DISTRIBUTION OF ABO BLOOD GROUP TYPES AND THEIR ASSOCIATION WITH MALARIA AMONG CHILDREN UNDER 5 YEARS ATTENDING OUTPATIENTS CLINIC AT BUNDIBUGYO HOSPITAL, WESTERN UGANDA

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DECLARATION

This research report is original and has not been submitted to the school of Allied Health Sciences (SAHS), CIU or any other university before

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APPROVAL

This research report titled" Distribution of ABO blood group types and their association with malaria among children under 5 years attending outpatient's clinic at Bundibugyo Hospital, western Uganda" has been submitted to International Health Sciences University as a final copy for filing following its examination by University examiners and the approval by the following supervisor

Signature……

MS. CATHERINE N. LWANIRA

SUPERVISOR

Date …….

DEDICATION

The report is dedicated to Clarke International University for its focus on building the health work force and all health workers for their struggle in eliminating malaria from Uganda

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

OPERATIONAL DEFINITIONS

ABO Blood group; is a system that classifies the human blood based on the inherited properties of red blood cells (erythrocytes) as determined by the presence or absence of an antigen A and B, these antigens are carried on the surface of the red cells. Therefore a person may have blood group type A, B, O, or type AB

Prevalence of Malaria; This is the proportion of the population in an area with common characteristics that have been classified as malaria based on signs, symptoms and laboratory confirmation in a given period of time

LIST OF ACRONYMS

ABO	-	Blood Grouping System
ANC	-	Antenatal Care
B/S	-	Blood slide
BMLS	-	Bachelor of Medical Laboratory Sciences
CIU	-	Clarke International University
IAHS	-	Institute of Allied Health Sciences
DHIS	-	District Health Information System
HDFN	-	Hemolytic Disease of fetus and Newborn
HDN	-	Hemolytic Disease of the Newborn
MOH	-	Ministry of Health
Rh (D)	-	Rhesus factor D
VWF	-	Von Willebrand Factor
HIV/AIDS	-	Human Immune Virus/Acquired Immune Deficiency Syndrome
TB	-	Tuberculosis
SPR	-	Slide Positivity Rate
DHS	-	Demographic and Health Surveys
MIS	-	Malaria Indicator Survey
WHO	-	World Health Organization
CDC	-	Centre for Disease Control
G6PD	-	Glucose 6 Phosphate Dehydrogenase
HMIS	-	Health Management Information System
MRDT	-	Malaria Rapid Diagnostic Test
OPD	-	Out Patients Department
IPD	-	Inpatients Department

MUAC	-	Mean Upper Arm Circumference
DBL	-	Duffy Binding Like-gene
PfEMP1	-	Plasmodium falciparum Erythrocyte Membrane Protein 1
EDTA	-	Ethylene Diamine Tetracetic Acid
IRS	-	Indoor Residual Spraying
ENABEL	-	Enabling
N/O	-	Nursing Officer
REC	-	Research and Ethics Committee
CIUREC	-	Clarke International University Research and Ethics Committee
WBC	-	White Blood Cells

ABSTRACT

Background: Infection with malaria under five remains a burden in sub Saharan African, and is associated with numerous preventable deaths. The clinical outcome of *Plasmodium* infection in endemic areas is influenced by erythrocyte polymorphisms including the ABO blood groups. This study reports the association of Malaria with the ABO blood group in children under 5years attending the outpatient's clinic at Bundibugyo hospital.

Methods: This was a cross-sectional prospective study that carried out during the months of July to August, 2018, in Bundibugyo hospital. The study population comprised of children 7 months-5 years presenting with a febrile condition. Clinical and laboratory investigations were done using malaria rapid diagnostic tests and microscopy; Blood grouping was done by hemagglutination methods. Statistical analysis was done at bivariate and multivariate levels using SPSS Version 23.0 and P-values less than 0.05 were considered significant.

Results: The prevalence of malaria was 14%. The most predominant blood group type were Rhesus (D) positive 'O' at 54%; A' at 26.2%, B' at 18.6% and AB' at 1.3%;. Blood group A were 1.24 times more susceptible to have positive malaria test than those with blood group O (OR= 1.24, 95%C.I (1.01 – 2.48) ; P-value =0.003. B were 1.29 times more susceptible to have positive malaria test than those with blood group O blood (OR= 1.29, 95% C.I (0.88 – 3.23); P-Value = 0.011). The statistically outcome indicated that: blood groups A and B patients are more likely to have a positive malaria test compared to blood group O

Conclusion and recommendations

The prevalence of malaria is lower than the national average, Blood group O" Rh (D) positive was the most predominant; there is an association between ABO blood group and Malaria. The **recommendation** is that the current malaria control/prevention measures should be intensified and while managing malaria cases, client's blood groups should be determined to help in management of vulnerable groups.

CHAPTER ONE: BACKGROUND

1.0 Introduction

This chapter is presenting the background of the study; explaining the burden of malaria globally, regionally and nationally. The chapter further expounds on the rationale between malaria and certain body markers such as ABO blood groups and why this study was important

1.1Background

Malaria remains among the leading causes of morbidity and mortality especially among children under five years in sub-Saharan Africa. It's a protozoan parasitic disease caused by genus *Plasmodium* with five species however *Plasmodium falciparum* has epidemiologically been proven by World Health Organization to have caused numerous severe cases leading to high mortality as compared to others *Plasmodium* species (WHO,2017).

The World Health Organization report of 2017 indicated that malaria disease is still endemic in some parts of Africa, South America, and Asia. In 2016, an estimated 216 million malaria cases were recorded as compared to the 211 million cases that were present in 2015 worldwide, which represents 5 million new cases (2.4%) of annual increase between 2015-2016 alone (WHO,2017). The African region reported 90% of the total world malaria cases followed by 7% in South-East Asia and 2% in Mediterranean region, with 90% of the mortality due malaria occurring in children under five years (WHO, 2017).

Like other countries in Tropical and Subtropical areas, Uganda has the third highest number of *Plasmodium falciparum* infections in Sub-Saharan Africa. In 2015, Uganda Ministry of

Health reports indicated that; 34% and 28% of the outpatient's visits and hospital admissions respectively constituted for malaria-related illnesses, with the majority being children less than five years. Although the prevalence of malaria over a period of 5years (2009 to 2014) had been indicated to drop from 40% to 19%, the number of children currently dying due to malaria is a big concern in Uganda. Notably, the WHO report of 2017 recorded an increase in the number of malaria cases by greater than 50,000 cases between 2015 and 2016 especially in districts where indoor residual spraying (IRS) was withdrawn (WHO, 2017); thus it becomes important to note that each region in Uganda has varying prevalence figures with the highest burden registered in rural areas as compared to urban areas (MOH, 2017).

Bundibugyo district which is part of the albertine region records a relatively high prevalence of 43%. According to the district health survey that was done in 2016/2017, Bundibugyo district recorded a total of 5,627 (38%) confirmed malaria cases; 40% of which occurred in children less than five years. Pediatric records at Bundibugyo Hospital ward where this study is going to be carried out show that between Nov 2017 and Jan 2018, over 458 severe malaria cases were treated. This represents a significant number of children suffering from severe malaria in the region (MOH, 2017).

In areas of high malaria transmission, development of naturally acquired immunity to malaria takes time after repeated exposure to malaria parasites (Doolan, Dobaño and Baird, 2009). Infections with Plasmodium falciparum often display a wide scope of clinical features ranging from asymptomatic infections, mild malaria to more fatal malaria syndromes including severe anemia, metabolic acidosis, coma, multiple organ failure and even death (WHO, 2000). Children under five years are often the most vulnerable to severe malaria because of their insufficiently developed acquired immunity. Although the greatest burden of disease is generally seen among children below the 5 years of age, the fact that some children

do become ill with malaria from time to time than other children suggests that some parasites are well tolerated better than others. The observable variability in the clinical presentation of malaria is attributable to numerous factors including parasite virulence factors, transmission intensity, host genetic factors and ABO blood groups (Laishram *et al.*, 2012).

In numerous studies, blood group O has been shown to confer some degree of protection against severe forms of malaria (Pathirana *et al.*, 2005; Panda *et al.*, 2011; Zerihun, Degarege and Erko, 2011).

The mechanism of this protection is demonstrated by the lack of a surface tri-saccharide, which acts as a receptor in the rosetting process, a key event in the pathogenesis of severe malaria. On the other hand, blood groups A and B express the surface tri-saccharides; A (which is structurally made up of GalNAcα1-3 (Fucα1-2) Gal1β1) and B (comprised of Gal1α1-3 (Fucα1-2) Galβ1) respectively, which are found attached to surface glycoproteins and glycolipids (Rowe et al., 1995). The presence of these surface molecules is believed to be important in the mediation of rosetting in infected red blood cells (RBCs) leading to severe disease in individuals with blood groups, A, B and AB than those of blood group O (Rowe *et al.*, 1995; Wahlgren, 2015).

Similar to other host factors such as the sickle cell trait, that have been selected for and whose prevalence is high in malaria endemic areas (Piel *et al.*, 2010), the distribution of blood group O also appears to be higher in areas historically known to be endemic for malaria. Worldwide, 33.6% of the people have blood group "O". A recent study conducted in Western Uganda found out that, blood group 'O' was more dominant in the population followed by 'A, B and AB (Apecu *et al.*, 2016). Even though available evidence from numerous studies points towards protection against malaria by especially blood group O, there is limited

information regarding the relationship between ABO group system and malaria in endemic areas of Uganda, including Bundibugyo district. In the present study, the influence of ABO blood group types on the clinical presentation of malaria among children below 5 years will be assessed. An understanding of this relationship may provide information emphasizing the importance of early detection of disease in vulnerable groups so that timely point of care and management is given to affected patients, so as to prevent malaria-related mortality in the region.

1.2 Statement of the problem

The World Health Organization report of 2017 recorded an increase in the number of malaria cases in Uganda, by greater than 50,000 cases between 2015 and 2016 especially in districts where indoor residual spraying (IRS) was withdrawn (WHO, 2017); whereas the national prevalence had dropped from 40% to 19% between (2009 to 2014); it's important to note that each region in Uganda has varying prevalence figures with the highest burden registered in rural areas as compared to urban areas (MOH, 2017). The albertine region where Rwenzori and Bundibugyo belong recorded a relatively high prevalence of 43%. According to the district health survey (data from the DHIS tool, MOH 2017), Financial Year 2016/2017, Bundibugyo district recorded a total of 5,627 (38%) confirmed malaria cases; 40% of which occurred in children less than five years.

