 µmole H_2O_2 eqv. /l) were significantly increased ($p < 0.05$) compared to low parasitemia and moderate parasitemic patients. However, the total antioxidant capacity level of high malaria parasitemic patients (9.6±1.2 Vs 2.4±0.4; $p = 0.01$) were exhibited significant decrease compared to low parasitemic patients.

This study showed that, the mean serum uric acid level of moderate parasitemic patients (5.0±0.2mg/dl) significantly increased (p=0.01) compared to lower parasitemic patients (4.0±0.1mg/dl) and the uric acid level of high parasitemic patients (5.8±0.2mg/dl) were

significantly increased (p = 0.01) compared to low parasitemic and moderate parasitemic patients.

The mean serum direct bilirubin level of high parasitemic patients (5.1±0.4mg/dl were significantly increased (p = 0.01) compared to low parasitemic patients (2.5±0.1mg/dl) and moderate parasitemia (3.8±0.2mg/dl). The mean serum direct bilirubin level of moderate parasitemic patients (3.8±0.2mg/dl also significantly increased (p = 0.01) than low parasitemic patients (2.5±0.1mg/dl) group.

The mean total bilirubin level of high parasitemic malaria patients (5.3±0.3mg/dl) were significantly (p=0.01) increased compared to the patients presenting with low parasitemia (1.8±0.1mg/dl) and moderate parasitemia (2.5±0.2mg/dl) and this level of moderate parasitemic (2.5±0.2mg/dl was significantly increased (p = 0.01) compared to low parasitemic patients (1.8±0.1mg/dl).

The mean levels of albumin were significantly decreased (p = 0.01) in high parasitemic patients than in low parasitemic patients. Whereas, the albumin level of moderate parasitemic patients (3.2±0.1mg/dl) were decreased compared to low parasitemic patients (3.8±0.2mg/dl) but it was not statistically significant (p > 0.05) (table 4).

Table 4: Serum parameters in pathologically confirmed malaria patients participated from Logia Health Center and Dubti Referral Hospital, Afar, Ethiopia, 2018

Serum parameter in malaria patients	Stages of malaria patients			ANOVA
	Low (+) N=32	Moderate (++), N=16	High (+++), N=12	p-value
TOS (µmole H_2O_2 eqv. /l])	2.4±0.4[b]	2.4±0.5[c]	6.2±1.8	**0.01***
TAC (nmole ascorbic acid eqv /l)	23.9±1.8[b]	17.8±1.7[c]	9.6±1.2	**0.01***
OSI (ratio of TOS/TAS) *100	13.3±3.6[b]	14.3±3.4[c]	97.9±36.5	**0.01***
Uric Acid (mg/dL)	4.0±0.1[ab]	5.0±0.2[c]	5.8±0.2	**0.01***
DB (mg/dL)	2.5±0.1[ab]	3.8±0.2[c]	5.1±0.4	**0.01***
TB (mg/dL)	1.8±0.1[ab]	2.5±0.2[c]	5.3±0.2	**0.01***
Albumin (mg/dL)	3.77±0.1[b]	3.2±0.1	2.7±0.3	**0.01***

**-indicates significant differences among stages of malaria patients at p < 0.05 as tested by one-way ANOVA.*

[a] mean difference between low (+) and moderate (++) stages of malaria at p < 0.05 as tested by Tukey post hoc multiple comparisons.

[b] mean difference between low (+) and high (+++) stages of malaria at p < 0.05 as tested by Tukey post hoc multiple comparisons.

[c] mean difference between moderate (++) and high (+++) stages of malaria at p < 0.05 as tested by Tukey post hoc multiple comparisons.

The oxidative stress index of malaria patients was analyzed and correlated with stage of malaria. There was significantly higher oxidative stress in high parasitemic patients than the other stages (p = 0.01).

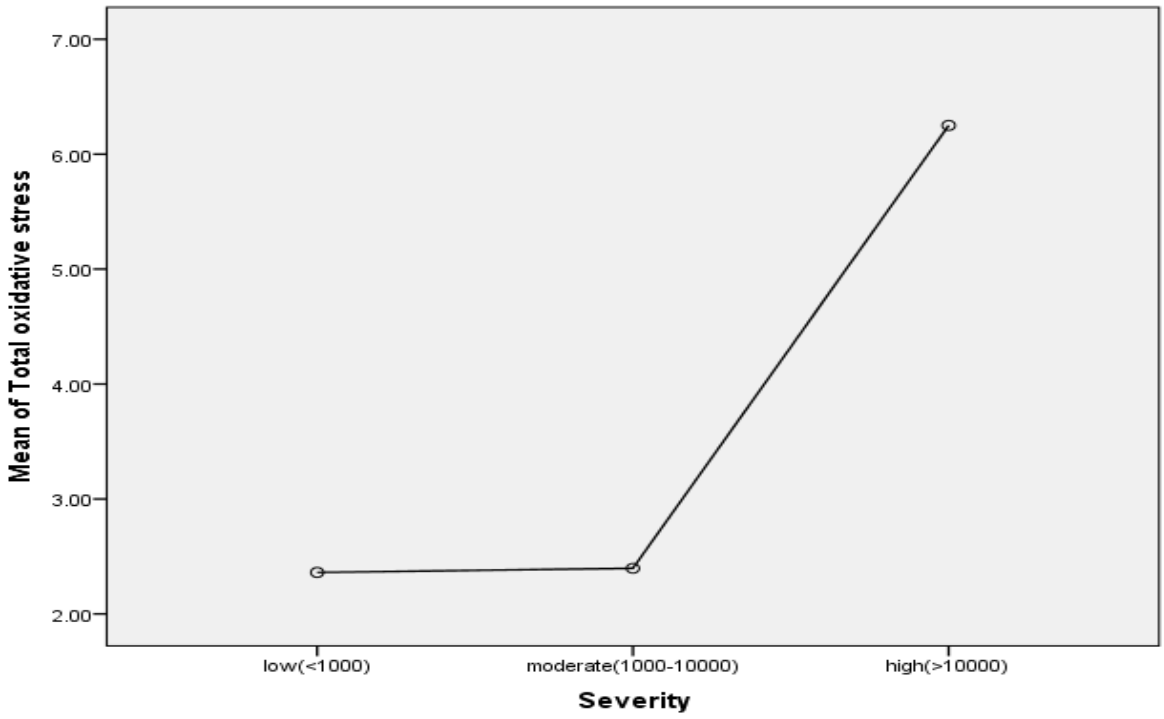

Figure 8: Mean plots of oxidative stress index in severity malaria patients

4.2.4. Correlations between total oxidant –antioxidant in malaria patients

The levels of TOS with TAC showed a negative correlation but there was not significant association (r= -0.213, p = 0.3) (Fig. 9a). Conversely, TOS showed a positive and significant correlation with levels of OSI among malaria patients (r= 0.584, p = 0.01) (Fig. 9b).

