DETERMINATION OF TOLL LIKE RECEPTOR-2 LEVEL AND OTHER NON-SPECIFIC IMMUNE PARAMETERS ASSOCIATED WITH *PLASMODIUM* INFECTION IN PATIENTS ATTENDING SELECTED HOSPITALS IN ZARIA, NIGERIA.

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JULY, 2023

DECLARATION

I declare that the work in this dissertation entitled "**DETERMINATION OF TOLL LIKE RECEPTOR-2 LEVEL AND OTHER NON-SPECIFIC IMMUNE PARAMETERS ASSOCIATED WITH *PLASMODIUM*INFECTION IN PATIENTS ATTENDING SELECTED HOSPITALS IN ZARIA, NIGERIA**" has been performed by me in the Department of Microbiology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

BALA USMAN ISA _____ _____

Signature Date

CERTIFICATION

This dissertation entitled "**DETERMINATION OF TOLL LIKE RECEPTOR-2 LEVEL AND OTHER NON-SPECIFIC IMMUNE PARAMETERS ASSOCIATED WITH *PLASMODIUM*INFECTION IN PATIENTS ATTENDING SELECTED HOSPITALS IN ZARIA, NIGERIA.**" by **BALA USMAN ISA**meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my parents; Mal. Bala Yahya Usman and Hajiya Zainab Kamaru Idris.

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ABSTRACT

Invading malaria parasites are recognized by TLR2 and engulfed by the innate immune cells. Malaria is a blood disease caused by the bite of female mosquito and accounts for more than one million deaths every year. The aim of this study was to determine TLR2 level and other non-specific innate immune parameters in patients with *Plasmodium* infection inselected hospitals in Zaria, Kaduna State, Nigeria. A hundred and eighty (180) bloodsamples were collected and analyzed.Malaria parasite antigen was detected using Rapid Diagnostic Test (Biosensor and Encode) while malaria parasite density,differential blood count and total white blood count (TWBC) were determined microscopically.The level of TLR2 concentration was determined using ELISA (Fine test).The overall prevalence of *Plasmodium* infection recorded was 17.7%. The mean and standard deviation of parasite density obtained from this study were 1878.94±289 parasite/µl. The mean concentrations of TLR2 recorded in malaria parasite positive and negative patients were 0.662ng/ml and 0.566ng/ml respectively. There was an inverse correlation of-0.149 between parasitemia and TLR2 which implies that an increase in parasitemia leads to a decrease in TLR2 level and vice versa. In this study, only the difference between PCV and TWBC in malaria parasite positive and negative patients were statistically significant ($p<0.05$). Age group of ≤10, herbal treatment for malaria and non-use of treated bed nets were factors associated with *Plasmodium* infection($p<0.05$). This research has shown that TLR2 and other non-specific innate immune cells could help in suppressing the level of *Plasmodium* infection in the body.

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ABBREVIATIONS

%	Percentage
CDC	Center for Disease Control and Prevention
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PRR	Pathogen recognition receptor
PAMP	Pathogen associated molecular pattern
DAMP	Danger associated molecular pattern
IL6	Interleukine6
TNF	Tumor necrosis factor
IFN	Interferons
MSP	Merozoite surface protein
AMP	Antimicrobial peptides
RBC	Red blood cell
TWBC	Total white blood cell
RDT	Rapid diagnostic test
CD	Cluster of differentiation
TCR	T-cell receptor
BCR	B-cell receptor
APC	Antigen presenting cells
PCV	Pack cell volume

EDTA	Ethylene diamine tetra acetic acid
NK	Natural killer
DC	Dendritic cells
TLR	Toll like receptor
CLR	C-type lectin receptor
NLR	NOD-like receptor
RLR	ROD-like receptor
GPI	Glycosylphosphatidylinositol
g	gram
hr	hour
mL	Milliliter
$^{\circ}C$	degree celsius
rpm	Revolution per minute
WHO	World Health Organization
μL	microliter
MHC	Major histocompactibility complex

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND TO THE STUDY

Malaria is one of the devastating diseases of human history. In many developing countries,malaria remains a significant health burden with around one million deaths per year, affectingmostlychildren under the age of five (Snow *et al*.,2005; Murray *et al*.,2012). *Plasmodium*, the causative agent of malaria, is an intracellular protozoan parasite, belonging to the genus *Plasmodium* and is transmitted by the bite of an infectedfemale *Anopheles* mosquito. Malaria is categorized into human and non-human malaria. The human malaria is caused by four species of *Plasmodium: P. falciparum,P. vivax, P. ovale, P. malariae,* with *P. falciparum* being the deadliest, causing cerebral malaria andanaemia mostly in sub-Saharan Africawhile the non-human malaria is caused by *P. knowlesi*, which is found in Macaques(Singh *et al.*,2004).

The immune system refers to a collection of cells,chemicals and processes that function to protect theskin, respiratory passages, intestinal tract and otherareas from foreign antigens (non-self) such as microbes (organismssuch as bacteria, fungi, and parasites), viruses, cancercells, and toxins. Beyond the structural and chemicalbarriers which protect hosts from infection, the immunesystem can be simplistically viewed as having two “linesof defense”: innate immunity and adaptive immunity.Innate immunity represents the first line of defense to anintruding pathogen. It is an antigen-independent (nonspecific)defense mechanism that is used by the hostimmediately or within hours of encountering an antigen.

The innate immune response has no immunologicmemory and, therefore, it is unable to recognize or“memorize” the same pathogen should the body beexposed to it in the future. Adaptive immunity on theother hand, is antigen-dependent and antigen-specific and therefore,

involves a lag time between exposureto the antigen and maximal response. The hallmark ofadaptive immunity is the capacity for memory whichenables the host to mount a more rapid and efficientimmune response upon subsequent exposure to the antigen (Turvey and Broide, 2010; Bonilla and Oettgen, 2010).

GPI (glycosylphosphatidylinositol) anchors link most surface proteins to the protozoan plasma membrane and are therefore essential for parasite viability, importantly, protozoan GPI anchors are potent stimulators of cytokine synthesis by macrophages. GPI anchors from *P. falciparum*merozoites have either two or three fatty acyl chains thattrigger the phosphorylation of mitogen-activated protein kinases and inhibition of nuclear Factor-Kb (NF-Kb) family members through the activation of TLR-2 (Ricardo *et al*., 2014).

Toll-like receptors (TLRs) are a classof proteins that play a key role in the innate immune system. TLRs received their names from their similarity to the proteinencoded by the tollgene in *Drosophila.* They are a family of pathogenrecognition receptors (PRR) that recognize Pathogen-Associated MolecularPatterns (PAMPs). PRR are classified into four families: TLRs, NOD-like receptors (NLRs), retinoic acid-inducible gene (RIG)-like receptors (RLRs) and C-type lectin receptors (CLRs) (Kumagay and Akira, 2010). Only 10 TLRs have been reported in humans. They include; TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and TLR10 while TLR11, TLR12 and TLR13 are found in mice(Medzhitov, 2001). TLRs 1, 2, 4, 5 and 6 are expressed on the cell surface, while TLRs 3, 7, 8, 9 and 10 are found at the cytoplasm. The main interactions of TLRs and their ligands are: TLR1/TLR2 recognize triacylated lipopeptides, TLR3 binds double stranded (dsRNA), TLR4 recognizes lipopolysaccharides(LPS), TLR5 binds flagellin, TLR2/TLR6 binds diacylated lipopeptides and lipoteichoic acid (LTA), TLR7 and

TLR8 recognizes single-strand (ssRNA) and TLR9 recognizes DNA and hemozoin (Shimada *et al*., 2012)

Toll-like receptor-2 is a membrane protein, a receptor, which is expressed on the surface of certain cells and recognizes foreign substances and passes on appropriate signals to the cells of the innate immune system. As a membrane protein, TLR-2 recognizes organisms and certain endogenous substances which result in the uptake of bound molecules by endosomes and in cellular activation; thus, such parameters of innate immunity such as monocytes, neutrophils, eosinophils and dendritic cells assume function of non-specific immune defense. The role played by TLR-2in the detection, recognition, activation of the innate immunity and the generationof an appropriate immune response to infection has not been well studied. So therefore, TLR-2 contribution to malaria associated pathogenesis has been difficult to determine andis a continued area of intense research (Zheng, 2015).

1.1 STATEMENT OF RESEARCHPROBLEM

Plasmodium infections continue to cause significant morbidity and mortality for residents of and travelers to endemic areas. This protozoan parasite is transmitted through the bite of an *Anopheles* mosquito and remains an important public health threat. Malaria occurs mostly in poor tropical and subtropical areas of the world (CDC,2021). In 2020, there was an estimated 241 million cases of malaria worldwide with an estimated death of 627 000. Africa was home to 95% of malaria cases and 96% of malaria death in 2020 and Nigeria accounts for 27% of those cases. Children under 5 accounts for about 80% of all malaria deaths in the region (WHO, 2021).

Most of the pathology of malaria is immune mediated, yet our knowledge of the regulation of innate immune responses during *Plasmodium* infection is poor. The mechanism by which the innate immunity and some of it cells (monocytes, neutrophil, eosinophil) work is not well

understood. The parasite density level plays a major role in pathology of *Plasmodium* infection and the role Toll-like receptors play in its regulation is not well understood. The search for vaccine has been focusing on the adaptive immunity showing less interest in the innate immunity.

1.2 JUSTIFICATION

As the quest for malaria vaccine which focuses on the adaptive immunity continues, this study has helped to bring to fore the possible involvement of the innate immune system in the recognition and elimination of the malaria parasite. It becomes very important in the absence of vaccines to develop an alternative from the innate immune systemhence exploring the role of Toll-like receptor-2 in the regulation of innate immune response in patients with *Plasmodium* infection. The study also established the role parasite density level plays in the pathology of *Plasmodium* infection. The study has helped increating awareness and research data on the role of innate immunity in the control of *Plasmodium* infection.

1.3 AIM OF STUDY

To determine toll like receptor-2 level and other non-specific innate immune parameters associated with *Plasmodium* infection in patients attending selected hospitals in Zaria, Nigeria.

1.4 OBJECTIVES OF THE STUDY

The objectives are to;

1. detect the presence of *Plasmodium*spp in the blood samples of the febrile patients attendingthe hospitals usingRDT kit.
2. determine the parasite density among participants harboring the parasite.
3. determine the level of TLR-2 in the blood samples of the study participants using ELISA kit.
4. determine the total, differential blood count and PCVof the study population.
5. determine the socio-demographic, risk factors and clinical features associated with the infectionusing questionnaires.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of the Immune System

The term “immunity” in a biologic context has historically referred to resistance to pathogens; however, the reaction to some noninfectious substances including harmless environmental molecules, tumors and even unaltered host components is considered form of immunity (allergy, tumor immunity and autoimmunity respectively). The collection of cells, tissues, organs and molecules that mediate this reaction is called the immune system. The most important physiological function of the immune system is to prevent and eradicate infections. The immune system is amazingly complex and is categorized into innate and adaptive immune system. It can recognize, keep memories and activate cells to clear up invading pathogens. The key to a healthy immune system is its remarkable ability to distinguish between the body’s own cells (self) and foreign cells (non-self). The body’s immune defenses normally coexist with cells that carry distinctive “self” marker molecules. But when they encounter “foreign” cells or organisms, they engulf them (Bonilla and Oettgen, 2010; Schroeder and Cavacini, 2010).

The immune system uses many potenteffector mechanisms to have the ability to destroy a broad range of microbial cells and to clear a broad range of both toxic and allergenic substances. Therefore, it is critical that immune response is able to avoid unleashing these destructive mechanisms against the mammalian host’s tissues. The ability of the immune system to avoid damaging self-tissues is referred to as self-tolerance. Failure of self-tolerance by the immune system causes autoimmune diseases (Bonilla and Oettgen, 2010; Schroeder and Cavacini, 2010).

2.2 Non-specific (Innate) Immunity

Innate immunity can be viewed as comprising fourtypes of defensive barriers: anatomic (skin and mucousmembrane), physiologic (temperature, low pH andchemical mediators), endocytic and phagocytic, andinflammatory. Innate immunity to pathogens relieson pattern recognition receptors (PRRs) which allowa limited range of immune cells to detect and respondrapidly to a wide range of pathogens that share commonstructures, known as pathogen associated molecular patterns (PAMPs) (Murphy *et al*., 2007; Turvey and Broide, 2010).

An important function of innate immunity is therapid recruitment of immune cells to sites of infectionand inflammation through the production of cytokinesand chemokines (small proteins involved in cell–cell communication and recruitment). Cytokineproduction during innate immunity mobilizes manydefense mechanisms throughout the body while alsoactivating local cellular responses to infection or injury.Key inflammatory cytokines released during the earlyresponse to bacterial infection are: tumor necrosisfactor (TNF), interleukin 1 (IL-1) and interleukin 6(IL-6). These cytokines are critical for initiating cellrecruitment and the local inflammation which isessential for clearance of many pathogens. They alsocontribute to the development of fever. Dysregulatedproduction of such inflammatory cytokines is oftenassociated with inflammatory or autoimmune disease,making them important therapeutic targets

(Murphy *et al*., 2007; Turvey and Broide, 2010)

The complement system is a biochemical cascade thatfunctions to identify and opsonize (coat) bacteria andother pathogens. It renders pathogens susceptible tophagocytosis, a process by which immune cells engulfmicrobes and remove cell debris, and also kills somepathogens and infected cells directly. The phagocyticaction of the innate immune response promotesclearance of dead cells or antibody complexes andremoves foreign substances present in organs, tissues,blood and lymph. It can also activate the adaptiveimmune response through the mobilization andactivation

of antigen-presenting cells (APCs) (Murphy *et al*., 2007; Turvey and Broide, 2010).Numerous cells are involved in the innate immuneresponse such as phagocytes (macrophages andneutrophils), dendritic cells, mast cells, basophils,eosinophils, natural killer (NK) cells.

2.3 Specific (Adaptive) Immunity.

The development of adaptive immunity is aided by theactions of the innate immune system and is criticalwhen innate immunity is ineffective in eliminatinginfectious agents. The primary functions of the adaptiveimmune response are: the recognition of specific "non-self"antigens, distinguishing them from "self" antigens,the generation of pathogen-specific immunologiceffector pathways that eliminate specific pathogensor pathogen-infected cells and the development ofan immunologic memory that can quickly eliminate aspecific pathogen should subsequent infections occur.Adaptive immune responses are the basis for effectiveimmunization against infectious diseases. The cells of theadaptive immune system include: antigen-specific T cells,which are activated to proliferate through the action ofAPCs, and B cells which differentiate into plasma cells toproduce antibodies (Bonilla and Oettgen, 2010).