The Uganda Ministry of Health has a vision of a malaria free Uganda and reduction of malaria related deaths (mortality) to near zero by the year 2020. Despite the currently available strategies for malaria management, including; indoor residual spraying, use of malaria prophylaxis medicines in pregnant mothers, malaria test and treat policy, the number of children being affected and still dying from malaria continues to be high (WHO, 2017). This therefore calls for more efforts and studies in exploring additional factors that might be facilitating the malaria disease. In previous studies conducted in Ethiopia, Nigeria,

Cameroon, Mali, and India , host factors such as ABO blood group have been shown to have significant association with malaria (Pathirana *et al.,* 2005; Panda *et al.,* 2011; Zerihun, Degarege and Erko, 2011); especially blood group O that was cited to be having some form of protection against malaria. Whereas such claims existed, there was limited data (literature) on the subject in reference to Uganda and more particularly in the Bundibugyo region of Western Uganda. Against this back ground, this study aimed to determine the influence of ABO blood group types on malaria disease among children below 5 years attending outpatient's clinic at Bundibugyo hospital. It's believed that the information generated from this study would be useful in identification of vulnerable groups that might require special care and attention towards provision of timely treatment to the affected individuals thereby minimizing the consequences of malaria disease in children

1.3 General objective

To determine the distribution of ABO blood group types and their association with malaria in children less than five years attending the outpatients clinic at Bundibugyo Hospital.

1.3.1 Specific Objectives

i) To establish the prevalence of malaria in children under 5years attending the outpatients clinic at Bundibugyo hospital

ii) To determine the distribution of ABO blood group types among children under 5years attending the outpatient's clinic at Bundibugyo hospital.

iii) To assess the association between ABO blood group types and malaria in children less than five years attending the outpatients clinic at Bundibugyo Hospital.

1.4 Research Questions

i) What is the prevalence of malaria in children under 5 years attending the outpatient's clinic at Bundibugyo hospital?

ii) What is the distribution of ABO blood group types among children under 5years attending outpatient's clinic at Bundibugyo hospital?

iii) Is there an association between ABO blood group types and malaria in children less than five years attending outpatient's clinic at Bundibugyo Hospital?

1.5 Significance of the study

Having better understanding of the host factors responsible for the infection and eventual progression of *Plasmodium* infection into severity is very useful as a knowledge tool that will help in the clinical management and prevention of severe malaria in vulnerable groups like the children below 5 years of age. If the risk group is identified early, then timely point of care can be given thereby preventing progression of disease into severity. The study outcome may have recommendations that can be integrated into the government malaria treatment policy and these will be a contribution from the study in support of malaria free Uganda

1.6 Scope

The study targeted children 7months to 5 years that had had a febrile condition for the past one day and came to attend to outpatients department at Bundibugyo Hospital

1.7 Conceptual frame work

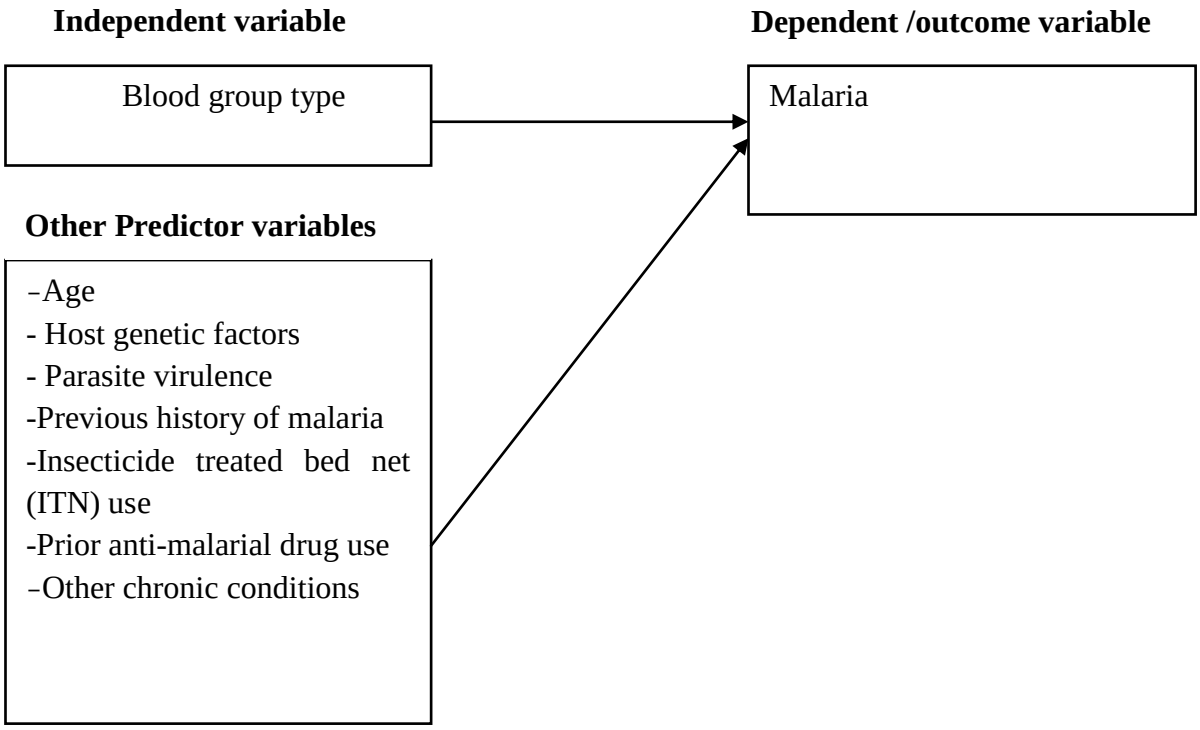


Figure 1: Conceptual frame work

In this study, it was conceptualized that susceptibility and eventual progression of malaria into severity (dependent variable) may depend on the blood group type an individual has **(**independent variable**).** However, there are other independent predictors of malaria outcome, in this case treated as the predictor variables including age, host genetic factors, parasite virulence, and previous history of malaria, ITN use and prior anti-malarial drug use. The effect of the measurable cofounders **w**ill also be determined to see how these influence malaria and its severity. Significant predictors of malaria will be treated in a multivariate regression analysis model to determine the relationship between the dependent/outcome variable and the independent variable.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter presents a review of existing literature from previous studies conducted in line with the objectives of the study. The sub themes are presented according to the specific objectives of the study.

2.1 Prevalence of malaria in children under 5 years.

Globally**;** Malaria has continued to heavily impact negatively on children under five as shown in a majority of recent field reports. According to the WHO, world Malaria report of 2016, 303,000 children under five years died due to malaria in the year. with majority of the affected living in sub Saharan Africa (World Health Organization, 2017). On global scale malaria affects Africa, Asia and South America; however most countries in Europe do get some limited cases of malaria connected to importation by travelers nevertheless; the children are the most affected compared to adults (Zanotti, 2016). In between 2010 to 2013, a prospective study that was conducted in Chhattisgarh Central, India to understand the epidemiology of complicated malaria in patients attending a referral hospital; it was found that up to 40,924 patients had malaria as confirmed by microscopy. After testing and data analysis, the malaria prevalence was found to be at 6% and *Plasmodium falciparum* was the dominant species identified (Jain *et al.*, 2014).

In a more recent retrospective study that was conducted in Italy over a period of 10yrs (2005 to 2015) to compare the imported malaria in adults and children at a teaching hospital of Padua, a total of 172 imported malaria cases with (124 adults and 48 children), of which 96.5% were from travelers to Africa were studied. The results indicated that (93%) of all the patients developed uncomplicated malaria but children showed higher parasitemia, anemia and severe malaria compared to adults (Luise *et al.*, 2017).

Regionally**;** Africa is the most affected continent with malaria with a number of studies having reported high prevalence's of malaria and mortality among children under five. According to the recent world malaria report released in 2018, Africa alone contributed up to 285,000 malaria deaths of the annual mortality rate for children under five years with a child dying every 2 minutes (World Health Organization, 2017), although there was also a reported significant increase in the number of malaria cases in the Eastern Mediterranean region from previous 3.9million by 2015 to 4.3million in 2016 accompanied with 8200 deaths.

In an earlier cross-sectional Beyond Garki project baseline survey that was conducted in Ethiopia and Uganda in 2012 with the aim of monitoring the changes in malaria epidemiology and how much impact the interventions had achieved, a total of 234 households were selected from the study sites of Aduku in Apac district and Butemba in Kyakwanzi district of Uganda and 571 households were studied in the study sites of Hembecho in Boloso Sore district and Guba in Halaba district in Ethiopia. From the baseline survey, Ethiopia had the lowest incidences of malaria of up to 1.4% in a setup of Guba area and Uganda had the highest malaria incidences of up to 9.9% at Butemba area at the peak of the transmission season. Whereas *Plasmodium vivax* was the dominant species accounting to 52% of infections in Ethiopia, Uganda had *Plasmodium falciparum* as the dominant species accounting to 95%. In both countries, malaria prevalence was moderately higher in children under five years as compared to those above 5years (Abeku *et al.*, 2015).

In a cross-sectional health facility based study that was conducted in Ethiopian Eastern Shewa zone of Oromo region also done in 2012, a total of 830 participants in five health facilities were enrolled and blood samples taken for microscopic diagnosis of malaria. Results of the study indicated that 20.5% of the children were positive for malaria with *Plasmodium vivax* being the dominant species at (11.7%), followed by *Plasmodium*

falciparum at (8.4%) and mixed infection at (0.4%). Overall, there was an observed increased prevalence of malaria among children aged 10yrs to 15yrs without mosquito nets as compared to children less than 2yrs (Haji, Fogarty and Deressa, 2016).

Another study that was done in Nouakchott Mauritania in 2011 aimed at measuring the malaria prevalence and morbidity among 301 children received at health facilities, 105 (34.9%) positive samples were confirmed by polymerase chain reaction (PCR) with *Plasmodium vivax* representing (97.1%) and *Plasmodium falciparum* (2.9%). Overall, the prevalence of malaria was high in children in Nouakchott Mauritania (Lekweiry *et al.*, 2011).

A similar prospective Cross-Sectional study was done in Nigeria, Tertiary Hospital Benin City in Edo state; aimed at measuring the prevalence of malaria and anemia in children less than five years. The study was conducted in 2009 with a total of (1325) participants enrolled and parasitemia in blood smears measured microscopically.

The results indicated an overall malaria prevalence of 75.8% and 87.3% for anemia among children under five years (Akinbo *et al.*, 2009).

In the Gambia, the malaria indicator survey that was conducted in 2010/2011 with an aim of assessing the socio-economic status and malaria infection in children and the rest of the population at large among 4,500 households, the malaria prevalence among children aged less than 5 years was at 10% (1,248) with (95% Confidence Intervals: 7.8-12.7%) and the children from the poorest families had 8.2 times risk of acquiring malaria as compared to their counter parts from the richest families (Sonko *et al.*, 2014).

In Tanzania, a sentinel non-probability study consisting of 51,467 pregnant women and 35,155 of their infants from 54 facilities was conducted with an objective of monitoring the prevalence of malaria in the lake zone, blood samples from the pregnant mothers and their

infants were tested by Rapid Malaria kits. After testing and analysis of data, the malaria prevalence was found to be at 12.8 % [95 % confidence interval (11.3–14.3] among pregnant women and 11.0 % (95 % CI 9.5–12.5) among infants (Willilo *et al.*, 2016).

In a systematic data review and meta-analysis that was conducted with the aim of comparing the prevalence of malaria infection in pregnant women and children in Sub-Saharan Africa, pooled prevalence data was taken from 18 sources and 57 data points. The results indicated a linear relationship between malaria prevalence in pregnancy and children aged (0-59months). Conclusively the prevalence was higher in children as compared to all gravidae (Van Eijk *et al.*, 2015).