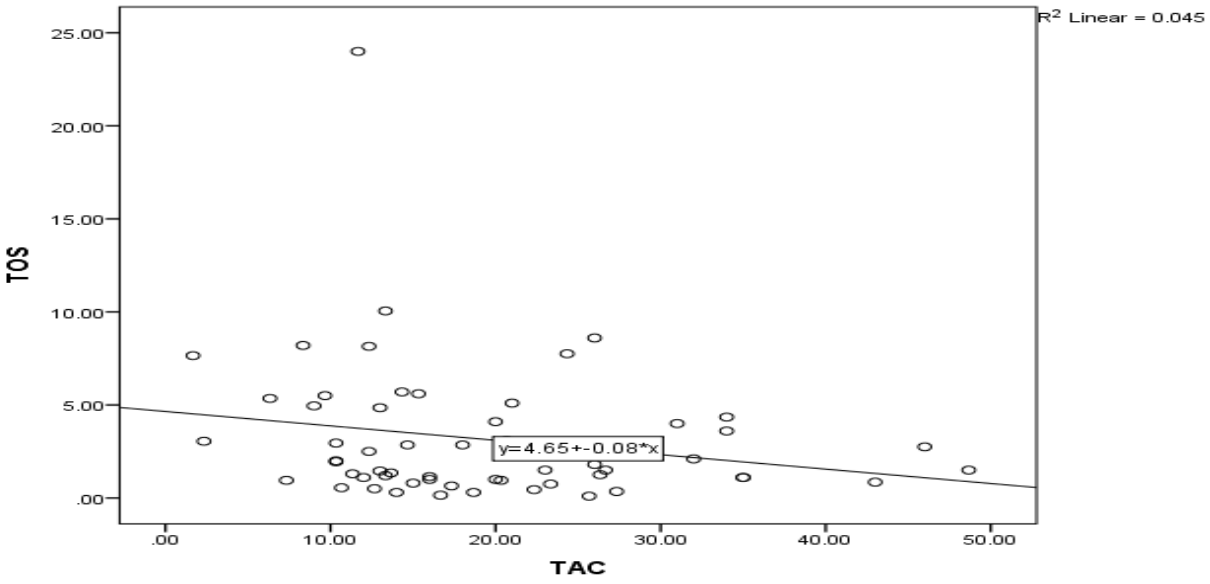

Figure 9a: Regression fit of TAC Vs OSI

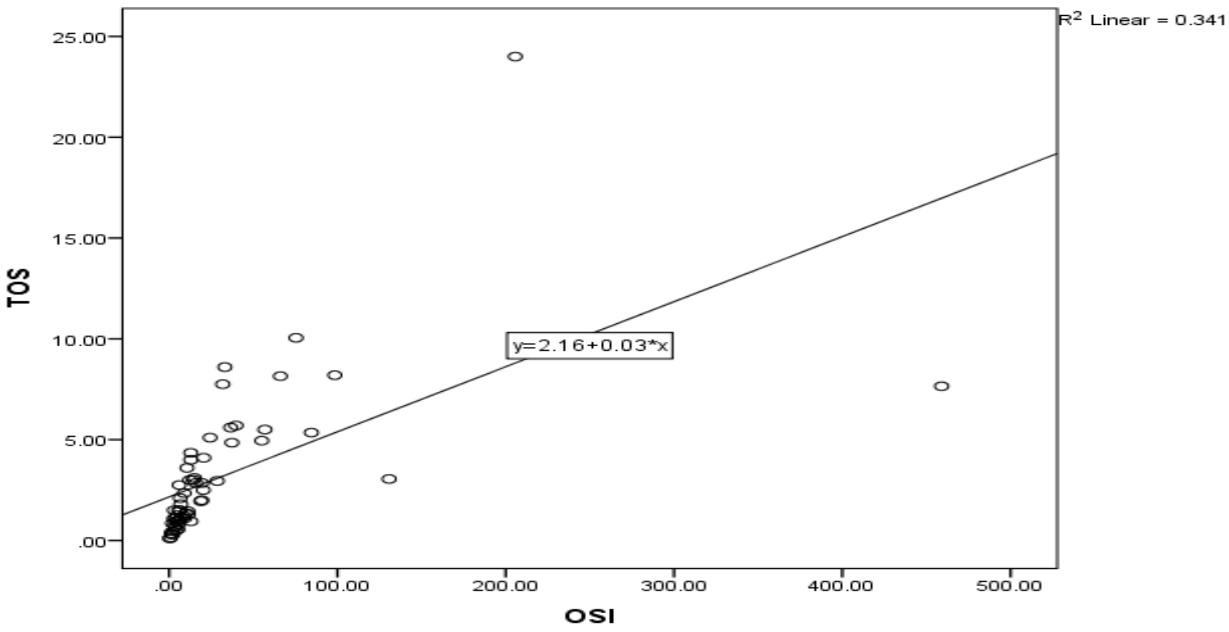

Figure 9b: Regression fit of TOS Vs OSI

4.2.5. Correlations between Total oxidant –Antioxidant and severity of disease

From the three stage of malaria patient, the serum level of total antioxidant capacity was negatively correlated with oxidative stress index. Low (+) and high (+++) parasitemia patients exhibited significant ($p<0.05$) correlation, whereas, moderate (++) malaria patients exhibited

insignificant (P>0.05) correlation. On the other hand, a positive correlation was found between TOS and OSI among the three stages with significant correlation in low (+) and moderate (++) ($p < 0.05$) whereas, high malaria patients exhibited insignificant ($p > 0.05$) correlation (Table 5).

Table-5: Correlations of TOS and TAC with OSI levels among the malaria patients

Variables	Low (+)	Moderate (++)	High (+++)
TOS and OSI	0.860[a]	0.790[a]	0.426[b]
TAC and OSI	-0.40[a]	-0.167[b]	-0.653[a]

Results are expressed in Pearson correlation coefficient among variables a -p<0.05, b-p>0.05 TOS= Total Oxidative status, OSI= Oxidative Stress Index and TAC=Total Antioxidant capacity.

5. DISCUSSION

Malaria, the disease caused by *Plasmodium* continues to be the disease with the highest mortality rate(Isaac *et al.*, 2011). In response to infection caused by *Plasmodium* parasites, the natural host defense mechanism is activated with involvement of phagocytes (macrophages and neutrophils). These, in turn, generate large amounts of ROS and RNS, causing an imbalance between the formation of oxidizing species and the activity of antioxidants which triggers oxidative stress (Percário *et al.*, 2012). Oxidative stress plays an important role in the development of malarial anemia that leads to production of ROS by *Plasmodium falciparum*-infected red cells (Narsaria *et al.*, 2012). So, the aim of this study was to investigate the level of total oxidative stress and antioxidant capacity and their relation to other biochemical parameters among malaria patients.

