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COVID-19 IN PATIENTS PRESENTING WITH MALARIA-LIKE SYMPTOMS AT KORLE BU POLYCLINIC, ACCRA.

BY

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THIS THESIS IS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, UNIVERSITY OF GHANA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL IN MEDICAL MICROBIOLOGY.

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DECLARATION

I, Isabella Asamoah do hereby declare that this thesis is the result of research undertaken by me towards the award of the Master of Philosophy in the Department of Medical Microbiology, Medical school, College of Health Sciences, University of Ghana. I certify that, with the exception of adequately referenced references to literature, this thesis has not been presented anywhere in whole or in part.

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DEDICATION

This thesis is dedicated to my parents Mr. and Mrs. Asamoah, my entire family, supervisors, colleagues, at the Department of Medical Microbiology, University of Ghana Medical School. Without your supervision, effort, and support, this project would not have been a success.

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LIST OF ABBREVIATIONS

SARS CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

COVID-19: Coronavirus Disease 2019.

HCoV: Human coronavirus.

SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus.

MERS-CoV: Middle East Respiratory Syndrome Coronavirus

RNA: Ribonucleic Acid.

DNA: Deoxyribonucleic Acid.

cDNA: complementary Deoxyribonucleic Acid.

PCR: Polymerase Chain Reaction.

IgM: Immunoglobulin M

IgG: Immunoglobulin G

IgE: Immunoglobulin E

EBV: Epstein Barr virus

IL-2: Interleukin-2

IL-6: Interleukin-6

IL-7: Interleukin-7

TNF: Tumor Necrosis Factor

EDTA: Ethylenediaminetetraacetic acid

TTM: Transfusion transmitted malaria

RDT: Rapid Diagnostic Test.

PBS: Phosphate Buffer Solution

HPM: Human population movement

WBC: White Blood cell

FBC: Full Blood Cell

CT: Cycle threshold

ABSTRACT

Malaria is one of Ghana's most frequent illnesses and the most common cause of febrile sickness. The prevalence of malaria in patients who visit the Korle Bu Polyclinic in Accra with malaria-like symptoms is around 8%. It is therefore essential to look into COVID-19's contribution to malaria-like symptoms. Most infectious diseases including COVID-19 and arboviral infections mimic malaria due to the overlapping of non-specific symptoms they both share. This study investigated COVID-19 in patients presenting with malaria-like symptoms at the Korle Bu Polyclinic, Accra.

A cross-sectional study was conducted among patients presenting with malaria-like symptoms at the Korle Bu polyclinic from June to August, 2021. A total of 300 patients who qualify for Malaria Rapid Diagnostic Testing, and met the criteria for the study, were selected using simple random sampling. Two to three millilitres of whole blood, nasopharyngeal and oropharyngeal swab samples was collected for screening of *Plasmodium falciparum* and SARS-CoV-2 respectively. The whole blood sample was also used for COVID-19 antibody test and full blood count.

Sixty out of three hundred (20%) study patients were positive for SARS-CoV-2 using Real-time PCR and twenty six out of three hundred (8.7%) tested positive using COVID-19 Rapid Antigen test. Majority were females (42/60; 70%), and participants had mean age of 28 years. Seven out of three hundred (2.3%) tested positive for *Plasmodium falciparum* using Malaria RDT and microscopy and eight out of three hundred (2.7%) using Nested Conventional PCR. Two coinfection of *Plasmodium falciparum* and SARS-CoV-2 were reported. Most of the patients came from the Ablekuma South district. Most patients with CT's below 30 (High viral load) presented with 5 or more symptoms (p value <0.01). The most common symptom experienced by the study patients at the polyclinic was headache (95%; 57/60) followed by general body weakness and pain (78.3%;47/60), tiredness (60%; 36/60) and fever(51.7%;

31/60) Comorbidities reported were hypertension, diabetes, Asthma, hypertension and diabetes and none had sickle cell diseases. Most of the study patients had been previously exposure to SARS CoV-2 (113/300) and 66.7% (34/51) Astrazeneca vaccinated patients had no antibody.

Due to the synergy of symptoms, screening for COVID-19 in patients presenting with malaria-like symptoms is vital for immediate diagnosis and treatment.

Keywords: COVID-19, Korle Bu, Accra

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Coronaviruses are single positive-sense enveloped RNA virus with 26,000 to 32,000 nucleotides of the genome which encodes 14 open reading frames (Loeffelholz and Tang, 2020; Wu *et al*., 2020). The outer surface of coronavirus is made up of 4 major structural proteins which includes the membrane protein, envelope protein, nucleocapsid protein and spikes protein (Shereen *et al*., 2020; Naqvi *et al*., 2020; Kadam *et al*., 2021; Pooladanda *et al*., 2020). The Membrane protein (M protein) provides shape to the virion and is the most abundant protein (Fehr and Perlman, 2015; Kadam *et al*., 2021). Hemagglutinin esterase (HE) is the fifth structural protein found on the surface of the virus which the spike protein access into the host (Boopathi *et al*., 2020).

The spike proteins are made up of two subunits, S1 and S2, which are glycoproteins that bind to angiotensin converting enzyme 2 (ACE2) as a means of entry into host cells (Devaux *et al*., 2020; Shang *et al*., 2020). ACE2 is a cellular receptor found on the surface of lung epithelial cells as well as other tissues and organs (including the kidney, liver, lung, Type I and II alveolar cells, small intestine heart enterocytes, and oral mucosa epithelial cells) that aids in the regulation of the rennin-angiotensin aldosterone system, which controls blood pressure (Devaux *et al*., 2020; Gheblawi *et al*., 2020). The term Corona was derived due to the crown-like appearance of spike protein (Shereen *et al*., 2020; Li, 2016). Coronaviruses belong to the order Nidovirales and Coronaviridae family (Shereen *et al*., 2020).

In December 2019, a new coronavirus was discovered in Wuhan, Hubei Province, China, and has been named severe acute respiratory syndrome coronavirus 2. (SARS-CoV-2) (Loeffelholz and Tang, 2020; Hassan *et al*., 2020). According to a genomic study, SARS-CoV-2 is the 7th coronavirus to infect humans and one of the most important pathogens that primarily targets the human respiratory system (Loeffelholz and Tang, 2020). SARS-CoV-2 is closely related to bat-borne betacoronaviruses (88 percent sequence identity), but it differs from SARS-CoV-1 (79 percent sequence identity) (Loeffelholz and Tang, 2020).

Apart from SARS-CoV-2, six other human coronaviruses have been found, including MERS-CoV, HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, and HCoV-HKU1 (Rabi *et al*., 2020). Direct contact with an infected individual, as well as exposure to respiratory aerosols from coughing, sneezing, shaking hands, and touching contaminated surfaces, results in human to human transmission (Shereen *et al*., 2020; Rothan and Byrareddy, 2020). Diarrhoea and loss of appetite are early signs of the disease, and while SARS and MERS do not cause gastrointestinal symptoms, SARS CoV-2 can cause gastrointestinal symptoms such as diarrhoea (Holshue *et al*., 2020; Zhang *et al*., 2020).

Infection with the Coronavirus Disease 2019 (COVID-19) causes severe pneumonia, RNAaemia, and ground-glass opacities, as well as fast heart damage (Byraddey and Rothan, 2020; Hagan *et al*., 2020). Patients infected with COVID-19 experienced an increase in blood cytokines, causing a cytokine storm and chemokines (Byraddey and Rothan, 2020; Liu *et al*., 2020). In severe cases, a high level of pro-inflammatory cytokines such as Interleukin-2 (IL-2), Interleukin-7 (IL-7), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Granulocyte colony-stimulating factor (G-CSF), Interferon-induced protein 10 kDa (IP 10), Monocyte chemoattractant protein 1 (MCP 1), Macrophage inflammatory protein 1 alpha (MIP 1), and Tumor necrosis factor (Byraddey and Rothan, 2020; Qin *et al*., 2020; Tan *et al*., 2020). Hyper-inflammation caused by cytokine storms forces immune cells to kill healthy cells

(Mustafa *et al*., 2020). Acute respiratory distress syndrome (ARDS), hyperinflammatory conditions associated with hypercytokinaemia is observed in SARS-CoV-2 afflicted patients (Xu *et al*., 2020; Huang *et al*., 2020).

Inflammatory substances drive the liver to generate proteins during COVID-19 (blood C-reactive protein) that defend the body from infections (Mustafa *et al*., 2020; Tan *et al*., 2020). These proteins blocks blood vessels in heart and other organs preventing nutrients and oxygen into these organs resulting in multi-organ failure which is a common cause of death (Mustafa *et al*., 2020). Acute respiratory distress syndrome (ARDS) develops in severe COVID-19 patients, necessitating ventilator support and intubation (Chiappelli, 2020; Ferrarese *et al*., 2020; Frontera *et al*., 2020). SARS-CoV-2 is a virus that not only causes respiratory illnesses, but also harms the liver, kidneys, and heart (Mustafa *et al*., 2020; Araya *et al*., 2021). Fourteen to 30% of COVID-19 patients required dialysis or kidney transplants due to the lost of their kidney function (Mustafa *et al*., 2020).

Viral infections trigger oxidative stress which plays a critical role in the pathogenesis of these viral infections (Cecchini and Cecchini, 2020). COVID-19 infection symptoms develop after a period of incubation ranging from 6 to 41 days, with a median of 14 days, depending on the patient's age and immune system health (Byraddey and Rothan, 2020). Patients above the age of 70 have a shorter recovery time than those under the age of 70. Fever or chills, cough, exhaustion, shortness of breath, sputum production, vomiting or nausea, haemoptysis, diarrhoea, sore throat, lymphopenia, and headache are all symptoms of COVID-19, as well as a body temperature of 39 degrees Celsius and a high level of pro-inflammatory cytokines (Byraddey and Rothan, 2020).

In Ghana, Azithromycin in combination with Chloroquine or Hydroxychloroquine, Remdesivir or Tocilizumab and vitamin C and other analgesics were used in treating

COVID-19 patients (Ashinyo *et al*., 2020). Sex, concomitant illnesses, and age are all important determinants of disease progression and severity in the host (Chen *et al*., 2020). The most important risk factor for COVID-19-related severe disease and death is getting older (Chen *et al*., 2020). SARS CoV-2 causes haematological, respiratory, gastrointestinal, neurological, and cardiovascular symptoms (Araya *et al*., 2021).

Malaria is caused by protozoan parasites of the genus *Plasmodium* and species that causes human malaria include *P. falciparum, P. vivax, P.ovale, P. malariae* and *P. knowlesi* (Phillips *et al*., 2017; Singh and Daneshvar, 2013; Tangpukdee *et al*., 2009). *Plasmodium falciparum* is a serious public health problem, with the vast majority of infections occurring in Africa and the second most serious public health issue is *P. vivax* (Phillips *et al*., 2017; WHO, 2021). *Plasmodium falciparum* has a strong transmission zone across the entire country and between 2000 and 2013, over 20,000 malaria deaths were recorded in Ghana, with febrile sickness accounting for more than half of all clinic visits (Stoler and Awandare, 2016). Even when malaria isn't present, fever is frequently misdiagnosed as malaria. As a result, the true burden of diseases like dengue fever, influenza, and typhoid in Sub-Saharan Africa is likely underestimated (Stoler and Awandare, 2016). Different illnesses such as bloodstream infections, bacterial and fungal infections, viral infections, and arboviruses are all presumptively diagnosed as malaria based on clinical signs due to the heterogeneous etiology of febrile sickness (Stoler and Awandare, 2016).

Because of the non-specificity of symptoms that overlap with other common life-threatening diseases, clinical diagnosis of malaria remains difficult. This impairs diagnostic specificity, which promotes indiscriminate antimalarial drug use and compromises the care of patients with non-malarial fevers in endemic areas (Mwangi *et al*., 2005). In the tropical areas such as Ghana, Malaria continues to be a major leading infectious disease and the most common diagnosis for febrile patients (Awandare and Stoler, 2016). Malaria contributes to high

mortality among children at age 5 and below and significantly impacts the health system. The pathogenesis, clinical manifestations, and infection routes are still being studied through extensive research in several countries, and COVID-19 investigations among patients presenting with malaria-like symptoms are critical for the early detection, control, and treatment of COVID-19 infected individuals.

1.2 Problem statement and justification

Malaria is endemic in Ghana and globally there is an estimated 241 million malaria cases in 2020 in 85 malaria endemic countries with most originating from countries in the WHO African regions (WHO, 2021). In 2020, deaths associated with malaria increased by 12% compared to the previous years due to COVID-19 pandemic which disrupted management of malaria (WHO, 2021). In low-resource health-care settings, malaria is the most prevalent diagnosis for feverish individuals (Awandare and Stoler, 2016). Some infectious diseases and arboviral infections including dengue fever, zika virus and chikungunya virus in the tropics mimic malaria in locations where malaria is endemic and the similarity in clinical presentation make the diagnosis more difficult (Yong and Koh, 2013).

Adults with severe malaria are more likely to experience multi-organ failure, while children with malaria are more likely to experience respiratory distress, which is similar to what is seen in COVID-19 patients (Chanda-Kapata *et al.*, 2020).

Both malaria and COVID-19 share some symptoms such as fever, fatigue, headache, and other acute onset (Chanda-Kapata *et al.*, 2020, Sherrad smith *et al.*, 2020). The prevalence of malaria in patients who visit the Korle Bu Polyclinic in Accra with malaria-like symptoms is around 8%, it is essential to look into COVID-19's contribution to malaria-like symptoms. Thus, COVID-19 cases that present with malaria-like symptoms may be misdiagnosed and

difficult to distinguish from malaria based on patients' signs and symptoms during this emergency period (Chanda-Kapata *et al*., 2020).