More recently, in a study was conducted in three sub-Saharan Africa countries; Democratic Republic of Congo, Uganda and Kenya aimed at estimating the true prevalence of malaria in children under five using a Bayesian modeling framework, data from district health system (DHS) reports, malaria indicator survey (MIS), that had information on 13,573 children for malaria testing using Rapid Diagnostic Test and or Blood Slide was analyzed by Bayesian model. In this study, data was collected from the DHS tool 2014-2015 for Uganda, then DHS Tool 2013-2014 for Democratic Republic of Congo and MIS tool 2015 for Kenya. The results of the data analysis indicated that Uganda had the highest malaria prevalence in children less than five years at 22%, followed by Democratic Republic of Congo at 20% and Kenya at 1% (Mfueni *et al.*, 2018).

Nationally; Uganda is reported to be having high malaria prevalence, with figures differing with the level of transmission in the different regions. Below are some of the studies that presented malaria prevalence in children less than five years.

In a study utilizing secondary data on the 2014 Malaria Indicator Survey dataset of children less than 5 years in Uganda, with an aim of investigating the factors that are associated with the malaria prevalence and its relationship with anemia, 5345 households were included. Of the 4930 children under 5 years of age who had their blood samples tested, 19.04% (95% Confidence Interval [16.63–21.71]) children between 0-5years had malaria. The research data also indicated that, the malaria prevalence greatly increased the incidences of anemia (Wanzira *et al.*, 2017).

Earlier, a retrospective study involving 2471 children less than five years hospitalized was conducted in Rakai Uganda in between 2011 and 2012 at Kalisizo Hospital and Bikira Health Center to establish the prevalence of malaria parasitemia and anemia. Of the (2471) cases enrolled, malaria prevalence was found to be at 54.6% and of the children diagnosed with anemia, 76.8% had malaria. The report concluded that there was high prevalence of malaria parasitemia and anemia among hospitalized children less than five years (Kiggundu *et al.*, 2013)

In another study by Ministry of Health Malaria Technical Working Group investigating the malaria prevalence and associated factors with anemia among children less than five years using the malaria indicator survey data of 2009, it was found that 60% of the children less than 5years were anemic and over a half of them had a positive malaria test using rapid diagnostic kit, this therefore revealed that the malaria prevalence among children under five was still high (Menon, Yoon and Group, 2015).

A cross-sectional prospective study was conducted in 2017 in Uganda to identify the contributing malaria species other than *Plasmodium falciparum*. Up to 176 participants blood specimen were stained by Giemsa and 323 specimens by Rapid Malaria diagnostic kit. Each

of the 10 regions of Uganda chosen provided 50 samples used for species identification. The results of the 499 samples studied showed that, 474 of them had plasmodium infection by polymerase chain reaction amplification of 18S ribosomal RNA genes. This plasmodial infections included; P. *falciparum* in 472, *P. Malariae* in 22, *P. Ovale* in 15, *and Vivax* in four; 435 were pure *P. falciparum*, two did not contain *P. falciparum*, and the remainder were mixed infections including *P. falciparum*. These results suggest that whereas *Plasmodium falciparum* is the most common species in Uganda, other non falciparum species significantly contribute the malaria infection (Rosenthal, 2017) and thus should not be ignored.

2.2 Distribution of ABO blood group types

ABO blood grouping is a system of determining the presence or absence of antigens in the red cells surface. Each individual has a particular blood group and the prevalence of the different blood groups in different ethnic groups is variable. Worldwide, the distribution of ABO blood group is not the same, as seen by the following studies done in different communities.

In a cross sectional study that was conducted in China in 2010/2012 involving 3,832,032 volunteers from 220 counties in 31 provinces with an objective of determining the frequencies of ABO and Rhesus blood group distribution in the ethnic populations, there was great variability in ABO and Rhesus blood group distribution based on different ethnic populations ($P<0.001$). The greatest proportion of O' blood group was found among the Zhuang ethnic group at 41.8%, followed by the Miao group (37.7%); whereas blood group A' was more prevalent in the Yi group at 34.0%; the Manchu and Mongolian ethnic groups had more B phenotypes at 33.7% and 33.3% respectively; AB phenotypes were more frequent in the Uygur ethnic group (10.6%) but lower in the Zhuang group (5.5%) (Liu *et al.*, 2017).

In Switzerland, a study examining immigration patterns against the distribution of ABO blood groups using large cross-sectional Swiss samples spanning 70 years analyzed a total of 275,664 Swiss army personnel ABO blood group from 1940 to 1945. In addition, the ABO data of 122,925 individuals that were recruited into the army from 2004 to 2015 was also analyzed and the validation sample of 175,202 patients from a university hospital. Blood group distributions of the historical army were: ('A' 47%, B' 8.4% and AB' 3.0%), and the ABO blood group phenotypes of the recent army were; (A' 45%, B' 9.8% and AB' 4.1%). According to this study, ABO blood group phenotypes had remained stable irrespective of the migration patterns over time (Volken, 2017).

Between 2012 and 2013, a retrospective study was conducted at Shushila Tewari Hospital, Haldwani Uttarakhad region in India with an aim of determining the distribution pattern of the ABO blood group types among 12,701 blood donors. In this study, blood group B was more dominant followed by O, A, and the least was AB (Garg *et al.*, 2014).

One other study that was conducted in Mexico in 2011 with an objective of describing the DNA haplogroups, ABO and Rhesus blood group systems among 3 Mexican communities that included the Tepehua,Otomi and Zapotec, blood group O' was more prevalent among the three communities in the ratios of 97.2, 94.7, and 86.2%, respectively (Sánchez-Boiso *et al.*, 2011).

Meanwhile in Africa, the distribution of ABO blood groups in different communities also varies; as reported by some studies below.

In one such a cross-sectional study that was done in Cameroon in 2014 with the goal of understanding the differences in the distribution of phenotypic, alleles and Rhesus (Rh) ABO blood groups in various ethnic communities, a total of (14,546) students was included. In this

study, blood group O' was more significant predominant at 48.6% followed by group A' (25.1%), B' (21.9%) and AB the least blood group was found at a prevalence of 4.5% (Ndoula *et al.,* 2014).

Another cross-sectional study that was conducted at Al-Jabal Al-Gharbi, University, and Zawia, Libya in 2010 among 305 students with the aim of understanding the correlation between physical characteristics like fingerprints and blood group in different individuals, most participants had blood group O' (48.9%) closely followed by A' (33.1%), B' (12.8%) and AB' (5.2%) being the least prevalent (Fayrouz, Farida and Irshad, 2012).

Elsewhere, a retrospective study was carried out in Mauritania and analyzed data from 5-years in the National blood transfusion Centre with an aim of determining the frequencies of ABO and Rhesus phenotype distribution in various ethnic groups. A total of 10,116 blood donors' volunteer's records were analyzed. In this study, blood group O' was the highest at 49.1% followed by A' (28.3%), B (18.6%) and lastly blood group AB (4.1%). This order of ABO blood group prevalence in North Africa was also noticed to be concordant with the global proportions (Hamed *et al.,* 2012).

In the horn of Africa (Ethiopia), a retrospective cross-sectional study was conducted in 2016 at Jimma Town blood bank to determine the distribution of ABO blood group system and Rhesus factor. A total of 6,922 participants had been screened and their blood group types determined with up to 76.6% consisting of males and 23.4% females.

The study results indicated that blood group type O' was more predominant representing up to 43.1% followed closely by type A ' (31.9%), then B' (21.5%) and the least being blood group type AB''at 3.5% (Zerihun and Bekele, 2016).

Nationally, ABO blood group distribution has been studied in some communities and the distribution pattern is not the same. One prospective cross-sectional study involving 23,504 blood donors of average age of 21yrs mostly males was conducted in southwestern Uganda in 2016 with the main objective of determining the ABO blood group distribution among the blood donors. The blood group typing was done using various methods used in the blood bank system. The study results indicated that blood group O (50.3%) was more predominant followed by A' (24.6%); B' (20.7%) and blood group AB (4.5%) the least (Apecu *et al.*, 2016).

2.3 Association between ABO blood group type distribution and malaria severity

Studies on malaria have been conducted in different parts of the world but most notably in Africa, a place where malaria prevalence has proven very high and the association between malaria and ABO blood types has been mentioned in some studies. Particularly, blood group O has been shown to confer some degree of protection against severe forms of malaria. The mechanism of this protection is demonstrated by the lack of a surface tri-saccharide, which acts as a receptor in the rosetting process, a key event in the pathogenesis of severe malaria. On the other hand, blood groups A and B express the surface tri-saccharides; A (which is structurally made up of GalNAcα1-3 (Fucα1-2) Gal1β1) and B (comprised of Gal1α1-3 (Fucα1-2) Galβ1) respectively, which are found attached to surface glycoproteins and glycolipids (Rowe et al., 1995). The presence of these surface molecules is believed to be important in the mediation of rosetting in infected red blood cells (RBCs) leading to severe disease in individuals with blood groups, A, B and AB than those of blood group O, (Rowe *et al.*, 1995; Wahlgren, 2015).

One such study from Ghana among 293 children of less than 6yrs old attending Korle-Bu Teaching Hospital in Accra, Ghana, a case-control approach was used to determine the relationship between malaria severity and blood group type. In this study, blood group O' was present in about 16.1% of complicated malaria cases compared to 40.9% of uncomplicated controls. Individuals with complicated malaria were about twice more likely to be of blood groups A and B compared to group O persons suggesting that individuals with blood group O' had a significant advantage over other blood groups in regard to malaria infection and severity (Afoakwah *et al.*, 2016).

Another cross-sectional study conducted in Northwestern Ethiopia, Felegeselam Health Center in 2011 had also indicated that persons possessing red blood cell surface antigens that give rise to phenotypes of blood group A, B and AB were more at risk of developing severe malaria infection as compared to other individuals of blood group O. The cross-sectional study aimed at assessing the association between severe malaria and ABO blood among patients showing signs of malaria sampled a total of 398 patients with febrile signs. Malaria test was done by microscopy and their blood groups determined using monoclonal A and B grouping anti-sera, (Tadesse and Tadesse, 2013). Additionally, a cross-sectional study that was done in Dore Baafano Health Center, southern Ethiopia, in the period of December, 2010 to February, 2011 to examine the association between ABO blood group and *Plasmodium falciparum* malaria in 1,065 malaria suspected febrile cases also found that individuals with blood group A 'were more susceptible of developing anemia compared to persons of O' blood group and non-A phenotypes among *Plasmodium falciparum* malaria patients (Degarege *et al.*, 2012).

Elsewhere, a more controlled cross-sectional study was done earlier in Mali involving 567 children rosette frequencies were found to be significantly lowered in parasite isolates from patients with blood group O' compared with isolates from patients with groups A, B, and AB.

In addition, they also noticed that there was more rosetting in patients of blood groups A' B' and AB having severe malaria as opposed to patients of blood group O' having severe malaria (Rowe *et al.*, 2007).