2.4Contrasting Innate and Adaptive Immunity

Differences between the innate and adaptive immune systems can be seen on anumber of levels. The adaptive immune system is organized around twoclasses of cells: T cells and B cells, while the cells of the innate immune system aremuch more numerous, including natural killer (NK) cells, dendritic cells (DCs) and macrophages. The receptors of innate system cells are entirely germlineencoded,in other words their structure is determined by the genome of thecell and has a fixed, genetically-determined specificity. Adaptive immune systemcells possess somatically generated variable-region receptors such as the TCR andBCR (T and B cell receptors) with

varying specificities, created by a complexprocess of gene segment rearrangement within the cell. On a population level,this leads to a non-clonal distribution of receptors on innate immune system cells,meaning that all cells of the same type have receptors with identical specificities.Receptors on adaptive immune system cells however, are distributed clonally inthat there are subpopulations of a specific cell type (clones) which all possessreceptors with identical specificities, but that generally, cells of the same typehave receptors with different specificities (Medzhitov and Janeway 2002; Janeway*et al.,* 2001).

The molecules which a receptor is able to recognize and bind with are knownas ligands. While all receptors at the most basic level recognize molecules, ligandsare often discussed in terms of higher-level structures. The variable-region receptorsof adaptive immunity recognize features of pathogen structure, with BCRsdirectly recognizing peptide sequences on pathogens, such as components of bacterialcell membranes, and TCRs recognizing peptide sequences which have firstbeen processed by DCs. These receptors are selected for over the lifetime of the organism by processes such as clonal expansion and deletion and are underadaptive not evolutionary pressure. Conversely, innate immune system receptorsrecognize a genetically-determined set of ligands under evolutionary pressure.One key group of innate receptors is the pattern recognition receptor (PRR)superfamily which recognizes evolutionary-conserved pathogen-associated molecularpatterns (PAMPs). PRRs do not recognize a specific feature of a specificpathogen as variable-region receptors do, instead recognize common featuresor products of an entire class of pathogens. The immune system utilizes adaptationof variable-region receptors to keep pace with evolutionary more rapidpathogens (Medzhitov and Jane 2002).The environment of a cell in vivo is the tissue in which it is located. Tissueis formed by specialized groups of differentiated cells, and itself forms majorcomponents of organs. A substantial part of tissue volume is extracellular

spaceand filled by a structured network of macromolecules called the extracellularmatrix. Many of the molecules found in the extracellular matrix are activelyproduced by cells and involved in intercellular signaling (Lodish*et al*., 1999; Albert *et al*., 2002).

Cytokines aresecreted molecules which mediate and regulate cell behavior, two importantsubsets of which are tissue factors, inflammation-associated molecules expressedby tissue cells in response to pathogen invasion, and chemokines, cytokines whichstimulate cell movement and activation. Cytokines bind to germline-encodedcytokine receptors present on all cells and are widely produced and consumedby both innate and adaptive immune system cells during an immune response.Recognition by the innate immune system leads to the immediate initiation ofcomplex networks of cytokine signaling which orchestrate the ensuing immuneresponse. Adaptive responses additionally involve processes of cell selection suchas clonal expansion, deletion and allergy, which take several days (Medzhitov and Jane 2002).

2.5 Pathogen Recognition Receptors (PRR)

Acritical first step in initiatingan innate immune response to infection is the sensing of thepathogens by host cells. This is mediated by the recognitionof specificmicrobial molecule by a limited array of dedicatedhost receptors. The microbial ligands corresponding toessential components of the pathogen are PAMPs and theircognate PRRs.PRRs are classifiedinto four main families: TLRs and CLRs, transmembraneMediators found in the plasma membrane, RLR and NLR proteins located in intracellular compartment (Takeuchi and Akira, 2010).

2.5.1 Toll-like receptors (TLRs)

Toll-like receptors (TLRs) are a classof proteins that play a key role in the innate immune systemand received their name from their similarity to the proteinencoded by the *toll* gene in

Drosophila (Hashimoto *et al*., 1988). DrosophilaToll is involved in both embryonic development and theimmune response to fungi (Lemaitre *et al*., 1996; Hashimoto *et al*., 1988).

The TLRs include TLR1–TLR10 and TLR11–TLR13,though the latter three are not found in humans.TLRs are able to recognize a variety of PAMPs includinglipoproteins and di- triacyl lipopeptides (TLR2/1 andTLR2/6), peptidoglycan, lipoteichoic acid, fungal zymosan(TLR-2), double-stranded RNA (TLR-3), flagellin (TLR-5),unmethylated CpG DNA (TLR-9), and a variety of synthetic molecules such as imidazoquinolones and guanosineanalogues (TLR-8) (Kawai and Akira, 2011)

2.5.1.1 Toll-like receptor-2

Since the discovery of TLR-2, it has gained much attention due to its ability to identify diversified array of pathogenic ligands including parasites (Kawai and Akira, 2011). TLR-2 gene leads to the differential expression of TLR-2 with different affinity and specificity to pathogen associated molecular pattern (PAMP) which may be the reason behind resistance or susceptibility to infectious diseases (Kimbrell and Beutler, 2001). As a surface TLRs, TLR-2 shapes pathogen specific innate immunity through distinct ligand binding which in turn develops antigen specific acquired immunity as well. TLR-2 is best characterized for identifying invading pathogens outside the cell and also intracellular pathogen captured in endosomes or lysosomes (Park and Lee, 2013). They can sense PAMP from various infectious microorganisms. TLR-2 is expressed in various immune cells such as neutrophils, monocytes, macrophages and dendretic cells.

2.5.2 C-type lectin receptor (CLRs)

CLRs possess a transmembranePRR with a carbohydrate-binding domain. CLRs recognizecarbohydrates on pathogens and are mainly expressed bymonocytes, macrophages,

andDCs (Takeuchi and Akira, 2010). Pathogen recognitionby CLRs leads to pathogen internalization and degradationand subsequent antigen presentation. CLRs recognize mannose,fucose, and glucan carbohydrate structures present inbacterial, viral, and fungal components. They are crucialfor controlling both innate and adaptive immune responses.Some CLRs induce signaling pathways that modulate TLRinducedgene expression (Geijtenbeek and Gringhuis, 2009). Inflammatory responses are crucial in innate immunityagainst infectious disease, but the factors that determine thedominant cellular component have not been identified.

2.5.3 Nod-like receptors (NLRs)

The nucleotide-binding oligomerizationdomain receptors, in short NLRs, are intracellularsensors of PAMPs that enter the cell via phagocytosis or poresand of danger-associated molecular patterns (DAMPs) thatare associated with cell stress.They are pattern recognitionreceptors and play key roles in regulating the innate immuneresponse. NLRs can cooperate with TLRs and regulateinflammatory and apoptotic responses. They are found inlymphocytes, macrophages, andDCs and also in non-immunecells, for example, in epithelia. NLRs are characterized bytheir cytoplasmic location and the possession of a nucleotidebindingdomain (NBD), which is also emerging as an importantcomponent of the innate immune response (Schroder and Tschopp, 2010). NLRsconstitute a large family of intracellular PRRs, such as NOD1, NOD2, and NALP3.

2.5.4 RIG-like receptors (RLRs)

RLRs are intracellular receptors for RNA viruses (Akira *et al*., 2006; Kawai and Akira, 2009).Virus infection of mammaliancells triggers innate immune defenses through thePRRs for PAMPs within viral products that engage the intracellularsignaling pathways to initiate an antiviral response.Viral RNA is a potent inducer of this host response and isrecognized by specific TLRs or by cytoplasmic RNA helicases(Saito *et al.*, 2007). TheRLR family is composed

of at least 3 members: RIG-I,melanoma differentiation factor-5 (MDA5), and laboratoryof genetics and physiology-2 (LGP-2). Studies of humancells defective in RIG-I signaling orcells from micewith a targeted deletion of RIG-I orMDA5 have revealed a remarkable degree of specificity of virus recognition betweenthe individual helicases that could reflect differences in RNAbinding and PRR function. Recognition by RLRs activatesinnate antiviral responses, mainly through the rapid inductionof type I IFNs and inflammatory cytokines that limitviral replication and coordinate an antigen-specific, adaptiveimmune response (Akira *et al*., 2006; Kawai and Akira, 2009).

2.6 Pattern Associated Molecular Pattern (PAMP)

Pattern Associated Molecular Pattern (PAMP) are diverse and complex array of molecular components including protein, lipids, carbohydrate, DNA and RNA. PAMP possess the ability to activate Pathogen Recognition Receptor (PRR). The recognition of microbial molecules by PRR activates intracellular signal cascades which lead to the expression of anti-inflammatory cytokines (Takeuchi and Akira, 2010). *Plasmodium* PAMPs includes glycosylphosphatidylinositol (GPI) anchors and parasite nucleic acids.

2.6.1 GPI anchors

GPI anchors of *Plasmodiumfalciparum* are glycolipids that link several proteins to the surface of the merozoite plasma membrane which are needed for erythrocytes invasion, and thus GPI is essential to parasite survival (Gilson *et al*., 2006). GPI was the first component identified as a malarial PAMP and is abundantly expressed on the surface of the parasite both linked to proteins and in free form (Naik *et al*., 2000). GPI signaling in monocytes is mediated mainly through the recognition of TLR-2 heterodimers and to a lesser extent by TLR-4 heterodimers. Structural variations of*Plasmodiumfalciparum*GPIs determine differential binding by an auxiliary receptor

(TLR-1 or TLR-2) for recognition by TLR-2. GPIs with three fatty acyl groups are preferentially recognized by TLR2/TLR1 while GPIs with two fatty acyl groups are preferentially recognized by TLR2/TLR6 (Krishnegwoda*et al*., 2005)

2.7 Biology of *Plasmodium* species

There are over 200 species of *Plasmodium* that produce disease in non-human primates, reptiles, rodents and birds in addition to humans. About 30 species naturally infect non-human primates (monkey, great apes and others), while only five identified species *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodiummalariae and Plasmodiumknwolesi* causes malaria in humans (Singh *et al*., 2004).

2.7.1 *Plasmodiumfalciparum*

Among all the febrile illnesses, malaria is the deadliestworldwide, especially in the tropical nations of the world and accounted for the highest causes of fever in Nigeria (WHO, 2019). According to World Malaria Report by WHO (Akinlua*et al*., 2015) Nigeria accounted for the highest global malaria burden (25%), malarial death (24%) and 99.7% of the malaria cases are caused by *Plasmodium falciparum* (WHO, 2019). The trophozoite mature into schizont, divides into merozoites and the erythrocytes rupture and then releases the merozoites into the blood stream. Repeated cycles lead to exponential increase in parasite burden. These high levels of parasite burden are linked to the pathogenesis of *Plasmodiumfalciparum* which is characterized by haemolyticanaemia (Panichakul*et al*., 2007).

2.7.2 *Plasmodiumvivax*

Plasmodium vivax is the most geographically widespreadspecies of *Plasmodium* causing human disease, with mostcases reported in Central and Southeast Asia, in the hornof Africa and in Latin

America (Mendir*et al*., 2001). It is considered to bea potential cause of morbidity and mortality amongst the2.85 billion people living at risk of infection, excludingthe large African populations who are mostly Duffy negativeand, therefore, naturally less susceptible to this infection. Studies suggest that the parasite isevolving and may use alternative receptors other thanDuffy (DARC) for erythrocyte invasion (Mendes *et al*., 2011). It is estimatedthat 5.5% of the populations at risk live in the Americas (Guerra *et al*., 2010).

The major biological characteristic of this parasite is the presence of liver hypnozoites responsible for the frequentrelapses. This adds a substantial number of cases tothe general burden of the disease, and considered asone of the most challenging bottlenecks for vivax malaria eradication (Mueller *et al*., 2009).Although often regarded as causing a benign infection,there is increasing evidence that the overall burden,economic impact, and severity of *P. vivax* havebeen underestimated, in part due to a bias in the scientificliterature which traditionally devoted most of itsattention to the more lethal parasite *Plasmodium falciparum*,probably as a reflection of a more substantial funding toward the study (Price *et al*., 2007).

2.7.3 *Plasmodiumovale*

Plasmodium ovale has long been considered to have a dormant hypnozoite stage that can persist in the liver and cause relapses. *Plasmodium ovale* has a low prevalence and found mostly in Africa (especially West Africa) where it accounts for up to 10% of malaria (Cheesebrough, 2009). Interestingly,it has been demonstrated by molecular methodsthat *P. ovale*essentially consists of two distinct sympatricspecies termed *P. ovalecurtisi*and *P. ovalewallikeri*(Sutherland *et al*., 2010).

2.7.4 *Plasmodiummalariae*

Plasmodium malariae is distributed over most of the malaria endemic areas and is the only human malaria species that has quartian (72 hours) cycle (Lucci*et al*., 2010; Polley*et al*., 2013). *Plasmodium malariae* has a much lower prevalence than *P. falciparum* and *P.vivax.* It is found in tropical and subtropical regions. In tropical Africa, it accounts for up to 25% of *Plasmodium* infection (Cheesebrough, 2009). Untreated, *P. malariae* often causes a long-term chronic infection which often remains latent and may probably last a lifetime. Chronically infected patients with *P. malariae* could experience serious complications (Petel*et al*., 2014).

2.7.5*Plasmodium knowlesi*

Plasmodium knowlesi, originally known to cause simian malaria, is now recognizedas the fifth human malaria parasite (White, 2008). Human knowlesi infections have been reportedin nearly all the countries in Southeast Asia and in travelers returning from these countries (Singh and Daneshver, 2013; Yusof *et al*., 2014).It is not surprising that the geographical distributionof *P. Knowlesi*is confined to Southeast Asia since it follows the limits of natural distributionof both its natural hosts and vectors (Singh and Daneshver, 2013). It is a natural pathogen of long and pig tailed macaques i.e old world monkeys of genus *Macaca*(White, 2008). The pattern of knowlesi infectionin humans does not indicate that they are transmitted via human–mosquito–human (Kantele and Jokiranta, 2011; Lee *et al*., 2011; Imai *et al*., 2014) Several factors have led to an increased reporting of *P. knowlesi*cases. Theseinclude better diagnostic capacity, decreasing human malaria cases which in turn reduce relative immunity, increased awareness to *P. knowlesi*, and close proximityof humans with natural reservoir hosts or infected vectors due to changes in humanland use (Abesinghe, 2017).

2.7.6 Life cycle of *Plasmodium*

The natural history of malaria involves clinical infection of humans by female Anopheles mosquitoes. In human, the parasite grows and multiplies first in the liver and then in the red blood cells. In the blood, successive broods of parasites grow inside the red blood cells and destroys them, releasing daughter parasite (merozoites) that continue the cycle by invading other red blood cells.

2.7.6.1 Exo-erythrocytic Stage

The malaria parasite life cycle involves two hosts. During a blood meal, a *Plasmodium*infected female Anopheles mosquito inoculates sporozoites into human host. Within minutes of entering the blood circulation, Sporozoites infect liver cells, replicates and mature into liver schizonts. The sporozoite will develop to form trophozoite and then mature into tissue schizonts. Tissue schizonts rupture and release several infectious merozoites into the blood stream which invades red blood cells to initiate a new cycle. In *P. vivax* and *P. ovale*, a dormant stage (hypnozoite) can persist in the liver and cause relapse by invading the bloodstream for week or even years if untreated (CDC, 2020)

2.7.6.2 Erythrocytic Stage

In this stage, the parasite undergoes asexual multiplication in the merozoite infected red blood cells. The ring stage trophozoite mature into schizonts, which rupture releasing merozoites. Some parasite differentiate into gametocytes. The blood stage parasites are responsible for the clinical manifestation of the disease. The gametocyte, male (microgametocytes) and female (macrogametocytes), are ingested during blood mealby female Anopheles mosquito and begin a cycle of growth and multiplication in the mosquito. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become

motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocyst. The oocyst grows, rupture and release sporozoites, which make their way to the salivary gland of the mosquito and the cycle continues (CDC,2020).