In this study, the serum level total oxidative stress (TOS), total antioxidant capacity (TAC), uric acid, direct bilirubin, total bilirubin and albumin were determined were determined in search of a potential biomarker for diagnosis, prognosis and treatment target for malaria disease in malaria patients. This study was done on 100 (60 malaria patients and 40 age-sex matched apparently healthy control groups) participants. The study showed that significantly higher serum level of total oxidative status, oxidative stress index, uric acid, total and direct bilirubin were observed in malaria patients compared to control groups.

The total oxidative status in malaria patients were significantly ($p < 0.05$) higher than apparently healthy controls (Table 2). This significantly elevated total oxidative status could be due to high free radical load and insufficient antioxidants which consequently indicates a high oxidative stress condition in malaria patients. These finding agreed with the study of (Ozcan *et al.*, 1997; Idoniji *et al.*, 2011; Percário *et al.*, 2012; Abubakar *et al.*, 2016). This increment of oxidative stress might be the imbalance between the generation of reactive oxygen species and the antioxidant defense system (Ramazan *et al.*, 2012).

In the present study, total oxidative status was significantly higher in patients with high parasitaemia counts than those with low parasitaemia count. This implies a higher oxidative stress status in high parasitaemic patients. However, the serum TOS level was higher in patients with moderate parasitaemia than in low parasitaemic patients, although the difference was not

statistically significant ($p > 0.05$). This might have been due to high parasite load in the red blood cells resulting in reduction of antioxidant level as a survival strategy. These finding agreed with the study of (Idoniji *et al.*, 2011; Olisekodiaka *et al.*, 2017) (Table 4). These change also happened because malaria parasite is capable of generating Reactive Oxygen Species (ROS) with in erythrocytes and damaging the uninfected erythrocytes by activation of immune cells (Douglas *et al.*, 2011) which agrees with our study in which there is an increased total oxidative stress in malaria patients .

The possible reason for high oxidative stress in malaria patients, may be due to invasion of human erythrocytes by malaria parasite resulting in vulnerability to damage of the erythrocytes due to toxic metabolites derived from both the host cells and parasites. Reactive oxygen species (ROS) generated in the host-parasite interaction could lead to the lysis of erythrocytes and possible alterations in the antioxidant defense system (Ozcan *et al.*, 1997).

Adiel and co-workers stated that, haem part of haemoglobin is a vital factor for a diverse set of proteins involved in various physiological functions such as respiration, oxygen transport and drug detoxification. The accumulation of free haem has deleterious effects on the normal physiology. Haem has capacity to combine lipid bilayers. It also catalyses lipid peroxidation, inhibit various enzymatic activity, lyses cells and parasites (Adil *et al.*, 2013).

In malarial infection, erythrocytes are exposed to the oxidative stress from the intra- and the extraerythrocytic environments. The intraerythrocytic malaria parasite is presumed to exert an oxidative stress on its host erythrocyte (Ozcan *et al.*, 1997). Another study revealed that reactive, oxygen species generated in host-parasite interactions causes the lysis of erythrocytes and alteration in antioxidants (Prasannachandra *et al.*, 2006).

Malaria patients had a significantly lower concentration of total antioxidant capacity (TAC) than serum samples of control group ($p < 0.01$) in (Table 3). The lower values observed in total antioxidant levels in malaria may be attributed to increased utilization of the host's plasma antioxidants by the malaria parasites to counteract oxidative stress. Additionally, increased consumption and degradation of antioxidants and antioxidant enzymes as well as haemoglobin

by malaria parasite to produce its own proteins has been reported by Jessica and her colleagues (Jessica *et al.*, 2016). This finding is in agreement with the findings of several previous studies where people with malaria parasite have been shown to have a higher oxidative stress (Claudio *et al.*, 2008; Oakpotuzor *et al.*, 2012; Ayodele and Oyedele, 2014; Olisekodiaka *et al.*, 2017). TAC is a biochemical parameter suitable for evaluating known and unknown antioxidants and their synergistic interaction and consumption by normal or increased levels of ROS production that gives an insight into the delicate balance between oxidants and antioxidants in living cells (Bansal and Bilaspuri, 2010; Jessica *et al.*, 2016).

Enzymatic and non-enzymatic antioxidants play an important role in counteracting oxidative stress. These compounds prevent the formation of ROS, and thus inhibit their interactions with cellular components and stop free radical reactions (Magdalena *et al.*, 2017). An individual antioxidant level or activity indicates the antioxidant characteristics of only one antioxidant, whereas TAC may represent the total antioxidant characteristics of all antioxidants found in the serum (Dokuzeylul *et al.*, 2015) . Deficiencies in any of the antioxidant defense system can cause a reduction in the total antioxidant status of an individual (Wagener *et al.*, 2013). Tyagi and his colleagues, (2017) reported that a fall in the erythrocytic SOD and CAT in malarial patients indicated that there was a decreased utilization of reduction potential in detoxication of ROS in the patients.

In this study, the total antioxidative capacity in high parasitemic malaria patients exhibited significantly decreased ($p < 0.01$) compared to low parasitemic patients and moderate malaria parasitemic patients (Table 4). The serum level total antioxidant capacity in moderate malaria parasitemic patients were lower than the patients presenting with low parasitemic but not statistically significant ($p > 0.05$) (Table 4). This implies a higher oxidative stress status in high parasitaemic patients.

The decreased TAC may be possibly because *plasmodium* parasites produces active redox products, free haem, and H_2O_2 leading to oxidative stress in infected cells. In addition, it could be due to depressed state of antioxidant system or due to the exaggerated inflammatory processes and oxidative stress in these patients. This finding is supported by another previous

work (Isaac *et al.*, 2011). These mechanisms lead to the decrease of the antioxidant capacity of the body therefore reflecting the severity of *P. falciparum* malaria (Tiyong *et al.*, 2008).
The OSI estimated in this study was significantly ($p < 0.05$) higher in malaria patients than in healthy controls (Table -2). This indicates the exact degrees of imbalance of oxidative stress and anioxidant status. On the basis of these results, we can deduce that a compromised antioxidant defense mechanism, accompanied by increased oxidant levels and OSI values in malaria patients, might play an important role in the pathogenesis and severity of this disease.

This study showed that there was statistically significant increase in OSI value in low ($p < 0.01$), moderate ($p < 0.01$) and high ($p < 0.01$) parasitemia case of malaria patients compared to healthy controls. In addition, high parasitemia patients exhibited significant increase in OSI ($P<0.01$) as compared to low and moderate parasitemic patients. But, low and moderate parasitemic patients have not had significant difference ($p > 0.05$) (Table-4)

The decrease in TAC and increase in TOS were more pronounced in high parasitemic (+++) patients comparing to low (+) and moderate (++) indicating that the anti-oxidants were nearly completely utilized to scavenge the free radicals produced during this disease.
When the oxidant/antioxidant balance is tilted towards oxidants, oxidative stress arises, There is a significant negative correlation between the TAC and TOS values between controls and malaria patients (Dokuzeylul *et al.*, 2015).