In a cross-sectional study that was done in Kenya in 2017 involving 154 children diagnosed with malaria to determine the relation between formation of rosettes in vitro and malaria severity, blood groups for all the children included in the study were examined. It was found that isolates from blood group O' patients had a median rosette frequency of 2% (range 0 to 45%) as compared to those from blood group A' (median, 7%; range 0 to 82%; Mann-Whitney U test, $P < 0.01$) or group AB (median, 11%; range 0 to 94%; Mann-Whitney U test, $P < 0.03$). Rosetting was more stronger in blood group AB' followed by B' then A' but least in blood group O, suggesting that rosetting is influenced by ABO blood group type and associated with severe malaria (Rowe *et al.*, 1995).

In Uganda, there is limited information regarding the influence of ABO blood group types on progression and severity of malaria.

CHAPTER THREE; METHODOLOGY

3.0 Introduction

This chapter contains the systematic processes that explain how the study was conducted; it describes the study design, target population, data sources and how the consent was obtained among other things.

3.1 Study design

This was a cross-sectional prospective study that was carried out during the months of July - August, 2018. This study was aimed at determining the distribution of ABO blood group types and their association with malaria among children less than 5 years, at that specific point in time; as such a cross-sectional study design was appropriate in determining the variables under consideration in the study population.

3.2 Study Area

The study was conducted in Bundibugyo hospital. Bundibugyo hospital is located in Bundibugyo town council in Bundibugyo district. Geographically, Bundibugyo district is located in the slopes of the western part of Mount Ruwenzori, approximately 380km from Kampala city the Capital of Uganda. It sits at an altitude of 900 metres (3,000 ft.) and has an Area of 848 km² with a density of 285.2/km². The estimated population of Bundibugyo district is about 224,387 people and has a population growth rate of +2.68% per year (Uganda Bureau of Statistics, 2017). Malaria transmission is this area is meso-endemic with transmission peaks seen following the major rains, which normally occur between April to June and September to December.

Bundibugyo Hospital is government facility with 100 bed capacity. The hospital provides both preventive and curatives services notably; antenatal care (ANC), immunization, theatre operations, management of malaria, HIV/AIDS, TB and other illnesses including specialized departments like laboratory testing and diabetic/sickle cell clinics. The hospital acts as a referral for all health 31 health units in the district and also serves patients from the neighboring DRC Congo since it's a border district. Of the 14,808 fever cases that were tested for malaria in 2016/2017, a total of 5,627 (38%) were confirmed malaria cases; 40% of which occurred in children less than five years (Bundibugyo Hospital biostatistics records). Between January to April 2018, laboratory records indicated an average of 83 suspected malaria cases tested per day of which 47% were children under five years of age.

3.3 Study population

The study population comprised of children 7 months-5 years presenting with a febrile condition.

3.4 Inclusion and Exclusion criteria

I. Inclusion criteria

Only Children aged 7months to 5years with febrile conditions and written informed consent from parent or guardian were included.

II. Exclusion criteria

The following are among the category of Children that were excluded from the study;

- Children aged 7 months to 5years with febrile conditions but had taken some antimalarial drug prior to the time of selection to the study
- Children with known history of chronic conditions such as HIV/AIDS, sickle cell disease, Diabetics and others

- Severely malnourished children (below −3z scores of the median WHO growth standards).

3.5 Data sources

Primary data sources of the study included the patient's bio-data that was collected from the child's parent or guardian and result from the clinical and laboratory examinations carried out by the; Doctors, Clinical Officers, and the Laboratory staff (Researcher, Research Assistant and External Quality Officer).

3.6 Sample size determination

The formulae of Kish and Leslie (1965) were used to determine the sample size for this study as shown below.

$$n = \frac{Z^2 PQ}{d^2}$$

Where;-

n = sample size

Z is the confidence level of 95% (1.96).

P= the estimated proportion of an attribute that is present in the population

d, is the margin of error at 5% (standard of +/- 0.05)

Q = (1-P)

Therefore;-

Z = 1.96, P = Estimated prevalence of malaria in Uganda of 19% (WHO, 2017)

Calculation

n = 1.96 x1.96 x 0.19 (1-0.19) / 0.05x0.05

= 3.8416 x 0.19 x 0.81 / 0.0025

= 0.5912222 / 0.0025

= **236.5**

Hence a minimum of 237 children will be included in the study

3.7 Sampling Technique

The participants were selected by consecutive sampling or total enumerative sampling where by every participant that met the inclusion criteria was picked until the required sample size was obtained

3.8 Study variables

The dependent variable in the study was malaria

The independent variable in the study was ABO blood group type.

3.9 Data Collection tools and method

Quantitative data was collected from the participants through clinical examination and laboratory investigations.

3.10 Clinical Examination and Treatment of Malaria

Clinical investigations on eligible participants were done by Clinicians and it involved physical examination, requisition for Laboratory tests and eventual diagnosis, drug prescription for malaria. For each eligible child, bio data including the child's socio-demographic information was recorded using the study data collection form. Each child was

examined for fever (tympanic temperature of ≥37.5°C) or history of recent fever (within the last 24 hours).

Malaria was determined as specified by the WHO criteria. Accordingly; uncomplicated malaria was determined as having any *P. falciparum* parasitemia plus fever or a history of fever within the past 24 hours (WHO, 2006). While severe malaria was identified by having hyper parasitemia (>5 % parasitized erythrocytes or >250,000 parasites/µl) and the presence of any one or more of the following danger signs including a core body temperature>40 °C, severe anemia (hemoglobin<5 g/dl), hyperbilirubinemia (total bilirubin >2.5 mg/dl), prostration or weakness, impaired consciousness, respiratory distress, hypoglycemia (blood sugar <40 mg/dl), acidosis or cerebral malaria with no other diagnosed cause of the illness (WHO, 2006; WHO, 2000b). Any severe case identified was managed with a matter of urgency

3.11 Laboratory Blood sample collection and Microscopy

Ethylene diamine-tetracetic acid (EDTA) anticoagulated blood was used for subsequent laboratory investigations including Rapid malaria test, microscopy and blood grouping. Thick and thin blood smears stained with Giemsa, Parasite densities were determined by microscopic examination of thick films using x100 oil immersion objective through counting the number of asexual parasites per 200 white blood cells (WBC) in high parasitemia or up to 500 WBC in low parasitemia and assumption of an absolute WBC count of 8,000/ µL of blood as shown in the formula below (WHO, 1998).

$$\text{Parasite density/ µL of blood} = \frac{\text{Number of parasites counted in a thick smear}}{\text{200 WBC}} \text{ X 8000 WBC absolute count}$$

(Refer to the Appendixes; VI, VII, VIII, IX)

3.12 ABO Blood grouping

Blood groups of the study children were determined using the direct hemagglutination test; agglutinating A and B monoclonal anti-sera as described by (Rowe, 2007). Approximately 20µL of EDTA blood was applied onto a clean white blood grouping tile using micropipette and mixed with an equal amount of antisera A, B and Rhesus (D) using the applicator sticks. The mixture was racked for an average of 2-3minutes under room temperature, any possible hemagglutination reaction was observed and the results recorded the patient lab result form

3.13 Quality control and quality assurance

Participant's biodata and clinical information written in the laboratory request form were verified by the research assistant before the samples are collected. All blood smears for malaria and ABO blood group results were read microscopically by two competent laboratory personnel (Bundibugyo Hospital Laboratory was undergoing accreditation process supported by Ministry of Health and the standard operation procedures prescribed that; Laboratory staff performing examination have to undertake competency assessment on every test procedure yearly. The Senior Laboratory Technologist from Infectious Disease Institute(Mr. Musinguzi Moses) incharge Malaria External Quality Assurance for Rwenzori Region2018reviewed and re-examined the discordant results plus 10% of all the study samples as a measure of ensuring quality of the Laboratory testing.

The data collected was reviewed and analyzed by the Principal researcher, Research assistant, Study Supervisor and two other data experts to ensure quality and validity. The research supervisor randomly sampled some data points to verify the reliability of the data analyzed.

3.14 Data presentation and Analysis

i. Data analysis

Data was cleaned, coded and entered into STATA 13 software. All descriptive statistical, bivariate and multivariate analysis was carried out using SPSS 23.0 statistical software.

ii. Data presentation

The descriptive statistics, frequencies and means from the collected data was calculated and presented in the form of frequency tables, bar graphs or pie charts.

The relationship between dependent and independent variable was determined in a bivariate analysis, odds ratios and their corresponding confidence intervals were calculated. The effect of the cofounding variables was controlled for in the multivariate regression analysis. All statistical tests were two tailed and P-value less than 0.05 were considered significant.

3.15 Ethical consideration

The research proposal and study protocols were presented for approval by the CIU research and ethics committee. Permission to conduct the study was sought from IAHS, CIU and Bundibugyo district Health Office (DHO) / Hospital authority.

The staff that supported the study among them; Clinicians, Nurses and Lab staff / Research assistant underwent introductory orientation training on how the study would be conducted. The informed consent process was administered to all presumptive participants (parents/guardians of the children) by the Research assistant, Clinician, Chief Researcher and (parents/guardians) was requested to sign or write a name on the consent form indicating acceptance of voluntary participation in the study.

3.16 Information dissemination

The data analyzed was then submitted to Clarke International University for marking

CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter gives a detailed outcome of the findings after data analysis

4.1 Demographic and Clinical characteristics of the study participants

A total of 237 children aged (0.58 – 5years) were recruited in the study. Majority (46%) was between 0.58 - 1years, followed by those aged 2 - 3years (32.5%), and the least proportion was those of 4 - 5years (21.5%). By gender, males were the majority at 54 % while females constituted 46 % only. The mean weight for the participants was 10.9 ±2.6.

Out of 237 participants recruited in the study, 92.0%(218) had history of fever in the past 24hours as compared to 8% (19) that did not have the history fever in the past 24 hours. Study further found out that 76.8 %(182) presented with fever at the time of selection while 23.2(%) did not have fever at the time of selection.

Other clinical presentation included respiratory distress of which 8.4% (20) of participants presented with it.

For Nutritional status; all the participants selected were measured and only those above 12.6cm MUAC as per the WHO minimum cut off value for normal growth in children under 5yrs were included in the study. Details of the clinical and demographic characteristics of the study children are given in the Table 1 below.