2.8 Immune Response to Malaria

Malaria is a mosquito borne infectious disease of humans andother animals caused by parasitic protozoan of genus *Plasmodium*(WHO, 2014) causing huge number of deaths each year (Carabolla and King, 2014). The host responds during malaria infection by the expression of highlevels of many pro-inflammatory cytokines, including TNF, IL-1, IL-6, IL-12 and IFN-gamma that have important roles in controllingparasite growth (Punsawad, 2013).Such types of responses are primarilyoriginated from interaction of malaria parasite molecules andTLRs of host immune cells. The search for TLR activatingPAMPs from malarial parasites have been the major highlightedobjective for last few decades. In this connection,a glycolipid moiety, glycosyl phosphatidylinositol (GPI) from *P.falciparum* has been reported to be a ligand of TLR-2 (Gun *et al*., 2014). This malarialigand from infected erythrocytes interacts with the TLRs located on Macrophages or DCs and contribute to theimmunopathology of the infection by inducing the expressionof pro-inflammatory cytokines, such as TNF, IL-6 andIL-1. In addition, GPI anchored proteins, merozoite surfaceprotein 1 (MSP1) and MSP2, two major asexual blood stagesurface antigens have endotoxin activity that indeed resultsin decreasing frequency of the parasitic load from host (Gun *et al*., 2014).

2.8.1TLR-induced Anti-malaria Responses

TLRs are expressed by a wide range of immune cells, though arepredominantly present on innate cells. Consequently,malaria parasiteshave the potential to be targeted by multiple cell populations. However,a lot of focus has been on DC and other antigen presenting cells as thesecells have been reported in several studies to play an important roleduring *Plasmodium*infection, and are also crucial in shaping the subsequent adaptive immune response (Luyendyk*et al*.,2003; Leisewitz*et al*.,2004;Ing*et al*., 2006; Chua *et al*.,2013). Evidence suggests that TLRmediatedsignaling is required for DC maturation, cytokine production and upregulation of co-stimulatory molecules. Additionally, it hasalso been demonstrated that involvement of TLR4, TLR9, MyD88and NF-κB signaling, as well as cell-cell contact and internalization ofinfected RBC, are required for DC activation (Seixas*et al*., 2009). However, it appearsthat the DC cytokine profile progressively changes with infection. DCisolated early following infection produce pro-inflammatory cytokines,whereas DC isolated later during infection are anti-inflammatory IL-10-producing cells (Perry *et al*., 2005).

A distinct *Plasmodium*mediated enhancement of TLRresponsiveness in immune cells has also been described. Peripheralblood mononuclear cells of malaria naive individuals that wereexperimentally infected with *P. falciparum* exhibited increased pro-inflammatoryand anti-inflammatory cytokine production in responseto TLR4 and TLR2/TLR1 ligation ((Mc call *et al*., 2007). Similarly, acute infections inGhanaian schoolchildren and a Brazilian cohort both presented withaugmented reactivity to TLR stimulation (Hartgers*et al.*,2008; Franklin *et al*., 2009), although theseresponses included not only stimulation with TLR2 and TLR4 ligands,but were attributed to all TLR ligands (Franklin *et al*., 2009). Further findings of this studyindicated that IL-12, TLR9 and IFN-γ were contributing components toTLRhyperreactivity.

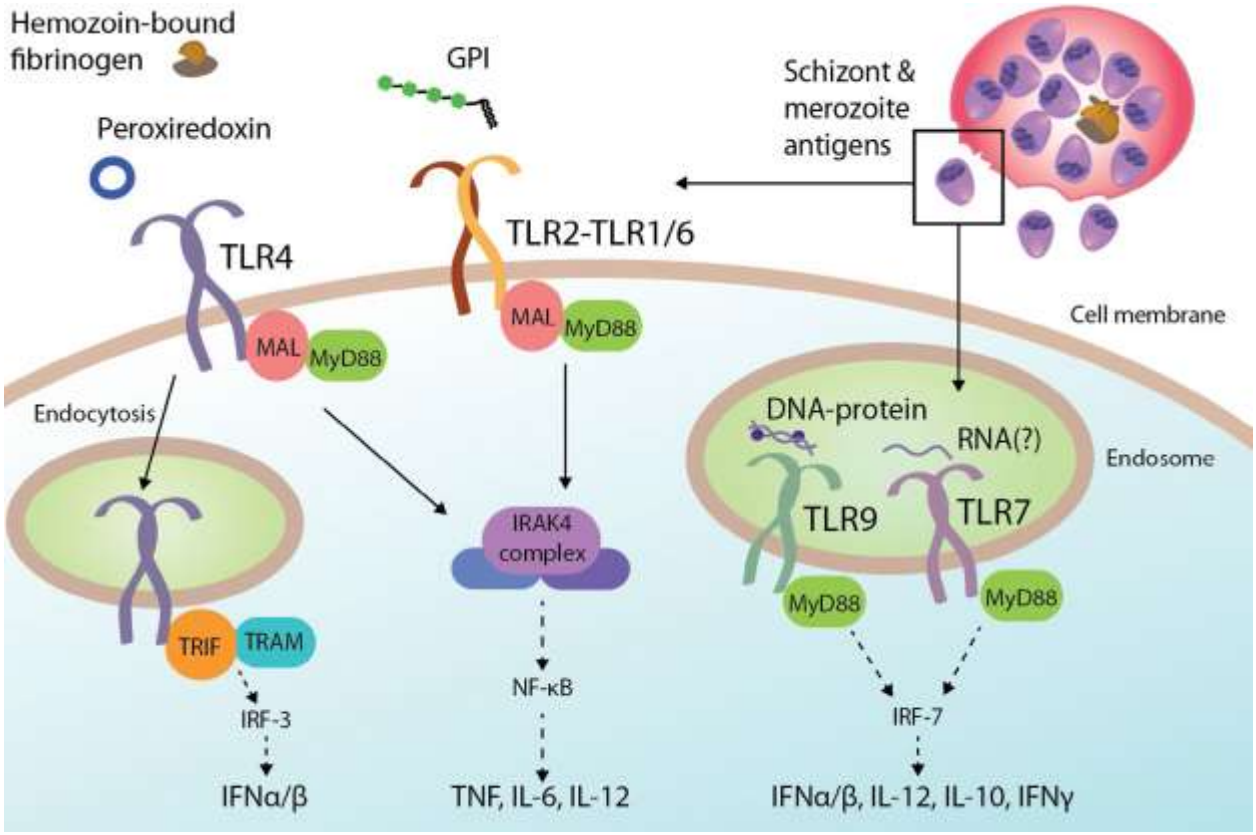


Fig 2: TLR detection of parasite antigen (Eriksson *et al*., 2013)

2.8.2TLR detection of parasite antigens

TLR2, TLR4 and TLR9, and most recently also TLR7, are activatedby *Plasmodium* antigens (Figure 2.2). The first demonstration thatparasite antigens could be detected by TLRs was by Campos *et al*. (2001) who showed that Glycosylphosphatidylinositol (GPI) anchors from*Trypanosoma cruzi*activate TLR2. A later study demonstrated thatTLR2 and, to a lesser extent, TLR4 are capable of detecting malarialGPI via a MyD88-dependent pathway, and that the TLR1/2 and TLR2/6dimers preferentially bind to distinct structural forms of GPI (Krishnegowda*et al*., 2005).

2.8.3 Innate immunity and malaria

Innate immunity as the first line of defense has evolved multiple evolutionary conserved germline-encoded pattern-recognition receptors (PRRs) to ensure detection and rapid response to a variety ofpathogens. These receptors include Toll-like receptors (TLRs), retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs) and nucleotide binding oligomerization domain (NOD)-like receptors (NLRs).These receptors activate distinct transcriptional programs and induce the synthesis of cytokines andchemokines not only to clear pathogens but also to induce the adaptive immune response. PRRsrecognize conserved Pathogen-associated molecular patterns (PAMPs), which distinguish foreignorganisms from self. PRRs reside in different cellular compartments. While TLRs detect PAMPs on thecell membrane of endosomes or the cell surface, RLRs and NLRs are cytosolic (Mogensen, 2009; Takeuchi *et al.*, 2010; Brubaker *et al*.,2015;Dolasia*et al*., 2018).

Innate immune responses begin in the early pre-symptomatic stages of plasmodial infection, whenthe parasitemia is still very low. At this stage, innate immunity genes such as TLRs, PRRs and manypro-inflammatory cytokines are already upregulated(Ockenhouse*et al*.,2006). Pro-inflammatory cytokines are upregulatedin the serum of mice infected with various *Plasmodium* species at days 5–9 of infection (Coban*et al*., 2007). Type I IFNis upregulated in the blood of malaria infected mice as early as 24–36 h post-infection(Spaulding *et al*., 2016).

The profile of the pro-inflammatory cytokine responses appears to correlate with thepathophysiological and clinical symptoms of malaria (Walther *et al.*, 2006). Thus, the innate immune response appears to occurearly and is apparently important in the ultimate outcome of the disease.Pathogen recognition byTLRs induces rapid activation of innate immunity by triggering the production of pro-inflammatorycytokines and the up-regulation of co-stimulatory molecules (Medzhitov, 2001). TLRs have beenreported to detect components derived from parasites such as *Plasmodium* (Campos *et al.*, 2001;Krishnegowda*et al*., 2005; Coban*et al*., 2005;Gazzinelli*et al*., 2006;Parroche*et al*., 2007). Years of studies onmalaria have shown a critical role for pro-inflammatory cytokines in pathogenesis of the disease (Hunt *et al*., 2003; Clark *et al*., 2006).

2.8.4 The roles of TLR signaling in innate immunity:

Microbial infection initiates TLR responses, and this interactionbetween TLRs and pathogen-associated molecularpatterns (PAMPs) results in the induction of an array ofantimicrobial immune responses. Various cytokines andchemokines, including TNF-α, cytokines of the IL-1 family(IL-1β, IL-18), IL-12, and IFN-γ, can be induced by therecognition of PAMPs by TLRs. The appropriate activationof these inflammatory cytokines and antimicrobial proteinsis required for the induction of host defense against diversemicrobial infection (Kumar *et al*., 2011).

TLR signaling pathways are tightly controlled to prevent excessive and uncontrolled inflammatory responses thatoften lead to deleterious pathogenesis with an increasedmortality rate. In TLR signaling, several negative regulatorsthat function through the prevention of ligand-receptorbinding, degradation of the target protein, and inhibition ofrecruitment or transcription of intermediates, have beenidentified (Iwami *et al*., 2000).Soluble receptors play a central role in the regulation ofinflammation in various conditions. Earlier studies demonstratedthat the soluble forms of TLRs (TLRs), includingTLR2 and TLR4, function as a feedback mechanism for theinhibition of excessive TLR activation. The soluble form ofTLR4 (sTLR4) significantly abrogates LPS-mediated TNFproduction and NF-κB activation via blockade of TLR4-MD2 interactions in mouse macrophages (Iwami*et al*., 2000).

2.8.5 Innate immune system cells

The cells of the innate immune system have several functions that are essential for defenseagainst pathogens. Some cells form physical barriers that impede infections. Several cell types express the various PRRs that recognize PAMPs and DAMPs, which respond by producinginflammatory cytokines to kill microbes or infected cells. These cells include non-myeloid, myeloid and some lymphoid cells.

2.8.6 Non-myeloid cells

Non-myeloid cells include epithelial cells and fibroblasts that basically form a barrierbetween the internal and external environment. These cells produce antimicrobial substancesthat hinder the entry of pathogens (Willian, 2011; Koendermanet *al*., 2014). These antimicrobial substances are called antimicrobial peptides (AMPs), and they are essential components of the innate immune response, which contribute to the first line of defense against infections (Chung and Khanum, 2016). In humans,AMPs are classified into three main families: defensins (α and β), cathelicidin,

and statins.AMPs have a wide spectrum of antimicrobial activity, exerting their functions throughelectrostatic interactions between their positive charge and the negative charge that certainpathogens have on their cell wall. AMPs mediate the inflammatory response allowingcytokine release, cell proliferation, angiogenesis, wound healing, and chemotaxis (Cesar de la Fuente-Nunez *et al*., 2017)

2.8.7 Myeloid cells

Myeloid cells include monocytes, macrophages, dendritic cells (DCs), neutrophils, eosinophils,basophils, mast cells and platelets. All these cells have specialized functions for defenseagainst invading pathogens (Koendermane*t al*., 2014).

2.8.7.1Monocytes

Monocytes are innate immune cells essential for host protection against malaria. Upon activation, monocytes function to help reduce malaria burden through phagocytosis, cytokine production and antigen presentation. Following infection with *Plasmodium*, early secretion of pro-inflammatory cytokines by monocyte lineage cells help to control parasite multiplication and resolution of infection, but excess production contributes to pathogenesis (Dobbs *et al*., 2020).

Monocytes are cells that develop in the bone marrow. They are released into the blood stream ocirculate for approximately 72 hours and then emigrate to different tissues wherethey differentiate into macrophages or DCs. They represent the major type of mononuclearphagocytes found in blood and are members of the myeloid cell family (Franc *et al*., 2017).

In humans, monocytesare classified into classical and non-classical depending on their surface expression ofcluster of differentiation (CD)-14 and CD16. Classical monocytes with phenotype CD14+CD16−are considered inflammatory cells representing more than 92% of total monocytes. In contrast,non-classical monocytes with CD14+CD16+ phenotype can eliminate debris from the

vascularsystem and produce low levels of pro-inflammatory cytokines, as well as high levels of anti-inflammatory factors (De Jong *et al*., 2017). Monocytes are involved in innate immune responseagainst pathogens during inflammatory processes. Monocytes migrateto the blood where they mature into macrophages or DCs toparticipate as phagocytes (Jakubzick*et al*., 2017).

2.8.7.2Macrophages

Macrophages are a very heterogeneous cell population, such as effector cells of the innateimmune system, which play an important role in a host's defense and inflammation. In general,macrophages can be divided into two populations: resident and inflammatory macrophages(Raggat*et al*., 2014). During *Plasmodium* infection, macrophages protect the host by inducing phagocytosis, antibody-dependent cell inhibition and chemokine production. The blood stage of malaria begins after the release of merozoite from the liver. Circulating merozoites invade RBCs and develop into trophozoite forms. Humoral immunity is the main type of response against blood stage malaria, but cell-mediated and innate immune response play a supplementary role in the protection of the host from parasite-induced injuries. These responses are mediated by circulating macrophages (Ryg-Cornejo *et al*., 2016).

Resident macrophages are found in almost all tissues and contribute to theirdevelopment, as well as immunological surveillance, homeostasis, and tissue repair. On the other hand, inflammatory macrophages are derived from circulatory monocytes andrapidly infiltrate tissues compromised by injury or infection (Gu *et al*., 2017).