In the present investigation a negative correlation was demonstrated between the TAC and TOS, which is one of the scales indicating the severity of malaria disease. These findings may indicate the importance of the anti-oxidant system in the pathogenesis and severity of malaria.
Serum TAC and OSI levels in malaria patients showed a negative correlation with the severity of malaria which indicated that the severity of disease was positively associated with oxidative stress levels. This finding is supported by other findings (Carlos and Bucalen, 2008) (table 5).

Higher level of serum uric acid was observed in malaria patients, which was statistically significant ($p = 0.01$). This laboratory finding agrees with the other reports on a significantly increased serum uric acid concentration in acute oxidative stress (Table 3).(Ozcan *et al.*, 1997;

Olisekodiaka *et al.*, 2017).The possible reason for increasing uric acid in malaria patients may be associated with increasing rate of purine metabolism and production of ureate during malaria infection, which is a general phenomenon during malaria infection because of haemolysis and oxidation of nucleic acid by reactive oxygen species produced (Siddiqi and Alhoida, 1999).

This laboratory finding agrees with the other reports on a significantly increased serum uric acid concentration in acute oxidative stress condition (Eghwrudjakpor and Allison, 2010; Nilgün, 2012). A possible suggested mechanism for this observation was the presence of hypoxanthine and xanthine in both erythrocytes and the *Plasmodium* parasite. High concentrations of soluble uric acid induces the release of inflammatory mediators from different cell types including immune cells suggesting that the soluble uric acid formed via hypoxanthine degradation could also contribute to the malaria induced inflammatory response (Kouam *et al.*, 2008; Olisekodiaka *et al.*, 2017).

This current study revealed that the serum level of uric acid was increased significantly ($p=0.001$, $p=0.017$) (table-4) in high malaria parasitemic patients compared to low and moderate malaria parasitemic patients. This finding agrees with other studies done earlier (Gallego-Delgado *et al.*, 2014; Olisekodiaka *et al.*, 2017). The possible reason for high uric acid level in serum in malaria patients may be due to the fact that the *Plasmodium* parasite imports excessive hypoxanthine and xanthine into the infected erythrocytes and further increase with disease severity (Neida *et al.*, 2013; Reyes *et al.*, 1982). The intraerythrocytic human malaria parasite requires a source of hypoxanthine for nucleic acid synthesis and energy metabolism, but, it is unable to synthesize purine *de novo*, and hence uses host-derived hypoxanthine preferentially as purine source. Purine salvage pathway in parasitized erythrocytes is more active approximately ten times than parasite-free erythrocytes (Ozcan *et al.*, 1997). The increased concentrations of uric acid might be a compensatory mechanism that confers protection against increased free radical activity.

Multifaceted mechanism of action of uric acid as an anti-oxidant has been proposed in the body fluids. It can act as an oxidisable co-substrate for any Reactive Oxidative Species (Piyali *et al.*, 2009; Gallego-Delgado *et al.*, 2014); by inactivating an oxidant via an electron transfer before the oxidant can act with the targeted biological membrane (Waring *et al.*, 2003) and thus can protect the important biomolecules from oxidative damage (Gallego-Delgado *et al.*, 2014).

Moreover, uric acid maintains the cell membrane integrity by inhibiting per-oxidation of membrane lipids (Piyali *et al.*, 2009).

Uric acid is also said to help in stabilization of ascorbate in biological fluids and because its serum concentration is higher than that of ascorbate, it is thought to potentially have a higher antioxidant property than ascorbate (Lawal *et al.*, 2012). Therefore, both uric acid and ascorbic acid are strong reducing agents (electron donors) and potent antioxidants. In humans, over half the antioxidant capacity of blood plasma comes from uric acid (Baillie *et al.*, 2007).

In this study, the level of total and direct bilirubin was significantly ($p = 0.01$) higher in malaria patients compared to with healthy control group (Table 2). The observed trend towards the increase in serum total and direct bilirubin level in malaria patients were in line with the fact that malaria parasitemia causes increased red blood cell haemolysis, which is associated with increase in bilirubin biosynthesis, hepatocellular damage, biliary tract obstruction, haemolysis and jaundice (Obimba *et al.*, 2014). In another study, it was noted that the possible causes of hyperbilirubinemia were multi-factorial and include intravascular haemolysis of parasitized RBCs as well as haemolysis of non-parasitized RBCs (Olisekodiaka *et al.*, 2017). Previous works suggest that hyperbilirubinemia in malaria patients occurs as a result of intravascular haemolysis of red blood cells and degradation of hemoglobin, a major nutrient source used by the malaria parasite (Kouam *et al.*, 2008).

Bilirubin has aantioxidant as well as prooxidant properties. At low concentrations, it acts as a scavenger of reactive oxygen species, reducing the damage caused to the cells (Fabbri *et al.*, 2013). Keshavan *et al*. (2005) suggested that bilirubin inhibited the cellular production of ROS in response to vascular cell adhesion molecul-1 (VCAM-1) stimulation as an antioxidant (Keshavan *et al.*, 2005). Bilirubin was found to be a free radical scavenger by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical and an inhibitor of superoxide production by mechanism employing the inhibition of NADPH oxidase (Valášková and Muchová, 2016). Recent studies reported that bilirubin is highly lipophilic which protects the diseases from lipid peroxidation and capable of protecting cells from a 10,000-fold increase in oxidative stress generated by hydrogen peroxide (Ai-Ching *et al.*, 2014).

In this study, the serum level of total bilirubin and direct bilirubin were significantly ($p = 0.01$) higher in patients with high parasitaemia count than those with low parasitemia count and moderate parasitaemia count. This indicated that high parasitaemic patients have developed a high amount of oxidative stress status. This is in agreement with previous report that patients with high parasitaemia count are more predisposed to oxidative stress than those with low parasitaemia count (Isaac *et al.,* 2011). Adelakun Ayodele A *et al* revealed that in malaria parasitemia especially by *P.falciparum*, large numbers of erythrocytes are infected and they are eventually destroyed by the spleen, thus resulting in haemolytic anaemia and there is also increased plasma level of total bilirubin (Adelakun *et al.*, 2015).

Previous studies revealed that hepatic injury is associated with malaria infection. Hepatic dysfunction may be due to alteration in vascular flow through the organ as parasitized erythrocytes adhere to endothelial cells blocking sinusoids and leading to the decrease in excretion of conjugated bilirubin through bile canaliculus (Kouam *et al.,* 2008). These findings are also supported by other works, where the plasma conjugated bilirubin was elevated in severe *falciparum* malaria infected individuals due to haemolysis (Devarbhavi *et al.,* 2005). Previous studies have also reported that in parasitized erythrocytes, increase in the production of hydrogen peroxide and free oxygen radicals leads to a decrease in antioxidant enzymes (Isaac *et al.*, 2011). It has been shown that intact *Plasmodium falciparum* trophozoite infected human red cells produce H_2O_2 and OH^- radical about twice as much as the normal erythrocyte (Claudio *et al.*, 2008).