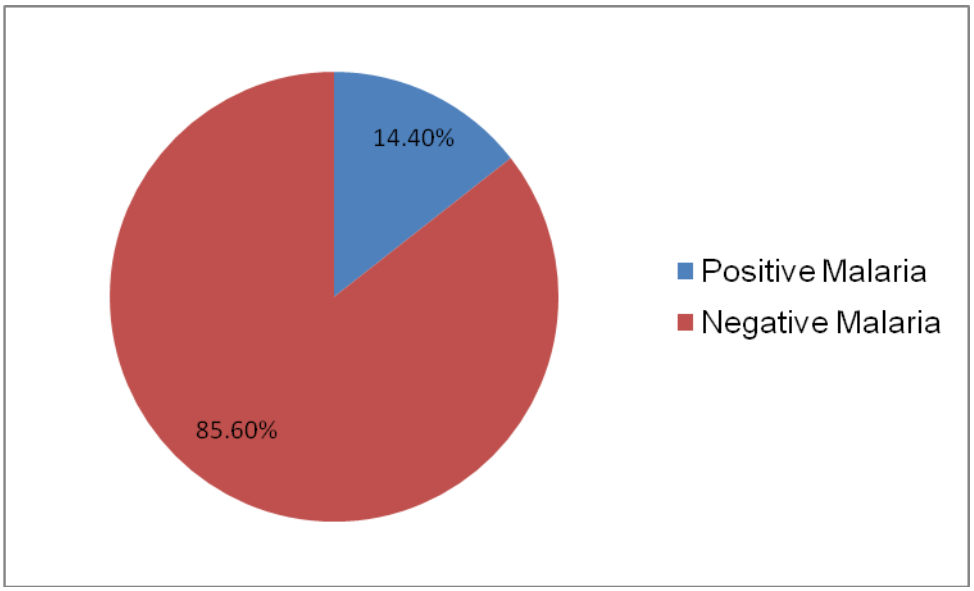
Table 1: Demographic and Clinical characteristics of the study participants

	Range	Frequency	Percentage (%)	Mean	SD (±)
AGE (years)	0.58 – 1yr	109	46.0	1.8	0.78
	2 – 3yrs	77	32.5		
	4 – 5yrs	51	21.5		
SEX	MALE	128	54.0		
	FEMALE	109	46.0		
WEIGHT (Kg)				10.9	2.6
FEVER IN PAST 24HRS	YES	218	92.0		
	NO	19	8.0		
FEVER AT TIME OF TEST	YES	182	76.8		
	NO	55	23.2		
RESPIRATORY DISTRESSES	YES	20	8.4		
	NO	217	91.6		
NUTRITIONAL STATUS	All the participants were above 12.6cm MUAC(inclusion criteria)				

4.2 Prevalence of malaria among children aged (0.58 to 5years)attending the outpatient's clinic at Bundibugyo hospital

Out of 237 participants enrolled in the study, only 14.4% (34) had Clinical and Laboratory confirmed malaria while 85.6% (203) that tested negative (Figure 1). Only 20.5% (07) out of the total positives satisfied the World Health Organization definition of severe malaria. All the positive malaria cases were of *Plasmodium* falciparum species as showed in the figure below.

Figure 2: Malaria prevalence in children aged (0.58 to 5years) at Bundibugyo Hospital

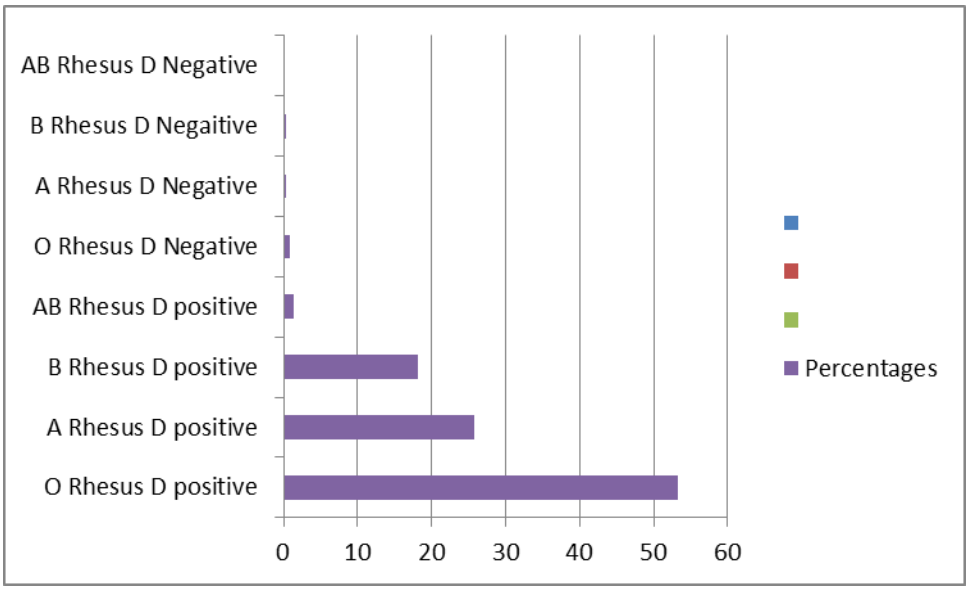


4.3 Distribution of blood groups among the study participants

The ABO blood group distribution among the study population showed that, Blood group O Rhesus (D) positive were the majority (126) 53.3%, this was closely followed by blood group A Rhesus (D) positive at (61) 25.7%. Blood group B Rhesus (D) positive was (43) 18.1% and blood group AB Rhesus (D) positive was (03) 1.3%.

The Rhesus negative blood group were very few; O Rhesus (D) Negative were (02) 0.8%, Blood group A Rhesus (D) negative were (01) 0.4% and blood group B Rhesus (D) negative were (01) 0.4%. There were no blood group AB Rhesus (D) negative participants found.

Bar graph: below shows the ABO blood group percentage distribution among study population for children under five years in Bundibugyo Hospital



4.4Association between ABO blood group types and malaria in children less than five years

Table 2: Malaria status and ABO blood group Crude values (Bivariate)

Variable	Malaria		
Sex	**Positive**	**Negative**	**p-value**
Male	15(44.1)	113(55.7)	.211
Female	19(55.9)	90(44.3)	
Age group			
0-18 Months	7(20.6)	79(38.9)	.098
19-36 Months	14(41.2)	72(35.5)	
>37 Months	13(38.2)	52(25.6)	
ABO Blood group			
O	17(50.0)	111(54.7)	.003
A	10(29.4)	52(25.6)	
B	7(20.6)	37(18.2)	
AB	0(0.0)	3(1.5)	
Fever -24 hours			
Yes	33(97.1)	185(91.1)	.002
No	1(2.9)	18(8.9)	
Fever at test			
Yes	30(88.2)	152(74.9)	.001
No	4(11.8)	51(25.1)	
Temperature			
Normal (<36.3^{o}C)	11(32.4)	131(64.5)	.000
High (>36.4^{o}C	23(67.6)	72(35.5)	
BMI index			
<18.5Kg/M^{2}	30(88.2)	163(80.3)	.252
18.6-24.9Kg/M^{2}	3(8.8)	38(18.7)	
25.0-29.9Kg/M^{2}	1(2.9)	2(1.0)	
MUAC			
Mild-moderate	0(0.0)	1(.5)	.751
Normal	4(11.8)	17(8.4)	
Over weight	30(88.2)	185(91.1)	

Table 3: Relationship between ABO blood group and Malaria

ABO Blood group	**Malaria Pos**	**Malaria Neg**	**Adjusted OR**	**95%C.I. OR**	**P-Value**
A	10(29.4)	52(25.6)	1.24	1.19 (1.01 – 2.48)	0.003
O	17(50.0)	111(54.7)	0.79 (Ref)		
B	7(20.6)	37(18.2)	1.29	1.17 (0.88 – 3.23)	0.011
O	17(50.0)	111(54.7)	0.81 (Ref)		

From a multivariate logistic regression analyses that controlled for other independent predictors of malaria susceptibility, participants of blood group A were 1.24 times more susceptible to malaria than those with blood group O (OR= 1.24, 95%C.I (1.01 – 2.48) ; P-value =0.003. Also, children carrying blood group B were 1.29 times more susceptible to malaria treated than those with blood group O blood (OR= 1.29, 95% C.I (0.88 – 3.23); P-Value = 0.011). The outcome statistically indicated that: blood groups A and B patients are more likely to have a positive malaria test compared to patients with blood group O.

Since we had only very few of children with severe malaria, we could not evaluate the association between ABO groups and malaria severity.

CHAPTER FIVE: DISCUSSION

5. 0: Introduction

This chapter discusses the outcome of the study results in details

5.1: Prevalence of Malaria for children aged 7 months to 5 years

In this study, the prevalence of Malaria among children under years in Bundibugyo Hospital was found to be at 14 %. Whereas in 2015, Uganda Ministry of Health reported that the Hospitals outpatients' clinic had 34% prevalence and in general the malaria prevalence in the whole country had dropped from 40% to 19% by 2017. According to the district health survey (DHIS and MOH-Uganda) data that was presented in 2016/2017, Bundibugyo district recorded a total of 5,627 (38%) confirmed malaria cases; 40% of which occurred in children less than five years. The possible explanation for this varying prevalence could be that the seasons of data collection may have differed. The present study was done during July and August before the peak of malaria transmission in Bundibugyo district, and this could explain the low prevalence of malaria that was found in the study population.

A multicentre study that was conducted in three sub-Saharan Africa countries; Democratic Republic of Congo, Uganda and Kenya aimed at estimating the true prevalence of malaria in children under five using a Bayesian modeling framework found out that overall Uganda had the highest malaria prevalence in children less than five years at 22%, followed by Democratic Republic of Congo at 20% and Kenya at 1% (Mfueni *et al.*, 2018). Despite the fact that this Bundibugyo hospital research had study population of 237 participants only, the outcome is consistent with the previous Ugandan studies which show that the prevalence of Malaria disease is generally on the decline.

The study also found out that, 100% of the positive malaria cases were of Plasmodium

falciparum and this has not differed much with the earlier cross-sectional "Beyond Garki project baseline survey" that was conducted in Ethiopia and Uganda in 2012 with the aim of monitoring the changes in malaria epidemiology and show how much impact the interventions had achieved. In Uganda the sites studied were Aduku in Apac district and Butemba in Kyakwanzi and they all showed that *Plasmodium falciparum* was the most predominant species at 95% (Abeku *et al.*, 2015)

The study results has shown that the prevalence of Malaria in Bundibugyo and Uganda is generally dropping down and this is a sign of positive score for the interventions such as test and treat, use of treated mosquito nets, malaria prophylaxis medicines and others that were put by MOH and other stakeholders. This therefore calls upon the stakeholders continue investing in the same methods of malaria control and prevention since the impact is visibly seen.

The study outcome has also shown that the most predominant species of Plasmodium in Bundibugyo and Uganda is *Plasmodium* falciparum; having this information is very important tool to the MOH and other stakeholders responsible for procurement of malaria test kits in Uganda and treatment should continuously be focused on *Falciparum* malaria.

5.2: Distribution of ABO blood group types among children under 5years attending outpatient's clinic at Bundibugyo hospital

The study found out that blood group; O Rhesus (D) positive was the most predominant in Bundibugyo at 54% followed by blood group; A Rhesus(D) positive at 26.2%, B Rhesus(D) positive at 18.6% and AB Rhesus (D) positive being the least at 1.3%. The people without Rhesus (D) antigen were very few (1.7%) only.

In comparison; One prospective cross-sectional study involving 23,504 blood donors of average age of 21yrs mostly males was conducted in southwestern Uganda in 2016 with the main objective of determining the ABO blood group distribution among the blood donors in the blood bank. The study results indicated that blood group O (50.3%) was more predominant followed by A' (24.6%); B' (20.7%) and blood group AB (4.5%) the least (Apecu *et al.*, 2016). The two independent outcomes are not far different from one another.