2.8.7.3Dendritic Cells

Dendriticcells (DCs) are antigen presenting cells which have been found to play an important role in initiating immune responses against malaria. They play a major role in internalizing *Plasmodium* antigen, processing the antigen and presenting it to the CD4+ T cells at early stages

upon activation (Chakravaty*et al*., 2007). Dendritic cells are a heterogeneous population of antigen-presenting cells that are crucial to initiate and polarize the immune response. Althoughall DCs are capable of capturing, processing and presenting antigens to T cells, DCs subtypes differ in origin, location, migration patterns and specialized immunological roles (Boltjes and Van Wijk, 2014).

There are mainly two subtypes of DCs: classical DCs and plasmacytoid DCs. Classical DCs are highly migratory cells that can move from tissues to the T cell and B cell zones of lymphoid organs. Classical DCs regulate T cell responses both at steady state and during infection. They are usually short-lived and replaced by blood-borne precursors (Waskow*et al*., 2008; Liu *et al*., 2009). On the other hand, plasmacytoid DCs differ from classical DCs in that they are relatively long lived (Corcoran *et al*., 2003). Plasmacytoid DCs are present in the bone marrow and in all peripheral organs, and they are specialized to respond to viral infection with massive production of type 1 interferons (IFNs). However, they can also act as antigen presenting cells and control T cell responses (Collona*et al*., 2004).

2.8.7.4 Neutrophils

In humans, about 100 billion neutrophils enter the bloodstream each day (Borregaard, 2010). One of the ways by which neutrophils play a role in the clearance of malaria parasite is by phagocytosis. Phagocytosis of the released sporozoite during *Plasmodium* infection are facilitated by the FCgamma (Ig binding) receptors and by the presence of antibodies against the sporozoites protein, one of the main surface antigens on sporozoites (Feng *et al*., 2021). Neutrophils may also be involved in the control of parasite growth through antibody-dependent respiratory burst(ADRB) which exhibit high activity in malaria patients and help promote parasite clearance.

Neutrophils originatefrom hematopoietic stem cells in response to both extracellular stimuli and intracellularregulators. They come from the myeloid cell line in the formation of granulocytes. Neutrophils play a major role in the resolution of microbial infections.After pathogens break into epithelial barriers, neutrophils are the first cell line of defense forthe innate immune response, which are recruited from the bloodstream to the site of infection.Neutrophils cross the blood vessels and migrate to the infection site with the help of chemotacticfactors and cytokines, which are produced as inflammatory signals during the tissue damagecaused by the invading pathogens. Neutrophils reach the infection site and initiate the phagocytosisprocess through recognition of PAMPs by their receptors such as TLRs. Neutrophilsexert their antimicrobial actions through the release of reactive oxygen species and cytotoxiccomponents contained in their granules such as AMPs (Kobayashi *et al*., 2017).

2.8.7.5 Eosinophils

Eosinophils are produced in the bone marrow from pluripotentstem cells, which first differentiateinto a precursor for basophils and eosinophils and then differentiate into an eosinophiliclineage (Boyce *et al*., 1995). Eosinophils are multifunctional leukocytes involved in the pathogenesis of numerousinflammatory processes (Rothenburg, 2004), including parasitic helminths infections and allergic diseases(Rothenburg, 2004; Gliech and Loegering, 1984).

Eosinophils play a role in protecting against malaria by inducing parasite elimination and was associated with hastened recovery from the effect of malaria infection and interpreted as signifying the onset of protective response in malaria individuals (Waters *et al*., 1987). Acute malaria in adults with or without limited previous exposure to *Plasmodium* infection is usually associated with high eosinophil count, followed by persistent eosinophilia (Davies, 2003). In addition to the multiple effector actions of eosinophils, these cells can initiate antigen-

specificimmune responses by acting as APCs (Shi *et al*., 2000; Mackenzei*et al*., 2001), as they can process and present a variety of parasitic antigens (Shi, 2004). Though investigations demonstrated adirect association of eosinophils with parasitic helminths infections, establishing the hypothesisthat eosinophils are the classic effector cells in a host's defense (Butterworth, 1977).

2.8.7.6 Basophils

Basophils are cells derived from the myeloid hematopoietic progenitors in the bone marrow, and they are phenotypically and functionally distinct from other leukocytes, including mastcells, since mast cells reside in tissues while basophils reside in the circulation and can berecruited to the tissues (Hida*et al*., 2005; Kim *et al*., 2009; Siracusa*et al*., 2011; Wakahara*et al*., 2012). Basophils have the ability to bridge innate and adaptive immunity,including the capacity to induce and propagate Th2 immune responses (Knol and Olszewski, 2011).

2.9Epidemiology of Malaria

Malaria is a mosquito borne disease in humans and animals (Ukaegbu*et al*., 2014). It is caused by parasitic protozoans of the genus *Plasmodium* and the mosquitoes which act as vector for this disease are female *Anopheles funestus, Anopheles mou-cheti, Anopheles gambiae, Anopheles arabiensis*(Kar*et al*., 2014; World Malaria Report, 2015). The disease was spread globally but it was successfully eliminated from many countries with temperate climate during twentieth century. Approximately 40% of the world population, mostly those living in the world poorest countries are at risk of malaria. This is mostly in the tropical and subtropical regions of the world. The vast majority of deaths occur in sub-saharan Africa where malaria also presents major obstacles to social and economic development. There are 241 million cases of malaria

every year, globally resulting in more than a million deaths, about 96% of these deaths occurs in Africa (WHO, 2021).

2.10 Pathogenesis

Clinical illness is caused by the erythrocytic stage of the parasite. No disease is associated with sporozoite, the developing liver stage of the parasite, the merozoites released from the liver, or gametocytes. The first symptoms of malaria are associated with the rupture of erythrocytes when erythrocytic-stage schizont matures. This release of parasite materials presumably triggers a host immune response. The cytokines, reactive oxygen intermediates and other cellular products released during the immune response play a prominent role in pathogenesis, and are probably responsible for fever, chills, sweats, weakness and other systemic symptoms associated with malaria (WHO, 2015)

2.11 Clinical manifestation

The most characteristic symptom of malaria is fever. Other symptoms of malaria include abdominal discomfort,headache, joint aches, muscle pain, vomiting, lethargy, anorexia. If anti-malarial treatment is not given on time, then it leads to severe malaria particularly in cases of*Plasmodium falciparum* malaria. In severe malaria the parasite burden continues to increase in the body of the patient which may be lethal. If it is untreated for a long time, then it becomes fatal in most of the cases. The symptoms of severe malaria usually manifest with more than one of the following: Hypoglycaemia, metabolic acidosis,acute pulmonary oedema,anaemia, coma, acute renalfailure (WHO, 2019).

2.12 Laboratory Diagnosis

Due to the non-specific nature of malaria symptoms, diagnosis is typically suspected based on symptoms and travel history, then confirmed with parasitological test. In area where malaria is common, the WHO recommends clinicians suspect malaria in any person who reports having high fever or who has a current temperature above 37.5 degrees without any other obvious cause (WHO, 2015). In areas with little to no malaria, the WHO recommends only testing people with possible exposure to malaria (typically travel to a malaria-endemic area) and unexplained fever (WHO, 2015).

Malaria is usually confirmed by the microscopic examination of blood film or by antigen-based rapid diagnostic tests (RDT). Microscopy is the gold standard for malaria diagnosis. Microscopic diagnosis is relatively resource intensive, requiring trained personnel, specific equipment, electricity and consistent supply of microscopy slides and stains (WHO, 2015). In places where microscopy is unavailable, malaria is diagnosed with RDTs, rapid antigen test that detect parasite proteins in a finger-stick blood sample.

Serological tests to detect antibodies against *Plasmodium*from the blood have been developed. Highly sensitive nucleic acid amplification tests have been developed, but are relatively expensive and have poor specificity for active infections (WHO, 2015).

2.13 Treatment

Malaria is treated with anti-malarial medication; the ones used depend on the type and severity of the disease. Providing free anti-malarial drugs to households may reduce childhood death when used appropriately. Uncomplicated malaria may be treated with oral medication such as Artemisinin or in combination with other anti-malarial drugs (WHO, 2021).

2.14Prevention and Control

Methods used to prevent malaria include medication, mosquito elimination and the prevention of mosquito bites. The presence of malaria in an area requires a combination of high human population density, high Anopheles mosquito's population density and high rate of transmission from human to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite eventually disappears from that area. Vector control is a vital component in malaria control as it is highly effective in preventing infection and reducing disease transmission. The two core interventions are insecticide treated net(ITNs) and indoor residual spraying (IRS) (WHO, 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area was Zaria metropolis in Kaduna State located in the North westpart of the countrywith an area of 300km square. Kaduna State is located on latitude 11° 6' 40'' (11°06'67) N and longitude 07°43' 21'' (7°43'36) E. Zaria is the second largest city in Kaduna State. Zaria is a large heterogeneous city with a population of about 1,490,000people drawn from different ethnic mix in which Hausa and Fulani predominate. The city houses several Federal Institutions thus attracting people from different States andother parts of the world. It is the gateway to several states in the North and thus provides the unique and multicultural population ideal for the conduct of the study.

3.2 Study Design

A hospital based cross-sectional study was carried out among patients with malaria attending the selected hospitals in Zaria, Nigeria.The study pooled samples using convenient sampling fromMajor General Ibrahim Bello Hospital andHajiyaGamboSawaba General Hospital Zaria City.

3.3 Study Population

The target populationwere those that were selected by the clinician for malaria test atthe selected hospitals (Major General Ibrahim Bello Hospital and GamboSawaba General Hospital Zaria City) to access medicare.

3.4Inclusion Criteria

People who reported to the hospital and were recommended to undergo malaria testduring the cause of study and they gave their consent to the study.

3.5 Exclusion Criteria

People who did not visit the hospital during the cause of study, those who visited the hospital but not for malaria illness and those that declined consent.

3.6Ethical Approval and Consent

Ethical approval for the study was obtained from Kaduna State Ministry of Health. A copy is attached in the appendix 1.

3.7 Sample size determination

n = $\frac{z^2 p(1-p)}{e^2}$

d= Precision.

For this study,

p = 14.1% (Sandie *et al*., 2019) was used

n = $\frac{1.96^2 X 0.141\ X\ 0.859}{0.05^2}$

n = $\frac{0.4512}{0.0025}$

= 180.48 ~ 180 samples.However, 180 samples werecollected from 180 patients that reported to the Hospitals for medical care.

3.8.Questionnaire Administration

Data on socio-demographic characteristics,clinical features and Risk factors wereadministered using structured questionnaire.These include Age, educational status, marital status, use of anti-malaria or herbal therapyand clinical characteristics of the participants.

3.9.1 Collection of blood samples

Three milliliters (3ml) of blood were collected. 1ml of blood was dispensed into a bottle containing Ethylene Diamine Tetra Acetic acid (EDTA)aseptically by venipuncturefor *Plasmodium*species detection, parasite density and differential blood count while 2ml were dispensed into another EDTA bottle, then centrifuged, and plasma was harvested into micro-centrifuge tubes for analysisof the TLR2 level. The samples were stored immediately at -20°C at the Department of Microbiology, Ahmadu Bello University Zaria.

3.9.2 Malaria parasite antigen detection from blood samples using PAN- RDT

Blood samples collected from various hospitalswere tested for malaria parasite antigen using Rapid Diagnostic Test(RDT). This was done according to the instruction on the manual. Briefly, RDTs was brought to room temperature first prior to use (<30°C). The RDT packaging was removed and the desiccant was checked for any color changes (i.e. blue to yellow). Calibrated micropipettes were used to dispense blood onto the cassette. Result was recorded. Valid result showed lines on both control and test lines.Marked abnormalities or issues affectingthe interpretation such as poor blood clearance was also recorded. Absence of control line gave invalid while presence of control line alone gave negative result (Biosensor and Encode).

3.9.3 Determination of parasite density

A thick film was prepared on a free grease slide. The slide was stained with 5% giemsa stain at pH7.2 and examined microscopically using 100x (oil immersion) objective as described by (Cheesebrough, 2009). Parasite density per microlitre of blood (parasitemia) was estimated from thick film as follows:Parasite density = parasite count x 8000/Number of WBCs counted. The parasite density was classified as low (PD≤100parasite/µl), intermediate(100>PD<10,000parasite/µl) and high (PD≥10,000parasite/µl) as described by Hammami *et al.*(2013).

3.9.4 Assay procedure for determination of Toll-like receptor-2 level.

Standard working solution, test samples (diluted at least ½ with sample dilution buffer), control (blank) wells were set on the pre-coated plate respectively and the positions were recorded. The standard and sample were measured in duplicate. The plate was washed twice before adding standard, samples and control (blank) to the wells.100uL of properly diluted samples were addedinto thetest sample wells(100uL for each well). The plate was sealed with a sealer and incubated at37°C for 90 minutes. The sealer was removed and the plate content were discarded and washed twice with a wash buffer.100µL of biotin-labeled antibody solution was added into wells (standard, test samples and blank), covered with the sealer, gently mixed and incubated at 37°C for 60 minutes. The solution was decanted from each well,washed with a wash buffer. Microplate was blotted dry against clean absorbent paper.100µL of Horse RaddishPeroxidase-streptavidin (SABC) Conjugate working solution was then added to each well, covered with the sealer and incubated at 37°C for 30 minutes. The solution was decanted from each well and the washing process was repeated three times.90µL of substrate reagent was added to each well, covered with a new sealer and incubated at 37°C for about 30 minutes and protected from light.

A blue color was observed in the plate. Note: the reaction time can be extended to enhanceinitial color (blue) identification, but not for more than 30min. 50μLof stop solution was then added to each well and a color change (blue to yellow) was observed.Theopticaldensity (OD value) of each well was determined at once using a micro-plate reader set to 450nm.The concentration of TLR2 in the sample was determined by comparing the optical density (O.D) with the standard curve. (Fine test)

3.9.5 Determination of differential blood count test

Thin films were prepared from the whole blood on a grease free slide. The smeared blood films were then stained using 5%giemsa dye. The dye helps to differentiate the different type of white blood cells in the sample. The thin films were fixed with absolute methanol, air dried and washed using buffer solution. The films were then examined microscopically using 100x (oil immersion). Each number of white blood cells was counted by virtually identifying the number and size of the different cells on the slide.

3.9.5.1 Pack Cell Volume (PCV) procedure

The samples collected were mixed by inverting them gently at least three times. The sample tube was held at an angle of 45 degrees, a capillary tube was introduced into the tube and blood from the sample tube was allowed to flow into tube. Once the tube was about 2/3 full, the open end of the tube was covered using finger and the tube was removed from the blood sample. The exterior of the capillary tubes was cleaned, placed in a clean centrifuge in opposite position on the rotor and allowed to spin at 10,000 rpm for 5 minutes. The capillary tubes were removed from the centrifuge and placed on a hematocrit reader. The measurements (in percentage) were taken.

3.10. Data Analysis

Results obtained was recorded and presented in tables and charts where appropriate and analysed statistically using student T-test at 0.05 level of significance using SAS software and Chi-square Pearson product moment correlation was used for correlating TLR-2 and parasitemia. For the correlation of haematological parameters and parasitemia, 32 malaria positive and negative samples were used respectively using the Pearson product moment correlation.

