UNIVERSITY OF CAPE COAST

EFFECT OF DIABETES AND MALARIA COMORBIDITY ON NATURALLY ACQUIRED ANTIBODY RESPONSES AGAINST MALARIA VACCINE CANDIDATE ANTIGENS

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BY

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Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Infection and Immunity

APRIL, 2024

DECLARATION

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere

Candidate's Signature..... Date :.....

Name: Gideon Agyare

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of the thesis laid down by the University of Cape Coast.

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ABSTRACT

Diabetes impairs the capacity of the immune system to combat liver and blood-stage parasites as well as increases the *falciparum* parasites' access to glucose. Glycosylation can make antibodies produced by vaccination or natural exposure less biologically effective. Type 2 Diabetes is linked to lowered humoral and T-cell immunity.This research looked at how diabetes and malaria affect the production of antibodies against malaria vaccine candidate antigens.A case control study consisting of 146 individuals took part in the study, of whom (n=40) had diabetes only, (n=27) had both diabetes and malaria, (n=41) had malaria only, and (n=38) did not have any of these conditions. Detection of *Plasmodium falciparum* and parasitaemia was determined using malaria RDT and the light microscope. Serum samples were examined for the levels of antibody reactions against six malaria vaccine candidate antigens namely; MSP3, GLURP -R2, AMA-1, MSP1, GLURP -R0, and CSP.The type 2 diabetes and malaria comorbidity group significantly recorded higher *Plasmodium falciparum* parasitaemia (10815.8±2540.0, p = 0.02) than the malaria only group (1349.0±1373.6). Increasing age was significantly associated with increase in IgG response against MSP3 (r^2=0.027, p = 0.048). Individuals with malaria and type 2 diabetes had significantly higher antibody level to GLURP-R2 (β=0.47,95% CI=[0.10-0.83], p =0.013), GLURP-RO (β=0.36,95% CI =[0.03-0.68], P =0.034) and MSP1 (β=0.35,95% CI=[0.09-0.61], P=0.008) compared to the negative group.This research revealed that individuals with T2DM and malaria comorbidity had an increased antibody responses (IgG) against the malaria vaccine candidate GLURP-R2, GLURP-RO and MSP1.It was found that people with comorbid malaria and T2DM had significantly higher amounts of parasitaemia.

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First of all, I am grateful for my superiors' advice, counsel, and direction, Rev. Dr. Benjamin Amoani , Prof. Samuel Victor Nuvor and Prof David Courtin for being patient and helping me finish this thesis. I appreciate the immense support of individuals and organizations throughout my study. I duly acknowledge the support of and Naa Adjeley Frempong who guided me carrying out a sound academic research work.

DEDICATION

I dedicate this thesis to the almighty God and my daughter Phyllis Tettevi Agyare

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABREVIATION

T2DM	Type 2 Diabetic Mellitus
NIDDM	Non-Insulin-Dependent Diabetic Mellitus
IgG	Immunoglobulin G
HbA1c	Glycated haemoglobin
MSP	Merozoites Surface Protein
IDF	International Diabetes Federation
TNF	Tumor Necrosing Factor
IR	Insulin Resistance
GLUT2	Glucose Transporter 2
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
ROS	Reactive Oxygen Species
RYR	Ryanodine Receptors
CICR	Ca2+-induced release of Ca2+
cAMP	Cyclic Adenosine Monophosphate
FFAs	Free Fatty Acids
UPR	Unfolded Protein Response
IAAP	Insulin and Islet Amyloid Polypeptides
RBC	Red Blood Cells
CDC	Centre for Disease Control
WHO	World Health Organization
RDT	Rapid Diagnostic Test
ADA	American Diabetes Association
GIGT	Gestational Impaired Glucose Tolerance

TLR	Toll like Receptors
G6PD	Glucose-6-Phosphate Dehydrogenase
NK cell	Natural Killer Cell
PfEMP1	Erythrocyte Membrane Protein 1 Encoded by *P. falciparum*
TGF-B	Transforming Growth Factor B
CD4	Cluster of Differentiation 4
Ab	Antibody
NMIMR	Noguchi Memorial Institute for Medical Research
OD	Optical Density
AGE -el	Advance Glycation to end product of vascular elastin
SERCA	Sarco/Endoplasmic Reticulum Ca2+ ATPase

CHAPTER ONE

INTRODUCTION

This chapter includes descriptions of the problem statement, hypothesis, aim and objectives, significance of study, delimitation, limitations, organizational structure and Study's Background.

Background to the Study

In Sub-Saharan Africa, infectious illnesses continue to be the main contributor to morbidity and mortality. An estimated 1 million people per year die from *Plasmodium falciparum* malaria (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006). The bulk of reported cases of malaria in the world are centered in tropical and subtropical regions that are poor. Malaria greatly contributes to death and morbidity in areas where it is endemic (CDC, 2021).

Globally, T2DM is increasing, but in Sub-Saharan Africa, it is happening at a startling rate. Wild, Roglic, Green, Sicree, & King, 2004; Brancati, Kao, Folsom, Watson, & Szklo, 2000). Type 2 diabetes is a metabolic disorder characterized by persistently elevated blood sugar levels. This condition can be caused by insulin insufficiency or insulin resistance. Diabetes mellitus type 2 is the term used to describe this illness (Knowler et al, 2002). According to (Kalra et al., 2017), the prevalence of type 2 diabetes climbed globally from 4.7% in 1980 to 8.5% in 2010. Non-insulin dependent diabetes mellitus (NIDDM) is the most common type of diabetes mellitus, affecting persons over 40 and accounting for 70 to 90% of all cases of diabetes worldwide (Manoj,The rise in the prevalence of T2DM in developing nations coincides with urbanization trend and lifestyle modifications, most notably the adoption

of a "Western diet" (Sobngwi et al, 2001). There is also proof that the prevalence of T2DM would skyrocket in many industrialized and developing countries (Manoj, 2001).An estimated 6% of Ghana's urban population has the T2DM (Danquah et al, 2012).

Diabetes and malaria co-infection are very common in Ghana. Using 1,466 urban adults in Ghana as a case-control sample, Danquah et al. (2010) discovered that patients with type 2 diabetes mellitus had a 46% higher risk of contracting Plasmodium falciparum infection.They discovered that for every mg/dl rise in blood glucose, there was a 5% increase in the chance of infection with falciparum. A glucose concentration of 155 mg/dl was found to be a significant threshold for increased infection (odds ratio = 1.63; p-value = 0.02). Many things could be causing this, including not enough protection against liver and blood-stage parasites, a drop in T-cell-mediated immunity, and more glucose being available for *P. falciparum*. Because mosquitoes pick up on smells, it's also possible that they like to bite people who have high blood sugar.

A higher incidence of diabetes mellitus may increase the number of people at risk of contracting malaria. This may have an impact on morbidity and immunological response to malaria infections, which could have an impact on the effectiveness of *P. falciparum* vaccine. The rise in *P falciparum* infections may be caused by the parasite's ability to live for a long time and the immune system's inability to fight off liver and/or blood-stage parasites. According to studies, T2DM is linked to lowered humoral and T-cell immunity (Muller et al., 2005). Tumor necrosis factor-alpha (TNF-α) levels in the systemic circulation may be lowered by long-term anti-diabetic medication at an increasing dosage according to Amoani et al, 2021.Cytophilic antibodies (IgG1 and IgG3) can

neutralize parasites indirectly through a system that uses monocytes and parasite-opsonizing antibodies attaching to Fc receptor IIA, which results in the secretion of solubilized factors that can inhibit parasite growth, like nitric oxide or TNF-α (Jafarshad et al, 2007; Tebo, Kremsner, & Luty, 2001 Bouharoun et al,1995). According to Muller et al, 2005 Diabetes patients may become more susceptible to malaria as a result of decreased T cell-dependent immune response.

The study investigated the humoral (IgG) immune responses to certain malaria vaccine candidates, including MSP1 and MSP3, GLURP-RO, GLURP-R2, CSP, and AMA-1.

MSP-1 and MSP-3 are extensively characterized proteins in *Plasmodium spp*. MSP-1 is thought to be involved in the first binding to the erythrocyte surface since it is particularly prevalent on the merozoite surface. To develop antibodies (Abs) against the mutant MSP antigens (Ag), the parasite needs to be exposed to the body multiple times. Most of the antigens on the surface of the adult merozoite are produced by processing of the merozoite surface protein (MSP1 and MSP3), a critical glycoprotein that occurs during schizont rupture (Holder 1988). MSP1 and MSP3, two merozoite surface proteins that may be used as vaccine candidates since they are responsive to humoral immunity (McBride et al. 1985; Diggs et al. 1993). One of the most important surface antigens of the malaria parasite Plasmodium falciparum is GLURP, a glutamate-rich protein. It is an essential component of a therapeutic vaccination. Three unique segments make up the GLURP protein: the C-terminal repetitive region (R2), the core repetitive sequence (R1), and the non-repetitive N-terminal region (R0). As is often the case with secretory proteins, the R0 region is expected to be

hydrophobic and possibly to serve as a signal peptide. (1993, Hogh et al.). The main surface protein of the sporozoite, circumsporozoite protein (CSP), forms a dense coating on the surface of the parasite. Studies have indicated that CSP contributes to the facilitation of sporozoite adhesion to specific cell types (Sinnis et al, 2002). Furthermore, it's been discovered that CSP is required for the formation of mosquito sporozoites (Menard et al, 1997).

In spite of the widespread of malaria and type 2 diabetes co-morbidity in Ghana (Danquah et al,2010; Acquah et al,2014), there is a paucity of information about their immunological interaction and its potential impact on any malaria vaccine candidate that might be administered to such individuals. Therefore, this research aims to determine how T2DM affects naturally occurring antibody responses in Ghana to antigens that are potential candidates for the malaria vaccine.

Problem statement

In sub-Saharan Africa, malaria continues to be a significant health burden and Ghana continues to be one of the 15 African nations that account for 78% of fatalities and 80% of malaria cases worldwide. (Udoh et al.2020).

Insulin resistance and malaria infection have recently been linked in a study from Central Africa (Udoh et al, 2020). Despite the significant prevalence of both diseases in Africa's sub-Saharan region, There is not much data available about the immunological interaction that results from their co-occurrence and the clinical repercussions that are brought on in individuals.

Kalra et al, 2017 revealed that diabetes can weaken the ability of the immune system to combat liver and blood-stage parasites, increasing the glucose accessibility for *P. falciparum* and the severity of malaria .

The frequency of clinical episodes is reduced, but not completely eliminated, in endemic areas due to the naturally acquired immunity that arises from repeated infection. Partial immunity shields older children and adults from major sickness and death, especially in hyper- and holo-endemic locations; it also plays a significant role in malaria incidence patterns by lowering parasite burdens in infected individuals (Dooland et al, 2009). In the absence of intervention, we are aware of the correlations between exposure and age, but we don't know the exact processes of immunity acquisition and decline. A protective immunity can be established against serious diseases after only a few bites from infective mosquitoes (Gupta et al., 1999), but this immunity requires repeated infections before the host develops a clinical immunity, and continuous exposure throughout life does not provide adequate protection. Because of this, regions with more intense malaria transmission tend to have a younger and more limited age range for vulnerability to the disease overall, and a lower risk of severe malaria in later life (Mbogo et al., 1995; Trape et al., 1996)..

Antibodies are known to offer protection against clinical malaria induced by P. falciparum. By giving immunoglobulins from people who didn't have *P. falciparum* to people who did, symptoms and parasitemia were reduced (Mayxay et al,2001). Thus, it is necessary to evaluate the connection between antibody acquisition and age. People with diabetes may have impaired immune function, which makes it harder for the body to rid itself of contaminated red blood cells and, in turn, gives the parasites more time to live (Muller et al.,

2005). It is possible that the prolonged elevation of glucose levels in DM patients fuels the proliferation of malaria parasites, as these parasites rely on exogenous glucose (Jensen et al., 1983).

The effectiveness of malaria vaccination among people with T2DM may be reduced in patients with malaria- T2DM comorbidity. However, in the majority of the malaria vaccine trial studies, neither a study in Ghana nor anywhere else has taken into consideration the effects that diabetes has on the malaria vaccine. Therefore, This study seeks to establish how T2DM affects naturally occurring antibody responses to malaria antigens that are potential candidates for the malaria vaccine among Ghanaians.

Significance of the study

Millions of people around the world still suffer from malaria and T2DM. In a recent analysis, from an estimated 366 million in 2011. According to the International Diabetes Federation, there will be 552 million more individuals with diabetes in the world by the year 2030 than there are at this point (IDF).80% of them will live in countries with low and middle incomes. According to these figures, co-existing cases of malaria and diabetes are quite common in underdeveloped nations, which makes it imperative to investigate how the two diseases interact. According to a study by Danquah et al. (2012), the Ghanaian population T2DM patients had a higher chance of contracting *P. falciparum* infection than people without the disease. Compared to non-diabetics, adult T2DM patients had a greater prevalence of plasmodium infection, according to a study conducted in Ghana. The research also

discovered that a mg/dl rise in blood sugar raised the probability of falciparum infection by 5%.