In the horn of Africa (Ethiopia), a retrospective cross-sectional study was conducted in 2016 at Jimma Town blood bank to determine the distribution of ABO blood group system and Rhesus factor. A total of 6,922 participants had been screened and their blood group types determined with up to 76.6% consisting of males and 23.4% females. The study results indicated that blood group type O' was more predominant representing up to 43.1% followed closely by type A ' (31.9%), then B' (21.5%) and the least being blood group type AB''at 3.5% (Zerihun and Bekele, 2016). The rationale in the ABO blood groups across Africa remains the same showing blood group "O" as more predominant over others.

Different versions of Genes (alleles) determine specific traits do not appear in equal frequency in the gene pool, this means that a person expressing AA or AO will appear as A because "O" is recessive, a person expressing BB or BO will appear as B, AB will appear as AB. Therefore in a gene pool, there are more O alleles hiding as recessive as compared to AA or BB; this therefore increases the chances of OO pairing hence resulting to predominance of Blood group O in the population.

Also number of studies has shown that blood group O plays a significance defense against Malaria infection and severity, the mechanism of the protection involves the lack of a surface tri-saccharide, which acts as a receptor in the rosetting process, a key event in the pathogenesis of severe malaria. On the other hand, blood groups A and B express the surface

tri-saccharides; A (which is structurally made up of GalNAcα1-3 (Fucα1-2) Gal1β1) and B (comprised of Gal1α1-3 (Fucα1-2) Galβ1) respectively, which are found attached to surface glycoproteins and glycolipids (Rowe et al., 1995). The presence of these surface molecules is believed to be important in the mediation of rosetting in infected red blood cells (RBCs) leading to severe disease in individuals with blood groups, A, B and AB than those of blood group O (Rowe *et al.*, 1995; Wahlgren, 2015). It is thus possible that the blood group O could maintained in malaria endemic regions as a result of natural selection since individuals with this blood are shown to be protected from malaria.

5.3: Association between ABO blood group and Malaria

In this study, participants of blood group A were 1.24 times more susceptible to malaria treated than those with blood group O (OR= 1.24, 95%C.I (1.01 – 2.48) ; P-value =0.003. Also, children carrying blood group B were 1.29 times more susceptible to malaria treated than those with blood group O blood (OR= 1.29, 95% C.I (0.88 – 3.23); P-Value = 0.011). The outcome statistically indicated that: blood groups A and B patients are more likely to have a positive malaria test compared to patients with blood group O. whereas the number of severe malaria cases in this study were very few and did not give a significant statistical information, it's important to note that, the positive malaria test eventually progresses to severity if not managed with urgency.

A number of studies were done by different scholars (Rowe *et al.*, 1995; Wahlgren, 2015), and they suggested that, blood group O offered protection against malaria severity by the mechanism of demonstrated lack of a surface tri-saccharide, which acts as a receptor in the rosetting process, a key event in the pathogenesis of severe malaria. On the other hand, blood groups A and B express the surface tri-saccharides; A (which is structurally made up of

GalNAcα1-3 (Fucα1-2) Gal1β1) and B (comprised of Gal1α1-3 (Fucα1-2) Galβ1) respectively, which are found attached to surface glycoproteins and glycolipids (Rowe et al., 1995). The presence of these surface molecules is believed to be important in the mediation of rosetting in infected red blood cells (RBCs) leading to severe disease in individuals with blood groups, A, B and AB than those of blood group O, (Rowe *et al.*, 1995; Wahlgren, 2015).

One such a cross-sectional study was conducted in Kenya in 2017 involving 154 children diagnosed with malaria to determine the relation between formation of rosettes in vitro and malaria severity, blood groups for all the children included in the study were examined. It was found that isolates from blood group O' patients had a median rosette frequency of 2% (range 0 to 45%) as compared to those from blood group A' (median, 7%; range 0 to 82%; Mann-Whitney U test, $P < 0.01$) or group AB (median, 11%; range 0 to 94%; Mann-Whitney U test, $P < 0.03$). Rosetting was more stronger in blood group AB' followed by B' then A' but least in blood group O, suggesting that rosetting is influenced by ABO blood group type and associated with severe malaria (Rowe *et al.*, 1995).

Another such study was done in Ghana and it comprised of 293 children of less than 6yrs attending Korle-Bu Teaching Hospital in Accra. A case-control approach was used to determine the relationship between malaria severity and blood group type. In this study, blood group O' was present in about 16.1% of complicated malaria cases compared to 40.9% of uncomplicated controls. Individuals with complicated malaria were about twice more likely to be of blood groups A and B compared to group O persons suggesting that individuals with blood group O' had a significant advantage over other blood groups in regard to malaria infection and severity (Afoakwah *et al.*, 2016).

In relation to the explanations of different studies as stated above; the outcome of these Bundibugyo cross sectional study can thefore conclude that, there is significant association between ABO blood group and Malaria

5.4: Limitations of the study

The study was conducted in the months of July to Aug, the Malaria transmission is this area is meso-endemic with transmission peaks seen following the major rains, which normally occur between April to June and September to December. So it's possible that the study did not coincide with the pick of malaria transmission hence leading to the reduced malaria prevalence.

This study was limited to Bundibugyo Hospital, thus it may not be generalizable to other geographical settings of Uganda.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.0 Conclusion

We conclude that, there is an association between ABO blood group and Malaria.

6.1 Recommendations

We recommend that while managing malaria cases, client's blood groups should also be considered so as to identify vulnerable cases that need special care based on their blood group. And the measures such as malaria test and treat policy in Uganda, use of infected mosquito nets and use of malaria prophylaxis therapy that seem to be effective in controlling and preventing malaria disease should continue.

6.2 Further studies

More studies can be done in a different geographical setting to further understand the association between ABO blood group and Malaria

REFERENCES

Abeku, T. A. *et al.* (2015) 'Monitoring changes in malaria epidemiology and effectiveness of interventions in Ethiopia and Uganda: Beyond Garki Project baseline survey', *Malaria Journal*, 14, p. 337. doi: 10.1186/s12936-015-0852-7.

Afoakwah, R. *et al.* (2016) 'Relative Susceptibilities of ABO Blood Groups to Plasmodium falciparum Malaria in Ghana', *Advances in Hematology*, 2016, pp. 1–4. doi: 10.1155/2016/5368793.

Akimbo, F. O. *et al.* (2009) 'Prevalence of Malaria and Anemia Among Young Children in a Tertiary Hospital in Benin City, Edo State, Nigeria', *Fooyin Journal of Health Sciences*, 1(2), pp. 81–84. doi: 10.1016/S1877-8607(10)60004-5.

Apecu, R. O. *et al.* (2016) 'ABO and Rhesus (D) blood group distribution among blood donors in rural south western Uganda: a retrospective study', *BMC Research Notes*. BioMed Central, 9(1), pp. 1–4. doi: 10.1186/s13104-016-2299-5.

Degarege, A. *et al.* (2012) 'Association of ABO blood group and P. falciparum malaria related outcomes: a cross-sectional study in Ethiopia.', *Acta tropica*, 123(3), pp. 164–9. doi: 10.1016/j.actatropica.2012.04.012.

Doolan, D. L., Dobano, C. andBaird, J. K. (2009) 'Acquired immunity to malaria.', *Clinical microbiology reviews*, 22(1), p. 13–36, Table of Contents. doi: 10.1128/CMR.00025-08.

Van Eijk, A. M. *et al.* (2015) 'Prevalence of malaria infection in pregnant women compared

with children for tracking malaria transmission in sub-Saharan Africa: a systematic review and meta-analysis.', *The Lancet. Global health*, 3(10), pp. e617-28. doi: 10.1016/S2214-109X(15)00049-2.

Fatrouz, I. N. E., Farida, N. andIrshad, A. H. (2012) 'Relation between fingerprints and different blood groups.', *Journal of forensic and legal medicine*, 19(1), pp. 18–21. doi: 10.1016/j.jflm.2011.09.004.

Franchini, G. M. (2013). Beyond immunohaematology: the role of the ABO blood group in human diseases. *Blood Transfusion* , 2013 Oct; 11(4): 491–499.

Garg, P. *et al.* (2014) 'Prevalance of ABO and Rhesus Blood Groups in Blood Donors: A Study from a Tertiary Care Teaching Hospital of Kumaon Region of Uttarakhand', *Journal of clinical and diagnostic research: JCDR*, 8(12), pp. FC16-19. doi: 10.7860/JCDR/2014/9794.5355.

Haji, Y., Fogarty, A. W.andAND Deressa, W. (2016) 'Prevalence and associated factors of malaria among febrile children in Ethiopia: A cross-sectional health facility-based study', *Acta Tropica*, 155, pp. 63–70. doi: 10.1016/j.actatropica.2015.12.009.

Hamed, C. T. *et al.* (2012) 'Frequencies and ethnic distribution of ABO and Rh(D) blood groups in Mauritania: results of first nationwide study: Frequencies and ethnic distribution of ABO and Rh(D) in Mauritania', *International Journal of Immunogenetics*, 39(2), pp. 151–154. doi: 10.1111/j.1744-313X.2011.01064.x.

Jain, V. *et al.* (2014) 'Burden of Complicated Malaria in a Densely Forested Bastar Region of

Chhattisgarh State (Central India)', *PLoS ONE*. Edited by L. H. Carvalho, 9(12), p. e115266. doi: 10.1371/journal.pone.0115266.

Kiggundu, V. L. *et al.* (2013) 'High prevalence of malaria parasitemia and anemia among hospitalized children in Rakai, Uganda', *PloS One*, 8(12), p. e82455. doi: 10.1371/journal.pone.0082455.

Laishram, D. D. *et al.* (2012) 'The complexities of malaria disease manifestations with a focus on asymptomatic malaria', *Malaria Journal*, 11, pp. 1–15. doi: 10.1186/1475-2875-11-29.

Lekweiry, K. M. *et al.* (2011) 'Malaria prevalence and morbidity among children reporting at health facilities in Nouakchott, Mauritania', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 105(12), pp. 727–733. doi: 10.1016/j.trstmh.2011.09.004.

Liu, J. *et al.* (2017) 'Frequencies and ethnic distribution of ABO and RhD blood groups in China: a population-based cross-sectional study', *BMJ Open*, 7(12), p. e018476. doi: 10.1136/bmjopen-2017-018476.

Luise, D. *et al.* (2017) 'Comparing imported malaria in adults and children presenting to an Italian teaching hospital: A 10-year retrospective study', *Travel Medicine and Infectious Disease*, 17, pp. 56–61. doi: 10.1016/j.tmaid.2017.04.008.

Menon, M. P., Yoon, S. S. AND GROUP, U. M. I. S. T. W. (2015) 'Prevalence and Factors Associated with Anemia Among Children Under 5 Years of Age--Uganda, 2009', *The American Journal of Tropical Medicine and Hygiene*, 93(3), pp. 521–526. doi:

10.4269/ajtmh.15-0102.

Mfueni, E. *et al.* (2018) 'True malaria prevalence in children under five: Bayesian estimation using data of malaria household surveys from three sub-Saharan countries', *Malaria Journal*, 17(1). doi: 10.1186/s12936-018-2211-y.

Ndoula, S. T. *et al.* (2014) 'Phenotypic and allelic distribution of the ABO and Rhesus (D) blood groups in the Cameroonian population', *International Journal of Immunogenetics*, 41(3), pp. 206–210. doi: 10.1111/iji.12114.