CHAPTER FOUR

4.0 RESULTS

The overall prevalence of the *Plasmodium* species in this study was 17.7%. HajiyaGamboSawaba Hospital and Major General Ibrahim Bello Hospital recorded 17% and 18.75% prevalence (Table 4.1)

The parasitemia level recorded in this study among malaria positive patients from HajiyaGamboSawaba General Hospital and Maj Gen. Ibrahim Bello Hospital were mostly intermediate (100<PD<10,000 parasite/µl)with an average mean and standard deviation of 1878.94±289.1. HajiyaGamboSawaba General Hospital and Maj Gen. Ibrahim Bello Hospital recorded 17 and 15 cases of intermediate parasitemia respectively. Only Maj Gen. Ibrahim Bello Hospital recorded one case of high parasitemia (15,200 parasite/ml)(Table 4.2).

Table 4.3 shows the TLR-2 levels in the blood samples. The higher concentration of TLR2in samples positive to malaria parasite was 1.401ng/ml while the lower concentration was 0.027ng/ml. The mean concentration of the samples positive to malaria parasite calculated was 0.556ng/ml. The higher concentration of TLR2 in samplesnegative to malaria parasite was

2.005ng/ml while the lower concentration was 0.115ng/ml. The mean concentration of the negative samples calculated was 0.622ng/ml. Therefore, the mean concentration of the samples negative to malaria parasite was higher than the positive samples.

Table 4.1: Prevalence of*Plasmodium* species among the selected Hospitals in Zaria,Nigeria

Hospital	**Number Examined**	**Number Positive**	**Prevalence (%)**
HGSGH	100	17	17.0
MGIBH	80	15	18.75
TOTAL	180	32	17.7

Key:

HGSGH: Hajiya Gambo Sawaba General Hospital

MGIBH: Major General Ibrahim Bello Hospital

Table 4.2: Level of parasitemia among the study participant attending the selected Hospitals inZaria, Nigeria

Parasitemia level	HGSGH	MGIBH	Total
Low	0	0	0
Intermediate	17	14	31
High	0	1	1
Total	17	15	32

Key:

Low: PD≤100parasite/µl Intermediate: 100>PD<10,000parasite/µl

High: PD≥10,000parasite/µl HGSHG: HajiyaGamboSawaba General Hospital

MGIBH: Major General Ibrahim Bello Hospital

Table 4.3: Concentration of TLR-2 levels among the study participants in relation to *Plasmodium* infection

Plasmodium	**TLR-2 Conc ng/ml**		
Status	**Highest**	**Lowest**	**Mean**
Positive	1.401	0.027	0.556
Negative	2.005	0.115	0.662

Key:

Conc: Concentration

Table 4.4 shows the correlation between TLR-2 levels and parasitemia using the Pearson product moment correlation test to check the relation between the test parameters. Samples that were positive to malaria parasite recorded an inverse correlation of -0.149 while the samples that were negative to malaria parasite recorded an inverse correlation of -0. 231.

The result of the hematological parameters of patients in relation to *Plasmodium* infection is presented in Table 4.5. From the result, the mean±SD counts of the PCV(39.25±8.1) and neutrophil (68.50±8.33) of the positive malaria cases were lower than that of the negative samples 45.97±4.54, 70.88±4.27 respectively while the counts for TWBC(total white blood cell), eosinophils, lymphocytes and monocytes were higher than the negative samples. The difference in PCV and TWBC between malaria positive and malaria negative individuals were statistically significant ($p<0.05$). The difference in Neutrophils, eosinophils, lymphocytes and monocytes between malaria positive and negative individual were not statisticallysignificant ($p>0.05$).

Table 4.4: Correlation between levels of TLR-2 and parasitemia among patients attending the selected Hospitals in Zaria, Nigeria

	Level of parasitemia	**Concentration of TLR-2**
Level of parasitemia	1	1
Concentration of TLR-2	-0.149	

Table 4.5: Haematological parameters in relation to *Plasmodium* infection among patients attending the selected Hospitals in Zaria, Nigeria

Haematological parameters	Mp Positive	Mp Negative	t-value	Df	P-value
PCV	39.25±8.12	45.97±4.56	-4.080	62	*0.000
TWBC	11.86±4.07	8.041±3.01	4.267	62	*0.000
Neutrophil	68.50±8.34	70.88±4.27	-1.434	62	**0.156
Eosinophil	1.28±3.01	0.31±0.93	1.740	62	**0.087
Lymphocyte	28.28±7.41	1.03±1.86	1.850	62	**0.069
Monocytes	1.03±1.86	0.44±1.076	1.565	62	**0.123

Key:

MP: Malaria Parasite

PCV: Pack Cell Volume

TWBC: Total White Blood Cell

*: Statistically Significant

**: Not Statistically Significant

The results on socio-demographic factors of the patients who attended Hajiya Gambo Sawaba General Hospital Zaria in relation to *Plasmodium* infection is presented in the tables below. From the table, the distribution across sex showed that Males (17.7%) had higher prevalence than the females (15.8%) although the difference was not statistically significant. In relation to age, patients ≤10 recorded the higher prevalence of 66.7% and was statistically significant($p \leq 0.005$) while the lower prevalence was recorded for the ages between 21-30 (years). Marital status, educational status and the type of residence were not statistically significant $p \geq 0.05$ (Table 4.6).

Those that used bed net(22.2%), used herbal medicine (46.2%), had myalgia(42.9%) and whose area were water logged (23.8%) had the higher prevalence but only those that treated malaria was statistically significant $p \leq 0.05$ in relation to risk factors as shown in Table 4.7.

Male (20.0%)had higher prevalence than the female (16.0%) among patient who attended Maj Gen. Ibrahim Bello Hospital Zaria. In relation to age, marital status, educational status and type of residence, those at the age ≤10 (30%), married (22.4%), in tertiary schools (33.3%) and lives in rural area (24.0%) recorded the higher prevalence but were not statistically significant $p > 0.05$ (Table 4.8).

Patients that did not use bed net (30.3%) had the higher prevalence with a p-value of 0.027 which is statistically significant. Those that lived in areas with stagnant water and uses herbal medicines had prevalence of 20.7% and 21.1% respectively. Patients with body pain and fever had higher prevalence than the other symptoms in this study(Table 4.9).

Table 4.6: Relationship between*Plasmodium* infection and some socio-demographic factors among those attending HajiyaGambo Sawaba General Hospital Zaria

Demographic factors	**No. examined**	**No. positive (%)**	**Chi-square**	**P-value**
Sex				
Female	38	6 (15.8)	0.064	0.801
Male	62	11(17.7)		
Total	100	17(17.0)		
Age				
≤10	6	4(66.7)	19.297	0.001
11-20	22	2(9.1)		
21-30	42	3(7.1)		
31-40	20	7(35.0)		
≥41	10	1(10.0)		
Total	100	17(17.0)		
Marital status				
Married	53	10(18.9)	0.281	0.869
Single	40	6(15.0)		
Divorced	7	1(14.3)		
Total	100	17(17.0)		

Educational status				
Primary	20	4(20.0)	0.401	0.816
Secondary	56	10(17.9)		
Tertiary	24	3(13.0)		
Total	100	17		
Type of residence				
Rural	77	12(15.6)	0.003	0.954
Urban	23	5(15.2)		

Key: No=Number

Table 4.7: Relationship between *Plasmodium* infections, Risk factors and clinical features among patients attending HajiyaGambo Sawaba General Hospital Zaria

Risk factors	No. examined	No. positive	Chi-square	P-value
Use of bed net				
Yes	37	6(22.2)	0.280	0.597
No	63	11(17.5)		
Total	100	17(17.0)		
Reside in area with stagnant water				
	42	10(23.8)	2.830	0.123
	58	7(12.1)		
Yes	100	17(17.0)		
NO				
Total				
How you treat malaria				
			21.164	0.000
Orthodox	74	5(6.8)		
Herbal	26	12(46.2)		
	100	17(17.2)		
Signs and symptoms				
Headache	12	2(16.7)	5.234	0.256

Fever	42	7(16.1)
Nausea	8	0(0.0)
Vomiting	7	1(14.3)
Shivering	20	3(15.0)
Body pain	4	1(25.0)
Myalgia	7	3(42.9)
	100	17

Table 4.8: Relationship between *Plasmodium* infection and some socio-demographic factors among those attending Maj Gen. Ibrahim Bello, Hospital Zaria

Demographic factors	**No. examined**	**No. positive (%)**	**Chi-square**	**P-value**
Sex				
Female	25	4(16.0)	0.181	0.671
Male	55	11(20.0)		
Total	80	15(18.8)		
Age				
≤10	10	3(30.0)	3.040	0.551
11-20	18	2(11.1)		
21-30	32	7(21.9)		
31-40	12	2(25.0)		
≥41	8	1(12.5)		
Total	80	15(18.8)		
Marital status				
Married	49	11(22.4)	1.638	0.651
Single	27	4(14.8)		
Divorced	4	0(0.0)		

Total	80	15(18.8)		
Educational status				
Primary	31	5(16.1)	0.579	0.751
Secondary	46	9(19.6)		
Tertiary	3	1(33.3)		
Total	80	15(18.8)		
Type of residence				
Rural	50	12(24.0)	2.412	0.120
Urban	30	3(15.0)		

Table 4.9: Relationship between *Plasmodium* infections, Risk factors and clinical features among patients attending Maj Gen. Ibrahim Bello Hospital Zaria

Risk factors	No. examined	No. positive	Chi-square	P-value
Use of bed net				
Yes	47	5(10.6)	4.921	0.027
No	33	10(30.3)		
Total	80	1518.8)		
Reside in area with stagnant water	29	6(20.7)	0.112	0.737
	51	9(17.6)		
Yes	80	15(18.8)		
NO				
Total				
How you treat malaria			0.768	0.087
orthodox	61	11(18.0)		
Herbal	19	4(21.0)		
Total	80	15(18.8)		
Signs and symptoms				
Headache	29	5(17.2)	2.596	0.768
Fever	20	5(25.0)		

Nausea	4	0(0.0)
Vomiting	1	0(0.0)
Shivering	6	1(16.7)
Body pain	11	3(27.3)
Myalgia	9	1(11.1)
Total	80	15(18.8)

CHAPTER FIVE

5.0 DISCUSSION

The overall prevalence of *Plasmodium* infection in this study was 17.7%. Previous studies on the prevalence *Plasmodium* infection carried out in Ibadan and Zaria respectively by Anumudu*et al*.(2006),Sani *et al*.(2021) and Balogun *et al*.(2021) recorded similar prevalence of 17%, 17% and 17.5% respectively. The similar prevalence recorded may be as a result of increased awareness on malaria. It could also be as a result of season (dry) in which the studies were carried out reduces which the breeding site of the parasite Olayemi*et al.* (2011). It could also be as a result of parasites failing to produce HRP2 antigen thereby evading detection when using the Rapid diagnostic test kit (RDT) Gatton *et al*. (2017). Higher prevalence of 71.7% and 80.19% were reported during wet season by Awosulu*et al.* (2021) and Omoya and Ajayi (2020). This variation in prevalence could be as a result of climate difference.

The parasitemia levels recorded in this study were mostly intermediate (100<PD<10000 parasite/µl) based on Hammami *et al.* (2013) with a mean and standard deviation of 1878±289.7 parasite density. Previous study by Omoya and Ajayi (2020) reported that most patients had parasite densities >2000parasite/µl. This could be due to a weak immune system or the intake of anti-malaria drugs prior to their hospital visit.

The mean concentration of TLR-2 recorded in this study for malaria parasite positive and malaria parasite negative patients were 0.556ng/ml and 0.622ng/ml respectively. The malaria parasite negative patients had higher mean concentration than the malaria parasite positive patients. This could be due to low parasitemia as a result of the release of pro-inflammatory cytokines (IL6, TNF) by the macrophages to clear parasites after activation Zheng*et al*. (2015). This therefore indicates that the presence of TLR-2 suppresses the *Plasmodium* species.

The study recorded an inverse correlation (-0.149) between TLR2 and parasitemia levels. This implies that an increase in the TLR2 leads to decrease in parasitemia.This could be as a result of the functions of the effector cells of the innate immune system.

The result from this study showed that only the difference in Pack cell volume(PCV) and Total white blood coμunt(TWBC) between malaria parasite positive and malaria parasite negative individuals were statistically significant $p<0.05$. PCV was significantly lower in malaria parasite positive patients which may reflect anaemia as result of the destruction of red blood cells or probably due to subsequent development of neutropenic leukocytopenia in *Plasmodium* infection with a relative increase in mononuclear cells as reflected in this study by lower neutrophil (Francis *et al*., 2014). The low PCV count agrees with the study of Francis *et a*l. (2014) who also reported a significantly low PCV count in*Plasmodium* positive patients.

Total white blood count (TWBC) was also significantly higher in malaria parasite positive patients ($p<0.05$) than in the negative patients. This conforms with the research of Francis *et al.* (2014) who also reported higher level of TWBC among malaria parasite positive patients. The high TWBC could probably reflect the efficiency of the innate immune response system. Haematological parameters as an investigating tool for cases of early *Plasmodium* infection may

help to detect early complication associated with serious infection (Maina*et al*., 2010; Kayode *et al*., 2011).

There was a statistically significant association ($p<0.05$) between age(in years) and the prevalence of *Plasmodium* infection among patients who attended Major General Ibrahim Bello Hospital in this study. Children ≤10 had the highest prevalence (66.7%) which is in line with study carried out by WHO (2021) who reported that Children under 5 accounts for about 80% of all malaria deaths in the region. This may be due to the slow acquisition of active immunity to malaria (Perlmann and Troye-Blomberg 2000). As they grow, due to recurrent exposure to the parasite, they tend to acquire immunity and become relatively protected (WHO, 2021) hence lower prevalence in older patients.

The patients who used treated bed nets among those who attended Maj Gen. Ibrahim Bello Hospital had lower prevalence (10.6%) than those who did not. This difference was statistically significant ($p<0.05$). This agrees with the study of Sani *et al.* (2021) who reported low prevalence among participants that use treated bed net. This could be because the use of treated bed net prevents the bite of mosquitoes therefore reducing malaria incidence in endemic areas.

There was a statistically significant relationship ($p<0.05$) between how patients treated malaria and prevalence of *Plasmodium* infection among those who attended HajiyaGamboSawabaGenaral Hospital. Patients that used anti-malaria drugs had lower prevalence (6.8%) than those that used herb treatment (46.2%). This could be due to the lack of knowledge on the appropriate dose to be used.

Although the difference was not significant statistically, the prevalence of *Plasmodium* infection among malepatients for both hospitals was higher than that of the female patients. This conforms

with the findings of WHO (2021). The higher prevalence could be due to male's high work burden in malaria endemic areas than female making them more prone to mosquito bite.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The overall prevalence of the *Plasmodium* species in this study was 17.7%. Mean concentrations of TLR-2 were 0.556ng/ml and 0.622ng/ml respectively, for malaria parasite positive and negative samples. The study established a negative correlation (-0.149) between TLR2 and *Plasmodium* parasitemia. In relation to the hematological parameters analyzed, only the difference in PCV and TWBC between MP positive and MP negative individuals were statistically significant at ($p<0.05$). Socio-demographics like age showed significant association with the prevalence of the *Plasmodium* species at ($p<0.05$). Risk factor such as the use of bed netsshowed statistically significant association in this study at ($p<0.05$).The use ofherbal treatment for malaria also showed statistically significant association in this study at ($p<0.05$).