The concentration of serum Albumin was found significantly low ($p = 0.01$) in malaria patients as compared to healthy controls. The possible causes for the low serum albumin in these patients were considered to be nutritional factors and the presence of an acute phase response among *plasmodium falciparum*-infected patients (associated with a decrease in serum albumin and other plasma proteins. This study is in agreement with Ozcan and his colleagues who reported that, the serum albumin levels were decreased in patients with *vivax* and *falciparum* malaria (Ozcan *et al.*, 1997). This finding agrees with the study of Kwena and his colleagues who reported that plasma albumin is a negative acute phase protein, the level of which falls as a result of malaria infection, probably because of an increase in its trans-capillary escape rate and it was degraded by the peroxy radicals (Ozcan *et al.*, 1997; Kwena *et al.*, 2012).

Previous studies revealed that low albumin level in malaria may be due to a number of factors: these include inhibition of synthesis by increasing levels of cytokines such as TNF, ILI and IL6, reduced food intake as a consequence of loss of appetite, and redistribution into extravascular spaces as a result of inflammation. The albumin in extravascular spaces enhances antioxidant activity at these locations (Galloway *et al.*, 2000; Oakpotuzor *et al.*, 2012).

Albumin serves as an antioxidant in vascular compartment because of its scavenging of reactive oxygen and nitrogen species that are generated by basal aerobic metabolism as well as produced at increased rates during inflammation (Ozcan *et al.*, 1997; Prakash, 2017).The antioxidant property of albumin is reported to be due to the presence of sulfhydryl group (Mustafa *et al.*, 2013).

In this study showed the serum levels of albumin were decreased in the severity of the disease. Then in high parasitemia case of malaria patients significantly decreased($p<0.01$) than in low and moderate parasitemia. However, their mean difference was not statistically significant ($p > 0.05$) in case of moderate parasitemia. These results are supported by other studies which stated that the serum levels of albumin was significantly decreased in malaria patients (Amah *et al.*, 2011). The use of serum albumin levels as reliable biochemical marker for establishing sever pathologic conditions such as malnutrition and infectious diseases (Kwena *et al.*, 2012). However, the predominant plasma albumin concentration in malaria infection is dependent on the nutritional status of the affected individual and hepatic functionality (Ogbodo *et al.*, 2010).

Akaninwor *et al*., (2013) showed the significant ($p < 0.05$) decrement of albumin concentration while malaria density getting increased. Consequently use of albumin infusion in place of other colloidal solutions in severe malaria is recommended as good intervention (Akaninwor *et al.*, 2013).

6. CONCLUSION

The results of this work show that malaria patients are at a risk of oxidative stress that can lead to complications and worsening of the disease. The serum total oxidative stress, oxidative stress index, uric acid, total bilirubin and direct bilirubin were significantly higher in malaria patients compared to control groups. But, total antioxidant capacity and albumin were significantly deceased in malaria patients.

Increased total oxidative stress, the ratio of total oxidative stress, uric acid and bilirubin in malaria patients may be attributed to the increased peroxidation and haemolysis.

As the severity of the disease increases, an increased oxidative stress is detected in malaria patients. Therefore, it is possible to conclude that there is increased of oxidative stress in malaria patients as severity of the disease increases.

Measuring of total oxidative stress and total antioxidant capacity in early malaria patients may help to therapeutically decrease complication of the disease.

7. RECOMMENDATION

- Malaria patients have to modify their feeding habit with high intake of fruit and vegetables, legumes, grains and cereals, fish and antioxidant levels with their therapy.
- Monitoring or evaluation of free radical and antioxidant level in malaria patients will be important and can indicate disease progression and clinical outcome of treatments.
- It is recommended that supplementation of diet and antimalarial drugs with antioxidant is a part of treatment plan for the management of malaria patients.
- This study should be conducted in wider scale to confirm that high parasitaemia predisposes humans to oxidative stress and consequent risk factors such as anemia.
- Further research is needed in the area of malaria parasitaemia levels in relation to ROS levels in the host.

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8. STRENGTH AND LIMITATION OF THE STUDY

8.1. Strength of the Study

Measurement of total oxidative stress and total antioxidative capacity in malaria patients and correlations with its stages is attempted for the first time in Ethiopia.

Therefore, this study is expected to offer the baseline information for further studies of malaria patient in Ethiopian, in relation to stages and grades.

8.2. Limitation of Study

- Financial and time constraints were limitations in this work, affecting the sample size used which could have been increased and the different assays that would have been used.
- The present study also does not measure the glutathione and malondialdehyde level.
- The major limitation of this study was a small sample size of study participants and due to this the result of this study may not reflect total population of malaria patients in Ethiopia.

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10. ANNEXES

10.1. Annex 1: Information sheet (English Version)

Research Project: The Estimation of Total Oxidative Stress and Non-enzymatic Antioxidant levels in malaria patients in Logia Dubti Area, Afar, Ethiopia.

Sponsoring organization: Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University

Principal Investigator: Adem Ebrahim (BSC, MSc in biochemistry candidate)

Advisors: Dr. Solomon Genet, Dr. Natesan Gnanasekaren

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors; from Addis Ababa University biochemistry department. Please take as much time as you need to read or listen in the information sheet**.**

Purpose of the Research Project

We are asking you to take part in this study because we will try to compare the total oxidative index among malaria patients so that we will suggest best strategy for treatment option of malaria.

Procedures and what will be expected from you for participation

In order to perform the indicated study at Logia health center and Dubti Referral Hospital. you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected

by a principal investigator and nurses. Then, you are requested to give your consent to the sample collector. After consent, 5ml blood specimen will be collected from you by specimen collector and face to face interview for additional questions.

Potential risks and Discomforts

There will be minor discomfort during blood specimen collection. During collection of specimen from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you**.**

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential**.**

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. But based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of malaria. Hence, you are indirectly benefiting other patients and the society in this respect**.**

Participation and Withdrawal from the Study

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free**.**

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information**.**

Adem Ebrahim **Phone:** 0913925201 **E-mail:** ademebrahim1999@gmail.com

10.2. Annex 2: Informed consent (English version)

Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project.

Name of the study participant ……

Code number…….

I have clearly been informed about the research project that it aims to evaluate of Estimation of Total Oxidative Stress and Non-enzymatic Antioxidant levels in malaria patients in Logia Dubti Area, Afar, Ethiopia. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the malaria disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and blood sample as the doctors find best for me.