T2DM is thought to be an immunosuppressed condition that increases the risk of infection for those who have it. According to Kalra et al,2017 diabetes lowers the immune system's capacity to fight against liver and blood-stage parasites as well as increases the *falciparum* parasites' access to glucose. According to research, glycosylation can make antibodies produced by vaccination or natural exposure less biologically effective (Peleg et al., 2007). They also discovered that diabetic patients' IgG glycosylation rates are correlated with their HbA1c levels. In other words, a person who has both diabetes and malaria may not respond effectively to any malaria vaccination. Nevertheless, many research have been done to evaluate the effectiveness of the malaria vaccination in communities where the disease is endemic. However, there hasn't been any research to find out how well the malaria vaccine works among individuals with malaria diabetes comorbidity.

Rapid growth of T2DM in sub-Saharan Africa may make people more likely to get malaria. However, no research has been done to determine how T2DM affects immunity to malaria. In order to better understand how T2DM affects *P. falciparum* parasitaemia and naturally occuring antibody responses to potential malaria vaccine antigens in Ghana, our study seeks to find solution to these. If the study is successful, it will add new information about the effectiveness of malaria vaccine candidate antigens among T2DM patients in Ghana to the body of scientific knowledge.

Hypothesis: It is hypothesized that no differences exist between malaria patients with and without type 2 diabetes in the way their bodies naturally respond to possible malaria vaccine antigens.

Aim

This study examines the effects of T2DM and malaria comorbidity on naturally occurring antibody responses against malaria vaccine candidates antigens

Specific objectives;

1. To determine *P. falciparum* parasitaemia level among malaria patients with or without diabetes
2. To evaluate antibody (IgG) levels directed against malaria vaccine candidate antigens (MSP1, MSP3, GLURP-RO, GLURP-R2, CSP and AMA-1) among the study participants
3. To assess the association between antibody level and age

Delimitations

The study concentrated on residents of Cape Coast Metropolis and Agona West Municipal. The Cape Coast Teaching hospital and Agona Swedru Municipal hospital serves as a referral and treatment Centre for persons living with T2DM and malaria in the Metropolis and municipality respectively. Malaria is endemic in Agona west municipal , malaria tops the top ten diseases in the municipal year after year thereby justifying the selection. Total sampling techniques were employed in recruiting 146 participants for the study. Socio-demographics of the clients were obtained

Limitation

According to the deadlines set for finishing the academic exercise, the study was conducted over a 12-month period, restricting the number of study participants who may enroll. Additionally, those who gave their assent had little trouble withstanding the discomfort of the venipuncture required for the malaria test and antibody analysis.

Organization of the study

There are five (5) chapters in this study project. The first chapter (chapter one) provided the background of the complete investigation. The literature review in Chapter 2 covers topics such as kinds of diabetes, T2DM epidemiology and pathophysiology of T2DM, epidemiology and pathophysiology of malaria ,and laboratory diagnostic methods for *P. falciparum* infection and T2DM. The study design, study site, sample collection, analysis, and detection of immunological parameters are presented in the third chapter's research methodology. The findings of the study are discussed in chapter four, along with the main conclusions. In the final chapter, you will find a summary, a conclusion, and some recommendations.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter discusses a variety of publications and research that examine the effects of diabetes and co-infection with malaria on antibody responses to malaria vaccine candidate antigens that occur naturally. The literature study that examined the subject in terms of themes is in Chapter 2. These include different types of diabetes, T2DM, the pathophysiology of T2DM, effect of T2DM on innate, cellular and humoral immune system, malaria's lifecycle, the pathogenesis of malaria, the epidemiology of malaria,

laboratory diagnostics for type 2 diabetes, laboratory diagnostics for *Plasmodium falciparum*, humoral, innate and cellular responses to malaria, and more.

Epidemiology of T2DM

Epidemiology data on T2DM's future are worrying. According to projections made by the IDF, the number of people receiving diabetes diagnoses will increase to 463 million in 2019 and 700 million in 2045.Diabetes killed 4.2 million individuals this year. Diabetes healthcare cost $720 billion in 2019. One-third of diabetics (232 million people) were misdiagnosed, hence the true disease burden of T2DM may be underreported. Diabetes typically affects 40–59-year-olds.Treatment for type 2 diabetes is challenging because 80% of its patients reside in low- and middle-income countries. Cardiovascular disease, or CVD, is the main cause of morbidity and mortality in the patient population with type 2 diabetes (T2DM), and people with this condition have an overall 15% increased risk of dying (Gaede et al, 2003). Diabetes was linked to a higher risk of dying from heart disease, stroke, and other vascular diseases, according to a meta-analysis (Garcia et al, 2020).Genetic and environmental factors influence type 2 diabetes epidemiology. Hereditary risks are exacerbated by inactivity and excessive caloric intake. Studies of the whole genome have discovered shared glycaemic genetic variants for type 2 diabetes, although they only account for 10% of the disease's symptoms. Rare variations may be important (Grarup et al,2014). Hypertension, insulin resistance, and dyslipidemia are more common in people with distinctive phenotypes (Wong et al,2016).

Types of Diabetes

A person with diabetes, also known as diabetes mellitus (DM) has extremely high blood glucose levels caused by the body's incapacity to produce enough insulin or the cells' resistance to the insulin that is produced Polyuria, polydipsia, and polyphagia are the signs of this elevated blood sugar. Specifically, there are three forms of diabetes namely: Type 1 Diabetes , Type 2 Diabetes and the third form is Gestational Diabetes. Conditions such as cardiovascular disease, stroke, amputation, peripheral arterial disease, kidney failure, retinopathy, neuropathy, blindness, and other complications are all linked to having diabetes (Jothivel et al, 2007). Some examples of hypoglycemic drugs used to treat diabetes are sulfonylureas and biguanides.

Type 1 Diabetes

An endocrine system disorder is type I diabetes. A localized inflammatory response in and around the islets causes type 1 diabetes by selectively destroying cells that make insulin. It's a condition in which the body refuses to manufacture insulin. Insulin injections are necessary for individuals who have type 1 diabetes,

Gestational diabetes

Pregnancy-related hyperglycemia occurs when a woman who has never had diabetes previously has high blood sugar levels during her pregnancy (Deshmukh et al, 2015).

Type 2 Diabetes Mellitus (T2DM)

The IDF (2013) reports that T2DM, which is characterized by insulin resistance, accounts for 90% of cases.as well as insulin production from the pancreatic islet cells. This illness causes high blood glucose. Overweight, inactivity, and age increase insulin resistance, which causes T2DM (Alberti et al, 1998; Brestoff et al,2015). Pancreatic islets increase their cell density and insulin production in response to insulin resistance. (Donath et al,2011). T2DM develops when this compensating effort fails (Donath et al, 2011). After 10 years of insulin resistance, pancreatic cell dysfunction requires insulin therapy in over half of T2DM patients (Weyer et al,1999; Lim et al 2011). Long-term chronic insulin resistance in T2DM can cause nephropathy, neuropathy, and retinopathy, as well as atherosclerosis. Those with T2DM are initially treated with diet and lifestyle changes when it fails before they are given the oral antidiabetic drugs (Bastaki, 2005).

Pathophysiology of T2DM

Unusual blood glucose levels are caused by broken insulin feedback loops (Stumvoll et al., 2005). Insulin release is decreased as a result of malfunctioning β-cells, which makes it challenging for the body to maintain glucose levels within normal physiological ranges. Conversely, insulin resistance (IR) increases the amount of glucose produced by the liver while preventing fat and muscle from absorbing glucose. β-cell dysfunction is frequently more severe than insulin resistance (IR), despite the fact that all of these things occur early in the disease process.Disruption of β-cells and IR exacerbate diabetes mellitus and hyperglycemia. (Zhen and colleagues, 2018)

Causes and pathophysiology of type 2 diabetes

β-Cell Physiology

It is necessary to regulate the pathways and mechanisms involved in β -cell physiology to ensure that β -cells can continue to perform at their highest potential while keeping their cellular integrity intact (Cert et al,2013).

The precursor form of insulin, called pre-proinsulin, is synthesized by β-cells. Several proteins located in the endoplasmic reticulum (ER) facilitate the structural alteration that pre-proinsulin experiences as it matures, allowing for the production of proinsulin (Bunney et al,2017). The Golgi apparatus (GA) receives proinsulin from the ER and cleaves it into insulin and C-peptide inside of immature secretory vesicles (Fu et al, 2013; Halban et al 1994).

Once insulin reaches full maturity, it is stored in granule form until it is needed. Glucose spikes trigger the release of insulin. Keep in mind that hormones, fatty acids, and amino acids are only a few of the many factors that might trigger insulin secretion (Boland et al, 2017). Figure 1 shows normal signaling pathways in insulin-releasing β-cells (A) and defective ones (B). (A) Insulin is secreted when blood sugar levels rise, and GLUT2 conducts most of the work. The ratio of ATP to ADP rises during glucose catabolism, depolarizing membranes and activating voltage-dependent Ca2+ channels by inhibiting ATP-dependent potassium channels. Induced Ca2+ influx aids insulin exocytosis. High blood sugar and cholesterol levels increase oxidative stress and ROS generation, which inhibits Ca2+ mobilization and initiates apoptotic signaling. FFAs and hyperglycemia cause ER stress, which activates the UPR and apoptosis-triggering pathways. ROS are created when high glucose

levels boost proinsulin and IAAP production. P2X, P2Y, IP2, 1,4,5-trisphosphate, and glucose transporter 2 are purinergic receptors (GLUT2).

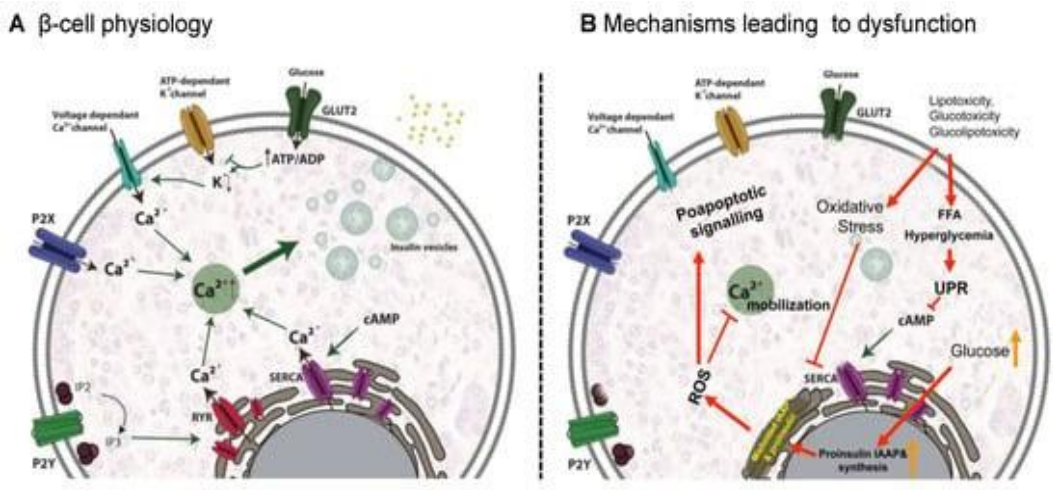

Figure 1: Mechanism leading to β-cell dysfunction

Source; Garcia et al 2020

Ryanodine receptors (RYR) can enhance Ca2+ signals and initiate Ca2+-induced Ca2+ release (CICR), which may link stimuli to insulin production. Following activation by metabolic messenger molecules in food or ligand interaction, RYR amplifies Ca2+ signals, which increases insulin production (Islam, 2002). 1A.

Nevertheless, β-cells absorb glucose via GLUT2, a solute carrier protein and glucose sensor. Catabolism begins with blood glucose. ATP/ADP overload inhibits plasma membrane ATP-dependent potassium channels. Ca2+ enters cells via depolarization and voltage-dependent channels. Intracellular Ca2+ priming and plasma membrane fusion of secretory insulin-containing granules exocytose insulin (Boland et al 2017; Rorsman et al,2018; Fu et al,2013; Seino et al,2011) 1A.

Figure 1 depicts normal signaling pathways in insulin-releasing β-cells (A) and faulty ones (B). (A) GLUT2 secretes insulin in response to high blood

sugar. During glucose catabolism, ATP/ADP ratios rise, depolarizing membranes and activating voltage-dependent Ca2+ channels while blocking ATP-dependent potassium channels. Increased Ca2+ helps insulin exocytosis. P2Y, P2X, SERCA, and RYR release Ca2+ and insulin. (B) Oxidative stress and ROS production from high blood sugar and cholesterol block Ca2+ mobilization and start apoptotic signaling. ER stress from FFAs and hyperglycemia promotes the UPR and apoptotic pathways. When glucose levels rise, proinsulin and IAAP produce ROS. Purinergic receptors P2X, P2Y, IP2, 1,4,5-trisphosphate, and glucose transporter 2 (GLUT2). Other cell signals help FFA, ROS, UPR, and SERCA cells release insulin. cAMP may be the most essential messenger for insulin production. Increasing data suggests that cAMP decreases intracellular Ca2+ reservoirs and increases intracellular Ca2+ concentrations to release insulin-containing secretory vesicles (Cuinas et al, 2016)

Mechanisms that contribute to the dysfunction of beta cells

Death of cells has been linked to cellular dysfunction in some studies (Christensen et al,2019). However, recent data suggests that a more intricate web of interactions between the environment and several biochemical processes related to cellular biology may be the cause of beta cell failure in type 2 diabetes. (Halban et al,2014). Increases in both IR and chronic inflammation are caused by hyperglycemia and hyperlipidemia, both of which are prevalent in an eating pattern that is comparable to obesity. Depending on the particulars of their

genetic make-up, Beta cells are vulnerable to a broad variety of toxic stressors, the most common of which are inflammation (Christensen et al,2019).