Failure to differentiate COVID-19 at the primary health care will cause delays in proper management, prolong hospitalization, potential transmission of SARS-CoV-2 especially among hospitalized patients and increase possible emergence of new variants (Nunthavichitra *et al*., 2020). The detection of COVID-19, as well as malaria, is critical for their correct care. (Guha *et al*., 2021), hence, there is the need to investigate COVID-19 in patients presenting with malaria-like symptoms at the primary health care.

1.3 Aim of study

To investigate COVID-19 in patients presenting with malaria-like symptoms at Korle Bu Polyclinic, Accra.

1.4 Specific objectives:

i. **To determine SARS-CoV-2 infections and co-infections with *Plasmodium sp.***
ii. To determine the anti-SARS-CoV-2 antibody presence in confirmed COVID-19 with and without malaria.
iii. To determine haematological parameters associated with COVID-19 patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Malaria in Ghana.

Malaria is caused by protozoan parasites of the genus *Plasmodium* and species that causes human malaria include *P. falciparum, P. vivax, P.ovale, P. malariae* and *P. knowlesi* but *Plasmodium falciparum* is the most deadly causative agent of malaria (Phillips *et al*., 2017; Singh and Daneshvar, 2013; Tangpukdee *et al*., 2009).

Plasmodium falciparum is a serious public health problem, with the vast majority of infections occurring in Africa (Phillips *et al*., 2017; WHO, 2021). The second most serious public health issue is *P. vivax* malaria, which is caused by the reactivation of hypnozoites in liver cells, resulting in malaria relapses and possibly the fatal rupture of an enlarged spleen (Clark and Schofield, 2000; Phillips *et al*., 2017).

Malaria is the most contagious illness in tropical and subtropical areas, and it is a global health issue (Tangpukdee *et al*., 2009). In the years 1900 to 1929 and 2010 to 2015, the prevalence of *Plasmodium falciparum* malaria fell by 40% and 24%, respectively (Smith and Whittaker, 2014). Malaria is endemic in Ghana and one of eleven high-burden nations, accounting for 70% of global malaria incidence and fatalities, and has seen an 8% increase in malaria cases (WHO, 2021). *Plasmodium falciparum* has a strong transmission zone across the entire country and between 2000 and 2013, over 20,000 malaria deaths were recorded in Ghana, with febrile sickness accounting for more than half of all clinic visits (Stoler and Awandare, 2016).

The percentage of children with positive malaria tests was higher among those from the poorest families (Nyarko, 2014), owing to a lack of health services and children are exposed to malaria transmission vectors as a result of inadequate housing conditions (Yankson *et al*., 2019). Even when malaria isn't present, fever is frequently misdiagnosed as malaria. As a result, the true burden of diseases like dengue fever, influenza, and typhoid in Sub-Saharan Africa is likely underestimated (Stoler and Awandare, 2016). Different illnesses such as bloodstream infections, bacterial and fungal infections, viral infections, and arboviruses are all presumptively diagnosed as malaria based on clinical signs due to the heterogeneous etiology of febrile sickness (Stoler and Awandare, 2016).

Fever, headache, weakness, myalgia, chills, disorientation, stomach aches, anorexia, vomiting, diarrhoea, pruritis, and nausea are just few of the early signs of malaria (Mwangi *et al*., 2005). Because of the non-specificity of symptoms that overlap with other common life-threatening diseases, clinical diagnosis of malaria remains difficult. This impairs diagnostic specificity, which promotes indiscriminate antimalarial drug use and compromises the care of patients with non-malarial fevers in endemic areas (Mwangi *et al*., 2005). Malaria results in anaemia, cerebral malaria and severe malaria if the use of currently available tools for malaria interventions is not intensified as part of the measures to eradicate and control malaria (Asante *et al*., 2011). The high transmission risk in places where malaria control has fallen, the presence of resistant strains, and increased migration to endemic areas all appear to be contributing to an increase in malaria incidence (Tangpukdee *et al*., 2009).

People living with HIV are one of the most vulnerable groups to malaria in Ghana, and parasitemia was seen more commonly among HIV positive people in the rural than in the urban areas (Owusu *et al*., 2018). During the rainy season in Ghana's forest zone, a high prevalence of asymptomatic *Plasmodium* infection was discovered, with 73% of adult residents of rural areas in the Ashanti region testing positive for at least one *Plasmodium*

species. Asymptomatic parasitaemia in adults is a reservoir for transmission and must be considered in efforts to eradicate malaria (Heinemann *et al*., 2020).

Humans with asymptomatic malaria serve as a large reservoir for *Plasmodium* transmission to uninfected Anopheles mosquitoes, driving malaria endemicity, and asymptomatic malaria can advance to clinical malaria (Okyere, 2020). By microscopy, Rapid Diagnostic Test (RDT), and nPCR, the prevalence of asymptomatic malaria among asymptomatic children in Ghana's Atwima Nwabiagya North district was 23.2 percent, 31.2 percent, and 36.8 percent, respectively (Okyere, 2020). Human population movement (HPM) and mobile populations, such as Head Porters, are a key problem for malaria control and elimination initiatives, as they pose a high threat of transmitting malaria to malaria-free areas (Ricci, 2012; Martens and Hall, 2000). Head porters do not have housing so they sleep outside and they move about to stores and other open spaces in commercial cities and towns which deprives them from the benefits of protection against malaria therefore are expose to malaria which drives transmission in Ghana (Snow *et al*., 2004; Ardayfio-Schandorf *et al*., 2012). *Plasmodium falciparum* was discovered as the significant parasite in all positive blood examinations in 12 percent and 9.6 percent of malaria infection among head porters at Agbogloshie market, respectively, using RDT and microscopy (Kwofie, 2020).

Other route of malaria transmission aside via the bite of a female *Anopheles* mosquito is through blood transfusion and recipient of such blood may develop transfusion transmitted malaria (TTM) (Adusei and Owusu-Ofori, 2018). TTM cases have been derived from the administration of infected packed Red blood cells (RBCs) or whole blood, fresh plasma, leukocytes and platelet concentrates (Garfield *et al*., 1978). One of the dangers of TTM is the inability to properly diagnose infected donors, particularly those with low parasitaemia levels, because a substantial number of donors may have been infected with malaria parasites in endemic locations (Kitchen and Chiodini, 2006). When malaria RDTs and microscopy

were used to analyze 200 blood samples taken at the Seventh Day Adventist Hospital in Kumasi, the rate of occurrence of *Plasmodium* parasitaemia in blood donors was 8% and 3%, respectively (Adusei and Owusu-Ofori, 2018).

Indoor residual spraying, long-lasting insecticide nets, intermittent preventative treatment, and test, treat, and track are some of the malaria control measures, yet despite efforts to eradicate malaria, asymptomatic parasite carriage drives transmission (Ndong *et al*., 2019).

2.2. Haematological parameters of malaria patients

White blood cells (WBC) play an essential role in the body's immune system (Adu-Gyasi *et al*., 2012). Depending on the disease conditions, the number of WBC may be decreased or increased and this could be mild, moderate or severe (McKenzie *et al*., 2005). WBCs are indirectly employed in assessing *Plasmodium* parasitaemia by counting them on microscopic examination of Giemsa-stained blood smears (Adu-Gyasi *et al*., 2012). White blood cells are vital in malaria patient's management and disease intervention studies (Adu-Gyasi *et al*., 2012). Thrombocytopenia, Leukopenia, and anemia are hematological abnormalities reported in malaria patients in Ghana (Anabire *et al*., 2018; Awoke and Arota, 2019). During malaria infection there is a high platelet destruction and reduced lifespan which occurs in parallel to Splenomegaly (Khan *et al*., 2008).

2.3. COVID-19 outbreak in Ghana

COVID-19 was declared a pandemic by World Health Organisation on 11th March, 2020 (WHO, 2020). Ghana recorded its first two cases on 12th March, 2020 and these were imported cases from Norway and Turkey (Kenu *et al*., 2020). Accra which is the first most

densely populated still remains the epicenter of the COVID-19 outbreak followed by the Ashanti region (Kenu *et al*., 2020). Majority of the reported cases are males and 75% of recorded cases are asymptomatic or present with mild symptoms (Kenu *et al*., 2020).

In Ghana among the symptomatic, cough is the most common symptom, followed by headache, muscle ache, sore throat and fever (Ashinyo *et al*., 2020; Oduro-Mensah *et al*., 2020; Adjei *et al*., 2020). Hypertension, Asthma and diabetes were the popular comorbidities reported and these are conditions that impact the severity of the illness (Ashinyo *et al*., 2020; de Almeida-Pititto *et al*., 2020). Combinations of Chloroquine and Azithromycin, or Hydroxychloroquine and Azithromycin, were utilized in Ghana as first-line treatment for asymptomatic and mild/moderate symptomatic cases of COVID-19 (Ashinyo *et al*., 2020). Bans on all social gatherings, temporary lockdowns, and limitations on movement in the Greater Accra and Ashanti regions have all been used in Ghana to combat the spread of COVID-19. Disinfection and fumigation activities were held in markets and various schools in the greater Accra region and all other regions across the country.

Viruses constantly change to form new variants which occur over time and these variants may go extinct or survive (CDC, 2021). Scientists have studied changes around the spike protein and reported some new variants which includes B.1.1.7 (United Kingdom), B.1.351 (South Africa), P.1 variant (Brazil) and B.1.617 (India),which affects the ability to be recognized by antibodies and become more transmissible (CDC, 2021).

Samples of some international passengers who arrived in Ghana were taken for COVID-19 test and scientist discovered the new COVID-19 variant after genomic sequencing (GHS, 2021). New variants may increase the number of cases, putting additional burden on health-care systems, resulting in more hospitalization and maybe more deaths (CDC, 2021). The majority of COVID-19 cases are located in the Greater Accra region, and wearing a mask in

public or private vehicles with more than one occupant, especially when leaving or returning to your place of abode, is a legal obligation. 'No mask, no admission' signs are erected in public places such as workplaces, stores, markets, malls, banks, and pharmacies (GHS, 2021). To enforce adherence to the wearing of masks, the Ghana police service has been given a broad list of sites where they can make arrests, including public venues such as car parks, markets, and the street (GHS, 2021). COVID-19 is indeed becoming alarming and having great impact on daily livelihood, there is the need to curb and control its transmission.

The Oxford Astrazeneca vaccine arrived in Ghana on the 24th February, 2021, being the first country to receive them in Africa (WHO, 2021). Six hundred thousand doses of the vaccine licensed to Serum Institute of India arrived in Accra, Ghana (WHO, 2021). The University of Oxford in collaboration with the British-Swedish company AstraZeneca developed coronavirus vaccine named as ChAd0x1 nCoV-19 or AZD1222 which has strong protection with efficacy of 76% after several clinical trials (Zimmer and Corum, 2021). Of all the potential vaccines and treatments, the spike protein is one of the major targets (Zimmer and Corum, 2021). The Oxford-AstraZeneca vaccine, unlike the Pfizer- BioN Tech and Moderna vaccines, employs double stranded DNA and it is based on the virus's genetic instructions for producing the spike protein. AZD1222 originates from several years of research on adenovirus- based vaccines (Zimmer and Corum, 2021).

The AstraZeneca vaccine is recommended for individuals with reported comorbidities such as obesity, diabetes, respiratory disease and cardiovascular disease and not recommended for persons with severe allergic reaction and younger than 18 years (WHO, 2021).The gene for the coronavirus spike protein has been introduced to an adenovirus which is a common virus that causes cold or flu-like symptoms (Zimmer and Corum, 2021). Adenoviruses have a strong protein that protects the genetic information within since DNA is not as fragile as RNA hence the Oxford team used a modified chimpanzee adenovirus which has been

engineered not to replicate inside cells (Zimmer and Corum, 2021). After one is being vaccinated, the host cell engulfs the adenovirus and travels to the nucleus and the coronavirus spike protein is copied into the messenger RNA which then begins to assemble spike proteins (Zimmer and Corum, 2021). These spike proteins migrate and protrude from the vaccinated cell, triggering an immune response that produces antibodies that marks the virus for destruction while also inhibiting the spike from connecting to its cellular receptor, preventing infection (Zimmer and Corum, 2021).

The delta variant of COVID-19 (B.1.617), a variant of concern, was first detected in India and has currently spread globally due to its high transmissibility and able to avoid the host immunity (Mallapaty, 2021).

2.4. Pathophysiology and Immunopathogenesis of COVID-19

COVID-19 is spread mostly through respiratory droplets by coughing, sneezing, shaking hands, and touching contaminated surfaces (Shereen *et al*., 2020; Rothan and Byrareddy, 2020; D'Amico *et al*., 2020). SARS CoV-2 has been found in faeces, pharynx swabs, and sputum of infected patients, raising the likelihood of fecaloral transmission (Meselson, 2020; D'Amico *et al*., 2020).

SARS CoV-2 virus inhaled interacts to ACE2, which is highly expressed in nasal epithelial cells (Wan *et al*., 2020; Hoffmann *et al*., 2020; Parasher, 2021). As the virus colonizes, multiplies, and moves down the respiratory system through the conducting airways, it infects other ciliated cells prompting an innate immune response (Wan *et al*., 2020; Hoffmann *et al*., 2020). This phase of the disease lasts for a few days and the immune response induced is a short one hence despite having a low viral load, the individual is infectious (Parasher, 2021). SARS CoV-2 then migrates across the conducting airways from nasal epithelial cells to the upper respiratory tract, triggering an immune response with the release of interferons and C-

X-C motif chemokines ligand (Parasher, 2021). Malaise, fever, and a dry cough are signs of the disease at this stage (Parasher, 2021).