Panda, A. K. *et al.* (2011) 'Association of ABO blood group with severe falciparum malaria in adults: case control study and meta-analysis.', *Malaria journal*, 10, p. 309. doi: 10.1186/1475-2875-10-309.

Pathirana, S. L. *et al.* (2005) 'ABO-blood-group types and protection against severe, Plasmodium falciparum malaria', *Annals of Tropical Medicine and Parasitology*, 99(2), pp. 119–124. doi: 10.1179/136485905X19946.

Piel, F. B. *et al.* (2010) 'Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis.', *Nature communications*, 1, p. 104. doi: 10.1038/ncomms1104.

Rowe, A. *et al.* (1995) 'Plasmodium falciparum rosetting is associated with malaria severity in Kenya', *Infection and Immunity*, 63(6), pp. 2323–2326. doi: 10.1086/373994.

Rowe, J. A. *et al.* (2007) 'Blood group O protects against severe Plasmodium falciparum

malaria through the mechanism of reduced rosetting.', *Proceedings of the National Academy of Sciences of the United States of America*, 104(44), pp. 17471–6. doi: 10.1073/pnas.0705390104.

Sánchez-Boiso, A. *et al.* (2011) 'Genetic structure of three Native Mexican communities based on mtDNA haplogroups, and ABO and Rh blood group systems', *Revista De Investigacion Clinica; Organo Del Hospital De Enfermedades De La Nutricion*, 63(6), pp. 614–620. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23650674.

Sonko, S. T. *et al.* (2014) 'Does socio-economic status explain the differentials in malaria parasite prevalence? Evidence from The Gambia', *Malaria Journal*, 13(1), p. 449. doi: 10.1186/1475-2875-13-449.

Tadesse, H. andTadesse, K. (2013) 'Assessing the association of severe malaria infection and ABO blood groups in northwestern Ethiopia', *Journal of Vector Borne Diseases*, 50(4), pp. 292–296. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24499852.

Wahlgren, M. (2015) *How blood group O protects against malaria*, *Karolinska Institutet*. Available at: https://www.sciencedaily.com/releases/2015/03/150309124113.htm (Accessed: 15 May 2018).

Wanzira, H. *et al.* (2017) 'Factors associated with malaria parasitaemia among children under 5 years in Uganda: a secondary data analysis of the 2014 Malaria Indicator Survey dataset.', *Malaria journal*, 16(1), p. 191. doi: 10.1186/s12936-017-1847-3.

Willilo, R. A. *et al.* (2016) 'Pregnant women and infants as sentinel populations to monitor

prevalence of malaria: results of pilot study in Lake Zone of Tanzania', *Malaria Journal*, 15(1). doi: 10.1186/s12936-016-1441-0.

WORLD HEALTH ORGANIZATION (2017) *World Malaria Report 2017*, *World Malaria Report 2017*. doi: 10.1071/EC12504.

Zerihun, T., Degarege, A. and Erko, B. (2011) 'Association of ABO blood group and Plasmodium falciparum malaria in Dore Bafeno Area, Southern Ethiopia', *Asian Pacific Journal of Tropical Biomedicine*, 1(4), pp. 289–294. doi: 10.1016/S2221-1691(11)60045-2.

APPENDICES

APPENDIX I: PARTICIPANT CONSENT FORM

Informed Consent to Participate in Research

We are asking you to take part in a research study called:

"Distribution of ABO Blood Group types and their Association with Malaria among Children under 5 years attending Outpatients Clinic at Bundibugyo Hospital, Western Uganda"

The person who is in charge of this research study is Mr. Ejoku Emmanuel. The research will be conducted here at Bundibugyo Hospital in Bundibugyo District - Uganda.

Purpose of the study

The purpose of this study is to investigate any association between the ABO blood group and the malaria disease among Children under 5 years. The outcome of the study may be used by the policy makers to help in the proper management of malaria among vulnerable category of people.

Study Procedures

You are being asked to participate in this study, as you are a Ugandan who may have had an encounter with clinical malaria at one point in life.

If you take part in this study, you will be asked to:

Take part in a one-time, one-on-one, semi-structured interview

The interview will take approximately 10 minutes

The interview will take place at a location most convenient to you as the participant

The interview will be transcribed, in the form of field notes, to ensure accuracy in reporting your statements

After the interview, you will be requested to provide a blood sample equivalent to 2ml, the

sampling will be done by the normal Hospital blood sampling procedures

Benefits

There are no financial/material benefits associated with your participation in the study, but the study team will provide you with your Malaria and ABO blood group type results and direct you where you can get further management of Malaria in the Hospital system.

Risks or Discomfort

This research is considered to be minimal risk. That means that the risks associated with this study are the same as what you face each day whenever you come for blood sampling for malaria diagnosis and ABO blood grouping. There are no known additional risks to those who take part in this study.

Compensation

No research participant will be compensated in this study

Privacy and Confidentiality

We will keep your study records private and confidential. Certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are:

The research team, including the Principal Investigator and those involved with the study.

I may publish what I have learnt from this study. If l do so, l will not include your name. l will not publish anything that would let people know who you are.

Voluntary Participation / Withdrawal

You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study. You are free to participate in this research or withdraw at any time. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in this study.

You can get the answers to your questions, concerns, or complaints

If you have any questions, or complaints about this study, or experience an adverse event or unanticipated problem, contact the researcher on (0782807386 Ejoku Emmanuel)

If you have questions about your rights as a participant in this study, general questions, or have complaints, or issues you want to discuss with someone outside the research, call the IHSU-REC Chairperson Dr. Samuel Kabwigu on 0779610100) & the executive secretary of UNCST on (0414 -705500) respectively.

Assessment of understanding

Please check which box best describes your assessment of understanding of the above informed consent document:

□ I have read the above informed consent document and understand the information provided to me regarding participation in the study and benefits and risks. I give consent to take part in the study and will sign the following page.

□ I have read the above informed consent document, but still have questions about the study; therefore I do not give yet give my full consent to take part in the study.

Signature or Thumb print of Person Taking Part in Study (Care taker of the child)

…… Date ……

Printed Name of Person Taking Part in Study (Care taker of the child)

……

Signature of Person Obtaining Informed Consent …….

Date ……

Printed Name of Person Obtaining Informed Consent ……....

APPENDIX II: DATA COLLECTION FORM

PATIENT ID #_____

Demographics of the patient

Age _____ Sex Male ☐ Female ☐

District of origin / residence _____

2. Anthropometry measurements of the patients.

Weight (kg) _____ Height (cm) _____ MUAC _____

BSA _____ BMI _____

3. Clinical Examination

History of fever in the last 24 hours ☐ No ☐

Presence of fever Yes ☐ No Temperature

Malaria present Yes ☐ No ☐

Uncomplicated malaria

Severe malaria (Tick which applies)

Cerebral malaria; Yes ☐

Hemoglobin level (g/dl) ☐

Blood glucose level (mmol) ☐

Any indication of Respiratory Distress; Yes ☐

Laboratory examination

Laboratory test information _____

Blood group _____

Malaria test by _____

MRDT

Malaria test by _____

Slide microscopy

Parasite density _____

Malaria species _____

Principal researcher; ----------

Signature ……. Date …….

Research Assistant Lab test/s examiner (Laboratory Technician) ----------

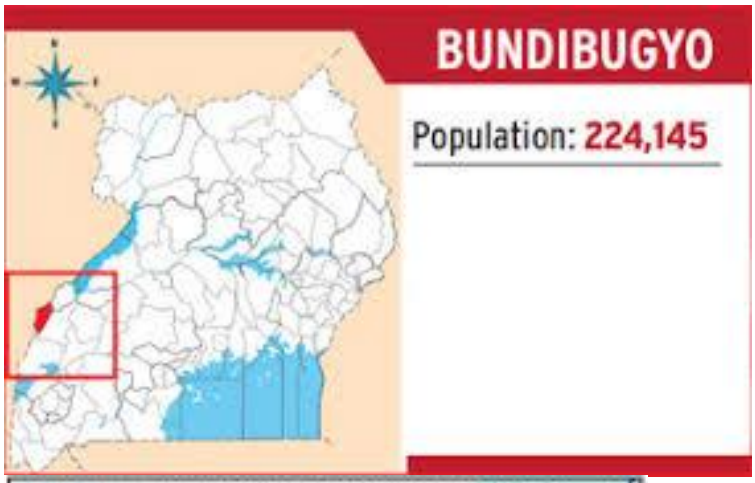
Signature ……. Date; …….

Data Evaluator (Medical Records Assistant) ----------

Signature ……. Date; ……

(Document source; Modification of the current Uganda MOH-OPD Pediatric HMIS 031)

APPENDIX III: MAP OF UGANDA AND BUNDIBUGYO



APPENDIX IV: INTRODUCTORY LETTER

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
sanitas per scientiam

making a difference to health care

Dean's Office-Institute of Allied Health Sciences

Kampala, Friday 23rd March 2018

TO THE MEDICAL SUPERINTENDENT
BUNDIBUGYO HOSPITAL
P.O. BOX 1148,
BUNDIBUGYO.

MEDICAL SUPERINTENDENT 03 JUL 2018 BUNDIBUGYO HOSPITAL

Permission granted. Get in touch with Lab I/c for help

Dear Sir/Madam,

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Ejoku Emmanuel** Reg. No. **2015-BMLS-PT-002** who is a student of our University. As part of the requirements for the award of a Bachelors Degree of Medical Laboratory Sciences of our University, the student is required to carry out research in partial fulfillment of his award.

His topic of research is: **Assessing the association between malaria and ABO Blood Group in children under five years in Bundibugyo Hospital.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to the student.

Sincerely Yours,

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
INSTITUTE OF ALLIED HEALTH SCIENCES
P. O. Box 7782, Kampala - Uganda

Dr. Okiria John Charles (PhD)
Associate Professor / Dean IAHS
(0772409126 / 0752409126)

The International Health Sciences University
P.O. Box 7782 Kampala - Uganda
(+256) 0312 307400 email: deanahs@ihsu.ac.ug / jokiria@ihsu.ac.ug
web: www.ihsu.ac.ug

APPNDIX V: CORRESPONDENCE LETTER

Medical Superintendent: 0772-453868
E-mail: amonbwambale@gmail.com
Administrator: 0772-358508
E-mail: francismdahura@gmail.com
Nursing Officer in-charge: 0782962135
Supplies Officer: 0787837371
Medical Social Worker:0782-487003

Bundibugyo General Hospital
Office of the Medical Superintendent

P.O Box 1148, Bundibugyo
Date: 5th Sept 2018
Our Ref:

TO; The Chairperson, Clarke International University Research and Ethics Committee (CIUREC)

Dear Sir/Madam

RE; MR. EJOKU EMMANUEL, RESEARCH TITTLED " DISTRIBUTION OF ABO BLOOD GROUP TYPES AND THEIR ASSOCIATION WITH MALARIA AMONG CHILDREN UNDER FIVE YEARS ATTENDING OUT PATIENTS CLINIC AT BUNDIBUGYO HOSPITAL, WESTERN UGANDA".