ER stress, which can be brought on by high blood sugar levels or an excess of free fatty acids (FFAs), can initiate apoptotic unfolded protein response (UPR) pathways, which in turn can cause beta cells to become dysfunctional (Yamamoto et al,2019

We have established that insulin secretion must be tightly regulated to meet metabolic requirements. The functionality of β-cells depends on the health of the islet, which must be preserved in order to meet metabolic demands. The aforementioned mechanism, when present in pathogenic contexts, can lead to a breakdown in islet integrity/organization, which disrupts pancreatic islet cell-to-cell signaling, impairs insulin and glucagon regulation, and worsens hyperglycemia. Insulin secretory dysfunction is the main cause of β-cell failure and type 2 diabetes. It can be caused by errors in insulin precursor or insulin synthesis, or by disruptions in the secretion process. Diabetes is often caused by proinsulin folding failure, and lower GLUT2 glucose transporter expression alters the downstream signaling cascade (Hoang et al, 2015; Liu et al,2018).

Life cycle of malaria parasite

Figure 2.4 depicts the life cycle of the malaria parasite. When a female mosquito infected with Anopheles bites a victim in order to feed on blood, it injects sporozoites into the wound. In the ensuing five to fifteen days, the sporozoites enter the circulation, assault liver cells, and develop into schizonts. The schizonts then burst, releasing between 10,000 and 30,000 merozoites that assault red blood cells (RBCs) in a cycle. This also kick-starts the growth of the

schizogony in the erythrocytes. While some merozoites continue to develop into trophozoites and the schizont stage, others separate into macrogametocytes (female) and microgametocytes (male).

The mosquitoes' midguts absorb the adult sexual form, which continues to develop into both large and small gametes. To fertilize larger gametes and create fertilized eggs, small gametes swell and move there (zygote). Within 18 to 24 hours, the fertilized eggs continue to grow and develop into motile ookinetes. The ookinetes enter the midgut epithelium's epithelial cells where they develop further to become sporozoites. A single oocyst develops into more than 10,000 motile sporozoites that travel into salivary glands before being transferred into a vulnerable host to start the life cycle all over again

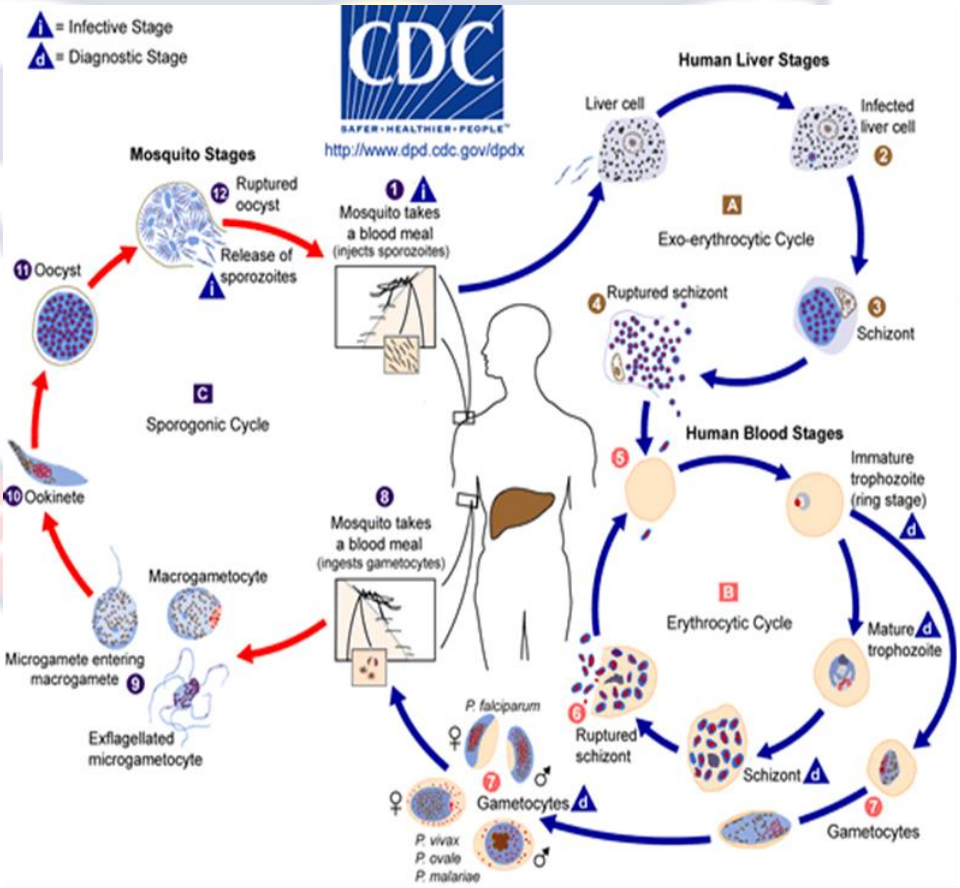

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Figure 2 Malaria life cycle-Adapted from (CDC, 2020a)

Pathogenesis of malaria

Plasmodium knowlesi, *Plasmodium* vivax, *Plasmodium* ovale, *Plasmodium* malariae, and *Plasmodium* falciparum are the five species of *Plasmodium* that can infect humans with malaria.*P. falciparum* on the other hand, is incredibly harmful and has the potential to cause cerebral malaria, a neurological disorder. Malaria can be broadly divided into two types: severe and simple.

The blood stage parasites start the pathophysiology and symptoms of malaria. However, most patients can easily recover from non-specific febrile malaria with the help of either host immune responses or anti-malarial medication (CDC, 2020b). The most common forms of complicated malaria are severe anemia and cerebral malaria that are considered to be life-threatening. High grade fever, hypoglycemia, renal failure, convulsions, pulmonary edema, circulatory collapse, and hepatic dysfunction are the chief clinical signs of severe malaria (White, 2017). The key factor contributing to severe anemia is the rapid erythrocyte damage caused by extensive hemolysis of parasitized RBCs (White, 2017). It's possible that tissue hypoxia, microvascular blockage, and other biochemical processes that have an impact on brain function are part of the etiology of unconsciousness in cerebral malaria patients (White, 2017).

Epidemiology of malaria

The bulk of reported cases of malaria in the world are centered in tropical and subtropical regions that are poor. Malaria greatly contributes to death and morbidity in areas where it is endemic (CDC, 2021). Pregnant women and newborns are especially susceptible to malaria in places with high

transmission. This is a result of children's low immunity to malaria and pregnant women's lower immunity. Residents might grow up without building a defense mechanism, making them susceptible to this disease as well as other dangerous and fatal diseases, even in areas with lower transmission rates, like in Asia and Latin America (CDC, 2021).

Many victories have been won in the fight against malaria, particularly in South-East Asia and Africa. According to WHO figures, there were 1.5 billion illnesses and 7.6 million deaths worldwide from 2000 to 2020. (WHO,2020b). Most cases (82%) and deaths (94%) were prevented in Africa, with Southeast Asia coming in second (10% and 3%, respectively) (WHO, 2020b). Similarly, a US CDC analysis revealed that from 2010 to 2019, more extensive interventions saved millions of lives globally and decreased malaria mortality by 44%, motivating the desire to eliminate and ultimately eradicate malaria (CDC, 2021).

Despite these successes, malaria incidence and mortality rates are generally rising. *P.falciparum* infection caused over 409,000 deaths in sub-Saharan Africa in just 2019 alone (CDC, 2021). 97% of the prevalence of malaria worldwide is due to *P. falciparum* (WHO, 2020b).

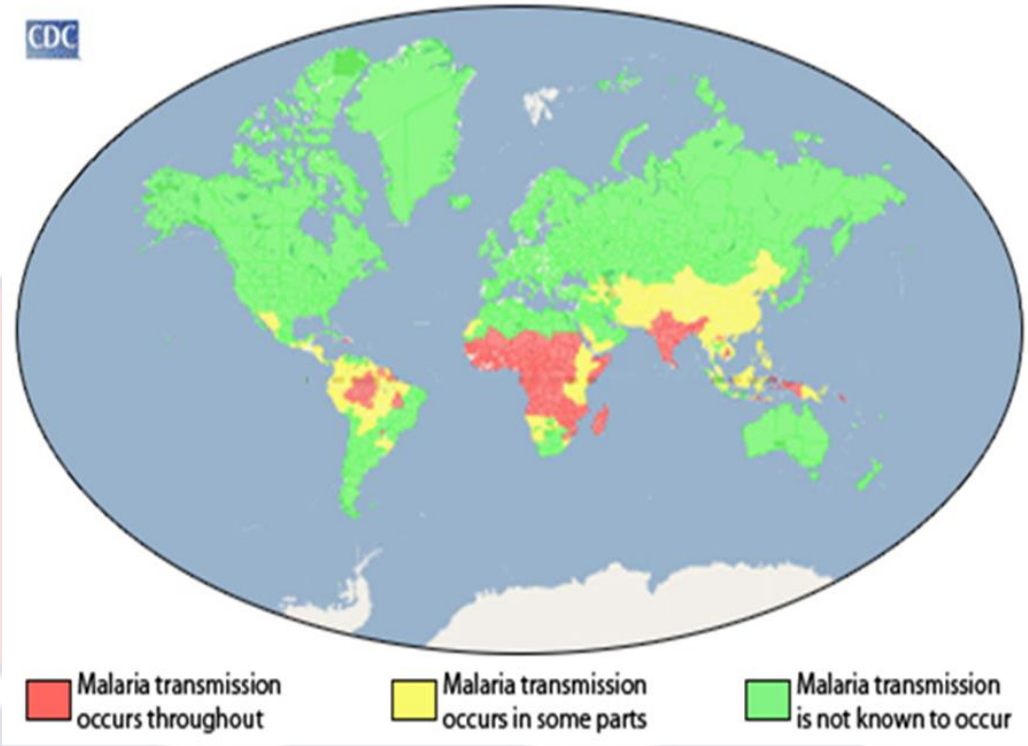


Figure 3*:* The Global distribution of malaria

Adapted from (CDC, 2021)

Laboratory diagnosis of malaria

The section's laboratory methods concentrate on identifying *Plasmodium falciparum* infections in people and then type 2 diabetes.

Techniques for diagnosing *P. falciparum* infection in the laboratory

The *P. falciparum* parasite is responsible for 97% of all malaria cases worldwide (WHO, 2020b). As a result, the bulk of laboratory tests conducted in Ghana and the majority of African nations concentrate on detecting the *P. falciparum* parasite. The most accurate method for determining the amount of parasitemia in venous blood is still microscopy utilizing thick and thin blood

smears. When detecting *P. falciparum* infections, RDT kits simply need blood samples from finger pricks.

Laboratory diagnosis of diabetes

The American Diabetes Association (ADA) recommends screening for diabetes with fasting blood glucose in addition to post-meal, random, and glucose tolerance testing. To make a diabetes diagnosis, at least one of the following requirements must be met:

Signs of diabetes (polydipsia, unexplained weight loss, polyurea, polyphagia, nocturia, etc.) Blood glucose was measured at 200 mg/dL (11.1 mmol/L), which is within the usual range. Fasting plasma glucose levels, measured after a period of no eating for at least 8 hours, should be within the typical range of 70-110 mg/dl.

Once known as gestational impaired glucose tolerance (GIGT) and now recognized as GDM, these two conditions are all part of the same grouping established by the World Health Organization (WHO).: prediabetes, impaired glucose tolerance, and diabetes. glucose in the blood while fasting 7. mmol/L (126 mg/dL)

Type 2 Diabetes and Immunity

Type 2 diabetes and innate immunity

How type 2 diabetes affect phagocytosis

The human body often employs remarkable defense mechanisms to prevent millions of parasites, viruses, bacteria, fungi, and poisons from invading. The malfunctioning of the immune system can be attributed to a great

deal of different causes and problems. It is quite tough for viruses to get through this defense system in conditions that are perfect. For instance, pus indicates that a wound is open and that bacteria can readily enter and develop an illness. Our defensive mechanisms work to prevent pathogenic invasion by producing reactive oxygen species, chemokines, and cytokines, as well as by maintaining intact skin and mucosal surfaces (Berbudi,2020). Unfortunately, Diabetes causes a disruption in the immune response of the host. In addition to the possibility of neuropathy lowering natural barriers, type 2 diabetes may potentially have an effect on the immune system's cellular defenses. This is brought on by insulin deficiency as well as elevated blood sugar levels (Tessaro, 2017). Infections are a serious issue for people who have diabetes, consistent with the recommendations of the American Diabetes Association This is due to the fact that people with diabetes have immune systems that are unable to successfully combat foreign pathogens that have invaded their bodies. Several studies have been done in an effort to pinpoint the specific mechanisms that are linked to diabetes and that lower the host's resistance to infection. These mechanisms include problems with phagocytosis and the inability to destroy germs, as well as an inhibition of cytokine manufacturing and immune cell dysfunction.