Signature: _____ Date_____

10.3 Annex 3: Questionnaire (English version)

Thank you for your willingness to participate; your cooperation is very important to the success of the study. This is a questionnaire you are asked to fill out. Please answer the questions as frankly and accurately as possible. All information obtained in the study will be kept confidential.

1. Personal identification:
   a) Full name of the subject: _____
   b) Subject code number: _____
2. Demographic detail
   a) Age: _____
   b) Gender:

      Male ☐ Female ☐

   c) Region: _____

d) Marital status:

Single ☐ Married ☐ Divorce ☐

e) Educational level: Illiterate ☐ High School ☐ College and above ☐

f) Occupation _____

3. Residential area: Rural ☐ Urban ☐
4. Group: study ☐ Control ☐
5. Income in a month A. <500 Birr B. 500-1000 Birr C. >1000 Birr
6. Does your house hold have any of the following mass media? A. Radio B. Television C. Internet D. No E. Other specify_____
7. Body Mass Index:
   a) Weight (in Kg) _____
   b) Height (m)_____
   c) BMI_(Kg/m^2) _____
8. Have you seen or heard any education messages pertaining to malaria from any source in the past? A. Yes B. No
9. What is the most important thing in your family to prevent getting malaria? A. use a bed net B. use insecticide sprays C. take tablet D. keep the house and surrounding clean E. destroy mosquito breeding sites F. other specify_____
10. Is there insecticide treated bed net in the house? A. Yes B. No
11. Are they currently being used? A. Yes B. No
12. Reasons for not using the available ITNs A. Nets do not prevent malaria B. Afraid of its toxicity C. Other (specify) _____
13. Reasons for unavailability of ITNs A. Not available B. Lost/stolen C. Used for other purposes D. Old; then thrown away F. Other (specify) _____
14. Main material of the Room's Roof? A. Thatch B. Corrugated Iron Sheet C. Other specify _____
14. Main material of the Room's Floor? A. Earth B. Local dung plasters C. Other specify_____

15. Main material of the Room's Wall? A. Mud blocks B. Sticks D. Corrugated Metal E. sticks and mud F. Other specify___
16. Is there any mosquito breeding habitat around the village? A. Yes B. No—
17. If yes, distance of the house from mosquito breeding habitat?

A. <1000 m B.1000m-2000 m C.>2000 m D. Other specify_____

18. What is the main source of drinking water for members of your household? A. Spring B. Dug Well C. Surface Water D. Public Tap/Standpipe E. Other specify_____
19. What kind of toilet facilities does your household use? A. No facility Bush/Field B. Pit Latrine (no cement slab) C. Other specify_____

For physician or clinician use only

Severity of malaria and percent in the body

o Low (<1000): _____

o Moderate (1000-10,000) _____

o Severe (>10,000) _____

20. If any underlying disease (specify)_____

10.4. Annex 4: Information sheet (Amharic version)

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

የጥናቱ ርዕስ Estimation of Total Oxidative Stress and Non-Enzymatic Antioxidant levels in malaria patients in Logia Dubti Area, Afar, Ethiopia.

እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቀሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ ኣይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን ኣይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም ኣይነት የከፋ ችግር አያጋጥምዎትም፡፡ ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል፡፡ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

ህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኝው ውጤት ግን ተጠቃሚ ነዎት፡፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን malaria disease ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ኣይነት የሆስፒታል አገልግሎት አይኖርም ፡፡ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም ኣይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

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10.5. Annex 5: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብ የተፈለኩት በዚህ ጥናት እንድሳተፍ ሲሆን የደም ግፊት ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠንና የደም ህዋሶች እንዲሁም ሌሎች ከደም ግፊት ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት የሚለው ጥናት አላማና ጥቅም ተገልፆልኛል፡፡ ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባክን እንደሚያዝም ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡ ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ፡፡

በተጨማሪም የምሰጠዉ የደም ናሙና ለTotal Oxidative Stress, Total Antioxidant Capacity, Uric Acid Bilirubin እና Albumin ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገርየመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለህኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤአለሁ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ

አረጋግጣለሁ፡፡

እኔ_____የተባልኩት ግለሰብ ይህን ሁሉ

በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ ቀን

ተሳታፊ _____ _____

10.6. Annex 6: Amharic questionnaire

የወባ ጠቋሚ ጥናት

የቤተሰብ መረጃ መሰብሰቢያ ቅጽ ቁጥር

መጠይቅ ክፍሌ አንድ፣ የቤተሰብ ሁኔታ

መኖሪያ አካባቢ ቀበላ

1. የቤት ቁጥር……. እድሜ…… ጾታ……. ክልል…….

2. የጋብቻ ሁኔታ ሀ. ያላገባ ለ.ያገባ ሐ. የፈታ/ች መ. ባለቤቷ/ቱ በህይወት የለሌ

3. የቤተሰብ ኃላፊ የትምህርት ደረጃ ሀ. ያልተማረ ለ. መጻፍና ማንበብ የሚችሌ ሐ. የመጀመሪያ ደረጃ መ. ሁለተኛ ደረጃ አና ከዛ በላይ

4. የቤተሰብ የስራ ሁኔታ ሀ. የመንግስት ሰራተኛ ለ. አርሶ አደር ሐ. ነጋዴ መ.ሌላ (ይግለጹ)

5. የቤተሰብ የወር ገቢ ሀ. ከ500 ብር ያነሰ ለ. ከ500-1000 ሐ. ከ1000 ብር የበለጠ

6. የመኖሪያ ቦታ ሀ. ከተማ ለ. ገጠር

7. የሰውነት ክብደት ልኬት (ኪ.ግ./ሜ2)……

8. ክብደት በኪሎ ግራም…….

9. በቤትዎ ውስጥ ከዚህ በታች ከተዘረዘሩት የመገናኛ ብዙሃን የትኞቹ አለ? ሀ. ሬድዮ ለ. ቴላቭዥን ሐ. ኢንተርኔት መ. የለም ሠ. ሌላ (ይግለጹ)

10. ከዚህ በፊት ስለወባ እና ስለሚያስከትሇው ችግር ትምህርታዊ መሌዕክት ሰምተው ወይም አይተው ያውቃለ? ሀ. አዎ ለ. የለም

11. ቤተሰባችሁ በወባ እንዳይያዙ የምትከሊከለበት ዘዴ ምንድን ነው? ሀ. አጎበር መጠቀም ለ. ፀረ-ነፍሳት ፍሉት መርጨት ሐ. ኪኒን መዋጥ መ. ቤትና አካባቢን በንጽህና መያዝ ሠ. የተጠራቀመ ውሃን መጥረግ