6.2 Recommendation

Increase awareness on malaria could help in further reducing the incidence of the disease. RDTs were effectively reliable.Bed nets distributed by thegovernment should be used appropriately. Hematological analysis of patients should also be encouraged because it also helps to determine the severity of the disease. Environmental sanitation should be encouraged so as to reduce breeding site for the parasite. More research should be carried out on the roles of innate immune system, TLRs and other non-specific immune parameters.

6.3 Contribution to Knowledge

1. The prevalence of *Plasmodium* infection among patients in this study was 17.7%
2. This study recorded a higher mean concentration of TLR-2 in malaria parasite negative patients (0.622ng/ml) than in malaria parasite positive patients (0.556ng/ml).
3. An inverse correlation was observed in this study between the TLR-2 concentration and parasitaemia level, indicating that TLR-2 down regulates parasitaemia.
4. The socio-demographic factors associated with *Plasmodium* infection was age $p<0.001$.
5. The risk factor associated with *Plasmodium* infection was the non-use of bed net $p<0.027$.The use of herbal treatment for malaria was also associated with *Plasmodium* infection $p<0.000$.

REFERENCE

Abeysinghe, R. (2017). Outcomes from the evidence review group on *Plasmodium knowlesi*. Presented at the Malaria Policy Advisory CommitteeMeeting; 22–24 March; Geneva.

Akinlua, J.T.,Meakin, R., Umar, A.M., Freemantle, N. (2015). Current Prevalence Pattern of Hypertension in Nigeria: A Systematic Review. *PLoSOne.* 13;10(10):e0140021. doi: 10.1371/journal.pone.0140021. PMID: 26461923; PMCID: PMC4603956

Akira, S.,Uematsu, S.,Takeuchi,O. (2006). "Pathogen recognitionand innate immunity," *Cell*, 124(4): 783–801.

Albert,B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter,P. (2002). Molecular Biology of the Cell. 4th edn. Garland Science.

Anumudu,C. I., Adepoju, A., Adeniran, M., Adeoye O, Kassim A, Oyewole I, Nwuba R (2006). Malaria prevalence and treatment seeking behaviour of young Nigerian adults. *Annals African Medicine.* 15(2): 82-88.

Awosulu,O. B.,Yahaya, S. Z., Hazikah, M.T.F (2021). Prevalence, parasite density and determinant of Falciparum malaria among febrile children in some peri-urban communities in southweathernNigeria.*Dovepress*https;//doi.org/10.2147/IDR.S312519.

Balogun, I,D., Inabo, H,I and Ella, E,E. (2021). Anti-plosmodial activities of some locallyprepared herbs against *Plasmodium falciparum* obtained from asymptomatic school children in Zaria, Kaduna state, Nigeria. *Nigerian Journal pure and applied science*. 34(2); 4101-4113. Doi10.48198/NJPAS/21A18.

Bonilla, F. A., Oettgen, H. C. (2010). Adaptive immunity. *Journal of Allergy Clinical Immunology*.125(2):S33–40.

Boltjes, A. and Van Wijk, F. (2014) Human dendritic cell functional specialization in steadystate and inflammation. *Frontiers in Immunology*. 5(131):1-13. DOI: 10.3389/fimmu.00131.

Borregaard, N. (2010). Neutrophils, from marrow to microbes. *Immunity*. 33(5):657-670.DOI: 10.1016/j.immuni.2010.11.011

Boyce, J.A., Friend, D., Matsumoto, R., Austen, K.F., Owen, W.F. (1995). Differentiation in vitro ofhybrid eosinophil/basophil granulocytes: Autocrine function of an eosinophil developmentalintermediate. *The Journal of Experimental Medicine*. 128(1):49-57. DOI:10.1084/jem.182.1.49

Brubaker, S.W., Bonham, K.S.,Zanoni, I., Kagan, J.C. (2015). Innate immune pattern recognition: A cell biologicalperspective. *Annals Review Immunology*. 33, 257–290.

Butterworth, A.E. (1977). The eosinophil and its role in immunity to helminth infection. *Current Topics in Microbiology and Immunology*. **77**:127-168 PMID: 336298

Campos, M.A., Almeida, I.C., Takeuchi, O., Akira, S., Valente, E.P., Procopio, D.O., Travassos, L.R., Smith, J.A., Golenbock, D.T.,Gazzinelli, R.T. (2001). Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchorsfrom a protozoan parasite. *Journal ofImmunology*. 167, 416–423.

Caraballo, H. and King, K. (2014). Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. *Emergency Medicine Practice*. 16(5):1–23.

Center for Disease Control and Prevention, (2020). Malaria Parasite. CDC: Malaria. U.S. Center for Disease Control and Prevention

Center for Disease Control and Prevention, (2021). Malaria Parasite. CDC: Malaria. U.S. Center for Disease Control and Prevention

Cesar De la Fuente-Núñez, C., Silva, O.N., Lu, T.K., Franco, O.L. (2017). Antimicrobial peptides: Role in human disease and potential as immunotherapies. *Pharmacology & Therapeutics*.S0163-7258(17)30105-5. DOI: 10.1016/j.pharmthera.2017.04.002

Cheesebrough, M. (2009). District laboratory practice in tropical countries, Part 2. (2^{nd} edition). *Cambridge university press*. Cambridge. Pp 454.

Chakravaty, S., Cockburn, I. A., Kuk, J., Overstreet, M. J., Sacci, J. B., Zavala, F. (2007). CD8+ T lymphocytes protective against malaria. Liver stage are primed in skin draining lymph node. *National Medicine*. 13(9), 1035-1041.

Chua, C.L., Brown, G., Hamilton, J.A., Rogerson, S., Boeuf, P. (2013) Monocytes and macrophages in malaria: protection or pathology? *Trends Parasitology* 29: 26-34.

Chung, P.Y. and Khanum, R. (2016). Antimicrobial peptides as potential anti-biofilm agents againstmultidrug-resistant bacteria. *Journal of Microbiology, Immunology, and Infection.*S1684-1182(17)30080-4. DOI: 10.1016

Clark, I.A. andRockett, K.A. (2006).The cytokine theory of human cerebral malaria .*ParasitologyToday.*10, 410–412.

Coban, C., Ishii, K.J., Uematsu, S., Arisue, N., Sato, S., Yamamoto, M., Kawai, T., Takeuchi, O., Hisaeda, H., Horii, Akira, S(2007). Pathological role of Toll-like receptor signaling in cerebral malaria.*InternationalImmunology*. 19; 67–79.

Coban, C., Ishii, K.J., Kawai, T., Hemmi, H., Sato, S., Uematsu, S., Yamamoto, M., Takeuchi, O., Itagaki, S., Kumar, N.H., Horii, T, Akira, S. (2005). Toll-like receptor 9 mediates

innate immune activation by the malaria pigment hemozoin.*Journalof Experimental. Medicine*. 201; 19– 25.

Corcoran, L., Ferrero, I., Vremec, D., Lucas, K., Waithman, J.O., Keeffe, M., Wu, L., Wilson, A.,Shortman, K. (2003). The lymphoid past of mouse plasmacytoid cells and thymic dendriticcells.*Journal of Immunology*.170(10):4926-4932. DOI:10.4049/jimmunol.170.10.4926

Colonna,M., Trinchieri, G., Liu, Y.J. (2004). Plasmacytoid dendritic cells in immunity. *Nature Immunology*. 5(12):1219-1226. DOI: 10.1038/ni1141

Davies, T.M.E (2003). Safety evaluation of drugs containing artemisinin derivatives for the treatment of malaria. *Clinical Infectious Diseases*. 36:1627-1628.

De Jong, E., Strunk, T., Burgner, D., Lavoie, P.M., Currie, A. (2017). The phenotype and function of preterm infant monocytes: Implications for susceptibility to infection. *Journal of LeukocyteBiology.*102(3):645-656. DOI: 10.1189/jlb.4RU0317-111R.

Dobbs, K. R., Crabtree, J. N. and Dent, A. E. (2020). Innate immunity to malaria. The role of monocytes. *Immunlogical Reviews*. 293(1), 8-24. Doi. Org/10.1111/imr.12830

Dolasia, K., Bisht, M.K., Pradhan, G., Udgata, A., Mukhopadhyay, S. (2018).TLRs/NLRs: Shaping the landscape ofhost immunity. *International Review. Immunology*. 37, 3–19.

Franç, C.N., Izar, M.C.O., Hortêncio, M.N.S., Amaral, J.B., Ferreira, C.E.S., Tuleta, I.D., Fonseca, F.A.H. (2017). Monocyte subtypes and the CCR2 chemokine receptor in cardiovascular disease.*Clinical Science* (London, England). 131(12):1215-1224. DOI: 10.1042/CS20170009

Francis U, Isaac Z, Yakubu A, Enosakhare A, Felix E (2014). Haematological Parameters of Malaria Infected Patients in the University of Calabar Teaching Hospital, Calabar, Nigeria. *Journal Hematology and Thromboembolic Disease* 2: 171. doi:10.4172/2329-8790.1000171

Franklin, B.S., Parroche, P., Ataide, M.A.,Lauw, F., Ropert, C., de Oliveira, R.B. Pereira, D., Tada, M.S., Nogueira, P., da Silva, L.H.*et al* (2009). Malaria primes the innate immune response due to interferon-gammainduced enhancement of toll-like receptor expressionand function. *Proceedings of the National Academic of Science*. USA. 106,5789–5794.

Gatton, M. L., Dunn, J., Chaudhry, A., Ciketic S., Cunningham, J and Cheng, Q. (2017). Implications of parasite lacking *Plasmodium falciparum* HRP-2 on malaria morbidity and control when Rapid diagnostic tests are used for diagnosis. *Journal of infectiousDiseases.*2017; 1156-1166

Gazzinelli, R.T. and Denkers, E.Y. (2006). Protozoan encounters with Toll-like receptorsignaling pathways: Implicationsfor host parasitism. *NatureReviewImmunology*. 6 : 895–906.

Geijtenbeek, T.B.H., Gringhuis, S,I. (2009). "Signalling throughC-type lectin receptors: shaping immune responses," *NatureReviews Immunology*. 9(7) 465–479.

Gilson, P.R., Nebl, T, Vukcevic, D, Speed, T.P., Schofield, L., Crabb, B.S (2006). Identification and stochiometry of glycosylphosphatidylinositol-anchored membrane proteins of the human malaria parasite *Plasmodium falciparum*. *Molecular Cell Proteomics*. 5(7):1286–1299.

Gleich, G. and Loegering, D.A. (2004). Immunobiology of eosinophils. *Annual Review of Immunology*.**2**(1):429-459. DOI: 10.1146/annurev.iy.02.040184.002241.

Guerra, C.A., Howes, R.E., Patil, A.P., Gething, P.W., Van Boeckel, T.P., Temperley, W.H., Kabaria, C.W., Tatem, A.J., Manh, B.H., Elyazar, I.R., Baird, J.K., Snow, R.W., Hay, S.I. (2011). The international limits and population at risk of *Plasmodium vivax* transmission in 2009. *PLoSNegleted Tropical Diseases*. 4:774.

Gu, Q., Yang, H., Shi, Q. (2017). Macrophages and bone inflammation. *Journal of Orthopaedic Translation.*10:8.6-93. DOI: 10.1016/j.jot.2017.05.002

Gun,S.Y., Claser, C., Tan, K.S., Renia, L. (2014). Interferons and interferon regulatory factors in malaria. *Mediators of Inflammation*. 243713.64

Hammami, I., Nuel, G., Garcia, A. (2013). Statistical properties of parasite density estimators in malaria. *PLos ONE*. 8(3):51987. DOI:10.1371/journal.pone.0051987

Hartgers, F.C., Obeng, B.B., Voskamp, A., Larbi, I.A., Amoah, A.S. (2008). Enhanced Toll-like receptor responsiveness associated with nitogen-activatedprotein kinase activation in *Plasmodium falciparum*-infected children. *InfectiousImmununology* 76: 5149-5157.

Hashimoto, C., Hudson, K. L., Anderson, K. V. (1988). "The*Toll*geneof drosophila, required for dorsal-ventral embryonic polarity,appears to encode a transmembrane protein," *Cell*,52(2); 269–279.

Hida. S., Tadachi, M., Saito, T., Taki, S. (2005). Negative control of basophil expansion by IRF-2 criticalfor the regulation of Th1/Th2 balance. *Blood*.106(6):2011-2017. DOI: 10.1182/blood-2005-04-1344

Hunt, N.H. and Grau, G.E. (2003). Cytokines: Accelerators and brakes in the pathogenesis of cerebral malaria. *Trendsof Immunology*. 24, 491–499.

Imai, N., White, M.T., Ghani, A.C., Drakeley, C.J. (2014). Transmission and control of *Plasmodium knowlesi:* a mathematical modelling study. *PLoS NeglectedTropical Diseases*. 8(7):2978

Ing, R., Segura, M., Thawani, N., Tam, M., Stevenson, M.M. (2006). Interaction ofmouse dendritic cells and malaria-infected erythrocytes: uptake, maturation,and antigen presentation. *Journal of Immunology.* 176: 441-450.

Iwami, K.I., Matsuguchi, T., Masuda, A., Kikuchi, T., Musikacharoen, T., Yoshikai, Y. (2000). Cutting edge: naturally occurring soluble form of mouse Toll-like receptor 4 inhibits lipopolysaccharide signaling. *Journal of Immunol*ogy: 165:6682-6.

Jakubzick, C.V., Randolph, G.J., Henson, P.M. (2017). Monocyte differentiation and antigen-presentingfunctions. *Nature Reviews Immunology*. 17(6):349-362. DOI: 10.1038/nri.2017.28

Janeway, C.A. and Medzhitov, R. (2002). Innate immune recognition. *Annal Reviews of Immunology.* 20: 197–216

Janeway, C.A.,Travers, P., Walport, M., Shlomchik, M. (2001). Immunobiology. 5th edition edn. Garland Science.

Kantele, A. and Jokiranta, T.S. (2011). Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clinical Infectious Diseases*. 52(11):1356–1362.Kabelitz, D.,Wesch, D., Oberg, H.H. (2006) "Regulation of regulatoryT cells: role of dendritic cells and toll-like receptors,*Critical Reviews in Immunology*, vol. 26, no. 4, pp. 291–306.

Kar, N.P., Kumar, A., Singh, O.P., Carlton, J.M., Nanda, N. (2014). A review of malaria transmission dynamics in forest ecosystems. *Parasite Vectors*, 7:265. doi:10.1186/1756-3305-7-265.

Kawai, T. and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity.* 34:637–50.

Kawai, T and Akira, S. (2009). "The roles of TLRs, RLRs and NLRs inpathogen recognition," *International Immunology*, 21(4); 317–337.