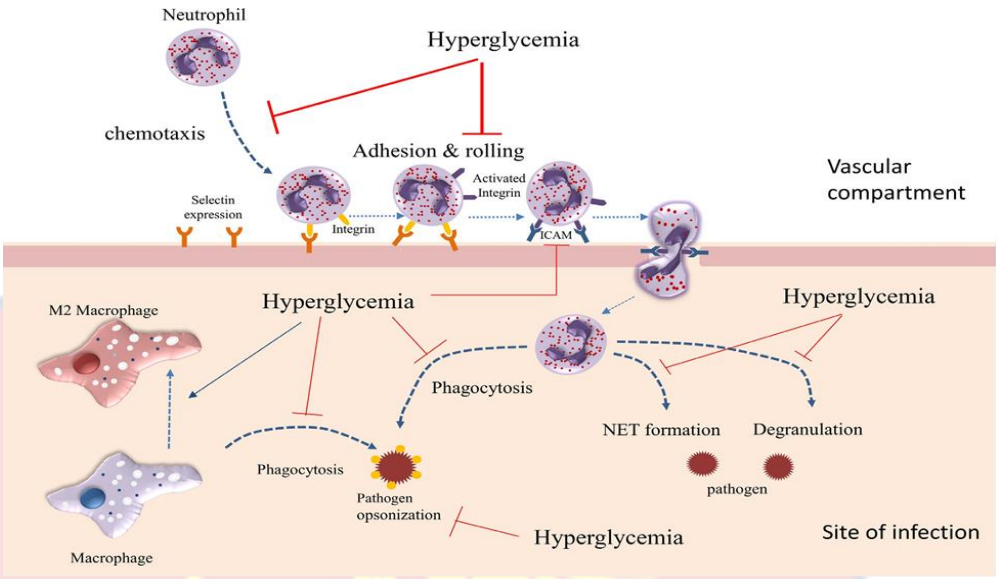


Figure 4 Image showing how type 2 diabetes affect phagocytosis

Berbudi 2020

Defects in T2DM Pathogen Recognition

Additionally, Martinez et al. (2016) discovered that the pathogen-recognition proteins Toll/IL-1R domain-containing adaptor protein (TIRAP) and Toll-like receptor 2 (TLR)-2 were expressed less in diabetic mice. Nonetheless, some investigations have demonstrated that separated neutrophils and monocytes from diabetics express more TLR than those from non-diabetics (Dasu et al,2008:Jafar et al, 2016;Gupta et al,2017). TLR expression was found to be up in patients who had well-controlled hyperglycemia without issues, according to a study that was conducted in 2017 by Gupta et al., while it was shown to be decreased in diabetic participants who had complications and poor glycemic control. As a result, the manner in which hyperglycemia influences TLR expression and the immunity associated with it in diabetic people is still a mystery.

Neutrophil Dysfunction by T2DM

Reactive oxygen species (ROS) were produced at reduced levels by isolated neutrophils from T2D TB patients in response to phorbol 12-myristate-13-acetate activation. When the serum was tested in a medium with a high glucose content, patients with T2DM showed elevated levels of resistance in their blood to this reduction in ROS generation (Chao et al,2015). Perner and associates found in 2003 that when neutrophils from healthy patients were isolated, the amounts of superoxide (O2-) decreased. Nicotinamide adenine dinucleotide phosphate synthesis was hampered by the inhibition of G6PD, which resulted in this impairment. (Et al., Perner, 2003). (Stegenga et al. 2008) found that when the blood of healthy individuals was exposed to components of bacterial walls after being made hyperglycemic, there was a reduction in the blood's neutrophil degranulation Hyperglycemia can cause C3-mediated complement suppression, which is another sign of neutrophil dysfunction in S. aureus phagoes (Hair et al, 2012).). In accordance with aforementioned results, Joshi et al. (2013) revealed that hyperglycemia reduced neutrophil action to generate neutrophil extracellular traps (NETs), resulting in vulnerability to infections. These investigations collectively demonstrated that hyperglycemia leads to neutrophil dysfunction, including abnormalities in ROS generation and impaired neutrophil degranulation (Chao et al., 2015; Stegenga et al,2008). suppression of opsonization caused by immunoglobulins reduce phagocytosis and abnormalities in NET formation (Joshi et al ,2013; Jafar et al,2016)

Damage to Macrophages by Type 2 Diabetes

Macrophage activity is also negatively impacted by hyperglycemia According to a study, isolated monocytes with abnormalities in Fc receptors and complement. receptors had impaired phagocytosis due to chronic elevated blood sugar levels (Restrepo et al,2014). In an in vitro study using macrophages isolated from mouse bone marrow, high glucose treatment resulted in decreased phagocytosis and antimicrobial activity (Pavlou et al,2018). Low phagocytosis was also found in the peritoneal macrophages of diabetic mice. This could be because of the persistent hypersensitivity to glucose that macrophages have, which limits their glycolytic reserve and ability.

Type 2 Diabetes and Natural Killer Cell Dysfunction

Berrou et al. (2013) demonstrated that Natural Killer (NK) cell malfunction is essential for suppressing invading pathogens. The study found deficiencies in the NK cell activating receptors NKp46 and NKG2D in T2D patients, which were associated with impairments in NK cells' ability to degranulate.

Effect of diabetes on cellular immune response.

Mooradian et al. (1991) found that after stimulation with lipopolysaccharides, Type 2 diabetic PBMCs and isolated monocytes released less interleukin 1 beta (IL-1). Another study by Ohno et al. (1993) found that monocytes from T1D patients' PBMCs created lower levels of IL-6 and IL-1 than those from healthy donors. Reinhold et al. found that after stimulation with anti-CD3 antibodies and high blood sugar, non-diabetic PBMCs produced less IL-10, IL-2, and IL-6 (1996).

IL-6 helps microbial defense and adaptive immunity by promoting antibody formation and effector T cell maturation (Tanaka ,2014).

Reinhold et al. (1996) discovered that suppressing these cytokines in individuals with high blood sugar levels may affect the immunological response to invading microbes. According to Spindler et al,2016 dextrose octreotide treatment lowered IL-17 and IL-6 expression, particularly in CD16+ and CD14+ intermediate monocytes, indicating altered immunological responses as a result of hyperglycemia. Price et al (2010) found that elevated glycation levels in myeloid cells suppress IL10 secretion. Further, they demonstrated that interferon gamma (IFN) and tumor necrosis factor a (TNFa) production by T

cells were decreased (TNF-A) High-fat diet-induced hyperglycemic animals and obese leptin-receptor-deficient (db/db) mice both have decreased levels of the cytokine IL-22 compared to normal mice (Wang et al,2014). Poly I:C activation of PBMC cultured in high glucose medium inhibited type 1 IFN release, according to another investigation (Hu et al ,2018). Interferon (IFN) and interleukin (IL)-12 synthesis by peripheral blood mononuclear cells (PBMC) cultures from diabetes patients infected with *Burkholderia pseudomallei* was lower than that of PBMCs from healthy donors, as discovered by Tan et al (2012) In addition, the PBMCs of diabetes patients contained a larger number of intracellular bacteria than those of healthy controls, suggesting that elevated blood sugar reduces the host's resistance to bacterial invasion. Recombinant IFN and IL-12 drastically lowered the bacterial load in diabetic patients' peripheral blood mononuclear cells (PBMCs), indicating that reduced IFN and IL-12 production in diabetes impairs immune cells' capacity to regulate the growth of pathogens during infection. It is thought that diabetics' elevated blood sugar levels reduce macrophage and other leukocyte capacity to eradicate infections. Insulin infusion significantly elevated the amount of IL-6 and TNF produced by bone marrow-derived macrophages obtained from diabetic mice following LPS stimulation. Tessaro and associates (2017) looked at how low insulin levels affected immunological response. Another rat investigation showed that the release of cytokines and alveolar macrophage phagocytosis are both disrupted in the absence of insulin, but both are restored following insulin administration (Ferracini et al,2010). This result suggested that giving exogenous insulin to diabetics might boost immune cell function and hence protect them from

infections since IL-6 and TNF-a are involved in leukocyte activities against pathogens. (Berbudi 2019)

Humoral responses to Type 2 diabetes.

Diabetic patients' sera had a lower capacity to opsonize S. aureus than control subjects' sera., according to research by Da Costa and Beardsley published in 1907. In a 1973 study of 46 patients, Farid and Anderson discovered that diabetics receiving insulin had lower IgG levels than those receiving only oral medications or diet alone. In a research of 66 type 1 diabetic patients conducted it was found that Uncontrolled diabetics had lower total IgG levels, as measured by HbA1c. (Liberator et al, 2005). Additionally, humoral rather than cellular variables appear to be the cause of the apparent deficiency in neutrophil phagocytosis. Studies on vaccines provide the strongest support for a humoral abnormality in diabetic patients. Diabetics had agglutinin deficiencies following subcutaneous typhoid immunization as early as 1930. (Moen et al,1933; Richardson et al,1933). Diabetes patients are less likely to produce a protective antibody response to hepatitis B immunization, according to numerous research (Ficicioglu et al,1995; Bouter et al,1992). leading some authorities to advise diabetes patients to routinely add a booster dose to the recommended course of treatment (Ficicioglu et al, 1995; Douvin et al, 1997). The research on the flu shot is more contradictory (reviewed by Brydak and Machala ,2000). Following influenza vaccination, type 2 diabetic patients had less activated lymphocytes, but no variations in antibody responses, according to Pozzilli et al analysis 's of 52 diabetic patients in 1986. In a 2003 research by Muszkat et al. on an older population, they discovered that people with type 2

diabetes had reduced antibody responses. Despite the fact that the initial response to the tetanus vaccination appears to be normal, diabetes is also linked to a shortening of the duration of protection it provides (Tamer et al,2005; Kilic et al,2003). Pneumococcal polysaccharide vaccines are well-received by diabetics; nevertheless, the length of their protection has not been studied.(Beam and others, 1980). There are no studies that explicitly link diabetes to humoral responses in sepsis (Koh et al, 2012).

Complement abnormalities

Type 1 diabetes has been linked to inherited impairments of component 4 (C4) (Mijovic et al, 1987; Jenhani et al, 1992), however it is unknown if this makes type 1 diabetics more susceptible to infection. In contrast, according to Hernandez et al. (2007), excessive levels of C3 are thought to be linked to obesity and high insulin levels (such as those found in T2DM). No matter the cause of the diabetes, complement C5 and C8 levels are elevated. Karlsson et al. (2008) looked for biomarkers of young adults who got diabetes as they got older. One potential explanation for these abnormalities is that glycated immunoglobulins have been shown to stimulate complement activation. It's possible that glucose assaults complement C3's thioester bond and inhibits it from adhering to the bacterial surface, which would explain why diabetic sera are less effective at opsonizing bacteria (Hosteller et al, 1990).

Innate immune response to malaria

Monocyte:

Lymphopenia, thrombocytopenia, and anemia are often observed hematological abnormalities in malaria. Children from Ghana who had a range of clinical *P falciparum* malaria were studied, and monocyte frequencies were constantly increased throughout the illness. The authors found that children with malaria had higher peripheral blood monocyte-to-lymphocyte (M:L) ratios, which correlated with parasitemia and malaria severity (Antwi et al,2018). Another study found that Kenyan toddlers with simple *P falciparum* malaria had an elevated M:L ratio that returned to normal 6 weeks after treatment (Dobbs et al,2017). Warimwe et al. (2013) found higher M:L ratios in Kenyan asymptomatic parasitemia toddlers (2013). Dobbs et al. 2017 found that ex vivo Kenyan child monocytes with acute malaria have weaker opsonic phagocytosis. than their own six weeks following recovery. Asymptomatic children with parasitemia had monocytes that were less able to phagocytose infected erythrocytes than those without parasitemia.

Macrophages:

The innate immune system's macrophages defend against infections in practically all tissues (Varol et al,2015; Davies et al,2013). Current research shows that most tissue resident macrophages are produced during pregnancy and self-renew without circulating monocytes (Sheng et al,2015; Ginhoux et al, 2016). Infection and inflammation diminish local macrophages but replenish them with circulating monocytes (Guilliams et al, 2017 ; Bleriot et al, 2015 ; Robinson et al, 2012).

According to human and mouse studies, mononuclear phagocytes can phagocytose infected erythrocytes without cytophilic or opsonizing malaria-specific antibodies. Mononuclear phagocytes may contribute to malaria-protecting innate immunity. Macrophages also combat malaria like APCs (Serghides et al,2003).

Kain and colleagues Serghides et al., 2003 found that monocytes from non-immune people phagocytose *P. falciparum*-infected erythrocytes without opsonin using the class B receptor CD36. Monocytes phagocytosing infected erythrocytes showed this role. It prevents monocytes and macrophages from producing pro-inflammatory mediators by targeting PfEMP1. Infected erythrocytes adhering to CD36 may alter adaptive immunity and infection severity. CD4+ T-cell-derived IFN-1-3 activates macrophages to cause antibody-dependent cellular inhibition or synthesize anti-parasite molecules like nitric oxide. making them more important during adaptive immunity. This may make macrophages more crucial in immune response. (2004).

Natural killer cells

NK cell IFN- responses are induced in the majority of volunteers when human PBMCs are exposed in vitro to *P. falciparum*-infected erythrocytes (Artavanis et al, 2002). In vivo NK cells have been demonstrated to be activated due to the fact that PBMCs obtained from children with acute P. falciparum infections exhibited enhanced lytic activity against the NK-sensitive cell line K562 (Theander et al., 1988). Prior to the onset of clinical signs, soluble granzyme A and IFN- levels rise simultaneously in mice infected with malaria (Hermsen et al., 2003). Because T and NKT cells release IFN- 24 to 48 hours

after the peak of the NK-cell reaction (12 to 15 hours), and because their activation is closely tied to it, NK cells may be the first step in a chain reaction of innate immune responses (Artavanis et al., 2002). NK cells might initiate the procedure.