About one-fifth of all SARS CoV-2 infected patients develop severe symptoms when the disease progress to this last stage (Parasher, 2021). Anti-inflammatory and pro-inflammatory cytokines are produced in response to SARS-CoV-2 infection when it is recognized by Toll-like receptors 7 or other receptors on lung resident cells (Masoomikarimi *et al*., 2021). COVID-19 lowers CD8+ T cell and Natural Killer cell numbers, both of which are linked to severe pneumonia (Patel and Jernigan, 2020). Cytokine storm is caused by an unexpected rise in pro-inflammatory cytokines, which produces severe lung injury, a systemic immune response, enhanced myocardial cell damage, hypoxia-related myocyte injury, and intestinal and cardiovascular abnormalities (Liu *et al*., 2020).

Shortness of breath, increased respiratory rate, and decreased oxygen saturation are caused by microscopic bilateral diffuse alveolar damages, interstitial mononuclear inflammatory infiltrates with lymphocyte domination, and cellular fibromyxoid infiltrates (Tian *et al*., 2020).

Elevated levels of other inflammatory cytokines and chemokines such as Interleukin-2 (IL-2) and interleukin-8 (IL-8) with a rise in numbers of eosinophils and neutrophils may induce COVID-19 patients with abnormalities (Masoomikarimi *et al*., 2021). In severe cases, a high level of pro-inflammatory cytokines including IL 2, IL 7, IL 10, GCSF, IP 10, MCP 1, MIP 1α and TNF α are showed and these contribute to disease severity (Byraddey and Rothan, 2020). Many studies have found that in individuals with severe COVID-19, both pro-inflammatory and anti-inflammatory plasma cytokines such as IL-10, IL-2, IL-6, IL-4, IL1, IL1RA, IL7, IL8, and tumor necrosis factor-alpha (TNF-) are raised (Peng *et al*., 2020; Li *et al*., 2020). In COVID-19 instances, the primary mediator for the development of cytokine

storm is IL-6 (Yip *et al.*, 2016). IL-6 is the major source of beta and lambda interferons is viral infected epithelial cells (Hancock *et al.*, 2018).

ACE2 has been found to be expressed in epithelial cells of the gastrointestinal tract. Diarrhoea, nausea, vomiting, and stomach discomfort have all been recorded as gastrointestinal signs of COVID-19, and the absence of fever does not exempt COVID-19 possibility, as 8.7% of patients had no fever at the time of admission (Mo *et al.*, 2020).

2.5. Immune Response of COVID-19

The immunological response of the host to SARS-CoV-2 appears to be important in disease development and clinical symptoms (Li *et al.*, 2020). The immune response to virus infection is usually controlled by both innate and adaptive immunity and intracellular receptors identify RNA viruses such as SARS-CoV-2, causing immune system dysfunction in extreme cases. (Saito and Gale, 2007; Bouayad, 2020; Kanto and Hayashi, 2006).

SARS-CoV-2 specific IgM and IgG were found 9 and 15 days after the beginning of COVID-19, respectively (Xiao *et al.*, 2020). Anti-SARS-COV-2 IgG can be detected on day 11 of sickness or 18–21 days after exposure (Lee *et al.*, 2020). Before clinical recovery, blood levels of IgM and IgG antibodies that can neutralize SARS-CoV-2 were found in serum (Thevarajan *et al.*, 2020). In other to treat or prevent SARS-CoV-2 infections, CR3022, a neutralizing antibody that binds to the SARS-CoV-2 Receptor Binding Domain (RBD), can be administered alone or in combination with other neutralizing antibodies (Tian *et al.*, 2020). Female patients with severe COVID-19 had a greater amount of anti-SARS-CoV-2 IgG antibody than male patients (Zeng *et al.*, 2020).

On day 80 after the onset of the disease, antiviral IgM and IgG were found to be absent (Liu *et al*., 2020). According to literature, passive transfer of neutralizing antibodies from SARS-CoV-2 treated patients to infected patients lowered viral load and patients fatality (Tiberghien *et al*., 2020). In addition, infusing 200ml of convalescent plasma with viral neutralising antibodies with titers greater than 1:640 into ten COVID-19 patients improved their clinical symptoms and reduced their C reactive protein levels in all of them (Duan *et al*., 2020). On a wide scale analysis of COVID-19 patients, the use of Convalescent sera indicates a reduction in the rate of significant adverse effects (Joyner *et al*., 2020).

2.6 Hematological parameters of COVID-19 patients.

Haematological findings have specific involvement in the early risk of grouping and prognosis of COVID-19 patients (Araya *et al*., 2021). The most common and prominent haematological abnormality found in admitted COVID-19 was lymphopenia because SARS CoV-2 infects lymphocytes which also express the cellular receptor Angiotension-converting enzyme 2 (ACE2) on their surface (Xu *et al*., 2020; Terpos *et al*., 2020). During the early stages of infection and incubation, SARS CoV-2 primarily affects all organs that express ACE2, therefore lymphocyte numbers are normal or lowered (Terpos *et al*., 2020). Following the onset of initial symptoms, pro-inflammatory cytokines (IL-6, IL-2, IL-7, granulocyte colony stimulating factor, and tumor necrosis factor) increase, resulting in cytokine storm (IL-6, IL-2, IL-7, granulocyte colony stimulating factor, and tumor necrosis factor), which promotes lymphocyte apoptosis (Singh *et al*., 2014). Severe COVID-19 patients are more likely to have anemia, neutrophilia, lymphopenia and leukocytosis than those with mild and moderate COVID infection (Araya *et al*., 2021; Liu *et al*., 2020).

Low lymphocyte and platelet count in the blood, raised liver transaminases, high C-reactive protein and erythrocyte sedimentation rate, elevated serum lactate dehydrogenase, and decreased or normal serum albumin were all found in COVID-19 individuals with clinical evidence (Azer, 2020; Liu *et al*., 2020).

2.7 Co-morbidities with COVID-19.

Co-morbidities elevates the chances of infection and people with other medical conditions such as diabetes, hypertension, liver, kidney and lung disease, smokers, transplant recipients, cancer patients on chemotherapy and patients taking steroids are at increased risk of contracting COVID-19 (CDC, 2020). Hypertension, Asthma and diabetes were the popular co-morbidities reported and these are conditions that impact the severity of the illness (Ashinyo *et al*., 2020; de Almeida-Pititto *et al*., 2020). Out of 1786 patients enrolled in a study, the most comorbidity was hypertension (15.8%), cardiovascular and cerebrovascular conditions (11.7%) and diabetes (9.4%) (Paudel, 2020; Zhou *et al*., 2020). Hypertension is the most common in COVID-19 patient with a range of 15% to 30% and it causes endothelial dysfunction where the vascular walls loses it vasorelaxation properties as a result of stiffness of the vascular walls (Wang *et al*., 2020; Zhou *et al*., 2020).The least common co-morbidities were respiratory illness (1.4%), renal disorders (0.8%), HIV and Hepatitis B (1.5%) and immunodeficiencies (0.01%) (Paudel, 2020; Zhou *et al*., 2020). In the USA, data reported by nursing homes, hospitals, and other health facilities revealed that the leading comorbidities among COVID-19 death were hypertension (55.4%), diabetes (37.3%), hyperlipidemia (18.5%), coronary artery disease (12.4%), renal disease (11%), dementia (9.1%) and cancer (8.1%) (Franki,2020).The ACE2 and SARS CoV-2 both require glucose to carry out its activities hence persons with high glucose concentration aids the virus entry into the host cells (Brufsky,2020).

2.8. Viral infection with malaria-like symptoms.

Aside COVID-19 there are other infectious diseases with similar clinical presentations with malaria and these include dengue virus infection, infectious mononucleosis, hepatitis virus infection and herpes virus infection. High grade fever, generalized body ache, nausea, arthralgia, myalgia, and maculopapular rashes are frequent flu-like symptoms in places where dengue is prominent. These symptoms of dengue may mimic other diseases such as Malaria and Leptospirosis, which are also common in areas where dengue is prevalent (Monath and Tsai, 1997). Early stages of Leptospirosis can be difficult to identify from other prevalent causes of acute febrile infections in the tropics, such as dengue fever, malaria, and typhoid (Levett, 2001; LaRocque *et al*., 2005).

Dengue virus infection is an arthropod-borne infection that is spread to people mostly by the bite of an infected Aedes mosquito (Tuan and Shuel, 2017). Viruses belonging to dengue virus species are grouped into four distinct serotypes (DENV 1, DENV 2 ,DENV 3 and DENV4) and one virus serotype infection does not confer protection against the other three (Beck *et al*., 2019). Dengue virus remains a major health problem and the most common arbovirus infection in humans in tropical and subtropical countries (Bonney *et al*., 2018). The primary vector of Dengue fever is the *Aedes aegypti* mosquito, but other species such as *Aedes albopictus* possess the ability to transmit the virus (Tuan and Shuel, 2017). The virus is transmitted when an Aedes aegypti mosquito feeds on an infected human during the viraemic phase of the illness, which begins 2 days before the onset of the fever and lasts 4 to 5 days after the commencement of the fever (Carrington & Simmons, 2014).

In three urban centres in Ghana, there has been evidence of recent dengue exposure among malaria parasite-positive children (Navrongo, Kintampo and Accra) among 218 children ages 2-14 years old (Stoler *et al*., 2015). The prevalence of dengue specific IgM was 3.2% of the

218 plasma sample tested across the 3 urban areas which indicates current exposure to dengue and dengue specific IgG was 21.6% which indicates previous exposure to dengue (Stoler *et al*., 2015). Dengue virus was detected in 2 children (3 and 14 years) suspected of Malaria in Accra, Ghana, which indicates acute dengue infection which was identical to DENV- 2 strains discovered in Burkina Faso (Amoako *et al*., 2018). Both children tested positive for dengue IgG and IgM which indicates infection was locally acquired. Dengue virus was molecular detected in patients suspected of Ebola virus disease in Ghana (Bonney *et al*., 2018). Out of 32 DENV IgM patients ,4 were detected positive (2 males and 2 females) and were characterized by 1 DENV -2 and 3 DENV-3 serotypes which suggest co-circulation and concurrent infection of 2 different DENV serotypes within patients suspected of Ebola virus (Bonney *et al*., 2018). Although DENV serotype 2 is known to be dominant in West Africa, DENV serotype 3 was in close homology to sequence from Senegal and India (Bonney *et al*., 2018).

Fever, sore throat, acute weariness, pharyngitis, adenopathy, malaise, and atypical lymphocytosis are frequent symptoms of infectious mononucleosis, which is caused by the Epstein-Barr virus (EBV). Infectious mononucleosis is most common in teenagers and children. Patients with infectious mononucleosis may develop splenomegaly, hepatomegaly, jaundice, and splenic rupture (Candy *et al*., 2006). Epstein-Barr virus is a human herpes virus that persists in a latent condition after primary infection and causes a chronic infection in 90% of the world's population (Chêne *et al*., 2007). In Africa most children infected with EBV have seroconverted by 3 years of age (Chêne *et al*., 2007). Malaria and EBV infections are recognized to play a role in the development of endemic Burkitt lymphoma, the most common childhood malignancy in equatorial Africa (Chêne *et al*., 2007). Hepatitis is a common disease which affect the liver and lower parasitaemia and higher viremia are mostly found in individuals coinfected with *Plasmodium* and Hepatitis B (Andrade *et al*., 2011).

2.9. COVID-19 and Malaria: great mimics.

Both malaria and COVID-19 shares some of the highly recognisable symptoms or travel history; fever, fatigue, headache, and others acute onset or history to affected areas or contact with an infected person (Chanda-Kapata *et al.*, 2020; Sherrad Smith *et al.*, 2020). As a result, a malaria case may be misclassified as COVID-19 if symptoms alone are used to diagnose a case during this emergency period (Chanda-Kapata *et al.*, 2020; Sherrad Smith *et al.*, 2020). The common symptom in both diseases is fever and there is a potential for misdiagnosis of malaria or COVID-19 and co-occurrence where COVID-19 patients may harbour malaria (Ewungkem and Ngwa, 2020). Malaria symptoms appear 10 to 15 days after an infective bite; multi-organ failure is typical in adults with severe cases, and respiratory distress is common in children with malaria, similar to what is seen in COVID-19 patients (Chanda-Kapata *et al.*, 2020).

Individuals afflicted with COVID-19 or Malaria, on the other hand, may go unnoticed for a long time while transmitting the infections through their respective transmission channels (Chanda-Kapata *et al.*, 2020). In Ghana, it has been recorded that 75% of confirmed COVID-19 cases are asymptomatic or present with mild symptoms and there is a likely possibility of some infected asymptomatic patients with malaria infections or parasitaemia and this leads to active community transmission.

In COVID-19 cases, developing fever goes up as one age whilst malaria fever declines with age (Sherrad smith *et al.*, 2020). The percentage of febrile children in whom fever is diagnosed as malaria is likely to be higher than COVID-19 fever in children (Sherrad smith *et al.*, 2020). Malaria infection induces neutralising antibodies and interferons released by lymphocytes which have both invitro and in vivo effects against COVID-19 (Du *et al.*, 2020). SARS CoV-2 in populations may have natural immunity due to widespread exposure to malarial infections or milder disease (Du *et al.*, 2020).

In Ghana, a female patient reported to the Korle-bu teaching hospital with a fever (37.9 C), overall body weakness, and chills, but no headache, cough, sore throat, running nose, or other symptoms typical with COVID-19 disease, therefore she was handled as a regular case (Vandyck-Sey *et al*., 2020). She tested negative for malaria and fever was unspecified hence she was treated with oral paracetamol and vitamin C. After 4 days, she later tested positive for COVID-19 (Vandyck-Sey *et al*., 2020).