Kayode O,T., Kayode, A.A.A., Awonuga O. O (2011). Status of Selected Hematological and andbiochemical parameters in malaria and malariatyphoid co-infection. *Journal of Biological Sciences* 11: 367-373

Kim, S., Shen, T., Min, B. (2009). Basophils can directly present or cross-present antigen to CD8lymphocytes and alter CD8 T cell differentiation into IL-10-producing phenotypes.*Journal of Immunology*. 183(5):3039. DOI: 10.4049/jimmunol.0900332

Kimbrell, D.A, Beutler B (2001). The evolution and genetics of innate immunity. *National Review of Genetics*. 2:256–67.

.Kita, H. (1996). The eosinophil: A cytokine-producing cell? *The Journal of Allergy and Clinical Immunology*. 97(4):889-892. DOI: 10.1016/S0091-6749(96)80061-3

Knol, E. F. and Olszewski, M. (2011). Basophils and mast cells: Underdog in immune regulation? *Immunology Letters.*138(1):28-31. DOI: 10.1016/j.imlet.2011.02.012

Kobayashi, S.D., Malachowa, N., DeLeo, F.R. (2017). Influence of microbes on neutrophil life and death. *Frontiers in Cellular and Infection Microbiology*. **7**:59. DOI: 10.3389/fcimb.2017.00159.

Krishnegowda, G., Hajjar, A.M., Zhu, J.,Douglass, E.J., Uematsu, S., Akira, S., Woods, A.S., Gowda, D.C. (2005).Induction of pro-inflammatory responses in macrophages by the glycosylphosphatidylinositols of*Plasmodium falciparum*: Cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement,and regulation of GPI activity. *Journal of Biological Chemistry*. 280, 8606–8616.

Kumagai, Y. and Akira, S. (2010). Identification and functions of pattern-recognition receptors, *Journal of Allergy and Clinical Immunology*. 125 (5) :985-992. doi.org/10.1016/j.jaci.2010.01.058.

Kumar, H., Kawai, T., Akira, S. (2011). Pathogen recognition by the innate immune system. *International Review ofImmunology*; 30: 16-34.

Lucchi, N.W., Damas, A., Narayanan, J., Sumari, D., Kabanyanyi, A., Kachurs, S.P., Barnwell J.W. and Udhayakumar, V. (2010). Real-time flrourescence loop mediated isothermal amplification for the diagnosis of malaria. *Public library of science one*. 5.13733/10.1371 journal. Pone. 0013733.

Lee, K.S., Divis, P.C., Zakaria, S.K.(2011). *Plasmodium knowlesi*: reservoir hosts and tracking the emergence in humans and macaques. *PLoSPathogens*. 7(4):e1002015

Leisewitz, A.L., Rockett, K.A., Gumede, B., Jones, M., Urban, B.*et al*. (2004). Response of the splenic dendritic cell population to malaria infection.*InfectiousImmununoly* 72: 4233-4239.

Lemaitre, B., Nicolas, E., Michaut, L.Reichhart, J.M and Hoffmann, J.A. (1996) "The dorsoventral regulatory gene cassettespatzle/Toll/Cactus controls the potent antifungal response in Drosophila adults," *Cell*. 86(6): 973–983.

Liu, K., Victora, G.D., Schwickert, T.A., Guermonprez, P., Meredith, M.M., Yao, K., Chu, F.F., Randolph, G.J., Rudensky, A.Y., Nussenzweig, M. (2009). In vivo analysis of dendritic cell developmentand homeostasis. *Science*. 324(5925):392-397. DOI: 10.1126/science.1170540.

Lodish,H., Berk,A. Matsudaira, P., Kaiser, C.A., Krieger, M., Scott, M.P., Zipursky, L., Darnell, J. (1999) Molecular Cell Biology. 4thedn. W. H. Freeman and Co.

Luyendyk,J., Olivas, O.R., Ginger, L.A., Avery, A.C. (2002) Antigen-presenting cellfunction during *Plasmodium yoelii* infection. *InfectiousImmununology* 70: 2941-2949.

MacKenzie, J.R., Mattes, J., Dent, L.A., Foster, P.S. (2001). Eosinophils promote allergic disease ofthe lung by regulating CD4(+) Th2 lymphocyte function. *Journal of Immunology*.167(6):3146-3155. DOI: 10.4049/jimmunol.167.6.3146.

Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J. (2010). Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malaria Journal* 9 (3): 1-11

McCall, M.B., Netea, M.G., Hermsen, C.C., Jansen, T., Jacobs, L. (2007). *Plasmodium falciparum* infection causes pro-inflammatory priming of humanTLR responses. *Journal of Immunology* 179:162-171.

Medzhitov, R. (2001). Toll-like receptors and innate immunity.*National Review Immunology* . 1, 135–145.

Medzhitov, R. and Janeway, C.A. (2002) Innate immunity. *The New England Journal of Medicine,* 343; 338–344

Mendis, K., Sina, B.J., Marchesini, P., Carter, R. (2001). The neglected burden of *Plasmodium vivax* malaria. *America Journal of Tropical Medical Hygiene* 64:97-106.

Mendes, C., Dias, F., Figueiredo, J., Mora, V.G., Cano, J., de Sousa, B., do Rosario, V.E., Benito, A., Berzosa, P., Arez, A.P. (2011). Duffy negative antigen is no longer a barrier to *Plasmodium vivax*-molecular evidences from the African West Coast (Angola and Equatorial Guinea). *PLoS Neglected Tropical Disease*. 5:e1192.

Mogensen, T.H. (2009). Pathogen recognition and inflammatory signaling in innate immune defenses.*Clinical Microbiology Review*. 22, 240–273.

Murphy, K. M, Travers, P.Walport, M. (2007). Janeway's immunobiology. 7th ed. New York: Garland Science;

Murray, C.J., Rosenfeld, L.C., Lim, S.S., Andrews, K.G., Foreman, K.J., Haring, D.,Fullman, N., Naghavi, M., Lozano, R., Lopez, A.D. (2012). Global malaria mortality between 1980 and 2010: Asystematic analysis. *Lancet.* 379; 413–431.

Mueller, I., Galinski, M.R., Baird, J.K., Carlton, J.M., Kochar, D.K., Alonso, P.L., del Portillo, H.A. (2009). Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. *Lancet Infect* Dis. 9:555-566.

Naik, R.S., Branch, O.H., Woods, A.S. (2000). Glycosylphosphatidylinositol anchors of *Plasmodium falciparum*: molecular characterization and naturally elicited antibody response that may provide immunity to malaria pathogenesis. *Journal of Experimental Medicine*;192(11):1563–1576

Ockenhouse, C.F., Hu, W.C., Kester, K.E., Cummings, J.F., Stewart, A., Heppner, D.G., Jedlicka, A.E., Scott, A.L., Wolfe, N.D., Vahey, M.,Burke, D.S. (2006). Common anddivergent immune response signaling pathwaysdiscovered in peripheral blood mononuclear cell geneexpression patterns in pre-symptomatic and clinicallyapparent malaria. *Infectious*. *Immunology*. 74; 5561–5573.

Olayemi, I.K., Ande, A.T., Ayanwale, A.V., Mohammed, A.Z., Bello, I.M., Idris, B., Isa, B.,Chukwuemka, V, and Ukubuiwe, A. C. (2011). Seasonal trends in epidemiological and entomological profile of malaria transmission in the north central Nigeria. *Parkistan journal of biological science* 14(4); 293-299. Doi 10.3923/pjbs.2011.

Omoya, F.O and Ajayi K.O (2020). Prevalence of Malaria among Febrile Patients attending Government Hospitals in Ondo State, South-West Nigeria. *American Journal of Epidemiology and Public Health*. 2020 Dec 03;4(4): 017-024. doi: 10.37871/ajeph.id40

Park, B.S. and Lee, J.O. (2013). Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med*. 45:e66.

Parroche, P., Lauw, F.N., Goutagny, N., Latz, E., Monks, B.G., Visintin, A., Halmen, K.A., Lamphier, M., Olivier, M., Bartholomeu, D.C. (2007). Malaria hemozoin is immunologically inert but radically enhancesinnate responses by presenting malaria DNA to Toll-like receptor 9. *Proceeds of National Academic. Science. USA* 104; 1919–1924.

Petel, J.C., Lucchi, N.W., Srivastava, P., Lin, J.T., Sug-aram, R., Aruncharus, S., Bharti,P.K., Shukla, M.M., Conpuong, K., Satimai, W., and Meshnick, S.R. (2014). Field evaluation of real- time fluorescence loop-mediated isothermal amplification assay, Real Amp, for the diagnosis in Thialand and India. *The journal of infection diseases*, 15(8), 1180-1187, doi:10.1093.

Panichakul, T., Sattabonkok, J.,Chotivanich, K., Sirichaisinthop, J., Cui, L., Udomsangpetch, R. (2007). Production of erythropoietic cells in vitro for continous culture of *Plasmodium vivax*. *Internatinal Journal of parasitology*. 37:1551-1557. Doi.11016/j para/2007. PMID17616880.

Perry, J.A., Oliver, C.S., Burnett, R.C., Avery, A.C. (2005). Cutting edge: the acquisitionof TLR tolerance during malaria infection impacts T cell activation. *Journal of Immunology.*174: 5921-5925.

Perlmann, P and Troye-Blomberg M (2000). Malaria Immunology. Perlmann P and Troye-Blomberg M (editors). Basel, Krager, 80: 229-242

Plebanski M, Hill AV (2000). The Immunology of Malaria Infection. *Current. Opinion. Immunology*. 12(4): 437-441.

Polly, S.D., Gonzales, I.J., Mohammed, D., Daly, R., Bowers, K., Mewse, E., Armstrong, M., Gray, C., Perkin, M.D., Bell, D., Kamda, H., Tomita, N., Kubota, Y. and Sutherland, C.J (2013). Clinical evaluation of a loop-mediated amplification kit for diagnosis of imported malaria. *The journal of infectious disease*, 208(4); 637-644, 10.1093.

Price, R.N., Tjitra, E., Guerra, C.A., Yeung, S., White, N.J., Anstey, N.M. (2007). Vivax malaria:neglected and not benign. *American Journal of Tropical Medical Hygiene*. 77:79-87.

Punsawad, C. (2013). Effect of malaria components on blood mononuclear cells involved in immune response. *Asian Pacific Journal of Tropical Biomedicine*. 3:751–6.

Raggatt, L.J., Wullschleger, M.E., Alexander, K.A., Wu, A.C., Millard, S.M., Kaur, S., Maugham, M.L., Gregory, L.S., Steck, R., Pettit, A.R. (2014). Fracture healing via periosteal callus formationrequires macrophages for both initiation and progression of early endochondral ossification.*The American Journal of Pathology*. 184(12):3192-3204. DOI: 10.1016/j.

Ricardo, G., Palisa, K., Katherine, A.F., Douglas, T.G. (2014). Innate sensing of malaria parasites. *Nature Review Immuno*logy. 14(11) 744–757 (2014). https://doi.org/10.1038/nri3742

Rothenberg, M.E. (2004). Eosinophilic gastrointestinal disorders (EGID). *The Journal of Allergy and Clinical Immunology*.113(1):11-28. DOI: 10.1016/j.jaci.2003.10.047

Ryg-Cornejo, V., Ly, A., Hansen, D. S. (2016). Immunological process underlying the slow acquisition of humoral immunity to malaria. *Parasitology*. 143(2), 199-207. Doi:10.1017/50031182015001705

Saito, T., Hirai, R., Loo, Y. M. (2007). "Regulation of innate antiviraldefenses through a shared repressor domain in RIG-1 andLGP2," *Proceedings of the National Academy of Sciences of the United States of America*, 104(2); 582–587.

Sandie, S.M., Sumbele, I.U.N., Tasah, M.M., Kimbi, H.K. (2019). Malaria parasite prevalence and haematoligical parameters in HIV seroprevalence patients attending the regional hospital Limbe, Cameroon. A hospital base cross-sectional studies. *BMC infectious disease*. 19(988). http://doi.org/10.1186/s12879-019-4624-4.

Sani, A., Doko, M.H.I., Aliyu, M.S. (2021). Detection of IgG Antibodies to Glycosylphosphatidylinositol (GPI) as a biomarker of immune status of *Plasmodium* species. *Nigerian Journal of Pure and Applied Science*.3(2): 2756-4045.

Siracusa, M.C., Saenz, S.A., Hill, D.A., Kim, B.S., Headley, M.B., Doering, T.A., Jessup, H.K., Siegel, L.A., Kambayashi, T., Dudek, M.C., Kubo, M., Cianferoni, A., Spergel, J.M., Ziegler, S.F., Comeau,M.R., Artis, D. (2011). TSLP promotes IL-3-independent basophil hematopoiesis and type 2inflammation. *Nature*477(7363):229-233. DOI: 10.1038/nature10329

Seixas, E., MouraNunes,J.F., Matos, I., Coutinho, A. (2009) The interaction between DC an *Plasmodium berghei*/chabaudi-infected erythrocytes in mice involvesdirect cell-to-cell contact, internalization and TLR. *European Journal of Immunology* 39: 1850-1863.

Schroeder, H.W., Cavacini, L. (2010). Structure and functions of immunoglobulins. *Journal Allergy of clinicalimmunolog*;125:S41-52

Schroder, K and Tschopp, J (2010). "Theinflammasomes," *Cell*, vol. 140,no. 6, pp. 821–832, 2

Sherman, I.W., Eda, S., Winigrad, E. (2003). Cyto-adherence and sequestration of *plasmodium falciparum*. Defining the ties that bind. *Microbe infect*. 5; 897-909. Doi:10.11

Shi, H.Z., Humbles, A., Gerard, C., Jin, Z., Weller, P.F. (2000). Lymph node trafficking and antigenpresentation by endobronchial eosinophils. *The Journal of Clinical Investigation*.**105**(7):945-953. DOI: 10.1172/JCI8945

Shi, H.Z. (2004). Eosinophils function as antigen-presenting cells. *Journal of Leukocyte Biology*.**76**(3):520-527. DOI: 10.1189/jlb.0404228

Shimada, k., Crother, R.T., Arditi, M. (2012). Innate immune responses to Chlamydia pneumoniae infection: role of TLRs, NLRs, and the inflammasome. *Microbes and Infection*.14(14):1301-1307. doi.org/10.1016/j.micinf.2012.08.004

Spaulding, E., Fooksman, D., Moore, J.M., Saidi, A., Feintuch, C.M., Reizis, B., Chorro, L., Daily, J.,Lauvau, G. (2016).STING-Licensed Macrophages Prime Type I IFN

Production by Plasmacytoid Dendritic Cells in the BoneMarrow during Severe *Plasmodium yoelii* Malaria. *PLoS Pathogen*. 12, e1005975.

Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y., Hay, S.I. (2005).The global distribution of clinical episodes of*Plasmodium falciparum* malaria.*Nature*. 434, 214–217.

Singh, B., Kim Sung, L., Matusop, A., Radhakrishnan, A., Shamsul, S.S., Cox-Singh, J., Thomas, A., Conway, D.J. (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings.*Lancet* 363; 1017–1024.

Singh, B. andDaneshvar, C. (2013). Human infections and detection of *Plasmodium knowlesi.Clin Microbiol Rev*. 26(2):165–184.Stevenson MM, Riley EM (2004): Innate immunity tomalaria. *Naure Review Immunology* 4: 169–180.