Immune response to *P. falciparum* infection

Regulatory T cells in *P. falciparum* infection

There is evidence that PBMCs from children who had a severe *P. falciparum* infection Malaria parasite infection is long-term and has been linked to "suppressed" immune responses to the parasite and to other antigens for a long time (Greenwood et al,1972). Malaria immunity must also be kept up with prevaccination which can be periodic reinfection or chronic, low-level parasitism (Striuk et al, 2004). Given how hard it is to control an overpowering infection and stop immunopathology at the same time,. Over the course of more than a decade, more and more evidence has shown that regulatory T cells are tempting to consider (Artavanis et al, 2003). These data suggest that regulatory T cells are crucial for immune-mediated disease regulation. T-cell cytokines regulate malaria immunity.

In a recent set of studies types 1 responses are modified by malaria in rodents, and both IL-10 and TGF-B were discovered to play significant roles in this process. Protective effects of IL-10 against Th1-driven pathogenesis and subsequent mortality were initially observed in *Plasmodium berghei* infections .When IL-10 is blocked in mice that are resistant to the disease, the disease gets worse and more mice die. Giving recombinant IL-10 to animals that are normally vulnerable to the disease stops them from dying (Kossodo et al,1997).

IL-10-/- mice develop greater pathology when infected with *P. chabaudi chabaudi* due to excessive TNF-α production (Li et al,2003

Humoral responses to *P. falciparum* infection

People who live in places where malaria is common and don't die from it before they are middle-aged will eventually become immune to it, but it takes time and repeated exposure (Marsh et al,2006). After some time , the capacity to treat parasitemia in the blood will have been established. No one knows anything at all about how disease resistance is built or what factors make a good defense. On the other hand, it is well known that antibodies protect against *P. falciparum*-caused clinical malaria. By giving immunoglobulins from people who didn't have *P. falciparum* to people who did, symptoms and parasitemia were reduced (Mayxay et al,2001).

With strong immunity, the risk of malaria severity decreases. However, defense mechanisms necessitate collaboration between cellular and antibody (Ab) responses (Gonzales et al, 2020). Antibodies are essential for reducing parasitemia and the clinical symptoms of malaria in humans and play a pivotal role in this process (Gonzales et al., 2020). The establishment of protective immunity is critically dependent on the interaction between monocytes and antibodies (Kana et al, 2018). Antimalarial antibodies that are cytophilic play a significant part in opsonization, phagocytosis, antibody-dependent cellular cytotoxicity, and/or antibody-dependent complement-induced cytotoxicity (ADCC) (Kana et al, 2018). The ratio of cytophilic Ab to non-cytophilic Ab influences immune activities against malaria (Adamou et al, 2019; Kana et al, 2018). In endemic populations, there is a strong correlation between a high

percentage of IgG1 and IgG3 cytophilic antibodies and a relatively lower parasitaemia. Conversely, non-cytophilic antibodies like IgG4 may control effector actions by competing with cytophilic antibodies (Kana et al, 2018)

Antibodies against proteins located on the surface of infected red blood cells or the parasite's merozoite form have been shown to be important components of innate malaria immunity (Richard et al., 2009).Reddy et al (2012) looked at the antibody affinities of MSP2 and AMA1, which are important immunological targets and the best candidates for a blood-stage *P. falciparum* vaccine, in serum. During follow-up in a part of Tanzania where the disease was very common, they found that people who had strong antibodies against MSP2 and AMA1 antigens were less likely to get sick. Also, the time between malaria attacks was longer in people with the strongest ties to MSP2-3D7. Both MSP2-3D7 and AMA1 got better with age. Research indicated that affinity is crucial for malaria protection and should be considered while selecting vaccine candidates.

Effect of *Plasmodium* falciparum and type 2 diabetes on the immune system

Danqua et al. (2010) found that people with T2DM had more *Plasmodium spp.* cases than people who did not have the disease. The cases were mostly caused by *P. falciparum* (16% vs. 10%; p = 0.001). These differences were not caused by new antimalarial drugs. A *P. falciparum* infection was linked to a number of factors that were different in people with and without T2DM. A multivariate study, on the other hand, that looked at age showed that people with T2DM were more likely to get *P. falciparum.* This risk rise was still very clear when the

same model was used to compare people with T2DM to people who didn't have diabetes.

The chance of getting *P. falciparum* increased by 5% for every mmol/L rise in blood glucose when glucose concentration was used instead of type 2 diabetes mellitus. A step-by-step method showed that people with T2DM were more likely to get sick when their glucose level was 8.6 mmol/L.

As type 2 diabetes and immune system problems get worse, partial immunity may not be able to control parasitemia as well (Muller et al., 2005). Fried et al. (1998) say that there is a link between the risk that was shown and glucose levels. Malaria may be more likely to infect children with serious type 1 diabetes and no immunity at all. Gestational diabetic pregnant women should be extra careful because their immune systems are weak for *P. falciparum*. Okell et al.2009 showed that type 2 diabetics with low-level infections may act as an unknown infectious reservoir in places where malaria is common. Jones et al. (2002) say that the fact that the number of people with *P. falciparum* malaria drops when they take metformin supports the idea that biguanides are effective at killing malaria parasites. Even though no one knows for sure what caused the rise in *P. falciparum* infections, Muller et al. (2005) say that the fact that the risk goes up as glucose concentration goes up shows that the theory is based on some biological truth. This risk may be higher if the immune system isn't strong enough to fight off liver or blood-stage parasites or if the infection lasts longer. Jensen et al. (1983) discovered that T2DM is connected to lowered T cell-mediated immunity but doesn't have a big impact on humoral reactions. Having more glucose available might, in theory, help *P. falciparum* grow in the lab. Takken et al. (1999) also showed that people with diabetes may get more

contagious mosquito bites because smells can change how attracted mosquitoes are to people.

Udoh et al. (2020) found that people with T2DM may harbor *P. falciparum* and that asymptomatic malaria makes it hard to manage blood sugar. Not a single study has looked into what happens to antibody reactions against malaria vaccine candidate antigens when someone has both diabetes and malaria at the same time.

Malaria vaccine candidates' antigens

Merozoite surface proteins 1 and 3 (MSP1 and MSP3) are highly described proteins in Plasmodium spp. MSP-1 is prevalent on the merozoite surface and is thought to play a role in the first binding to the erythrocyte surface. To acquire antibodies (Abs) against mutant MSP antigens (Ag), the parasite must be exposed to the body multiple times. The merozoite surface protein (MSP1 and MSP3) is a critical glycoprotein that is processed when the schizont ruptures, producing the majority of the antigens found on the adult merozoite's surface (Holder 1988). Merozoite surface proteins (MSP1 and MSP3) are responsive to humoral immunity and hence could be used as vaccine candidates (McBride et al. 1985; Diggs et al. 1993). GLURP,

Summary

In summary; There are three forms of diabetes namely Type 1 Diabetes also known as Insulin Dependent Diabetes, Type 2 Diabetes also known as non insulin Dependent Diabetes and the third form is Gestational Diabetes. T2DM patients have a 15% higher risk of death, Glucose spikes trigger the release of insulin, Increases in both IR and chronic inflammation are caused by hyperglycemia and hyperlipidemia. The five species of *Plasmodium* that are capable of causing malaria in people are *Plasmodium knowlesi, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium falciparum.* 97% of the prevalence of malaria worldwide is due to *P. falciparum (WHO, 2020b).* Diabetes causes a disruption in the immune response of the host. Macrophage activity is also negatively impacted by hyperglycemia. Antibodies are essential for reducing parasitaemia and the clinical symptoms of malaria in humans (Gonzales et al., 2020).

CHAPTER THREE

METHODOLOGY

Introduction

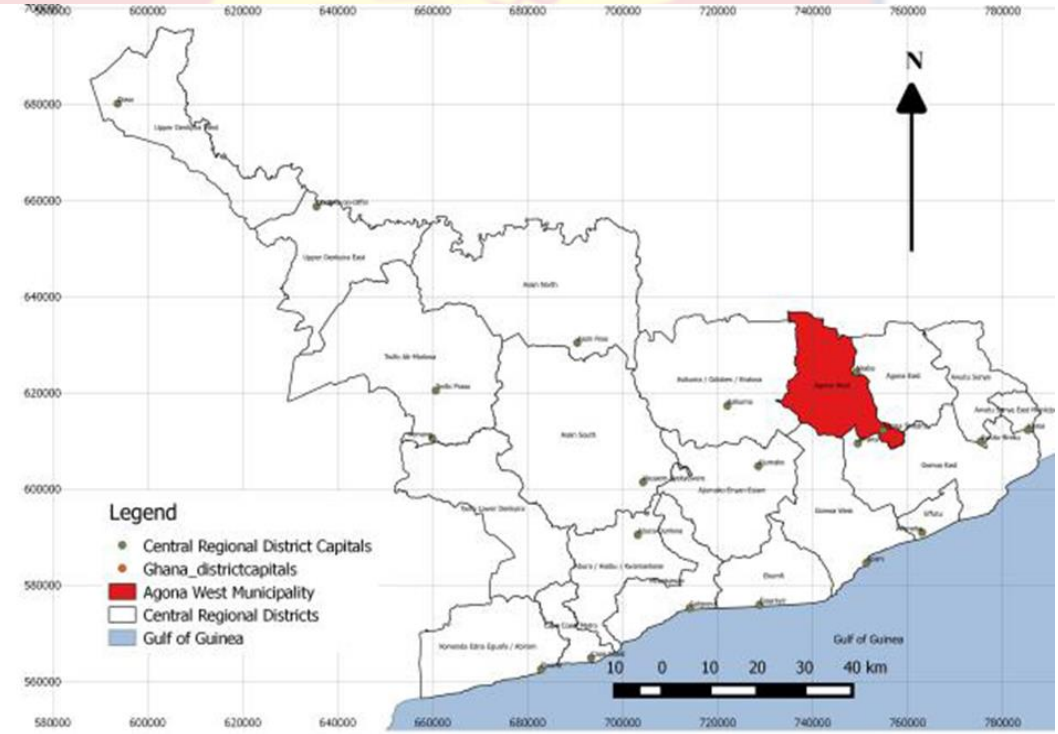
This chapter talks about where the study took place, how it was set up, who took part, how many people were in the sample, how the data was collected, how it was analyzed, and what the ethical issues were.

Study Settings

The study concentrated on residents of Cape Coast Metropolis and Agona West Municipality. In the easternmost portion of the Central Region, between latitudes 50 30' and 5 0 50'N and longitudes 00 35' and 00 55'W, is Agona West. There are 447 square kilometers of land there altogether. Six (6) Town/Area Councils or subdistricts make up the Municipality. To the north, south, and west of the municipality are the districts of Asikuma- Odoben-Brakwa, Ajumako-Enyan-Essiam, Gomoa East, and Agona East, respectively. To the east of the municipality are the districts of Gomoa East and Agona East. In the center of a network of roads leading to the Central Region's productive cocoa growing regions sits the municipal capital of Swedru. About twenty-four kilometers separate Swedru from Winneba. According to the Population and Housing Census of 2021, the Agona West Municipality is anticipated to have a total population of 136,882 people. The Municipal population's age and sex distribution reveals that women (53.1%) make up the majority of the population, with men making up roughly 46.9%.

The region's natural resources determine the Municipality's resource base. As a result, the municipality's economy is primarily based on agriculture.

The Municipality favors agriculture because of its land, climate, and labour force. As a result, agriculture is the primary source of all Municipality products. Cash crops including cocoa, citrus, oil palm, and coconut are the main focus of all farming activities in the municipality. A few clay and gold resources do exist, but not enough to support industrial production and exploitation. More than 64% of the population of Agona West is employed in agriculture, which is the area's primary economic activity. Every year in the municipality, malaria tops



the list of the ten most common diseases.

Figure 5: Shows the location Agona west Municipality in central regional

Source: 4-Year Integrated MTDP (2014-2017)

Cape Coast Metropolis, Ghana's tiniest city, covers 122 square kilometers. It's at 5°06'N and 1°15'W. It covers 124 km2. Cape Coast is the district's administrative hub. LI 1373 elevated it to town in 1987 and LI 1927 to metropolitan area in 2007. The Gulf of Guinea is south of Cape Coast Metropolitan, west of Komenda Edina Eguafo Abrem City, east of Abura Asebu

Kwamankese District, and north of Twifo Hemang Lower Denkyira District. According to the 2021 population and housing survey, the Metropolis has 189,925 residents, 92,790 men and 97,135. Secondary forest with 4.5-meter-tall thickets and bushes prevails. 13 kilometers of shoreline. 240 C to 320 C, 60%–80% relative humidity. May, June, and October have the greatest rains. Along the shore, annual rainfall is 90–110 centimeters; inland, 110–160.. There are dry spells (harmattan) between November and February. The terrain is typically hilly, with valleys separating the hills and a peak elevation of about 60 meters above sea level. Siwere and Kakum are the principal rivers and streams, respectively. The Fosu is one of the lagoons. Low-lying places are less than 60 meters above sea level. The Fosu Lagoon, which is located in Bakaano, and the sea, which is located in Abakam are where rivers and streams eventually drain after passing through wetlands **(**source; Ministry of food and agriculture-Ghana, 2022).