Mr. Ejoku Emmanuel reported to our office at Bundibugyo hospital on 8th July 2018, seeking for permission to conduct his research at the hospital using the hospital facilities; we granted him the opportunity, we supported him with all the necessary materials, human resource and participants he needed. As a hospital this research was very useful and important to us because we believe the outcome of the research will help inform the policy decisions on the management of malaria among children and other category of patients. He conducted his research between July and August 2018

We shall be grateful to receive the feedback of the research analysis

I wish him success in his academic field

Thankyou

MEDICAL SUPERINTENDENT
05 SEP 2018
BUNDIBUGYO HOSPITAL

Dr. Amon Bwambale
Medical Superintendent Bundibugyo Hospital
C.C: DHO Bundibugyo
C.C; Principal Nursing Officer- Bundibugyo Hospital
C.C; File

APPENDIX VI: STANDARD OPERATING PROCEDURES

Venous Blood collection equipment's and other requirements

All the equipment needed for the procedure were collected and placed within safe and easy to reach access and this included:

Sample tubes (EDTAtubes)

Gloves

A tourniquet

Butterfly needles and 2ml syringes

Alcohol swab (70% alcohol swabs for skin disinfection)

Gauze or cotton-wool ball that was applied to the puncture site;

Laboratory specimen labelling pencil

Laboratory forms

Biohazard leak-proof puncher resistant waste container for needles and syringes and the one for cotton swabs

The rack containing the sample tubes also placed closer

And a Laboratory Technician / Research assistant (Phlebotomist) for blood collection would get ready

Patient preparation would start by greeting the patient and verifying the names and the form by checking to ensure that it matched with the patient's identity.

The consent form would then be checked to be sure that it was consented

The patient attendant would be asked whether he/she has allergies, phobias or has ever fainted during previous injections or blood draws

The patients care taker and the child would then be reassured to create comfort

Any patient that refused later due to fear or any other concerns would be left/ not included in the study but given the usual patient care

The patient’s arm extended and antecubital fossa or forearm inspected to get the best vein

The tourniquet appliednot so tightly

The skin cleaned with antisepsis 70% absolute alcohol swab

Once the infant or child was immobilized, the skin was then punctured with a butterfly needle 3–5 mm distal to (i.e. away from) the vein, this allowed good access without pushing the vein away

If the needle entered alongside the vein rather than into it, the needle would then be removed slightly without removing it completely, and angle it into the vessel

Blood drowned slowly and steadily up to 2ml with the butterfly needle fixed to the vacutainer tube

Tourniquet released and the vacutainer detached from the butterfly “first “followed by removal of the needle from the vein and discarding immediately to the needle sharps container

Blood was then mixed in the vacutainer by inverting gently for 4 to 6 times to enable ETDA mix well with blood

The sample tube would then be labelled as per the provided procedures

APPENDIX VII: PROCEDURE OF MALARIA RAPID DIAGNOSTIC TEST (RDT)

RDT for malaria are strips coated with monoclonal antibody against malaria parasites antigen. Blood flows along the device and if the malaria parasites antigens are present in the sample, the antigen/antibody complex binds with the conjugate to form two lines. A control band is incorporated into the test to ensure validity

Specimen used included

Coagulated blood (EDTA)

Method

We would begin by checking the instructions in the test kit and check for expiry date (only non-expired kits were used

All requirements would be placed closer

We first put on protective gear

Labelle the cassette with patient identification (name or number)

Filled the capillary tube with blood up to its mark (approximately 20ul) or and sometimes we used the micropipette, pipetting 20ul of blood

Transferred blood into the sample well marked(S) on the cassette

Timed and Read results within a minimum of 15 minutes as (per manufacturer's instructions)

Documented /Recorded test results

Source; whereas each test kit manufacturer had its guidelines that were followed; the World Health Organization generic method for Malaria RDT were adapted in 2015)

APPENDIX VIII: MALARIA TEST USING BLOOD THICK AND THIN BLOOD SMEARS, GIEMSA STAIN AND MICROSCOPY

Preparation of Thick and Thin smears

Thin film

A clean spreader slide would be held at a 45° angle, towards the drop of blood on the specimen slide (a measure of 2ul of blood)

Waited until the blood spread along the entire width of the spreader slide

While holding the spreader slide at the same angle, we then pushed it forward rapidly and smoothly

Thick film

A drop of blood measuring 6Ul would then be placed on to the Centre of the clean glass slide, then spread in a circle the size of a button (diameter 1-2 cm) using the corner of another glass slide while ensuring that the smear wasn't too thick or thin . (We quality controlled the smear size by looking through it over the newsprint and be able to read the letters under it.)

Waited until the thin and thick films were completely dry before staining

Staining of Malaria blood smears using Giemsa stain

Begun by preparing the stock solution of Giemsafollowingstandard procedures as per the World Health Organization recommendation (version 1, effective date 01/01/2016) and Hospital Lab SOPs

Requirements included

For a total of 500ml stock solution, 3.8g of Giemsa powder wereused

Absolute methanol of high grade 250ml, Glycerol high grade pure 250ml

Spatula, weighing paper and weighing scale, Graduated cylinder, funnel, a storage clean clear glass for storing the stains

Protective gear such as gloves, lab coat and masks(all safety precautions would be taken not to inhale or come into contact with the chemicals)

Procedure for Measuring and preparing the stock solution of Giemsa

Measured 3.8g of Giemsa powder and poured into the bottle

Added 100ml of absolute pure methanol

Closed the bottle and mixed vigorously for about 2-3minutes

Added 250ml of glycerol closed the bottle and mixed for 2-3minutes

Added 150ml of absolute pure methanol

Mixed for about 2-3minutes

Labelled and documented on the quality control log

The stock Giemsa stain should was kept in dark glass bottle in cool locked cupboard protected from light

The stock Giemsa was kept for at least 24hours without shaking to avoid precipitates re-suspension

We did quality control on the stock solution before using it (we got a positive malaria sample and negative sample, made smears and stained with the Giemsa new stain and proved it was of quality)

The rapid 10% stain working solution staining method

Estimated the amount of 10% Giemsa working solution required for the number of slides to be stained. Each slide required approximately 3 mL of stain to cover it

Prepared 10% working solution using the formulae (Concertation required multiplied by volume needed divided by the original concentration of the stock solution). Buffered water of

pH 7.2 was used for dilution.

Fixed the thin smear using a Pasteur pipette or dipped the thin film for 2 seconds into a small container containing methanol.

Placed the slides on drying rack and allowed the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface.

Placed slides for staining blood films facing up since we used a staining rack.

Poured the stain gently into the top of the slidelying face upwards on a staining rackuntil each slide was covered with stain

Set the timer to 15 minutes and allowed the blood films to stain.

Quality control of Giemsa stock solution and buffered water was done everyday

At the end of the staining time, removed each slide individually and gently flushed the stain from the slide by adding drops of buffered water until all the stain had been washed away. The stains were not directly poured off the slides, as the metallic green surface scum would stick to the film, spoiling it for microscopy

When the stain had been washed away, the slides then were placed in the drying rack to allow thick film to drain and dry, ensuring that thick films were not scraped against the edge of the rack.

Discarded the remaining 10% Giemsa solution

Preparation and staining of malaria thin smears using 3% Giemsa

Estimated the amount of 3% Giemsa stain working solution needed for the number of slides to be stained

Prepared 3% working solution using the formulae (Concertation required multiplied by volume needed divided by the original concentration of the stock solution). Used buffered water of pH 7.2 for diluting the stock Giemsa solution

Fixed each thin film using a Pasteur pipette or by dipping the thin film for 2 s into a small container or beaker containing methanol

Allowed the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface

Placed the slides back-to-back in a staining trough

Poured the stain gently into the thin smears and set the timer for 45–60 minutes

Quality control of Giemsa stock solution was done daily

Gently poured buffered water into the stain to float off the iridescent "scum

Gently poured off the remaining stain, and rinsed with buffered water

Carefully removed the slides, one by one, and placed them vertically in the drying rack to dry

Discarded the remaining 3% Giemsa solution

Microscopic examination and interpretation of malaria results

Purpose; description of the procedure for correct detection and identification of malaria parasites in Giemsa stained blood films

Background; Identification of species and stages of malaria parasites and determination of their density is crucial in clinical management of malaria and drug efficacy trials, epidemiological surveillance and control of malaria. Therefore the diagnosis of malaria based on Giemsa stained blood films had to be performed correctly.

Requirements that were used; Compound binocular microscope with ocular (eyepieces); x10, x40 and x100 objective lens, Oil immersion, lens tissue paper, Giemsa stained blood slide smears, pen and pencil, tally counter and a malaria registry or log book

Procedure of examination; Placed a dry Giemsa stained slide on to the microscope stage, turned on x10 objective lens to help bring the specimen into focus using the coarse adjustment, switched on the light, placed a drop of oil immersion onto the stained film, turned on to x100 objective lens and ensured it touched the oil immersion, used the fine adjustment

knob to get the focus. Looked through the film while moving the slide at the stage from top to bottom and left to right systematically to ensure that all the fields were covered

For a positive smear, identified and counted all the forms of malaria parasites stages including; Merozoites, Trophozoites, Schizonts, Gametocytes and not forgetting the differentiation of species.

Interpretation of results; when no form of malaria parasites stage of any species was seen then the stained blood smear was reported as "No malaria parasites seen". On the other hand when some form of malaria parasites stage was seen then the "Number of parasite form was counted and reported as per the World Health Organization recommended reporting format adopted by the Bundibugyo Hospital Laboratory health facility.

Sources; (World Health Organization, 2016) and Bundibugyo Hospital Laboratory SOPs

APPENDIX IX: ABO BLOOD GROUPING BY TILE METHOD

Principle

The ABO blood group system is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes. In human blood transfusions it is the most important of the 35 different blood type (or group) classification systems currently recognized. When antisera containing corresponding antibodies are mixed with red blood cells containing specific antigen, agglutination takes place, whole blood, EDTA blood, equipment included Grouping tile, micropipette, container with 1% diluted Jik

Method

Used of commercially procured ABO blood grouping antisera(Provided by National Medical Stores, government agency for medical supplies), Drew and Labelled the tile with A, B, AB, and D, Placed 1 drop of anti-A, 1 drop of anti-B, 1 drop of anti-AB and 1 drop of anti-D on to the tile. Added 1 drop of test red cell suspension to each drop of the typing antiserum. Mixed the cells and reagent using a clean stick and spread each mixture evenly on the slide over an area of 10-15 mm diameter, Tilted the slide while mixing for about2 to 3minutes at room temperature, Inspected the mixer for agglutination, recorded the results accordingly

Table of results for ABO grouping

Cells	Antisera A	Antisera B	Antisera AB	Blood group
A-cells	A (agglutination)		A (agglutination)	A
B-cells		B (agglutination	B (agglutination)	B
AB-cells	A (agglutination)	B (agglutination)	AB (agglutination)	AB
O-cells				O
Control cells				

Source; (Nakasero blood bank- MOH, 2010) and Bundibugyo Hospital Lab SO

Principal Investigator;

Ejoku Emmanuel
2015-BMLS-PT-002
Tel; 0782807386, 0790411140
P.O Box 7782
Clarke International University