Plasmodial infections induce high Immunoglobulin E (IgE) in the blood of the majority of people living in malaria endemic areas (Perlmann *et al*., 1999). One reason for high IgE among populations in malaria endemic areas is due to the prevalence of parasitic infections such as helminths infections (Mulu *et al*., 2014). High levels of serum IgE was found in individuals co-infected with malaria and intestinal helminths (Mulu *et al*., 2014). The increase in T helper 2 cells favors IgE production and presence of helminths egg loads also induces elevated serum IgE levels (Mulu *et al*., 2014).

A study was done at a malaria clinic in India to investigate the incidence of SARS-CoV-2 infection among 262 febrile patients (Guha *et al*., 2021). All patients who participated in the study were tested for malaria parasite by examining rapid malaria antigen tests and thick and thin blood smears (Guha *et al*., 2021). Rapid antigen test and reverse transcriptase–polymerase chain reaction was used to detect SARS CoV-2 (Guha *et al*., 2021). Out of 262 patients, 45 (17.17%) were positive for *Plasmodium falciparum*, 3(10.34%) were positive for COVID-19 and 2(6.89%) were positive for both COVID-19 and *Plasmodium vivax* (Guha *et al*., 2021).

A similar case was reported in Dubai which describes a young man who presented to a healthcare facility complaining of generalised body aches with fever for 15 days (Eid Mahmood, 2021).Nasopharyngeal and oropharyngeal swab was taken and several blood test

was done and the results confirmed positive for Malaria aside having 28 mg/L C-reactive protein. Also, Urinalysis and chest radiography was clear and his condition improved hence he was discharged home with paracetamol and Azithromycin. 2 days later his swabs taken was positive for SARS CoV-2 indicating coinfection of malaria and COVID-19 (Eid Mahmood, 2021).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This was a single-centre- cross-sectional study.

3.2 Study site and population

The study was carried out at Korle Bu Polyclinic in Accra. Korle Bu Polyclinic is a government polyclinic in Greater Accra, Korle-Bu Ghana with coordinates (5.542078; -0.23448). Korle Bu Polyclinic is a part of the Korle bu teaching hospital which is one of the major health care center where most febrile and malaria cases are recorded and it offers primary health care to the Korle Bu community, its environs and the city Accra as a whole. The duration of the study was from 8th of June, 2021 to 2nd August, 2021.

Case definition: Patients with malaise, general body pains, general body weakness, headache and +/- fever (37.5° C).

The study population were all adults (18 years and above) who presented with malaria-like symptoms which has been captured in the case definition and qualified for malaria rapid diagnostic test to be performed.

Sample size was calculated from the formula $n = Z^2 P (1-P) / d^2$ and was determined to be a minimum of 260.

Where n is the sample size, Z is the statistic corresponding to the level of confidence 95% =1.96, P is expected prevalence =21.3% and d is precision =5% (Pourhoseingholi *et al.*, 2013).

For contingencies and errors the sample size was increased by 10% which gave a total of 300 sample size

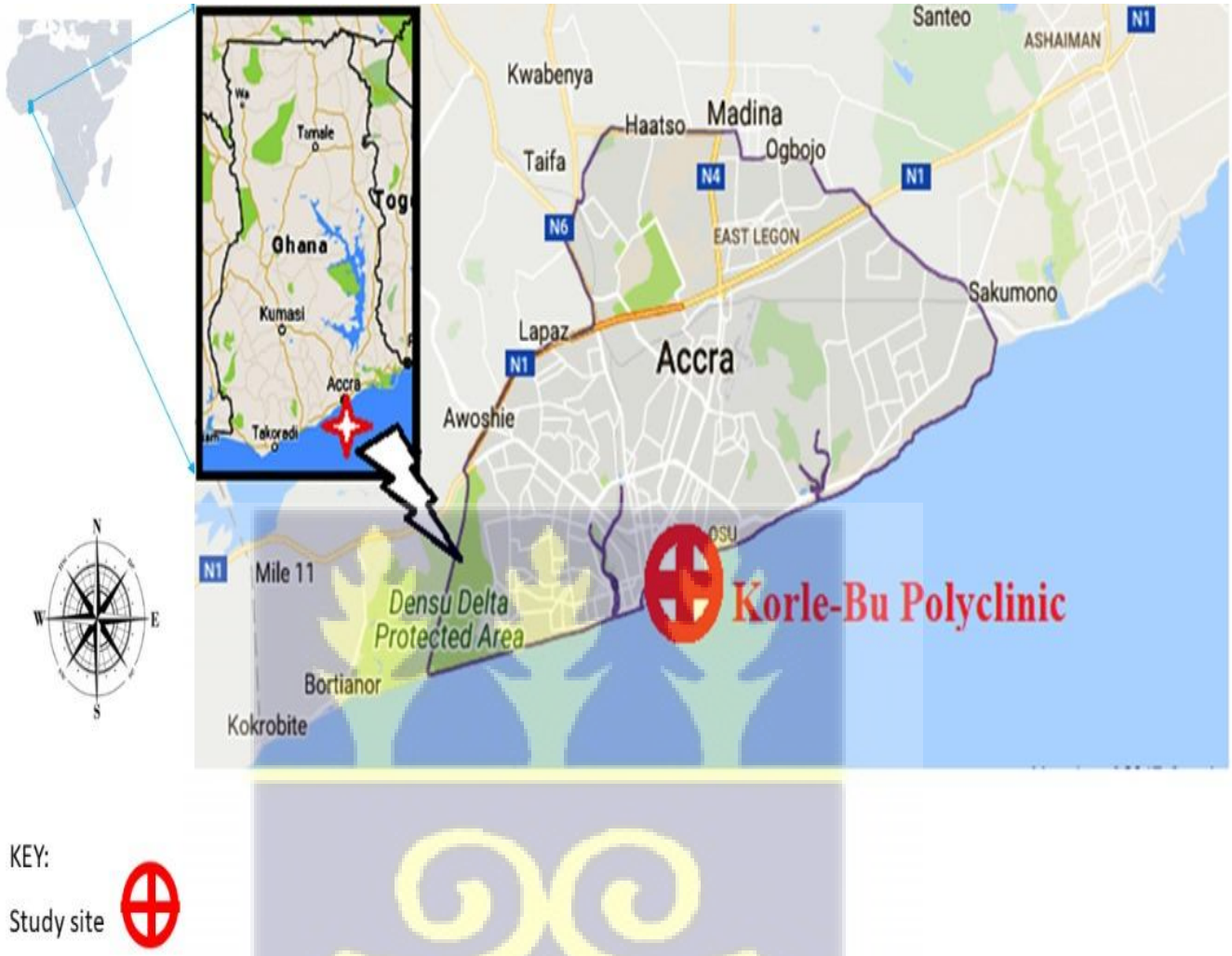

Fig 0.1: Map of Accra, Ghana showing Korle Bu Polyclinic.

Source:

(*https://www.researchgate.net/publication/339962220/figure/fig1/as:869878449844226@1584406592190/Map-of-Ghana-showing-the-three-hospitals.ppm*).

Inclusion Criteria: Adults, 18 years and above presenting with or without fever (≥37.5°C) in addition with malaise, general body pains, general body weakness and headache.

Exclusion Criteria: Pregnant women and persons below 18 years.

3.3 Sample collection

All patients who reported to the outpatient department of the Korle Bu Polyclinic and who had malaria-like symptoms and were asked by the clinicians to take a Malaria Rapid diagnostic test and who met the inclusion criteria for the study were consented and upon agreement, questionnaire was administered to obtain records of socio-demographics and clinical presentations of disease status as well as medical history. All qualified patients were selected by simple random sampling and maximum of 8 patients were selected in a day until the total sample size was obtained. Simple random sampling was utilised to give a true presentation of patients who visit the polyclinic with malaria-like symptoms to avoid bias in sampling.

For initial diagnostic test for COVID-19, Center for Disease Control (CDC) recommends specimen from the upper and lower respiratory tract of suspected or infected COVID-19 patients (CDC, 2020). Swabs from the respiratory mucosa of nasopharyngeal and oropharyngeal area and whole blood was collected.

Oropharyngeal swab was collected first followed by nasopharyngeal swabs. For oropharyngeal swab, the patient's mouth was opened wide and tongue protruding out then the swab was lowered gently to pick sample at the back wall of pharynx behind the uvula. For nasopharyngeal sample, all visible nasal mucus was cleared off and the patient sat in an upright position before insertion of swab. A straight line was measured from the corner of the nose to the front of the ear and the swab was inserted gently by rotating gently along the floor of the nose until it reached the posterior nares. The swab was allowed to sit in place for 2-5 seconds and rotated several times to dislodge the columnar epithelial cells. The swab was then withdrawn and placed in the viral transport medium or saline and kept wet and transport into the laboratory for further processing. Viral transport medium is labelled with a unique identification number which corresponds to the hospital identification number to ensure easy

verification and to eliminate errors of assigning wrong identification numbers to nucleic acid extract.

About 3ml of whole blood was collected from the arm into EDTA tube for full blood count and antibody analysis. Also, finger prick was done to collect 5µl of blood on a glass slide for thin and thick film microscopy examination of *Plasmodium falciparum*. Dry blood spot was blotted on a Whitman filter paper for nested conventional PCR.

Malaria Rapid diagnostic test and COVID-19 Antigen test was performed at the Korle Bu Polyclinic and malaria microscopy was done at the Parasitology laboratory of the department of Medical Microbiology, University of Ghana Medical School. Antibody test was done at clinical virology unit of the department of Medical Microbiology. COVID-19 PCR and malaria nested conventional PCR at Noguchi Institute for Medical Research, virology and immunology department.

3.4 SARS- CoV-2 ribonucleic acid (RNA) isolation

The kit used for the RNA extraction was the SPIN-X Viral RNA extraction kit (SD BIOSENSOR, Inc). The nasopharyngeal and oropharyngeal swab were collected into sterile tube containing 2-3ml of viral transport medium or normal saline and transported to the virology laboratory at Noguchi Memorial Institute for Medical Research (NMIMR), department of Virology for RNA extraction and Real-time PCR. The swab sample was homogenized using the vortex and 1.5ml of homogenized swab sample was aliquoted into cryotubes. The extraction was done based on four main steps; cell lysis, binding, washing and elution.

Ten microlitre (µl) of proteinase K and 200ul of aliquoted sample was dispensed into a labelled 1.5ml Eppendorf tube. Lysis buffer (300 µl) was added and incubated for 10 minutes

at 56-60° C at the same time the elution buffer. Isopropanol (300 µl) was added, vortexed for about 10 seconds and centrifuged for 5 seconds. Disruption of an infected cell was done by the use of lysis buffer which breaks open to release cell components such as protein, cell material and nucleic acid. BST solution (100 µl) was added to the binding column tube (fit in collection tube) and centrifuged for 30 seconds at 13,000 rpm then the solution was discarded from the collection tube and the collection tube was reused.

The lysate was transferred into the binding column and centrifuged at 13,000rpm for 1 min. It was centrifuged again until the liquid completely passes through and then the solution was discarded and the collection tube was reused. The binding column contains silica matrix which enhances selective binding due to the difference in polarity. Thus, the viral nucleic acid (negatively charged) binds to the solid phase of silica which contain silicon oxide (positively charged). Other cell components and inhibitors which are not bound to the silica membrane are washed away into the collection tube.

Five hundred microlitre (µl) of washing buffer was added and centrifuged at 13,000 rpm for 1 min and then discarded and the collection tube was reused. Washing buffer 2(600 µl) was added into the column and centrifuged at 13,000rpm for 1 min and the solution was discarded and the collection tube was reused. The washing buffer was repeated and dry spinned to remove all the remnant ethanol in the binding column. The washing buffer which contains absolute ethanol was added to maintain the binding conditions and removed the binding salts and other contaminants to produce a purified nucleic acid. Nucleic acid is insoluble in ethanol therefore the absolute ethanol in washing buffers precipitates the nucleic acid and several washing with increased ethanol was done to free the nucleic acid on the silica membrane from other contaminants.

The binding spin column was taken out and fitted in a labelled 1.5ml tube and 50ul of elution buffer was added and allowed to stand for 1 minute to allow the buffer permeate the column. To increase RNA yield, it was allowed to stand for about 5 minutes and eluted by centrifuge at 13,000rpm for 1 minute. The elution buffer broke the bond between the hydrophilic nucleic acid and the silicon oxide to obtain a purified nucleic acid. The eluted RNA solution was used directly for PCR.

3.5 Detection of SARS-CoV-2 gene using Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR).

Requirements of real-time reverse transcription Polymerase Chain Reaction (RT-PCR) used for the detection of SARS CoV-2 included a master mix which was composed of a probe/primer, reverse transcriptase to convert single strand RNA to cDNA template, internal positive control to detect the presence of nucleic acid, buffer to provide right PH and positive control and negative control to validate the PCR reaction. The RT-PCR is based on Reverse Transcription technique that employs the use of fluorescent probes which is composed of a quencher dye and a reporter fluorescent dye designed to bind to specific target gene sequence between the forward and reverse primer. PCR produces more copies of the SARS-CoV-2 gene under 3 cyclical conditions (annealing, denaturation and extension). In every cycle, as the cDNA template is being synthesized when it reaches the target gene sequence, the quencher is cleaved off and reporter dye is allowed to fluorescent and the intensity increase as the amount of target genome sequence in the specimen. Visualisation of results is interpreted by a sigmoidal or an S curve on a graph showing cycle threshold (Ct) values against change in reaction. The PCR Kit used was the Mico Biomed VERI-Q Prep kit (MiCo BioMed Co., Ltd) which comprises of probes that are designed to target the Open reading frame 3a (ORF 3a) and the Nucleocapsid gene (N gene) found on SARS CoV-2.