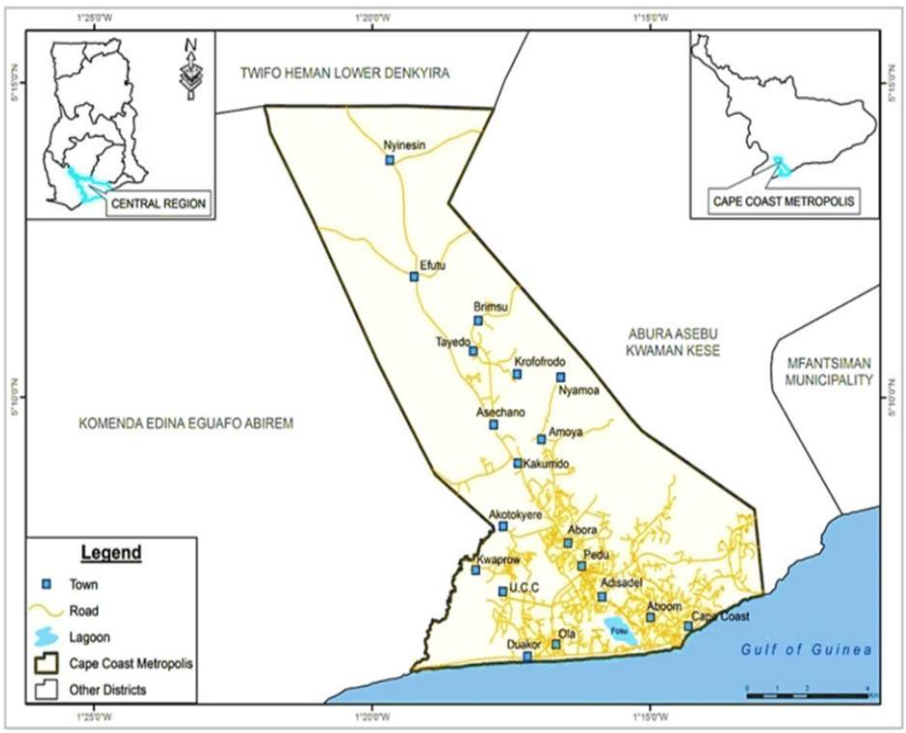


Figure 6: Map of cape coast metropolis

Study Design

To find out how having both diabetes and malaria affects the immune system's response to malaria vaccine candidate antigens., a case control study design was carried out in the cities of Cape Coast and Agona West.

Study Participants

Participants were chosen from patients seeking medical attention at the Cape Coast Teaching Hospital and the Agona Swedru Municipal Hospital, as well as from diabetic patients seeking evaluation at the institutions' diabetic clinic. Patients were informed of the study's procedure and goals, and those who agreed to participate were required to sign a consent form before being questioned using a questionnaire. Their blood was drawn into an EDTA tube in order to collect the necessary study samples. A total of 146 individuals took part in the study, of whom (n=40) had diabetes only, (n=27) had both diabetes and malaria, (n=41) had malaria only, and (n=38) did not have any of these conditions.

Sample Size

A minimum sample size of eighty-seven (87) was estimated for this study using the formula below.

The following formula determines the sample size:

Z is the standard normal variate with a 95% confidence interval of 1.96, and n = (Z2 p(1p))/e2.

In the earlier study that was conducted by Danquah et al. (2012), the prevalence T2DM was reported to be 6%, and the margin of error was reported

to be 0.05.n (minimum number of participants) = (1.96 x (0.06) (1 x 0.06), dividing by 0.05 yields 87. But this study recruited 146 respondents; comprising of 38 negative controls, 40 diabetes only group. 27 diabetics with malaria infection group and 41 malaria only infected group

The Inclusion criteria

Diagnosis of diabetes was based on glucose values, fasting plasma glucose of ≥ 126mg/dl or ≥ 7.0 mmol /dl or 2 hours postprandial > 200mg/dl or ≥ 11.1mmol/dl according to WHO criteria, and diagnosis of malaria was based on a positive result from RDT and confirmed with microscopy Negative control was based on individuals who visited the health facility and test negative for both malaria and diabetes and aged 40 and above.

Exclusion criteria

Any individual aged below 40 years was excluded from the study. Those above 40 years who refused to consent to the study were also excluded. Those diagnosed with type 1 diabetes and gestational diabetes were excluded from the study. Above all, individuals with diagnosed health conditions such as fibrosis, hepatitis A, B,C,D ,HIV, pancreatitis, viral and bacterial infections, rheumatoid arthritis, asthma and heart failure were excluded from the study based on their health records because these conditions can affect antibody production levels.

Ethical Consideration

Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC/EC/2021/058) approved the study. The study methods and confidentiality were explained to participants. Before being recruited, each participant agreed to the data and sample collection.

Data Collection

Collection of Socio-demographic Data

Information on age and gender were collected through interview with structured questionnaire. The questionnaire was structured into two sections, the socio-demographic characteristics, sample collection and laboratory investigation.

Blood Sample Collection

Five (5) milliliters of peripheral blood were withdrawn from participants before breakfast, all blood samples were centrifuged for 5 minutes at 1500 rpm to separate the plasma and kept at -80°C.Those with diabetes who tested positive for malaria as well as participants without diabetes who tested positive for malaria had their venous blood drawn with sterile hypodermal syringes and placed into a vacutainer EDTA tubes and centrifuged to separate the plasma. Additionally, participants who did not have diabetes or malaria were recruited as control group. Before their blood samples were collected, participants signed a consent form. The sera were transported to the Noguchi Memorial Institute for Medical Research (NMIMR) and stored at -80 degrees

Celsius until they were ready to be used for immunological assessments of antibody levels.

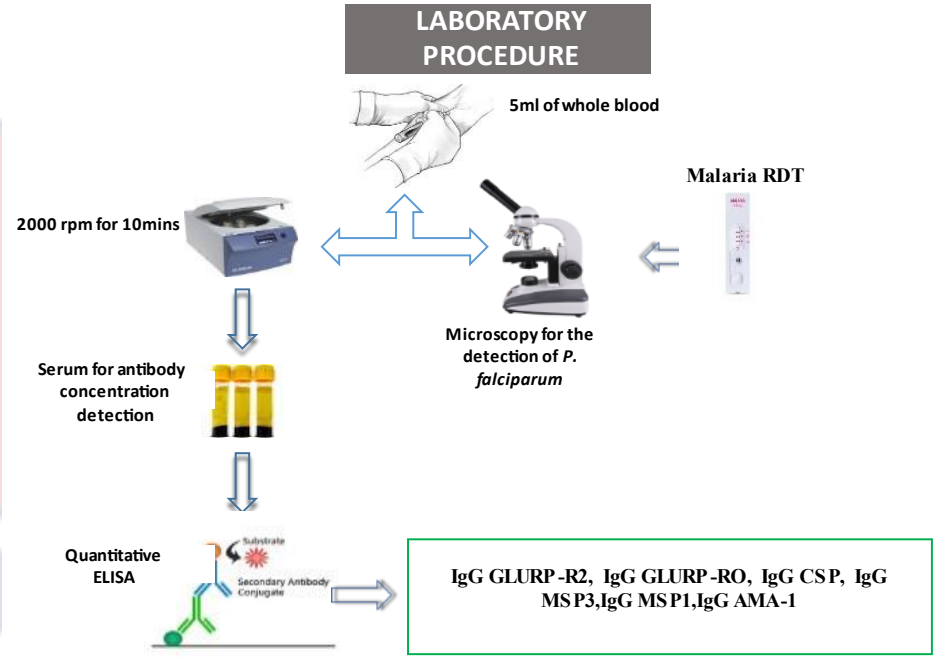


Figure 7*:* ELISA Laboratory procedure

***P. falciparum* estimation**

With a Rapid Diagnostic Test (RDT) kit (CareStart TM Malaria PfHRP2/pLDH Ag RDT, Access Bio, Inc., USA), blood from a finger prick was used to check for malaria infection. Based on the fact that there are 8,000 leukocytes per microliter of blood,200 leukocytes in a thick film were used to figure out the parasite density. Results of the test was interpreted following WHO guidelines(WHO, 2016). Two clean slides were used for each sample to make both thick and thin blood films. Afterward, the slides were stained using a 10% Giemsa stain solution (Cheesbrough, 2006). Slides were examined under oil immersion without knowledge about HRP2-RDT results. Parasitemia quantification was estimated following standard protocol. Thick blood films

were used for malaria diagnosis and quantification of malaria parasites if present. Thin blood films on the other hand were used for malaria specie identification; however, in cases of hyper-parasitaemia, thin blood films were used for quantification.

Plasma antibody quantification for malaria vaccine candidate antigens using the ELISA method

In all plasma samples, IgG quantification against *P falciparum* antigens (GLURP-R2, GLURP-RO, MSP3, MSP1 AMA-1, and CSP) was performed using indirect ELISA in accordance with the standardized ELISA protocol.Unless the optical density (OD) of the test samples was over the reading limit for the ELISA plate reader, at which point the test sample must be further diluted, the plasma dilution was in the range of (1:100, 1:200, or 1:2000).Positive Control and Negative Control dilutions of pools of plasma from clinically immune adult blood donors who had not previously been exposed to malaria were identical to plasma dilution

Using a polyclonal reference IgG (1.0 mg/ml), a calibration (standard) curve for antibody quantification was built for each test. For the standard curve, serial 2-fold dilutions of standard IgG proteins were directly coated onto duplicate wells in columns 1 and 2 using 100 per well. Standard IgG concentrations were 200, 100, 50, 25, 12.5; 6.25; 3.13; and 1.56 ng/ml.

Microtiter plates were coated in columns 1 and 2 with 100uL of 0.5ug/mL of desired antigen in PBS coating buffer and 100uL of serially diluted standard reference IgG solution in PBS coating buffer**.** For a test the next day, plates were sealed with plastic overnight. Antigen name, serial number, date, and personnel

initial were all labeled on each plate. The plates were kept in the refrigerator between 2 and 8°C.

Serum dilution buffer (1% BSA in PBST plus 0.02% NaAz) was used to dilute plasma samples (1: 100, 1: 200, and 1:2000). The dilutions were kept in the refrigerator between 2 and 8°C.The dishes were taken out of the refrigerator and rinsed in washing buffer four times (PBST with 0.5 M NaCl,). Each time we washed a dish, we filled the plate with washing buffer and left it there for 1 minute before emptying it. We padded the plates dry and left them at room temperature for an hour after adding 150 of blocking buffer (3% BSA in PBS-Tween 20 (PBST)).The plates were emptied, dried with a pad, and washed in a washing buffer four times. Each time we washed a dish, we filled the plate with washing buffer and left it there for 1 minute before emptying it. Dilution buffer 100 was added to the wells. A PBS blank, 100 each of the diluted positive and negative control samples, and 100 each of the test sample were added into the wells in duplicate. The samples were then incubated for 2 hours at room temperature on a rocker platform without shaking. Washing buffer was used to wash the plates four times. The plate was filled and allowed to stand with the washing buffer for 1 minute after each washing stage before being emptied.Each well was given 100 ml of goat anti-human IgG that had been diluted with dilution buffer to a concentration of 1:3000. rocker platform was used to gently rock the contents during the one hour incubation period at room temperature.

Washing buffer was used to wash the plates four times. The loaded plate was immersed in the washing buffer for one minute for each washing cycle before being removed. Each well gets 100 cc of color solution put to it. Incubate

for 10-30 minutes at room temperature in the dark. Each well received 100 ml of 0.2 M H2SO4, and a plate reader read the absorbance at 450 nm and 620 nm. The team received the results via email once they were saved on the lab's desktop PC. A curve-fitting tool based on the ADAMSEL software was used to convert absorbance values into A.U.

Data analyses

Version 8.0 of GraphPad Prism manufactured by Dotmatics USA was used to analyze the data. Mean and standard deviation were employed to present continuous values, whereas frequency and percentage were used for categorical variables. Creating Log10 units involved arbitrarily converting antibody units (AUs). With the aid of linear regression, the relationship between antibody levels and age was found. In addition, the impact of age and sex was taken into account while evaluating a multivariable linear regression analysis between infection status and antibody levels. P value less than 0.05 was considered statistically significant in all analyses.

Summary

In summary, the study concentrated on residents of Cape Coast Metropolis and Agona West Municipality .A case control study design was used for the study. A total of 146 individuals took part in the study. Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC/EC/2021/058) approved the study. Information on age and gender were collected through interview with structured questionnaire. IgG quantification against *P falciparum* antigens (GLURP-R2, GLURP-RO, MSP3, MSP1 AMA-1, and

CSP) was performed using indirect ELISA. Version 8.0 of GraphPad Prism manufactured by Dotmatics USA was used to analyze the data.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

In this work, the effects of diabetes and malaria co-infection are examined in relation to naturally acquired antibody (IgG) responses to malaria vaccine candidate antigens. in Cape coast metropolitan and Agona west municipal. Blood samples were analyzed to assess the parasite presence and levels, while measuring serum levels of IgG against the malaria antigens MSP1, MSP3, GLURP-R0, GLURP-R2, CSP, and AMA-1 among four groups, thus those with malaria and type 2 diabetes comorbidity, those with T2DM only, those with malaria infection only and those with neither malaria nor T2DM. The findings are given here in the context of other empirical findings that have been gathered previously. All of the statistical relationships were found to be statistically significant at the 5% significance level.