Sutherland, C.J., Tanomsing, N., Nolder. D., Oguike. M., Jennison, C., Pukrittayakamee. S. (2010). Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale*occur globally. *Journal of Infection Disease*. 201:1544–50.

Takeuchi, O.; Akira, S (2010).Pattern recognition receptors and inflammation.*Cell* 140, 805–820.

Turvey, S.E, Broide, D.H. (2010) Innate immunity. *Journal of Allergy and Clinical Immunology*.125 (2):S24–32.

Ukaegbu,C.O., Nnachi,A.U., Mawak,J.D., Igwe, C.C. (2014). Incidence of Concurrent Malaria and Typhoid Fever Infections in Febrile Patients in Jos, Plateau State Nigeria. *International Journal of Scientific and Technology Research.*3(4): 157-161.

Vijayan, D, Radford, K. J., Beckhouse, A.G., Ashman, R.B., and Wells, C.A. (2012). "Mincle polarizes humanmonocyte and neutrophilresponses to *Candida albicans*," *Immunology and Cell Biology*,vol. 90, no. 9, pp. 889–895, 2012.

Walther, M., Woodruff, J., Edele, F., Jeffries, D., Tongren, J.E., King, E., Andrews, L.Bejon, P., Gilbert, S.C., De Souza, J.B.(2006). Innate immune responses to human malaria: Heterogeneous cytokine responses toblood-stage *Plasmodium falciparum* correlate with parasitological and clinical outcomes. *Journal of Immunol*ogy 177,5736–5745.

Waskow, C., Liu, K., Darrasse-Jèze, G., Guermonprez, P., Ginhoux, F., Merad, M., Shengelia, T.,Yao, K., Nussenzweig, M. (2008). The receptor tyrosine kinase Flt3 is required for dendritic celldevelopment in peripheral lymphoid tissues. *Nature Immunology*. 9(6): 676-683.DOI: 10.1038/ni.1615

Waters, L.S., Taverne, J., Spry, T.J.F., Target, G.A.T., Playfair, J.H.L. (1987). Killing of *Plasmodium falciparum* by eosinophil secretory products. *Infectious Immunology.* 55:877-881.

World Health Organization (2014). World Malaria Report (2014). Geneva: World Health Organization

World Health Organization (2015). World Malaria Report (2015).

World Health Organization (2019). Malaria Report Sheet for 2018. WHO World Malaria factsheet. Geneva, Switzerland. 7-9.

World Health Organization (2021). World Malaria Report (2021). Geneva: World Health Organization.

Wakahara, K., Baba, N., Van, V.Q., Bégin, P., Rubio, M., Ferraro, P., Panzini, B., Wassef, R., Lahaie, R., Caussignac, Y., Tamaz, R., Richard, C., Soucy, G., Delespesse, G., Sarfati, M. (2012). Human basophilsinteract with memory T cells to augment Th17 responses. *Blood*. 120(24):4761-4771. DOI: 10.1182/blood-2012-04-424226

White, N.J. (2008). *Plasmodium knowlesi*: the fifth human malaria parasite. *Clinical Infectious Disease*. 46(2):172–173.

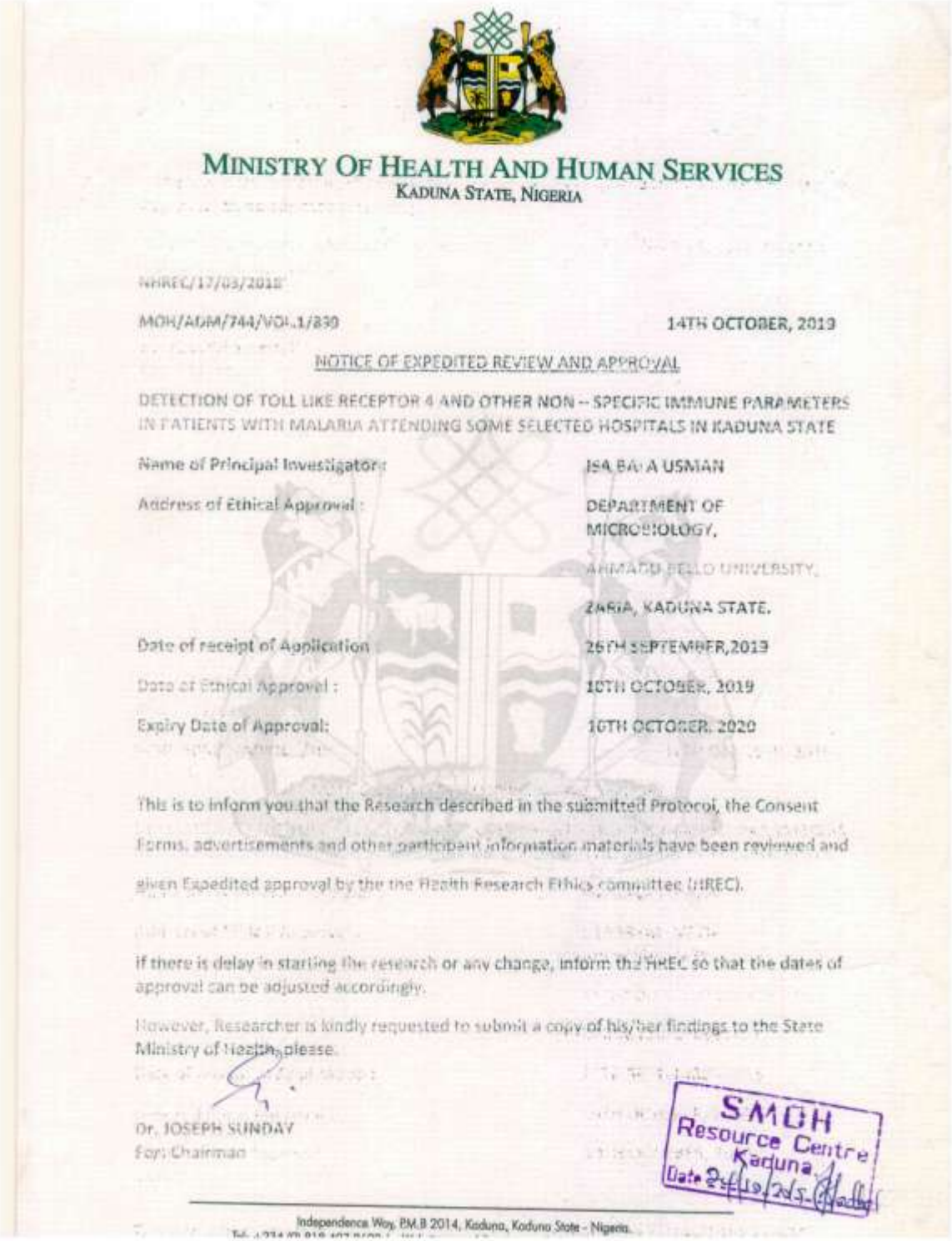
Williams, A.E. (2011). Basic Concepts in Immunology. In: Immunology: Mucosal and Body Surface Defences. *Chichester, UK*: John Wiley & Sons, Ltd; p. 1-19. DOI: 10.1002/9781119998648.ch1.

Yusof, R., Lau, Y.L., Mahmud, R., *et al.*, (2014). High proportion of knowlesi malaria in recent malaria cases in Malaysia. *Malaria Journal*. 13(1):168.

Zheng, H. (2015). The TLR2 is activated by sporozoites and suppresses intrahepaticrodent malaria parasite development. *Scientific Report.* 5; 18239; doi:10.1038/srep18239

APPENDICES

APPENDIX I: Ethical Approval



MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

NHREC/17/03/2018

MOH/ADM/744/VOL.1/839 14TH OCTOBER, 2019

NOTICE OF EXPEDITED REVIEW AND APPROVAL

DETECTION OF TOLL LIKE RECEPTOR 4 AND OTHER NON -- SPECIFIC IMMUNE PARAMETERS IN PATIENTS WITH MALARIA ATTENDING SOME SELECTED HOSPITALS IN KADUNA STATE

Name of Principal Investigator:	ISA BABA USMAN
Address of Ethical Approval :	DEPARTMENT OF MICROBIOLOGY, AHMADU BELLO UNIVERSITY, ZARIA, KADUNA STATE.
Date of receipt of Application	26TH SEPTEMBER,2019
Date of Ethical Approval :	10TH OCTOBER, 2019
Expiry Date of Approval:	10TH OCTOBER, 2020

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other participant information materials have been reviewed and given Expedited approval by the the Health Research Ethics committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

Dr. JOSEPH SUNDAY
For: Chairman

SMOH Resource Centre Kaduna Date 24/10/2019

Independence Way, PM.B 2014, Kaduna, Kaduna State - Nigeria.

APPENDIX II: Detection of parasite antigen using Rapid Diagnostic test kit



APPENDIX III: Questionnaire

M.Sc. Research Questionnaire (Strictly Confidential)

Research Topic: DETERMINATION OF TOLL LIKE RECEPTOR 2 LEVEL AND OTHER NON-SPECIFIC IMMUNE PARAMETERS ASSOCIATED WITH *PLASMODIUM* INFECTION IN PATIENTS ATTENDING SELECTIVE HOSPITALS IN ZARIA, NIGERIA.

Instruction: Please tick as appropriate and provide answers where necessary

Consent [ ]

1. Age _____
2. Marital Status. Single [ ] Married [ ] Divorced [ ]
3. Educational Status. Primary [ ] Secondary [ ] Tertiary [ ]
4. Sex. Female [ ] Male [ ]
5. Type of residence. Rural [ ] Urban [ ]
   CLINICAL FEATURES:
6. Which of these symptoms do you experience during malaria infection

   Head ache [ ]

   Fever [ ]

   Nausea [ ]

   Vomiting [ ]

   Shivering [ ]

   Body pain [ ]

   Myalgia [ ]

   RISK FACTORS:

7. Do you use bed net [ ] Yes [ ] No
8. Do you live in area with stagnant water [ ] Yes [ ] No
9. How do you treat malaria [ ] Orthodox [ ] Herbal

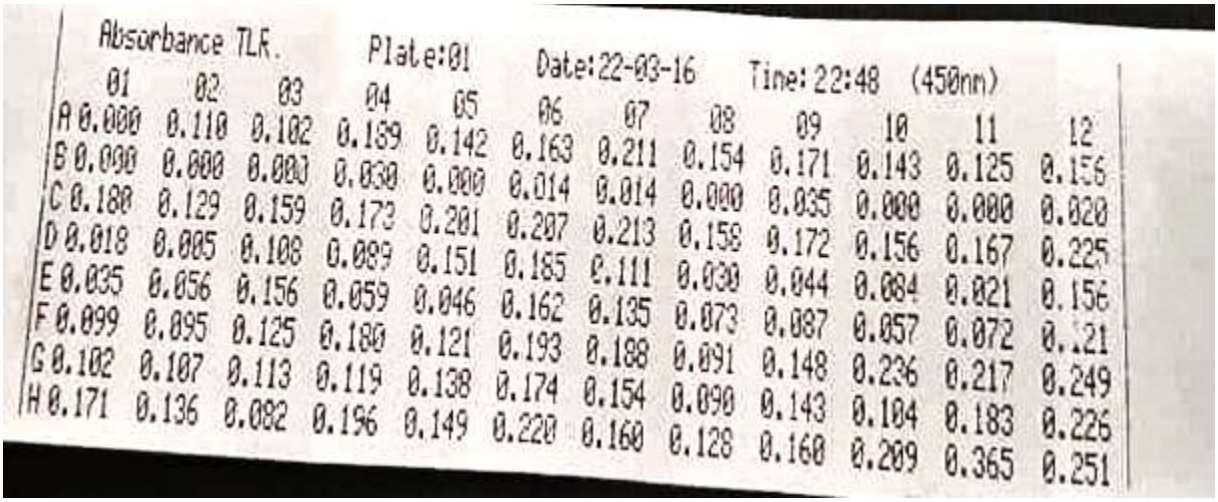
APPENDIX IV: Concentrationof Toll Like Receptor-2 Levels In Patients with *Plasmodium* Infection

10	**1.737**
5	1.03
2.5	0.538
1.25	0.291
0.625	0.155
9.543956	1.737
1.159341	0.211
0.846154	0.154
0.93956	0.171
0.785714	0.143
0.686813	0.125
0.857143	0.156
0	0
0	0
0	0
0	0
0	0
0.076923	0.014
0.076923	0.014
0	0
0.192308	0.035
0	0
0	0
0.10989	0.02
0.989011	0.18
0.708791	0.129
0.873626	0.159
0.950549	0.173
1.104396	0.201
1.137363	0.207
1.17033	0.213
0.868132	0.158
0.945055	0.172
0.857143	0.156
0.917582	0.167
1.401099	0.255
0.098901	0.018
0.027473	0.005
0.593407	0.108

0.489011	0.089
0.82967	0.151
1.016484	0.185
0.60989	0.111
0.164835	0.03
0.241758	0.044
0.461538	0.084
0.115385	0.021
0.857143	0.156
0.192308	0.035
0.307692	0.056
0.857143	0.156
0.324176	0.059
0.252747	0.046
0.89011	0.162
0.741758	0.135
0.401099	0.073
0.478022	0.087
0.313187	0.057
0.395604	0.072
0.664835	0.121
0.543956	0.099
0.521978	0.095
0.686813	0.125
0.989011	0.18
0.664835	0.121
1.06044	0.193
1.032967	0.188
0.5	0.091
0.813187	0.148
1.296703	0.236
1.192308	0.217
1.368132	0.249
0.56044	0.102
0.587912	0.107
0.620879	0.113
0.653846	0.119
0.758242	0.138
0.956044	0.174
0.846154	0.154
0.494505	0.09
0.785714	0.143
0.571429	0.104

1.005495	0.183
1.241758	0.226
0.93956	0.171
0.747253	0.136
0.450549	0.082
1.076923	0.196
0.818681	0.149
1.208791	0.22
0.879121	0.16
0.703297	0.128
0.879121	0.16
1.148352	0.209
2.005495	0.365
1.379121	0.251
17.78022	

APPENDIX V: Absorbance of Toll Like Receptor-2



Absorbance TLR. Plate:01 Date:22-03-16 Time:22:48 (450nm)

	01	02	03	04	05	06	07	08	09	10	11	12
A	0.000	0.110	0.102	0.139	0.142	0.163	0.211	0.154	0.171	0.143	0.125	0.156
B	0.000	0.000	0.000	0.030	0.000	0.014	0.014	0.000	0.035	0.000	0.000	0.020
C	0.180	0.129	0.159	0.173	0.201	0.207	0.213	0.158	0.172	0.156	0.167	0.225
D	0.018	0.085	0.108	0.089	0.151	0.185	0.111	0.030	0.044	0.084	0.021	0.156
E	0.035	0.056	0.156	0.059	0.046	0.162	0.135	0.073	0.087	0.057	0.072	0.121
F	0.099	0.095	0.125	0.180	0.121	0.193	0.188	0.091	0.148	0.236	0.217	0.249
G	0.102	0.107	0.113	0.119	0.138	0.174	0.154	0.090	0.143	0.104	0.183	0.226
H	0.171	0.136	0.082	0.196	0.149	0.220	0.160	0.128	0.160	0.209	0.365	0.251