Prior to RT-PCR, the reagents were completely thawed and centrifuged to remove drops from the inside of cap. The master mix was prepared under a safety cabinet with light off to completely protect the reagents from light. The pipette was used to collect a required amount of the pre-master mix, probes/primers and the internal positive control into a 1.5ml tube based on the number of reactions to run and vortex for 3 seconds. The reaction mixture (6 µl) was aliquoted in each well of the PCR plate. The extracted RNA (4µl) was added to each well followed by the negative and positive control respectively to avoid contamination. The RT-PCR mixture was mixed, centrifuged at 1,000 rpm for 30 seconds and placed into the wells of the real time PCR machine called BIOER thermocycler. Each well within the plate was defined and targets was assigned (ORF3a, N gene and the Internal positive control) and the right cyclical conditions (temperature and time) was setup in 4 steps (50° C for 10 minutes in 1 Cycle, 95° C for 3 minutes in 1 Cycle, 95° C for 9 seconds and 58° C for 30 seconds in 45 cycle). Visualisation of results was interpretated by a sigmoidal or an S curve on a graph showing Cycle threshold (Ct) values against change in reaction.

3.6 COVID-19 Antigen Test

The COVID-19 Antigen Test kit used was the SARS-CoV-2 Antigen Test Kit (Colloidal Gold Chromatographic Immunoassay) (Ultra-Diagnostics-Bio) which is based on colloidal gold immunochromatography method to detect SARS-CoV-2 Nucleocapsid (N) protein in respiratory secretion from the nasopharynx and oropharynx as described by Terpos et al (2021). . Specimen added to the test device was absorbed by capillary action and mixed with gold-labelled antibody and flowed across the precoated membrane. A coloured test band is produced in the Test Region (T) when the SARS-CoV-2 antigen in the specimen is captured by the gold-labelled antibody bound to antibody S1 immobilized in the Test Region (T).

The swab sample was mixed using the vortex in Viral Transport Medium / Saline to obtain a homogenized solution. 80ul of the swab sample was added into the extraction tube and mixed for 5 seconds and allowed to wait for 1 minute. The nozzle cap was pressed tightly onto the tube and the test card was taken out from the aluminium foil bag and laid flat on the test bench. 3 drops of the extracted specimen was added to the specimen well of the test device. The results were read within 15 minutes and interpreted following manufacturer's instructions.

3.7 Detection of *Plasmodium falciparum* in patients at the health facility

3.7.1 Malaria Rapid Diagnostic Test

SD Bioline Malaria Antigen *Plasmodium falciparum* test kit which is a Malaria Rapid diagnostic test (RDT) was used to detect *Plasmodium falciparum* in the patient's blood at the OPD unit. The Malaria RDT test targets the *P. falciparum* specific protein i.e histidine-rich protein II or lactate dehydrogenase (LDH) (Tangpukdee *et al*., 2009 and Abuaku et al.,2021).

The patient's 4thfinger on the left hand was disinfected using alcohol swab and allowed to dry. The lateral side of the 4th finger was then pricked using sterile lancet and a5µl capillary pipette was used to draw blood to the black line. The drawn blood (5µl) was dispensed to the round specimen well on Malaria test cassette. The lancet was discarded in the Sharps box immediately after pricking. Drops of the assay diluent (4 drops) was added vertically into the square assay diluent well hole and the test results were read in 15 minutes following manufacturer's instructions. A coloured band on both the Control and Test line indicates a positive result; hence the patient does have malaria. A coloured band on the Control line and no band on the Test line indicates negative result, hence the patients does not have malaria. The absence of a coloured band in the control line indicates invalid results. Malaria positive

RDT test was further analysed using microscopy and nested PCR for confirmation and speciation (Tangpukdee *et al*.,2009 and Abuaku *et al*.,2021).

3.7.2 Malaria Blood Thick Films

A drop **(5µl)** of blood was placed from the syringe onto the center of a pre-cleaned labelled glass slide and the corner of another slide was used gently to prepare a 6µl blood smear in a circular pattern and allowed to dry at room temperature. 10% Giemsa stain (1 ml of Giemsa to 9ml of distilled water) was added and allowed to stand for 10 minutes and the stain was washed in water. The slide was allowed to dry and a drop of immersion oil was added on the smear and observed under the light microscope using oil immersion magnification to detect the *Plasmodium* parasites.

The entire smear was first screened at a low magnification (X10 x X40 objective lens) to detect suitable fields and then examined using X100 oil immersion. Fields were examined at least 100 high power before a thick smear was declared negative or positive.

Parasite density was determined for each malaria positive slides. The total number of white blood cells (Neutrophils, Eosinophils, lymphocyctes and basophils) and *Plasmodium* parasites was counted in each field on the slides using a tally counter until a total of 200 white blood cells was obtained and the corresponding parasites count was recorded respectively. *Plasmodium* parasites were counted per 200 leukocytes which was used to estimate the parasite density.

$$\text{Parasite density} = \frac{Total\ number\ of\ Plasmodium\ parasite}{Total\ number\ of\ white\ blood\ cells} x\ 8{,}000\mu l$$

3.7.3. Detection of *Plasmodium falciparum* Using Polymerase Chain Reaction

3.7.3.1. Extraction of *Plasmodium* sp DNA from Blood Paper Blot Using Saponin/ Chelex DNA Extraction Protocol

The hole puncher was sterilized using 10% bleach, distilled water and 80% ethanol. Two punches of each labelled blood blot filter paper to a size 5-6mm into a sterile 1.5ml Eppendorf tube. The hole puncher was sterilized and 1 ml of phosphate buffer saline and 50µl of 10% Saponin was added to each labelled Eppendorf tube and was vortexed briefly and incubated overnight at 4°C while shaking to enhance the lysis of red blood cells to release the plasmodium parasites. The Eppendorf tube was vortexed and spinned briefly for 1 minute at 10,000rpm. The reddish PBS/Saponin supernatant was decanted and pad. Ice-cold PBS (1ml) was added to the tube containing the filter paper and was vortexed briefly and incubated at 4°C for 30 minutes. After 30 minutes the tubes were spinned at 10,000rpm for 1 minute and the supernatant was aspirated. After that, 50µl of 20% Chelex and 100µl of distilled water was added and incubated at 95°C for 10 minutes and vortexed at 2 minutes interval. The Chelex reagents prevent degradation of the nucleic acid (DNA) and traps contaminants. The tubes were then spinned for 6 minutes at 13,000rpm. The supernatant (DNA) was transfered into a sterile labelled 0.5ml microfuge tube and store at -20°C.

3.7.3.2. *Plasmodium* Speciation Using Nested Conventional Polymerase Chain Reaction.

The Nested Conventional Polymerase Chain Reaction was performed using two primer set and successive PCR using a thermocycler used to detect *Plasmodium* specie in the DNA extract. Nested Dream Taq Master Mix which is compose of $MgCl_2$, Taq Polymerase, Buffer and deoxynucleotide triphosphates (dNTPs) was used in addition with primers. Amplicons as a result of the first PCR was used as a template for the second PCR. The Dream Taq master

mix(7.5µl) , 3.7µl of double distilled water, 0.4µl of rPLU5, 0.4µl of rPLU6 and 3µl of template was used in Nest I to detect 18S rRNA genes s **as** previously described by Li *et al*. (2014). Polymerase chain reaction was performed using a thermocycler (Applied Biosystems™ SimpliAmp™) with reaction conditions consisting of cyclical conditions of 94ºC for 2 min, followed by 94ºC for 30 seconds, 54ºC for 1 min, 68ºC for 1 min and the reaction was subjected to 35 cycles. The final step is a 5 min extension at 68ºC.

Nest II reaction consisted of 7.5µl of the Dream Taq master mix, 6.20µl of double distilled water, 0.4µl ofrFAL2, 0.4µl of rFAL3 and 2µl of Nest I PCR product was used in Nest II to detect meroziote surface protein msp2 which is a specific target sequence for *Plasmodium falciparum* and has a band size of 200 base pairs. The second cycling conditions for Nest II was 94ºC for 2 min, followed by 94ºC for 30 seconds, 59ºC for 1 min, 68ºC for 1 min and the reaction was subjected to 35 cycles. The final step is a 5 min extension at 68ºC. Size-fraction of the PCR products was performed on 2% agarose gel stained with Ethidium bromide (Biotium, Hayward, California, USA). The gel was ran at 130V for 45 mins and visualized under ultraviolet light.

Table 3.1. Primers sequences for *Plasmodium* parasite identification.

Primer	Sequence: 5'-3'
rPLU5	CCTGTTGTTGCCTTAAACTTC
rPLU6	TTAAAATTGTTGCAGTTAAAACG
rFAL2	ACACAATGAACTCAATCATGACTACCCGTC
rFAL3	TTAAACTGGTTTGGGAAAACCAAATATATT

3.8 Detection of the anti-SARS-CoV-2 antibody presence

Whole blood specimen of each patient was separated into 4 aliquots; 2 plasma,1 buffy coat and 1 red blood cells and were stored in cryotubes. Genrui 2019-nCoV IgG/IgM Detection kit (Colloidal Gold-Based) is based on capture and solid-phase immunochromatography which helps in identifying individuals with an adaptive immune response to SARS CoV-2 indicating recent or past infection. The test panel contains one colloidial gold –labelled recombinant COVID-19 S1 spike protein antigen and a quality control colloidial gold marker, two detection lines; IgG and IgM and one quality control line. The test area of nitrocellulose membrane is coated with mouse-anti-human IgM/ IgG antibody immobilised at test IgG, IgM and Control respectively.

The test strips from the aluminium foil punch was placed on a horizontal and dry table. A dropper was used to add 1 drop (about 20 ul) of plasma and 3 drops of the dilution buffer to the sample loading position. The plasma mixes with S1 antigen colloidal gold of 2019-nCoV to form an immune complex of IgG/ IgM antibody and colloidal gold-labelled S1 antigen and migrated due to capillary action along the nitrocellulose membrane to a capture zone of nitrocellulose membrane-immobilized antibody (mouse-anti-human IgM/ IgG antibody) thereby generating a coloured IgM and IgG band respectively if the plasma specimen contains specific IgM/ IgG antibodies against COVID S protein. The test card was observed after 10 minutes and the results were analysed following manufacturer's instructions. A coloured band at the Control line was used as a procedural control if the test procedure was performed correctly.

3.9. Full blood count analysis

Two to three millilitres of venous blood was collected into EDTA tube and was mixed with the anticoagulant using a sample roller for 1 minute. The blood sample was analyzed using the Mindray Hematology analyser (Medsinglong CO LTD) which is based on the principle of flow cytometry to provide essential information about the kinds and numbers of cells in the blood (red blood cells, white blood cells and platelets) to help detect abnormalities (National Committee for clinical laboratory Standard, 1997). Blood sample was aspirated and run using the hematology analyser within few minutes of collection. The White blood cell, neutrophils, eosinophils, basophils, lymphocytes, red blood cells and platelet count data was extracted from the FBC-printed data.

3.9.1 Ethical Consideration

Ethical approval was sought from the Ethics and Protocol Review Committee (EPRC) of the College of Health Sciences (CHS) of the University of Ghana with protocol identification number: CHS-Et/M.3 -4.5/2020-2021 and the Ghana Health Service Ethics Review Committee with protocol identification number: GHS-ERC 028/03/21. Administrative approval letter from Korle Bu Teaching Hospital (KBTH-ADM/00652/2021) to conduct the study was also obtained.

3.9.2 Data Management and Statistical Analysis

Data was collected into data sheets and notebook on the field and laboratory. Data were later entered, stored and managed in Microsoft Excel, 2019. A test for normality using the Shapiro-Wilk test showed non-normal distributions and so non-parametric analyses were done. Proportions and percentages analysis was done using Excel. Paired T-test was also used to determine whether there was a significant difference between symptoms presented by COVID-19 patients before entry and at the primary health care (Appendix II). Chi-square test

was used to determine whether there is a statistical significant relationship between the categorical variables (Appendix III). Pearson's correlation test was used to determine the association between two continuous variables via SPSS version 2 (Appendix IV).

CHAPTER FOUR

4.0. RESULTS

4.1. Demographics and clinical characteristics of malaria patients.

There were 7 out of 300 patients who were positive for malaria using malaria rapid diagnostic test and microscopy representing 2.3% respectively while, 8 (2.7%) were positive using nested PCR (**Fig 4.1**). Majority of the malaria patients were females (6/8; 75%). Two (2) participants had coinfection of malaria and COVID-19 (**Fig 4.2**) and they were 1 male and 1 female each (2/300; 0.7%).