Results

Demographic and clinical Characteristics of the Study participant

The study recruited 40 participants with T2DM only, 27 participants with T2DM and malaria comorbidity, 41 participants with malaria only, and 38 individuals with neither malaria nor T2DM in the statistical analyses. Age did not differ significantly between the groups that were investigated ($p = 0.971$), as indicated by the statistic. There was no statistically significant difference in the distribution of gender between the research groups ($p = 0.243$). On the other side, there was a statistically significant correlation between the participant groups and the temperature ($p = 0.001$).Also, the group with T2DM and malaria

comorbidity had significantly higher *Plasmodium falciparum* parasitaemia (10815.82540.0) than the malaria only group (1349.01373.6) (p = 0.02) (Table 1

Table 1: Clinical and demographic details of the study participant

Variables	**Diabetes Only (N=40)**	**Co-Morbidity (N=27)**	**Malaria Only (N=41)**	**Negative Control (N=38)**	**P-value**
Age (Mean±SD)	57.2±105	57.2±12.7	58.3±130	57.4±10.3	0.97[a]
Gender					
Male	12(41.4)	5 (17.2)	5(17.2)	7 (24.1)	0.24[b]
Female	28 (23.9)	22 (18.8)	36 (30.8)	31(26.5)	
Temperature (Mean± SD)	36.4±0.8*	36.5±0.8	37.0±0.*	36.3±0.7**	**0.00[a]**
Pf Density (Mean± SD)		10815.8±2540	1349.0±1 373.6		**0.02[c]**

[a] One-way ANOVA; [b] Chi-square test of association; [c] Independent T-test. Values in bracket are percentages.

The relationship between age and antibody responses to malaria vaccine candidate antigens

Linear regression examined the age-IgG response association to malaria vaccine candidate antigens. Inversely, IgG reaction against MSP3 antigen increased with age (r2=0.027, p = 0.048). IgG reaction against GLURP-R2 (r2=0.005, p=0.390), GLURP-R0 (r2=0.009, p = 0.267), MSP1 (r2=0.001, p=0.699), CSP (r2=0.001, p=0.752), and AMA-1(r2=0.008, p=0.281) antigens showed no significant correlation with age (Figure 4.2).

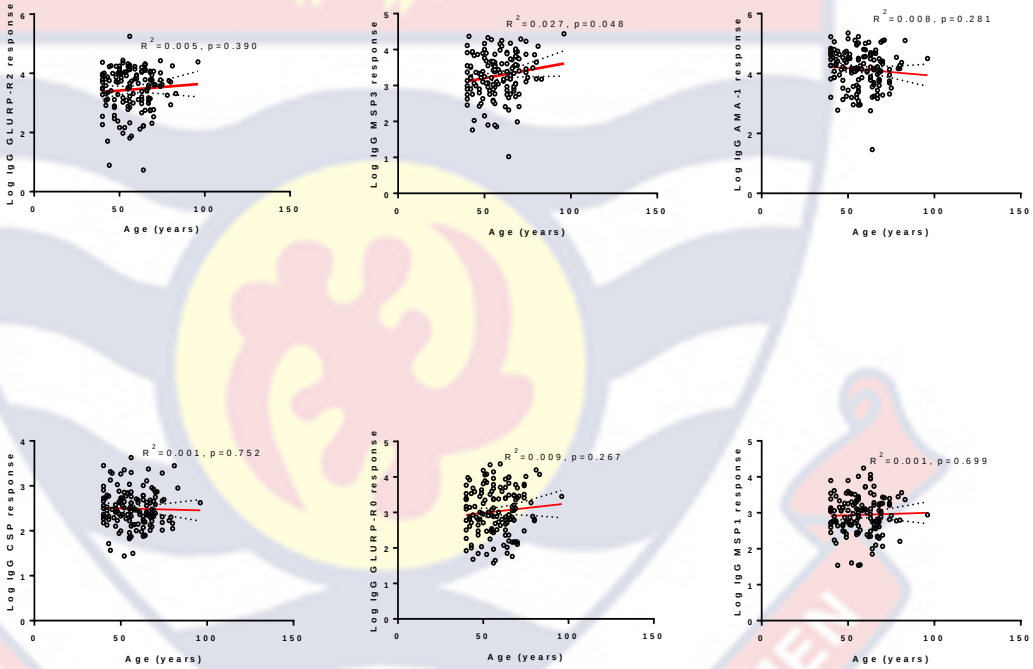


Figure 8*:* relationship between age and antibody responses to malaria vaccine candidate antigens

Antibody reactions against potential antigens for a malaria vaccination among the different study groups

Antibody reactions to potential antigens for a malaria vaccine were analyzed in relation to a variety of infection states. Antibody levels to GLURP-R2 (β=0.47,95% CI=[0.10-0.83], p=0.013), GLURP-RO (β=0.36,95% CI=[0.03-0.68], p =0.034), and MSP1 (β=0.35,95% CI=[0.09-0.61], p =0.008) were all significantly higher in those who had malaria and T2DM in comparison to the group that did not have either condition. Antibody levels to GLURP-R2 were significantly higher in individuals who had been infected with malaria compared to those who were in the control group (=0.36, 95% CI=[0.04-0.69], p=0.026). Antibody levels against GLURP-RO, MSP3, MSP1, CSP, and AMA-1 were not significantly different between people with malaria and the negative control group ($p > 0.05$). Similarly, antibody levels against all malaria antigens were not significantly different between people with T2DM and the negative control group ($p > 0.05$). There was also no discernible difference in the amounts of MSP3, AMA-1, or CSP antibody between the group that had type 2 diabetes and malaria coinfection and the control group ($P > 0.05$). (Table 4.2a and Table 4.2b)

After correcting for age and sex, the antibody levels against GLURP-RO (β=0.32, 95% CI=[0.07-0.59], p=0.027) and MSP1 (β=0.29, 95% CI=[0.02-0.47], p=0.048) were also significantly higher in the group with type 2 diabetes and malaria coinfection. There was no statistically significant difference ($P > 0.05$) in the amounts of antibodies against GLURP-R2, CSP, AMA-1, or MSP3. (Figure 4.3)

Table 2: Antibody responses to malaria antigens in the context of malaria and diabetes infection

Table 2 Crude analysis of the association between IgG response and Infection status

	Diabetes Only		**Co-infected**		**Malaria Only**	
IgG Response	β (95% CI)	P value	β (95% CI)	P-value	β (95% CI)	P-value
GLURP-R2	-0.002 (-0.32, 0.32)	0.991	**0.46 (0.10, 0.83)**	**0.014**	**0.37 (0.06, 0.69)**	**0.022**
MSP3	0.04 (-0.23, 0.31)	0.789	0.10 (-0.21, 0.41)	0.513	0.25 (-0.02, 0.52)	0.070
AMA1	-0.06 (-0.35, 0.23)	0.682	0.02 (-0.30, 0.35)	0.882	0.07 (-0.21, 0.36)	0.611
GLURP-R0	-0.13 (-0.41, 0.16)	0.392	**0.35 (0.02, 0.68)**	**0.037**	0.10 (-0.18, 0.39)	0.479
MSP1	0.05 (-0.18, 0.28)	0.669	**0.35 (0.09, 0.61)**	**0.008**	0.17 (-0.06, 0.39)	0.147
CSP	-0.09 (-0.27, 0.10)	0.354	0.03 (-0.18, 0.23)	0.795	0.03 (-0.15, 0.21)	0.717

Multivariate regression analysis without age and gender adjustments. , estimated covariate effect on antibody level; CI, confidence interval. Log10 transformations were performed on arbitrary antibody units. The model's reference was set to the negative control.

Table 3: Adjusted analysis of the association between IgG response and Infection status

	Diabetes Only		Co-infected		Malaria Only	
IgG Response	β (95% CI)	P-value	β (95% CI)	P value	β (95% CI)	P-value
GLURP-R2	0.01 (-0.31, 0.33)	0.946	**0.47 (0.10, 0.83)**	**0.013**	**0.36 (0.04, 0.69)**	0.026
MSP3	0.04 (-0.23, 0.31)	0.772	0.11 (-0.20, 0.42)	0.479	0.24 (-0.03, 0.51)	0.078
AMA1	-0.05 (-0.34, 0.23)	0.709	0.02 (-0.31, 0.35)	0.902	0.07 (-0.21, 0.36)	0.606
GLURP-R0	-0.12 (-0.41, 0.17)	0.428	**0.36 (0.03, 0.68)**	**0.034**	0.09 (-0.19, 0.38)	0.517

MSP1	0.05 (-0.18, 0.28)	0.638	**0.35 (0.09, 0.61)**	**0.008**	0.16 (-0.06, 0.39)	0.160
CSP	-0.08 (-0.27, 0.10)	0.374	0.03 (-0.18, 0.23)	0.802	0.03 (-0.15, 0.22)	0.723

Multivariate regression analysis with age and gender adjustments. , estimated covariate effect on antibody level; CI, confidence interval. Log10 transformations were performed on arbitrary antibody units. The model's reference was set to the negative control.

Table 4: Antibody responses against malaria antigens among comorbidity in relation to malaria infection

	Co-infected	
IgG Response	β (95% CI)	P-value
GLURP-R2	0.13 (-0.23, 0.48)	0.478
MSP3	-0.12 (-0.39, 0.16)	0.405
AMA1	-0.05 (-0.33, 0.24)	0.741
GLURP-R0	0.32 (0.07, 0.59)	**0.027**
MSP1	0.29 (0.02, 0.47)	**0.048**
CSP	0.004 (-0.22, 0.23)	0.975

Multivariate regression analysis with age and gender adjustments. estimated covariate effect on antibody level; CI, confidence interval. Log10 transformations were performed on arbitrary antibody units. The malaria-only infected group was used as the model's reference.

Discussion

The purpose of this study is to look into how diabetes and malaria comorbidity affect naturally occurring antibody (IgG) reactions to malaria vaccine candidate antigens in Cape coast metropolitan and Agona west municipal.

T2DM is thought to be an immunosuppressed condition that increases the risk of infection for those who have it. Diabetes impairs the capacity of the immune system to combat liver and blood-stage parasites as well as increases the *falciparum* parasites' access to glucose (Kalra et al., 2017). Glycosylation can make antibodies produced by vaccination or natural exposure less biologically effective (Peleg et al., 2014) They also discovered that IgG glycosylation occurs in relation to HbA1c levels in diabetic patients. In other words, a person who has both diabetes and malaria may not respond effectively to any malaria vaccination. Nonetheless, there has been several malaria vaccine effectiveness research in malaria-endemic areas. There has been no research to assess the efficacy of the malaria vaccine in individuals with autoimmune diseases like diabetes in Ghana. co-existing cases of diabetes and malaria are quite common in underdeveloped nations, which makes it imperative to investigate how the two diseases interact. According to a study by Danquah et al. (2012), the Ghanaian population with T2DM had a higher chance of contracting *P. falciparum* infection than people without diabetes. Kalra et al., 2017 revealed that diabetes can weaken the immune system's fighting capacity off liver and blood-stage parasites, increasing the glucose accessibility for *falciparum* and the severity of malaria .

Association of clinical characteristics among the study groups

It has been shown that temperature plays a role in parasitic or vector diseases like malaria. According to some models, the sensitivity of the mosquito maturation rate is lowest and highest at 24°C and 30°C, respectively (Agusto et al., 2015). According to a 2010 study by Oluleye and Akinbobola, Temperature strongly affects malaria spread, this result is consistent with recent study findings, which found a substantial correlation between temperature and malaria infection. The average temperature found is consistent with (Agusto et al., 2015), who showed that temperatures higher than 34°C negatively affect malaria parasite survival and result in low parasitaemia because the parasites' ability to survive depends on temperatures rapidly falling below 30°C and 32°C (Agusto et al., 2015).

The influence of age and antibody responses to potential antigens for a malaria vaccine

In order to develop a protective immunity against *P. falciparum*, naturally acquired antibody responses need to be exposed to the parasite repeatedly. The type of antigen and the age of the human host are two factors that affect the rate of antibody acquisition against *P. falciparum* proteins (Kobayashi et al,2019;King et al,2015; McCallum et al,2017; liu et al,2018).

The relationship between IgG response to malaria vaccine candidate antigens and age was evaluated, and it was found that increasing age was substantially associated with an increase in IgG response against the *Plasmodium falciparum* Merozoite Surface Protein-3 (MSP-3). In general, as people age and transmission intensity increases, so do antibody levels

(McCallum et al, 2017;King et al, 2015; Kobayashi et al., 2019; McCallum et al, 2017). Previous studies by Elbashir et al (2008) in eastern Sudan, Kwenti et al. (2019) in Cameroon and Reddi et al. (2012) also identified an increase in IgG response against other *Plasmodium falciparum* antigen (MSP)

Association between *P. falciparum* parasitaemia level among type 2 diabetes and malaria comorbidity

In various African research, it has been shown that diabetes and malaria are more frequently associated (Kalra et al,2017).