12. በቤትዎ ውስጥ አጎበር አለ? ሀ. አዎ ለ. የለም

13. በአሁኑ ሰዓት አጎበሩን ትጠቀማላችሁ? ሀ. አዎ ለ. የለም

14. አጎበር ካለዎ የማይጠቀሙበትን ምክንያት ይግለጹ? ሀ. አጎበር ወባን አይከላከልም ለ. መርዙን ስለምንፈራ ሐ. ሌላ

15. የትንኝ መከሊከያ አጎበር ከለሌዎት ምክንያቱ ምንድን ነው? ሀ. አጎበር የለም ለ. ጠፍቶናል ሐ. ለሌላ ጥቅም አውለዋለው መ. አሮጌ ስለሆነ ጥለነዋል ሠ.ሌላ (ይግለጹ)

16. የቤትዎ ጣሪያ በዋናነት ከምንድን ነው የተሰራው? ሀ. ከሣር ለ. ከቆርቆሮ ሐ. ሌላ (ይግለጹ)

17. የቤትዎ ወለል በዋናነት ከምንድን ነው የተሰራው? ሀ. ከአፈር ለ. በእበት የተለቀለቀ ሐ. ሌላ (ይግለጹ)

18. የቤትዎ ግድግዳ በዋናነት ከምንድን ነው የተሰራው? ሀ. ከጭቃ ጡብ ለ. ከእንጨት ሐ. ከቆርቆሮ መ. ከእንጨትና ከጭቃ ሠ. ሌላ (ይግለጹ)

19. በአካባቢያችሁ ለወባ ትንኝ መራቢያ አመች የሚሆን ቦታ አለ?

ሀ. አዎ ለ. የለም

20. መልሱ አዎ ከሆነ ቦታው ከቤትዎ በምን ያህል ይርቃል ? ሀ. < 1000 ሜትር ለ. 1000-2000 ሜትር ሐ. > 2000 ሜትር መ. ሌላ (ይግለጹ)

21. ለቤተሰብዎ የመጠጥ ውሃ በዋናነት የሚያገኙ ከየት ነው? ሀ. ምንጭ ለ. የጉድጓድ ውሃ ሐ. የከርሠ- ምድር ውሃ (ወንዝ፣ ኩሬ ወዘተ..) መ. ቦኖ ውሃ ሠ. ሌላ ይግለጹ

22. ቤተሰብዎ የሚጠቀሙበት የመጸዳጃ ቤት አይነት? ሀ. መጸዳጃ ቤት የለም (ጫካ/ሜዳ ላይ ነው) ለ. መፀዳጃ ጉድጓድ ሲሚንቶ የሌለው ወለል ሐ. ሌላ (ይግለጹ).

10.7. Annex 7: Information sheet (Afaregna version)

Edde yangleh yan marih fayxi kee caabinni cibta

Addis Ababah yuniversti Qaafiyat saynisih kolloji hikkiminna baayo kimistri Barittoh exxal Mastiret xigri Barteenit daqayso kusaaqat yangaloonuh Arciba geya sin mayaanh takusaaqat tangaleenimik Afal Tohaak gubal yanih yan qasir cubbusak kawisa siin qaddoowe wayte mabla tenek esserita.

Takusaqak qasir qasir **Estimation of total oxidative stress and non-enzymatic antioxidant leyels in malaria patients in logia, Dubti Area Afar, Ethiopia.**

Toh kee isin takusaaqal geytimak faxxiima mara akkuk doorimten isin takusaaqat liton gabah Agle inkih meqem Abittoh gabah agle kinni

Ta kusaaqat tangalepenim hinnammay Angale waytaanam madaqteenik lakal Soolissaanamah ta hospitaalal yamcawweh yan Ayfaaf masoola hinnammay mayaggiriqqa.

Ta kusaaqat tangaleenim madqteenik abteenih tannin bica tama bici cibtal kutbeh hinnammay gili warah daffessaanam faxximta.

Ta kusaaqat yangaloonuh faxximtam macaay?

Ta kusaaqat t yangaloonuh fayxi yeelleenik qablinaamuna Beyak takusaqaloonuh fayxi yeelleenik qablinaamuna Beyak takusaqat t akah Assannaah bictakah Assannaah bictaanam siinik faxximta beenih yanin qablal geytimtah yan xaloot

Ta hospitaalak irol geytimah yan moray ta taamat fantaaxaw leh yanih tan marah warsaanamah sade waytaanamal isinni geysissaanam faxximta

Takkay ikkah ta qaynatih oyti sin kinnaane Qaddossah tanih tan ceelalloh migaaqaay Adreesiy kee silki nebrooy tanna tannah tannin tan oytitte edde motana kaloh ta aytaafah dubuk nafqi yaceeh yan sin elle yaaxigeenih yan bedunebro nafqil akah Asah yan innah Akkele Tahak assitinah sin qaafiyat wagsiisaak inkiniki esseroh gacsa yaceenimi

Ta kusaaqat yangaleenih yaniinim katassah tan taqabitte macaa kee macaay?

Qabli naamana beyaanah yan waqdi kaxxa taqabih ta sinaamal mabahta takkay ikkah qabala sinaamak beyan waqdi daguu biyakah biyaakitaanah

Qabli naamana beyaanah yan waqdi kaxxa taqabih ta sinaamal mabahta takkay ikkah qabala sinaamak beyan waqdi daguu biyakah biyaakitaanah

Takkay ikkah qabli naamunah koboxah abak Raagle mehratleela Akah haynaamih sabbatah taxxiimah yanih yan cubbi Akah haynaamih sabbatah biyak mayaalla.

Dayli Oyti Sarri Dacrisok elle Sugam Mannaay?

Sinni wagsiisaak teceenih tannin faxe qaynatih Oyti kee Beenih Yanin Qabali naamunaa kee laboratoori xaloot elle asam Aiqam Ta kusaaqih Caagid Dubuk kinni ta cibto geytam dubuk ta Caagid wagtah yan mara Tahaak bisol Sin Wagsiisaak Taxe faynatih Baxaabaxsale sirri qangarle tan kompiitewa oyticibtih Addat Akah Daffayonnah Akkeele.

Ta kusaaqal yongaleenim geysisah yan nafqi macaay?

Ta kusaq mastres xigrih daqaysimo kinniimih sabbatah takusaqat tengeleenimih sabbatah lakqo gee waytaanam Faxeemimmay ta kusaaqak geytimah yan nafqit Ahlih qaso biyak yaaxigeenim kee kattaataanamah faxxa nafqile.

Ta kusaaqat tangaleenimih gar macaa kee macaay?

Ta kusaaqat tangaleenimih gar inki katuuk sinim kinniimih sabbatah faxxen saaqat soolissaanam xiqtaanah tohuuk toturak isin ta taama soolisseenimih sabbatah siinik Raaqah yan Hospital ayfaaq mayan Tahaak Ossitinah Ta kusaq wagsiisaak faxe qaynatih essero essertaanam kee Addaffakoot siinih Abanamih garliton.