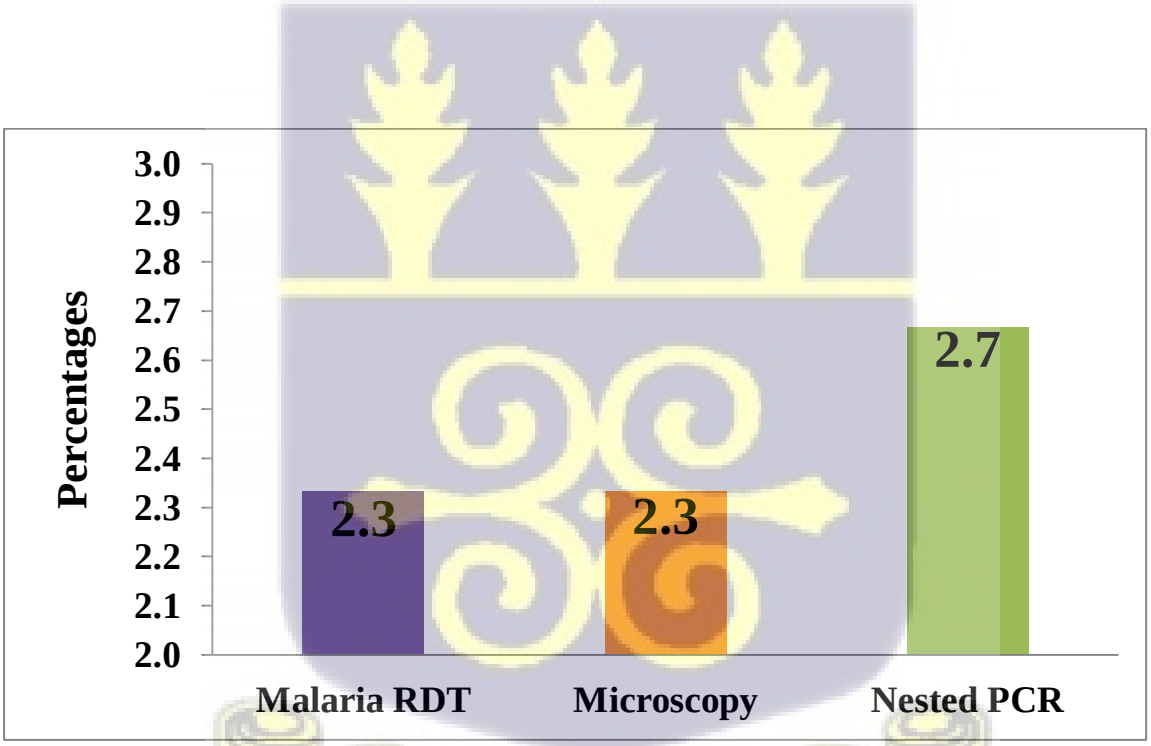

Fig 4.1. Malaria positivity by different diagnostic methods.

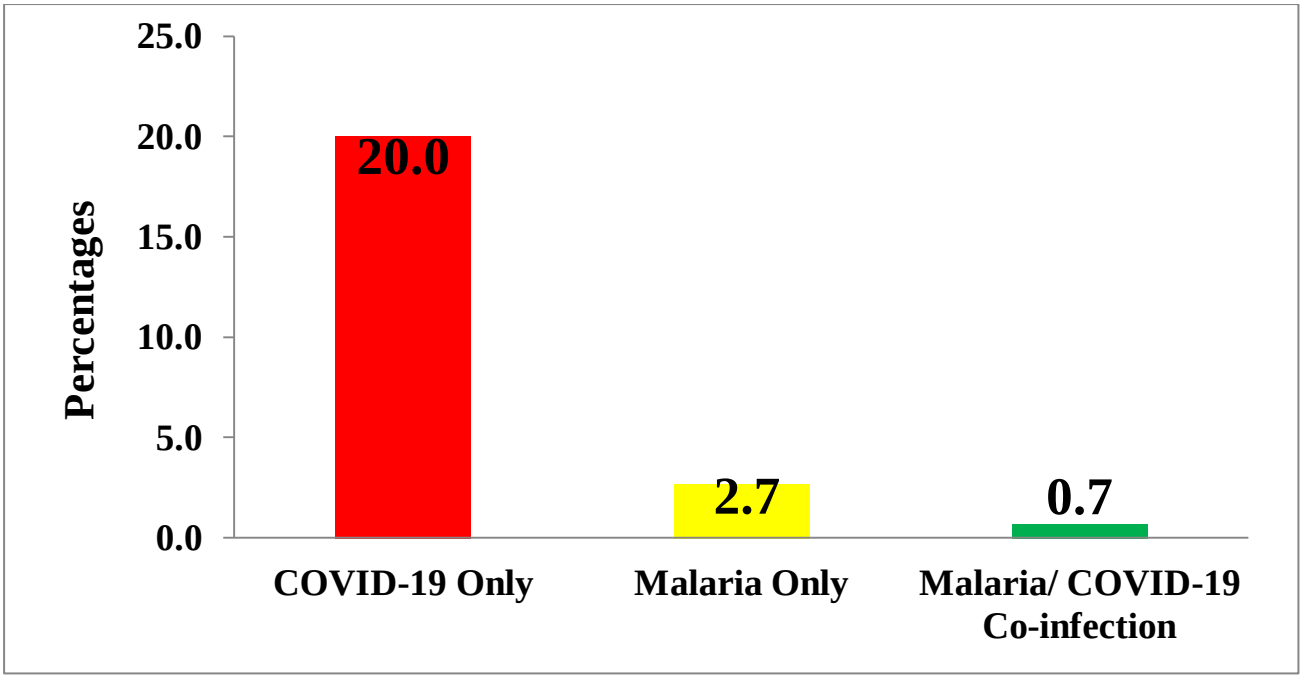

Fig 4.2. Infections with malaria and COVID-19.

The highest parasite count was 426 with a corresponding parasite density of 17,040 µl and the lowest parasite count was 139 with a corresponding parasite density of 5560 µl (**Table 4.1).**

Table 4.1. Parasite count and parasite density of microscopy malaria positives.

Parasite Count	**Parasite Density (µl)**
139	5560
426	17040
150	6000
280	11200
149	5960
152	6080
227	9080

Majority of the malaria patients were females (6/8; 75%) and most came from the Ablekuma South district (5/8; 62.5%) which included Chorkor, Dansoman, Korle Bu and Korle-gonnor. The highest temperature reported was 38.8^{o}C.

Majority of the malaria patients were between the age range of 21-40 (6/8; 75%) and most were SHS students (4/8; 50%). The most common symptom is headache and body weakness and pain (8/8; 100%). Hypertension was the only co-morbidity reported (1/8; 12.5%) (Table 4.2).

Table 4.2. Demographics and medical history of malaria patients.

Characteristics	**Malaria positives**
Gender	Females (6/8; 75%) and males (2/8; 25%)
Age	Highest age was 53 years Lowest was 18 years Majority were within the age range of 21-40 years (6/8; 75%)
Marital Status	Majority were single (7/8; 87.5%)
Occupation	Majority work in the public sector (3/8; 37.5%) Students (2/8; 25%) Traders / business men and women (2/8; 25%) Artisan (1/8; 12.5%)
Educational level	Most were SHS students (4/8; 50%) Tertiary students (2/8; 25%) Primary and (1/8; 12.5%) Middle school Form 4 (1/8; 12.5%)
Districts	Most came from the Ablekuma South district (5/8; 62.5%)
Temperature	Highest temperature reported was 38.8^{o}C and lowest is 35.6^{o}C
Co-morbidities	Hypertension (1/8; 12.5%)
Clinical symptoms	The most common symptom is headache and body weakness and pain (8/8; 100%) Tiredness (6/8; 75%) Fever (4/8; 50%) Bitter taste (3/8; 37.5%)

4.2. Demographics and clinical characteristics of COVID-19 Patients.

There were 26 out of 300 patients who were positive for SARS-CoV-2 using the SARS-CoV-2 Antigen Test representing 8.7% while, 60 tested positive using Real-time PCR test representing 20% (**Fig 4.3**).

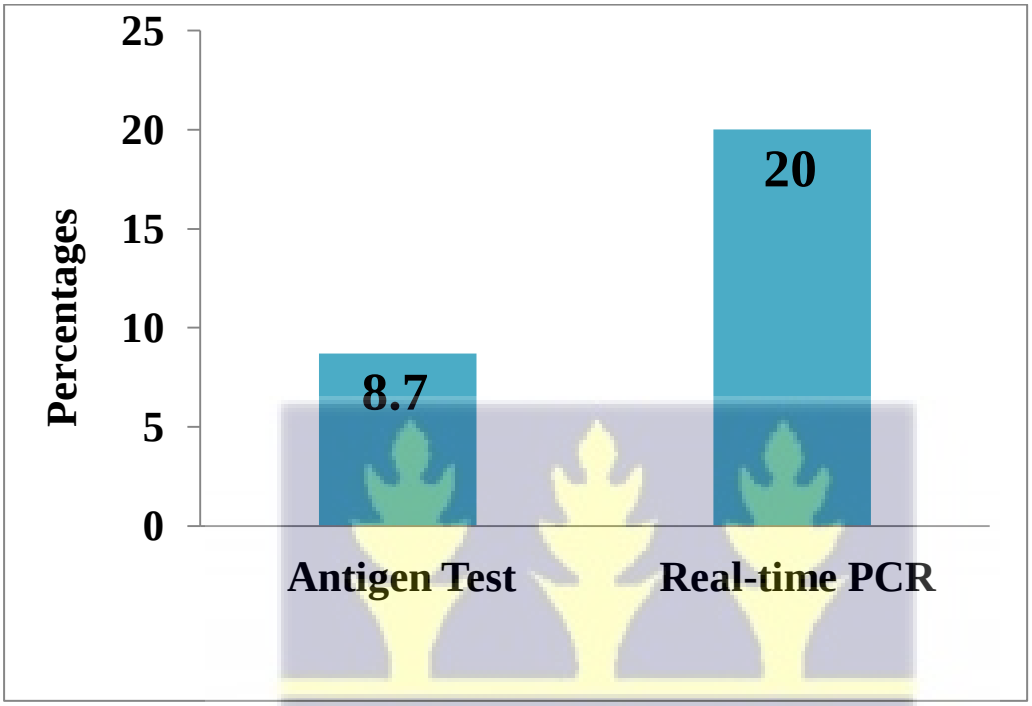

Fig 4.3. COVID-19 positivity by different diagnostic methods.

Majority of the COVID-19 patients were females (42/60; 70%) between the ages of 21-40 years (42/60; 70%). Most had no educational background (20/60; 33.3%). Majority were Traders/ Business men and women (20/60; 33.3%). Confirmed COVID-19 study patients came from diverse areas in Accra (**Fig 4.4**). Most of the confirmed COVID-19 patients came from Korle-gonnor, Dansoman and Korle Bu and all these areas are found within the Ablekuma south district (**Table 4.3**).

Table 4.3. Demographics of COVID-19 patients.

Characteristics	COVID-19 positives
Age	18-20 years (4/60; 6.7%) 21-40 years (22/60; 36.7%) 41-60 years (19/60; 31.7%) Above 60 years (15/60; 25%)
Gender	Females (42/60; 70%) and males (18/60; 30%)
Marital Status	Married (31/60; 51.7%) Single (22/60; 36.7%) Widowed (7/60; 11.7%)
Educational Level	No educational background (20/60; 33.3%) Tertiary students (18/60; 30%) Senior High School student (SHS) (11/60; 18.3%) Junior High School students (JHS) (10/60; 16.7%) Primary student (1/60; 1.7%)
Occupation	Public sector (11/60; 18.3%) Students (9/60; 15%) Unemployed (14/60; 23.3%) Artisans (6/60; 10%) Traders/ Business men and women (20/60; 33.3%) Retired (0/60; 0%)
Residence areas	Confirmed COVID-19 study patients came from diverse areas in Accra and majority were within the Ablekuma South District.

✦ = **Areas of COVID-19 patients,** Source: (Self).

✦ = **Ablekuma south district,**

✦ = **Korle Bu polyclinic.**

Fig 4.4. Map showing residence area of confirmed COVID-19 patients.

There were 35 out of 60 COVID-19 patients who had normal temperature ranging from 35.6°C -37.5°C representing 58% .The highest comorbidities reported were hypertension (9/60; 15%) and both hypertension and diabetes (4/60; 6.7%) (Table 4.4).

The most common symptom experienced by the COVID-19 patients was headache (95%; 57/60) (Table 4.4).

There was no significant difference between clinical symptoms presented by the patients before and at the polyclinic ($p > 0.001$) however negative correlation was reported between the cumulative number of symptoms and CT values of both the ORF3a and N protein of COVID-19 patients, r (58)= -0.744, $p < 0.001$ and r (58)= -0.754, $p < 0.001$ respectively (**Table 4.5**). This revealed that majority of COVID-19 patients with Cycle threshold (CT) values below 30 (high viral loads) (67.7%; 21/31) presented with 5 or more symptoms.

Table 4.4. Medical history of COVID-19 patients.

Medical history	**COVID-19 positives**
Temperature	Minimum temperature was 35.6° C Maximum temperature was 37.9° C Mean temperature was 37.2 °C Mode was 37.6° C and median was 37.5 °C 58% (35/60) had normal temperature ranging from 35.6° C -37.5°C 42% (25/60) had high temperature (fever) ranging from 37.6° C -37.9° C
Co-morbidities	Hypertension (9/60; 15%) Diabetes (2/60; 3.3%) Asthma (1/60; 1.7%) Hypertension and diabetes (4/60; 6.7%) and none had sickle cell diseases
Clinical Symptoms	Headache (95%; 57/60) General body weakness and pain (78.3%; 47/60) Tiredness (60%; 36/60) Fever (42%; 25/60) Cough (30%; 18/60) Bitter taste (36.7%; 22/60) Sore throat (18.3%; 11/60) Loss of taste (8.3%; 5/60) Loss of smell (10.0%; 6/60) Diarrhoea (3.3%; 2/60) Difficulty in breathing (1.7%; 1/60) Others (11.7%; 7/60) which included cold, dizziness and chills

Table 4.5 Correlation of the cumulative number of symptoms of COVID-19 patients and cycle threshold value the Open Reading Frame 3a (ORF3a) and N gene.