According to the findings of our investigation type 2 diabetes and malaria comorbidity group significantly recorded higher *Plasmodium falciparum* parasitaemia which agrees with the findings of an earlier study carried out in Ghana by Danquah et al (2010) and also with Katja et al (2017) in Sweden, both studies recorded higher *Plasmodium falciparum* parasitaemia in those with diabetes than their counterpart respondents without diabetes. However, these findings are contrary to studies done in Nigeria by Ndiok et al. (2016) and Park et al. (2010). Both studies recorded lower *P. falciparum* parasitaemia in diabetics than their non-diabetics counter parts. This disparity could be because of differences in where they live and level of malaria elimination activities being carried out at the study settings. Although the precise causes of the rise in *P. falciparum* infection are unknown. Udoh et al. (2020) showed that people with T2DM could be *P. falciparum* reservoirs and that asymptomatic malaria makes it very hard to control blood sugar. This risk may be increased by a weakened immune response against liver or blood-stage parasites, as well as by prolonged persistence of the infection. Jensen et al.

(1983) found that T2DM is related with a decline in T cell immunity, and it has only a minor impact on humoral responses. Increased glucose availability may, in theory, fuel the in vitro growth of *P. falciparum*. Additionally, Takken et al. (1999) showed that individuals with diabetes may experience more contagious mosquito bites because olfactory signals influence mosquito attraction.

Antibody responses of study groups to malaria vaccine candidates' antigens

It is critical to understand the relationship between the natural immune response to malaria and the potential protective advantage of naturally produced immune responses in order to properly build a vaccine. Human antibodies against CSP, AMA-1, MSP1, and MSP3 limit P. falciparum development in vitro, as do monocytes. This is due to the participation of GLURP (R0 and R2). Oeuvray et al.(1998;1994).

Passive transfer trials have demonstrated that antibodies can reduce morbidity and parasite density in malaria patients (Jouin et al., 1995; Cohen et al., 1961). Diabetes patients are less likely to produce a protective antibody response to hepatitis B vaccine, according to multiple studies (Ficicioglu et al., 1995; Bouter et al., 1992), leading some authorities to advise. Despite the fact that the initial response to the tetanus vaccination appears to be normal, diabetes is also linked to a shortening of the duration of protection it provides (Tamer et al,2005; Kilic et al,2003). Diabetes patients tend to respond well to the pneumococcal polysaccharide vaccination, despite the fact that there are no trials examining the duration of protection in this population. (Beam et al., 1980). Studies

directly relating humoral responses in sepsis for that matter malaria to diabetes do not exist (Koh et al,2012).

However in our study, comparisons were made between the levels of antibody reactions against malaria vaccine candidate antigens in people whose infection statuses varied. Individuals with malaria and type 2 diabetes comorbidity had significantly higher antibody responses to GLURP-R2, GLURP-RO and MSP1 in contrast to the control group. The increase in the antibody level could be due to the increase in the *P. falciparum* parasitaemia level observed in the type 2 diabetes and malaria comorbidity group and also the chronic nature of the T2DM could be a contributing factor. These findings are similar to a recent study by Nikolov et al,2020 who discovered that type 2 diabetes patients with hypertension comorbidity with microvascular problems had serum levels of total anti- advance glycation to end product of vascular elastin (AGEs) antibodies that were considerably greater than those of healthy individuals and patients without these issues .They investigated the amounts of IgM and IgG autoantibodies to AGEs of vascular endothelial cells (AGE EL) in the serum of people with severe types 1 and 2 diabetes and hypertension who are at an increased risk for cardiovascular disease. According to the results of the study, those with type 2 diabetes had significantly higher amounts of anti-AGE EL IgG antibodies than those in the control group. which can be attributed to the disease's chronic nature.

Additionally, patients with malaria infection exhibited considerably greater antibody levels to GLURP-R2 than the negative control group. This finding is consistent with the findings of a study conducted in Burkina Faso by Nebie et al. (2008), who discovered that antibodies to GLURP segments (R0

and R2) in children infected with malaria were higher during the peak of the malaria high transmission season in the communities of Tensobentenga and Balonghin. Antibodies are critical in lowering parasitemia and clinical symptoms of malaria in humans (Gonzales et al., 2020). Antibodies are well known to provide protection against P. falciparum clinical malaria. Reduced clinical symptoms and parasitemia.

Summary

In conclusion, age did not differ substantially across the tested groups (p = 0.971). There was no statistically significant variation in gender distribution throughout the research groups (p = 0.243). There was a statistically significant relationship between participant groups and temperature (p = 0.001). Furthermore, the group with T2DM and malaria comorbidity showed substantially greater Plasmodium falciparum parasitaemia (10815.82540.0) than the malaria-only group (1349.01373.6) (p = 0.02). Antibodies to GLURP-RO (β=0.32, 95% CI=[0.07-0.59], p=0.027) and MSP1 (β=0.29, 95% CI=[0.02-0.47], p=0.048) were substantially greater in the group with type 2 diabetes and malaria coinfection.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Introduction

This chapter provides a concise overview of the study's key findings and draws conclusions that are consistent with those findings. Our research led us to conclude with this chapter, where we make concrete suggestions for future work (both in policy and practice). Additionally, ideas for additional research in the studied field are provided**.**

Summary of the study

Despite the high prevalence of malaria and T2DM co-morbidity in Ghana, there is a paucity of information about their immunological interaction and its potential impact on any malaria vaccine candidate that might be administered to such individuals. As a result, the reason for this study is to gain a deeper comprehension of the effects that T2DM has on the naturally occurring antibody responses to malaria antigens that are prospective candidates for the development of a malaria vaccine.

To determine the impact of diabetes and malaria co-morbidity on naturally occurring antibody reactions to potential malaria vaccine targets. A case control research was carried out in the cities of Cape Coast and Agona West. The study also compared the levels of *P. falciparum* parasitaemia among people with or without diabetes condition.

A total of 146 individuals took part in the study; 40 had diabetes only, 27 had both diabetes and malaria, 41 had malaria only, and 38 were negative controls who visited the hospital without any of these conditions.

Participants who gave their consent had their venous blood samples (5mL) taken using sterile hypodermal syringes and collected into EDTA tubes. The blood samples were centrifuge and sera were transported for immunological evaluations of antibody levels at the Noguchi Memorial Institute for Medical Research (NMIMR). Diabetic people were tested for malaria infection using finger prick blood samples.

The findings are presented here in the context of other empirical findings that have been gathered previously. At the 5% level of statistical significance, every statistical association was deemed to be statistically significant.

Summary of Findings

Association between p. falciparum parasitaemia level among type 2 diabetes and malaria comorbidity and malaria only infection

The type 2 diabetes and malaria comorbidity group significantly recorded higher *plasmodium falciparum* parasitaemia (10815.8±2540.0) than the malaria only group (1349.0±1373.6) ($p = 0.02$)

The influence of age and antibody responses to potential antigens for a malaria vaccine

Increasing age was significantly associated with an increased IgG response against MSP3 (r^2=0.027, $p = 0.048$).

Antibody responses of study groups to malaria vaccine candidates' antigens

People who had both malaria and T2DM had significantly higher antibody levels to GLURP-R2 (β=0.47, 95% CI=[0.10-0.83], p=0.013), GLURP-RO (β=0.36, 95% CI=[0.03-0.68], p =0.034), and MSP1 (β=0.35, 95% CI=[0.09-0.61, p =0.008) than the negative group. Also, people with malaria had significantly higher antibody response to GLURP-R2 (β=0.36, 95% CI=[0.04-0.69], P=0.026) than people in the negative control group.

Conclusion

This study revealed that individuals with *Plasmodium falciparum* and T2DM comorbidity had significantly higher IgG response against the malaria vaccine candidate antigens GLURP-R2, GLURP-RO and MSP1. Malaria parasitemia level were found to be significantly higher in those with Type 2 diabetes comorbidity.

Recommendations

Recommendations for research to be conducted in the future

1. Future research to assess the functional activity of the antibody using monocytes cell line (THP-1 cell culture and Opsonic Phagocytosis assay)
2. Future research to assess the effect of duration and dosage of metformin on malaria parasitaemia and antibody levels against malaria vaccine candidate antigens among diabetes and malaria comorbidity patients.

3. We recommend a policy should be made to screen all diabetic patients for *P falciparum* any time they visit the diabetic clinic .
4. The Government of Ghana, Ministry of Health, and other partners should invest funds for universities and research centers to do research on a larger scale to find out how having both T2DM and malaria affects the immune system's response to malaria vaccine candidate antigens. This will help us assess malaria vaccine potency among Ghanaians with diabetics

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APPENDIX I

Study Questionnaire

STUDY QUESTIONNAIRE ON

EFFECTS OF DIABETES ON NATURALLY ACQUIRED ANTIBODY RESPONSES AGAINST MALARIA VACCINE CANDIDATE ANTIGENS.

Name (Surname in blocks): .....Date: .....Serial Number: .....

Contact Tel. Number: .....

Address: .....

VITAL SIGNS AND DEMOGRAPHICS

1. Age: ..... (40 years and above) 2. Height (m): .....

3. Weight (kg): ..... 4. BMI: ..... 5. BP: .....

6. Pulse: .....7. FBS/RBS.....8.*plasmodium falciparum* estimation.....9.Temperature.....

(pleases tick [√] the appropriate box where applicable)

9. Sex: A.[Male] B. [Female]

10. Marital status A.[Single] B. [Married] C.[Divorced] D. [Widowed]

11. Number of children: A.[None] B. [1 – 2] C. [3 – 4] D.[5 or more]

12. Level of education. A.[None] B.[Primary] C.[Secondary] D. [Tertiary]

13. Occupation: A. [Unemployed] B. [Government worker] C. [Trader] D. [Others].....

LIFESTYLE

14. What type of food do you mostly eat? Can choose more than one A.[More carbohydrates] B.[Less carbohydrates] C.[More meat or fish] D.[Less meat or fish] E.[More fruits] F. [Less fruits] G.[More vegetables] H. [Less vegetables]

15. How many times do you eat in a day? A.[once] B. [Twice] C.[thrice] D. [Four times]

1

E.[Five or more times]

16. Do you Exercise? A.[Yes] B.[No]

i. If yes to question 15, how many times a week do you exercise? A.[Once a week] B. [Twice a week] C.[thrice a week] D. [four or more times week]

MEDICAL HISTORY

17. Are you diabetic? A.[Yes]B.[No] If yes, how long have you had it? i.[less than a year] ii[1-2 years] iii [3-4 years] iv.[5-10 years] v. [more than 10 years]

18.which medications are you on A. [Biguanide only eg Metformin] B. [sulphonylureas only eg Glibenclamide or Glimepiride]C.[Insulin only] D. [Biguanide and sulphonylureas only] E .[herbal only] F. [herbal and orthodox](specify).....

G.others specify.....

19.How long have you been on the antidiabetics.....

.20. How long have you been on metformin? .....

21. What is the dosage? .....

22. Do you have the following diabetes-related comorbidities, You can choose more than one answer(s) i [diabetic nephropathy] ii [diabetic eye complications] iii [diabetic foot] iv [diabetic cardiovascular complications] v[diabetic cerebrovascular disease] vi[diabetic neuropathy] (7) others, _____ (8)[none]

23. Are you hypertensive? A.[Yes] , If yes, how long have you had it? ..... B. [No]

24 .Are you on antihypertensive drug(s) ? A. [Yes] , if yes which drug(s) are you taking.....B[No]

INFORMED CONSENT FORM FOR MEDICAL RESEARCH

Full Title of Project: Effects of diabetes and malaria comorbidity on naturally acquired antibody responses against malaria vaccine candidate antigens

This study seeks to investigate the effect of T2DM on *Plasmodium falciparum* parasitaemia and naturally acquired antibody responses against malaria vaccine candidate antigens in Ghana. The successful completion of the research will provide to the scientific knowledge the first-time information on efficacy of malaria vaccine candidate antigens among T2DM patients in Ghana.

Name of Principal Investigator: Gideon Agyare

Please tick box

1. I confirm that I have read and understand the subject information sheet dated ..... version ..... for the above study and have had the opportunity to ask questions which have been answered fully. [ ]
2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from university of cape coast or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to access my records that are relevant to this research.[ ]
4. The compensation arrangements have been discussed with me. [ ]
5. I agree to take part in the above study. [ ]

_____ _____ _____

Name of Patient/Participant Signature Date

_____ _____ _____

Name of Person taking consent Signature Date

_____ _____ _____

Principal Investigator Signature Date

.....

APPENDIX II

Ethical Clearance

P. O. Box CT 1363
Cape Coast
CC-071-9967
Tel: 03321-34010-14
Fax: 03321-34016
Website: www.ccthghana.org
email: info@ccthghana.com

In case of reply the reference number and the date of this letter should be quoted.

Our Ref: CCTH

Your Ref.:

30th July, 2021

Gideon Agyare
UCC/SMS
University of Cape Coast
Cape Coast

Dear Sir,

ETHICAL CLEARANCE – REF: CCTHERC/EC/2021/058

The Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC) has reviewed your research protocol titled, "**Effects of Diabetes and Malaria Comorbidity on Naturally Acquired Antibody Responses against Malaria Vaccine Candidate Antigens**" which was submitted for Ethical Clearance. The ERC is glad to inform you that you have been granted provisional approval for implementation of your research protocol.

The CCTHERC requires that you submit periodic review of the protocol and a final full review to the ERC on completion of the research. The CCTHERC may observe or cause to be observed procedures and records of the research during and after implementation.

Please note that any modification of the project must be submitted to the CCTHERC for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the CCTHERC within ten (10) days in writing. Also note that you are to submit a copy of your final report to the CCTHERC Office.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours sincerely

Prof. Ganiyu Rahman
Chairman, ERC