Laboratoor fakkaaqah xaloot meklamaleh geytaanam xiqtaanah Takkay ikkah isin teceenih taninoyti tataqbik fidnaane waasoonuh lowsiisoonuh nafqileemih sabbatah sin esseraanah yanin essero gitah yanih yan gacsa taceenim koxxa massakaxxa luk siinil esserna

Essero ellek hinnamnlay taqabi yot boodek maca abam faxximtaah?

Takusaq wagsiisaak hinnammay takusaq ceelah yan sin matrah yan taqabik hinnammay essero teeleenik tahaak gubal tablen Adreesil neh rubtaanam xiqtaanah.

Adam Ibraahim

Mobaayil 0913925201

IImeel ademebrahim1999@gmail.com

10.8. Annex 8: Informed consent (Afaregna version)

Edde yengeleh yan mariedde bicem diggoosah yan cibta

Cuumi nebro ----------

Edde yengeleh yan numih migaq ----------

Anu migaq Ahaak dagal kak xaggiimeh yan num kinnuk edde Akah engelem takusaaqat gabah Agle haysita ggidi kinnuk qabli manga leh tan dayla beya

Qablak Addal keenik tan subac manga kee Qabli Rimiidaa kee tonnah kaadn gersi qabli mangalluk Angaaraw leh tancaagiida Caddoo kee Caalat miizaanisaanaah intah tan kusaq wadba yoh qaddouteh Tonna kinnuk kusaq kee oytaay edde Biceemin Aceehan xagana Akah Aceeh anim. Takusaqak ugut kee nafqi Asmatuk inni tayxiik kinni ta esseril Aceeh anihan oytii finqitewaay

Qagittak aben kusaqih Elle angaal waam Faxek Cakly Dacarissak LakinFexe udur kusaqa Isin Abta tan margaqak edde anuk Cakly kinnim kee kusaqak Yawqenim wali Calwaay kataase

waytam Elle Faximtannal tah qaddowteh takkay Immay Caagida /gexsit Elle Faximanal kusaqiseemih taagah In Fayxik fukaq yangaalenimit Emeete.

Qagittak Yeceen Qbaali Fakaqo Tittat **total Oxidative stress, total anti-oxidant Capacity, uric acid, bilirubin** kee **Albumin** Fukaqo dubuk Elle Asannal Walaalal Itta geyne. Fexe Caagidiy yoo cule Wee Essernamin Samtoh yeeceenim kee Faxah An Afat Gacassa akak egeeh Qagitak Inkih tan Labraatori fokaqo Dalotle uddurt Daylaabel Yooh Yaceenim kee dalot Yaxigenim Faxek Geeyam Xiqam yo Warsenih. Amolladih Anu Caabinni cibtal xaggimteh tanim Inkih elle Faxximtannal kowiseh finniimih Sabbatah ta kusaaqat Angaluh waral figgoosa

Anu ----------Cubbussa heeh ta kusaqal yioytaakee. naamuna Acayuh edde Bicih

Wara Ayro

Edde yengele num ----------

10.9. Annex 9: Afaregna questionnaire

Qaso Yaybulluge kusaqa

Buxammarih oyta edde kobxisan cibtak Ixxima Esseriyyak inik haytoh exxal buxamarih caalat guubah elle yanin Arac (Awda)

1. Qariloowa ---------- karma ---------- Nado ---------- Rakaakay ----------
2. Rihim Caalat

   A. Degbeweenum B. Cabtentu / Cabtento

   C. Barra kak Rubte num / Baqlikak D. Baqlikak Rabe barra
3. Buxah Abbak Baritto Caddo

   A. ma bartinna B. Kawisah yak tube

   C. Qimboh Caddo D. 2hayto Caddo kee tohuuk Dage
4. Buxa Marih taama

   A. Doolat taama abeena B. Buqre abeena

   C. Kabxa Abeena D. gersim tekkek ersim tekkek (Qaddos)
5. Buxa mari Alsi culenta

   A. 500 biir Adda B. 500 – 1000 C. 1000 biir Daga
6. Siga buxa A. Magiaala B. Barri

7. Nabsi (Dagar) Quuki Yakkeh Gede (k.g/Miter^2)

8. Qilsa k.g ----------

9. buxah Adda taahak lakal tanimik maca tan?

A. Radiyo B. Televizion

C. internet D. gasamali E. gersim Qados

10 Ahaak dumal Qaso kee kataysa taqabittek Baritto Farmo toobbeh Aro tu – blehtaaxige?

A. Yeey B. Baleey

11. buxa mari Qaso 11. buxa mari Qaso BiyaaBiyaaBiyaakak edde kalahimta cagda Macaay?

A. kanina yanxuqeenim B. Agooberit yantifeqeenih

C. finit caxi D. buxa kee Dariifin Saytunaane Daarisaanama

E. keebimte qanan lee cax

12. buxah Addal Agoober yani?

A. Yeey B. Matan

13. Away Agooberit tantifiqeeni?

A. Yeey B. Matan

14. Agoober telleenik maca edde matan fiqen?

A. Agober Qaso Mawassa B. Summi mmeysitna C. Gersiimi

15. Qaso kalaluh agoober Alle wayteenik sabab macaay?

A. Agoober Mannu B. Beyteh

C. Gersi tu xxiiqil Asisna D. idooliteemih sabbatah Qidneh

E. Gersim (Qaddos)

16. Qarik Amo macay xissimteh tan

A. Qanshok B. Korkorrek C. Gersim tekkek (Qaddos)

17. Qari Baax macak xissiimeh yan

A. Bullaaqek B. gudeqtuk C. gersim tekkek (Qaddos)

18. Qari gidaar macak xissiimeh yan?

A. kauaytu B. Bocok (Caxxar) C. korkorrek

D. Caxxa kee kallaytuk E. Gersimtekkek (Qaddas)

19. sin Dariifal Qasoh Andoorrah Bica le Arac yani?

A. Yeey B. Mayan

20. Gacsi yey tekkek Arac Buxak magideh deddale?

A. < 1000 mtir B. 1000 – 2000 mitir C 2000 mitir gexsim tenek

21 Buxa marah maqub lee akak geyam an keey?

A. Buyyi B. Qeela lee C. Baaxok tewqelee daqaara

D. Dora E. gexsim tenek

22. Buxa mari edde yantifiqe daaco Buxah qaynat?

A. Daaco Buxa matanaa garbo, booxal Aban

B. Daaco Buxa saminto mahi

C. Gexsim tenek

10.10. Annex 10: Declaration

I declare that this research paper entitled: Estimation of Total Oxidative Stress and Non-Enzymatic Antioxidant levels in malaria patients in Logia Dubti Area, Afar, Ethiopia**, 2017/2018** is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research have duly been acknowledged.

Adem Ebrahim

Signature_____

Date_____