		Cumulative number of symptoms	**ORF3a**	**N**
Cumulative number of symptoms	Pearson Correlation	1	-.744**	-.754**
	P value Sig. (2-tailed)		.000	0.00
	N	60	60	60
**. Correlation is significant at the 0.01 level (2-tailed).				

4.2.1 SARS-CoV-2 antibody presence and vaccination status.

Measurement of SARS-CoV-2 antibodies in the study participants showed that 32% (113/300) of the study patients had COVID-19 IgG antibody, 12% (36 /300) had COVID-19 IgM and 50.3% (151/300) had no COVID-19 IgM and IgG (**Table 4.6**). There were 28 out of 60 (46.7%) COVID-19 patients who had COVID-19 IgG antibody, 45% (27/60) had COVID-19 IgM and 30% (18/60) had no antibodies.

Table 4.6. COVID-19 antibody presence and vaccination status.

	SARS-CoV-2 antibodies			**Vaccination status**	
	IgM	**IgG**	**None**	**Antibody**	**No Antibody**
Study Patients (N=300)	12% (36/300)	32% (113/300)	50.3% (151/300)	33.3% (17/51)	66.7% (34/51)
COVID-19 Positives (N=60)	45% (27/60)	46.7% (28/60)	30% (18/60)	0 (0/2)	100% (2/2)

There were 51 Astrazeneca vaccinated patients out of 300 study patients representing 17%. Out of the 51 vaccinated patients, 33.3% (17/51) had antibodies (**Fig 4.5**). There were 10 out of 51 vaccinated patients who had a single shot of the Astrazeneca vaccine and 70% (7/10) showed no antibodies. Also, 41 out of 51 vaccinated patients had the full dose of vaccination and 66% (27/41) showed no antibodies. Eighteen out of 51 showed evidence of their vaccination cards which included the dates and details of vaccine.

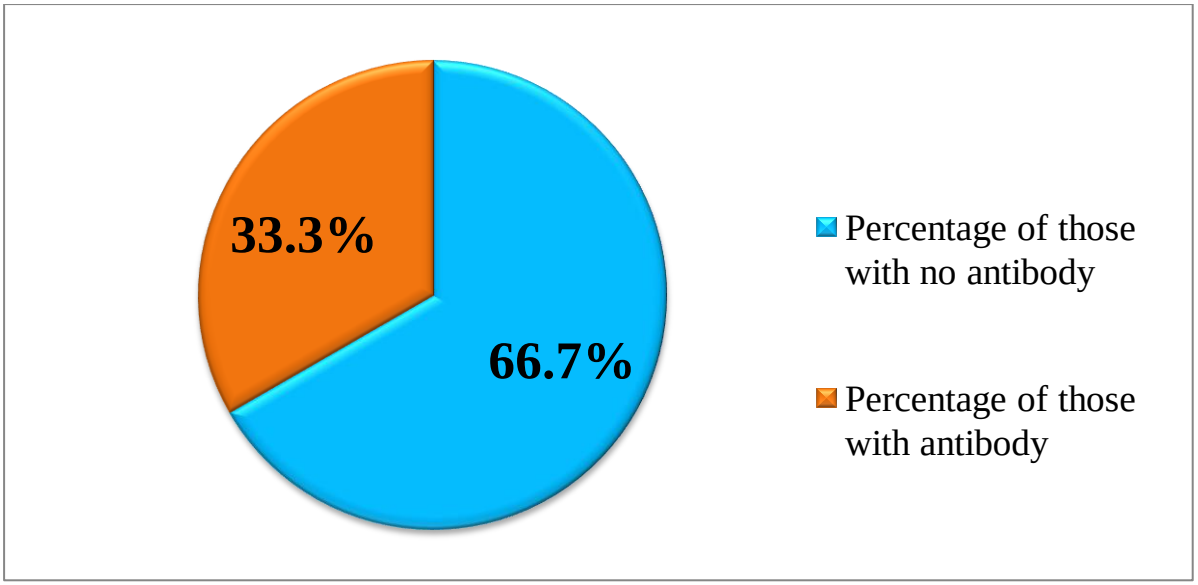

Fig 4.5. SARS-CoV-2 antibodies of Astrazeneca vaccinated persons.

There was significant association between COVID-19 IgM and ORF3a and N gene of PCR (X^2=17.475; *df* =1; p < 0.001; X^2=19.862; *df* =1; p < 0.001). This revealed that majority of confirmed COVID-19 IgM had low Cycle threshold (CT) values (CT < 30) (81% and 92.6%) (**Table 4.7**).

Table 4.7. COVID-19 IgM and PCR CT values of ORF3a and N gene.

IgM	ORF3a gene		N gene	
	Low CT	High CT	Low CT	High CT
COVID-19 IgM Positive	81% (22/27)	18.5% (5/27)	92.6% (25/27)	7.4% (2/27)

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4.3 Haematological parameter associated with COVID-19 patients.

There was a significant association between normal lymphocytes counts and majority of study patients (81%; 243/300) which included COVID-19 patients, malaria patients and those with no infections (X^2=31.97, *df* =2, p < 0.001). However no significant association was reported between the other red and white blood cells (basophils, monocytes, eosinophils and neutrophils) and all study patients. Majority of the COVID-19 patients (65%), malaria patients (75%) and patients with no infections (85.3%) had normal lymphocytes counts (**Table 4.8**).

Table 4.8. Lymphocytes count among study patients.

Lymphocytes Counts	**No Infections**	**COVID-19 positives**	**Malaria positives**
Low	7.8% (18/232)	35% (21/60)	25% (2/8)
Normal	**85.3% (198/232)**	**65% (39/60)**	**75% (6/8)**
High	6.9% (16/232)	0% (0/60)	0% (0/8)

There was a significant association between normal platelets counts and majority of study patients (70%; 210/300) (X^2=31.97, *df* =2, p< 0.001) however no significant association was reported between the other red and white blood cells (basophils, monocytes, eosinophils and neutrophils) and study patients. Majority of the COVID-19 patients (65%), malaria patients (75%) and patients with no infections (71.1%) had normal platelets counts (**Table 4.9**).

Table 4.9. Platelets count among study patients.

Platelets Counts	**No Infections**	**COVID-19 positives**	**Malaria positives**
Low	7.8% (18/232)	33.3% (20/60)	12.5% (1/8)
Normal	**71.1% (165/232)**	**65% (39/60)**	**75% (6/8)**
High	21.1% (50/232)	1.7% (1/60)	12.5 (1/8)

CHAPTER FIVE

5.0. DISCUSSION

Malaria is one of the killer diseases in Ghana which contributes significantly to morbidity and mortality in the public healthcare facilities. Due to the onset of COVID-19 pandemic, provisions to clinical services to malaria in most moderate and high malaria burden countries in 2020 has been interrupted (WHO, 2021) and this could increase the number of malaria cases, therefore, studying factors that could contribute to increasing the intensity of the pandemic is very essential. In this study, COVID-19 was investigated in patients who presented with malaria-like symptoms at the Korle Bu polyclinic in Accra. From the study, 20% of patients who presented with malaria-like symptoms had COVID-19. Co-infection of malaria and COVID-19 were low. Some of the study patients (37%) with malaria-like symptoms were reactive for SARS-CoV-2 IgG antibody. Headache, general body weakness, general body pains and tiredness were the top four common symptoms reported. Majority of the COVID-19 patients had normal lymphocytes and platelets counts.

In the study, 20% of the patients who presented with malaria-like symptoms were infected with COVID-19 and it was more likely that these patients were going to be treated for malaria only, however, they harboured SARS-CoV-2. Therefore, diagnosing malaria and COVID-19 together may be more efficient for immediate control and to contain transmission. These findings are similar to a study conducted in India which reported 10.34% of COVID-19 among febrile patients at a malaria clinic (Guha *et al.*, 2021). The difference in prevalence between this study and the study conducted in India is due to the reason that most febrile patients in India disagreed for nasopharyngeal and oropharyngeal swabs to be taken and this could contribute to the low prevalence of COVID-19 reported. Also, the anxiety and fear for

COVID-19 was alarming during the heat of the pandemic and most people were unwilling to attend to healthcare facilities and this could lead to less clinic visits.

Co-infection of COVID-19 and malaria were low (0.7%) and a couple of reasons may support this finding. Malaria has been shown to inhibit COVID-19 development in malaria endemic areas, as shown in a study done in Uganda (Achan *et al*., 2021) and Immunoglobulin E are higher in malaria endemic areas and it modulates immune responses to COVID-19 resulting in less severe outcome (Achan *et al*., 2021) and these could account for the low level of prevalence of co-infection of *Plasmodium spp* infection and COVID-19 reported in this study. This finding is similar to the study in India among febrile patients where 2 (6.89%) were positive for both COVID-19 and *Plasmodium vivax* (Guha *et al*., 2021). A low level of prevalence of *Plasmodium spp* and COVID-19 patients was also reported in a study conducted in Congo (Matangila *et al*., 2020). The lower prevalence of co-infection of SARS-CoV-2 and plasmodium reported in this study and similar in other studies indicates immunoglobulin E which is reported to be higher in malaria endemic areas could modulate immune responses to COVID-19 which could impede the development of both malaria and COVID-19.

Age, occupation and absence of fever were factors associated with COVID-19 transmission from the study. Age is one of the risk factors of COVID-19 and majority of the COVID-19 patients in this study were between the ages of 21 to 40 years and this indicates that individuals in this age range are more likely to interact more hence the likelihood of spreading SARS-CoV-2 to the vulnerable age group is high. Studies shows that older people are more prone and are likely to have severe COVID-19 and approximately 80% of the deaths associated with COVID-19 happen in persons over the age of 65 (CDC, 2021). Majority of the COVID-19 patients were traders and business persons and this suggests low adherence to COVID-19 protocols. Market places situated in Accra facilitate a fast spread of

infectious disease due to many exchanges of locally produced and imported goods (Asante and Mills, 2020). Highest rate SARS-CoV-2 antibody seropositivity (27%) was reported amongst participants in market and lorry stations by Quashie *et al*. (2021) in Ghana. Monitoring patient's temperature is important however measuring temperature alone is not informative enough because 58% (35/60) had normal temperature (< 37.5° C) therefore the absence of fever (≥ 37.5° C) does not exempt the possibility of COVID-19 and a similar finding was also reported in a study by *Mo et al*. (2020) where about 8.7% of COVID-19 patients had no fever at admission.

Some of the study participants (32%) and those with COVID-19 (46.7%) were reactive for SARS-CoV-2 IgG antibody which indicates previous exposure to SARS-CoV-2. Also, some of the study patients (12%) and COVID-19 patients (45%) were reactive for SARS-CoV-2 IgM antibody which indicates current infection to SARS-CoV-2. A study in Pakistan reported a similar finding of seroprevalence of SARS-CoV-2 IgG antibody (33%) among healthcare workers (Batool *et al*., 2021) and also, another study in Iran reported similar finding of seroprevalence of SARS-CoV-2 IgG antibody (33.3%) among healthcare workers. Similarity of the prevalence of SARS- CoV-2 IgG in this study and other studies noted indicates that quite a number of people have been previously exposure the SARS-CoV-2**.** Majority of COVID-19 patients (92.6%) who were reactive for SARS-CoV-2 IgM antibody had PCR CT< 30 and this key finding confirmed that antibody test can also be used as diagnostic and surveillance tool to assist screening of COVID-19 to provide vital information on how far the disease has spread and forecast on future hotspots areas. Also, not all AstraZeneca vaccinated patients had attained the full immune protection against SARS-CoV-2 hence they are at risk of been infected therefore there is the need to continuously observe all the COVID-19 protocols even after vaccination.

Hematological parameters and COVID-19 disease are clinical markers related to disease severity and progression. Most COVID-19 patients, malaria patients and patients with no infections had normal platelets and lymphocyte counts and this reveals that most of the confirmed COVID-19 patients had mild to moderate infection. These findings are in line with another study where majority of the COVID-19 patients presented with mild disease (80.9%) (Shang *et al*., 2020). Araya *et al*. (2021) and Liu *et al*. (2020) also reported that severe COVID-19 patients are more likely to have low lymphocyte and platelet counts than those with mild and moderate COVID-19 infection and this similarity with other studies confirms that majority of confirmed COVID-19 patients had mild to moderate infections.

Hypertension (15%), diabetes (3.3%), asthma (1.7%) and both hypertension and diabetes (6.7%) were the comorbidities reported among COVID-19 patients and these findings are in line with previous studies which reported hypertension, diabetes and asthma as comorbidities reported in COVID-19 patients (Ashinyo *et al*., 2020; de Almeida-Pititto *et al*., 2020; Paudel, 2020; Zhou *et al*., 2020). Also in USA, data reported by nursing homes, hospitals, and other health facilities revealed that the leading comorbidities among COVID-19 death were hypertension (55.4%) and diabetes (37.3%) (Franki, 2020). The most common symptom presented by COVID-19 patients was headache followed by general body weakness and pains and tiredness. Majority of the COVID-19 patients with CT values below 30 (High viral loads) (21/31) had five or more number of symptoms.

Diagnosing malaria and COVID-19 together at health facilities during the COVID-19 pandemic era is a great burden and challenge, due to the non-specific and wide range of signs and symptoms during the early stage of infection and from the findings of this study there is the need to pay detailed attention to proper screening of COVID-19 and malaria at the primary healthcare.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The prevalence of COVID-19 identified in patients presenting with malaria-like symptoms was 20%. Co-infection of malaria and COVID-19 were low. Some of the patients with malaria-like symptoms were exposed to SARS-CoV-2. Headache, general body weakness, general body pains and tiredness were the top four common symptoms reported for patients with SARS-CoV-2 infection. Majority of the COVID-19 patients had normal lymphocytes and platelets counts. Due to the synergy of symptoms, screening for COVID-19 in patients presenting with malaria-like symptoms is vital for immediate diagnosis, treatment to avoid serious complications and prevent transmission within the healthcare.

6.2 Recommendations

Based on the results of this research study, the following recommendations are given;

1. Screening of COVID-19 and malaria among patients presenting with malaria-like symptoms at primary health facilities will be a great idea.
2. Surveillance studies of COVID-19 should be done more frequently to keep in mind the seasonal prevalence of viral infections mimicking malaria-like or febrile diseases.
3. In future, studies on bacterial etiologies contributing to malaria-like systems must be included in such studies.

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APPENDICES

APPENDIX I: Occupational Background

Public Sector: Health workers, Teachers, mechanical engineer, manager, administrator and banker.

Student: Junior High School, Senior High School, Tertiary and vocational training.

Unemployed

Artisans: Seamstress, Caterer, Carpenter, Car sprayer, Video editor, Beauticians, Aluminium worker, Automechanic, Cleaner, Cooker and Farmer.

Trader/Business

Retired

APPENDIX II: Paired T Test Analysis

A) Paired T Test Analysis of clinical symptoms of COVID-19 patients before visiting the Polyclinic and at the Polyclinic.

		N	Correlation	Sig.
Pair 1	B_Fever & N_Fever	60	.905	.000
Pair 2	B_Headache & N_Headache	60	.552	.000
Pair 3	B_Cough & N_Cough	60	.921	.000
Pair 4	B_Sorethroat & N_Sorethroat	60	.887	.000

		N	Correlation	Sig.
Pair 1	B_Tiredness & N_Tiredness	60	.381	.003
Pair 2	B_Loss_of_smell & N_Loss_of_smell	60	.830	.000
Pair 3	B_Loss_of_taste & N_Loss_of_taste	60	.782	.000
Pair 4	B_Bitter_Taste & N_Bitter_Taste	60	.311	.015
Pair 5	B_Body_Weakness & N_Body_weakness_and_pain	60	.627	.000

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	B_Difficulty_in_breathing	.0167[a]	60	.12910	.01667
	N_Difficulty_in_breathing	.0167[a]	60	.12910	.01667
Pair 2	B_Diarrhoea	.0333[a]	60	.18102	.02337
	N_Diarrhoea	.0333[a]	60	.18102	.02337
Pair 3	B_Other	.0000[a]	60	.00000	.00000
	N_Other	.0000[a]	60	.00000	.00000

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	B_Fever - N_Fever	-.05000	.21978	.02837	-.10678	.00678	-1.762	59	.083
Pair 2	B_Headache - N_Headache	-.01667	.22487	.02903	-.07476	.04142	-.574	59	.568
Pair 3	B_Cough - N_Cough	.00000	.18411	.02377	-.04756	.04756	.000	59	1.000
Pair 4	B_Sorethroat - N_Sorethroat	-.03333	.18102	.02337	-.08010	.01343	-1.426	59	.159

		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	B_Tiredness - N_Tiredness	-.11667	.55515	.07167	-.26008	.02674	-1.628	59	.109
Pair 2	B_Loss_of_smell - N_Loss_of_smell	.03333	.18102	.02337	-.01343	.08010	1.426	59	.159
Pair 3	B_Loss_of_taste -	.00000	.18411	.02377	-.04756	.04756	.000	59	1.000

	N_Loss_of_taste								
Pair 4	B_Bitter_Taste - N_Bitter_Taste	-.16667	.52615	.06793	-.30259	-.03075	-2.454	59	.017
Pair 5	B_Body_Weakness - N_Body_weakness_and_pain	-.08333	.38142	.04924	-.18186	.01520	-1.692	59	.096

B) Paired T Test Analysis of clinical symptoms of COVID-19 patients before Visiting the Polyclinic and at the Polyclinic.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 2	B_MHeadache - N_MHeadache	-.12500	.35355	.12500	-.42058	.17058	-1.000	7	.351
Pair 5	B_MTiredness - N_MTiredness	-.12500	.35355	.12500	-.42058	.17058	-1.000	7	.351
Pair 6	B_MLoss_of_smell - N_MLoss_of_smell	-.12500	.35355	.12500	-.42058	.17058	-1.000	7	.351

Pair 7	B_MLoss_of_taste - N_MLoss_of_taste	.12500	.35355	.12500	-.17058	.42058	1.000	7	.351
Pair 8	B_MBitter_Taste - N_MBitter_Taste	-.25000	.46291	.16366	-.63700	.13700	-1.528	7	.170
Pair 11	B__Diarrhoea - N_MDiarrhoea	.12500	.35355	.12500	-.17058	.42058	1.000	7	.351

Appendix III: Chi-Square Test Analysis Between categorical groups

a) PCR (ORF3a) and platelets count.

Platelet_Variables * ORF3A_C Crosstabulation					
			ORF3A_C		Total
			low	high	
Platelet_Variables	low	Count	12	7	19
		Expected Count	9.8	9.2	19.0
	normal	Count	19	19	38
		Expected Count	19.6	18.4	38.0
	high	Count	0	3	3
		Expected Count	1.6	1.5	3.0
Total		Count	31	29	60
		Expected Count	31.0	29.0	60.0

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.254[a]	2	.119
Likelihood Ratio	5.424	2	.066
Linear-by-Linear Association	3.095	1	.079
N of Valid Cases	60		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.45.

b) PCR (N) and platelets count.

Platelet_Variables * N_C Crosstabulation					
			N_C		Total
			low	high	
Platelet_Variables	low	Count	13	6	19
		Expected Count	11.7	7.3	19.0
	normal	Count	24	14	38

		Expected Count	23.4	14.6	38.0
	high	Count	0	3	3
		Expected Count	1.9	1.2	3.0
Total		Count	37	23	60
		Expected Count	37.0	23.0	60.0

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.229[a]	2	.073
Likelihood Ratio	6.165	2	.046
Linear-by-Linear Association	2.303	1	.129
N of Valid Cases	60		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.15.

c) PCR (N) and lymphocytes count.

Lymphocyte_variables * N_C Crosstabulation

			N_C		Total
			low	high	
Lymphocyte_variables	low	Count	17	4	21
		Expected Count	13.0	8.1	21.0
	normal	Count	20	19	39
		Expected Count	24.1	15.0	39.0
Total		Count	37	23	60
		Expected Count	37.0	23.0	60.0

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.083[a]	1	.024		
Continuity Correction[b]	3.906	1	.048		
Likelihood Ratio	5.391	1	.020		
Fisher's Exact Test				.029	.022

Linear-by-Linear Association	4.999	1	.025		
N of Valid Cases	60				
a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.05.					
b. Computed only for a 2x2 table					

d) PCR (ORF3a) and lymphocytes count.

Lymphocyte_variables * ORF3A_C Crosstabulation					
			ORF3A_C		Total
			low	high	
Lymphocyte_variables	low	Count	16	5	21
		Expected Count	10.9	10.2	21.0
	normal	Count	15	24	39
		Expected Count	20.2	18.9	39.0
Total		Count	31	29	60
		Expected Count	31.0	29.0	60.0

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.781[a]	1	.005		
Continuity Correction[b]	6.343	1	.012		
Likelihood Ratio	8.089	1	.004		
Fisher's Exact Test				.007	.005
Linear-by-Linear Association	7.651	1	.006		
N of Valid Cases	60				
a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 10.15.					
b. Computed only for a 2x2 table					

e) IgM and ORF3a

COVID-19 IgM * ORF3A Crosstabulation					
			ORF3A		Total
			Low CT	High CT	
COVID-19 IgM	Negative	Count	9	24	33
		Expected Count	17.1	16.0	33.0
	Positive	Count	22	5	27
		Expected Count	14.0	13.1	27.0
Total		Count	31	29	60
		Expected Count	31.0	29.0	60.0

Chi-Square Tests

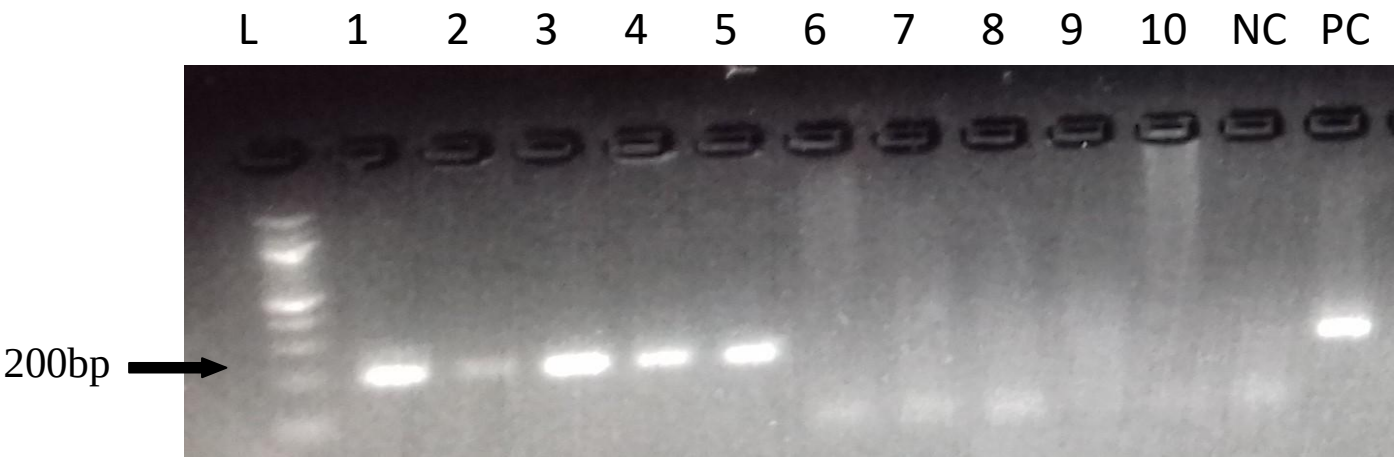
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	17.475[a]	1	.000	.000	.000	
Continuity Correction[b]	15.371	1	.000			
Likelihood Ratio	18.563	1	.000	.000	.000	
Fisher's Exact Test				.000	.000	
Linear-by-Linear Association	17.183[d]	1	.000	.000	.000	.000
N of Valid Cases	60					

f) IgM and N

COVID_19_IgM * N Crosstabulation					
			N		Total
			Low CT	high CT	
COVID_19_IgM	Negative	Count	12	21	33
		Expected Count	20.4	12.7	33.0
	Positive	Count	25	2	27
		Expected Count	16.7	10.4	27.0
Total		Count	37	23	60
		Expected Count	37.0	23.0	60.0

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	19.862[a]	1	.000	.000	.000	
Continuity Correction[b]	17.554	1	.000			
Likelihood Ratio	22.360	1	.000	.000	.000	
Fisher's Exact Test				.000	.000	
Linear-by-Linear Association	19.531[d]	1	.000	.000	.000	.000
N of Vali	60					

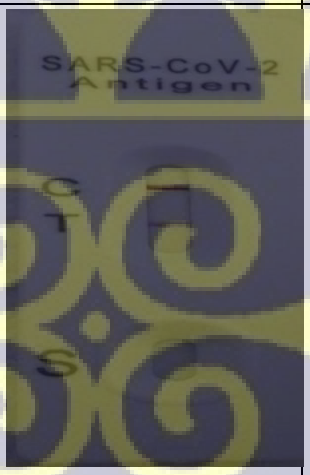
APPENDIX IV: Agarose Gel Showing DNA Bands Of *Plasmodium falciparum*.



L= DNA LADDER, NC= NEGATIVE CONTROL , PC= POSITIVE CONTROL, bp= base pair.

(2% Agarose gel image showing PCR amplification of *Plasmodium falciparum*).

APPENDIX V: Representative pictures of study cassettes showing SARS-CoV-2 antigen and antibody test and malaria rapid diagnostic test.

<table>
<tr><td> 
</td><td> 
</td><td> 
</td></tr>
<tr><td>A</td><td>B</td><td>C</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>D</td><td>E</td></tr>
</table>

A= Positive IgG, B= Positive IgM, C= Positive IgM and IgG, D= Positive malaria RDT and E= SARS-CoV-2 Antigen Test.

APPENDIX VI: College Ethical Clearance Form

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/APRIL /2021 April 28, 2021

Ms. Isabella Asamoah
Department of Medical Microbiology
University of Ghana Medical School
Korle-Bu.

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.3 –4.5 /2020-2021***

FWA: 000185779 **IORG: 0005170** **IRB: 00005220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on April 28, 2021 reviewed and approved your research protocol.

Title of Protocol: **"COVID-19 in Patients Presenting with Malaria-like Symptoms at Korle Bu Polyclinic"**

Principal Investigator: Ms. Isabella Asamoah

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid until April 21, 2022.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, University of Ghana Medical School
Head, Medical Microbiology

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APPENDIX VII: Ghana Health Service Ethical clearance form

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

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

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Fax + 233-302-685424
Email: ethics.research@ghsmail.org

My Ref. GHS/RDD/ERC/Admin/App[illegible]
Your Ref. No.

14th May, 2021

Isabella Asamoah
University of Ghana
Legon.

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC 028/03/21**
Study Title	Viral etiology in patients presenting with malaria-like symptoms at Korle-Bu Polyclinic
Approval Date	14th May, 2021
Expiry Date	13th May, 2022
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of a yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months.
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED..... .....
Dr. James Akazili
(Head, Ethics & Research Management Department)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra