INFLUENCE OF INTERMITTENT PREVENTIVE TREATMENT ON TRANSPLACENTAL TRANSFER OF ANTI-MEASLES IMMUNOGLOBULIN-G ANTIBODIES IN MOTHER-INFANT PAIRS FROM AHERO SUB-COUNTY HOSPITAL IN KISUMU COUNTY, WESTERN KENYA

BY

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DECLARATION

I declare that this thesis is my original work and has not been presented to any institution or university for a degree or other awards.

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DEDICATION

To my mother, Ruth Atieno Ariera for her encouragement and overwhelming support.

ABSTRACT

Measles still remains one of the leading causes of vaccine preventable deaths globally with 140,000 deaths in 2019. These deaths occur despite the existence of a safe and effective measles vaccine. In Kenya, annual measles incidence ranges from 2-65 cases per million persons with about 76% of these being reported in unvaccinated infants aged less than a year. Primary protection against measles during infancy is mediated by maternally derived antibodies prior to vaccination with the levels in infants correlating with maternal antibody levels. The efficiency of transfer of measles specific antibodies is dependent on a number of factors including malaria infection. World Health Organization recommends malaria chemoprophylactic treatment in pregnant women in malaria endemic areas like western Kenya which may improve or suppress the vertical transfer of antibodies against other infectious diseases like measles. This study investigated the levels and vertical transfer of anti-measles Immunoglobulin-G to infant in expectant women taking either Dihydroartemisinin Piperaquine(DP) or Sulphadoxine Pyrimethamine (SP) for malaria Intermittent Preventive Treatment in pregnancy (IPTp). Specifically, this study compared the levels of anti-measles antibodies in mother-infant pairs undertaking IPTp-SP; compared the levels of anti-measles antibodies in mother-infant pairs undertaking IPTp-DP; compared the levels of anti-measles antibodies in mother-infant pairs between the IPTp-DP and SP treated participants, and finally investigated the influence of confounding variables (placental malaria, maternal age, hyper/po-gammaglobulinemia, parity and gestational age)- on the vertical transfer of anti-measles antibodies in women attending Ahero Sub-County Hospital in Kisumu County, Western Kenya. Using convenient sampling, samples from 132 mothers and 66 infants' samples were included in this retrospective-prospective study design. The levels of antibodies against measles; EBV and malaria antibodies (internal positive controls) in maternal venous, cord (neonatal), and infants blood samples at one and six weeks were quantified using Luminex technology. Total IgG levels were determined in maternal venous blood by Enzyme-linked Immunosorbent Assay (ELISA). Wilcoxon paired test was used to compare the median levels of antibodies in mother-infant pairs to measles, EBV and malaria within the DP and SP treated groups. Using Wilcoxon paired test, the levels of MV (measles), EBV and malaria antibody levels were comparable between the mother-infant pairs in the SP treatment arm (*P= 0.47, 0.97, 0.16, 0.47 and 0.73* for MV, AMA1, MSP1, EBNA1 and ZEBRA respectively). The levels were also comparable for the mother-infant pairs treated with DP (*P= 0.71, 0.66, 0.96, 0.94, 0.71* for MV, AMA1, MSP1, EBNA1 and ZEBRA, respectively). Mann-Whitney U test comparing median antibody levels between the DP and SP treated mothers and their respective infants only showed a significant difference at enrollment (baseline) for MV and EBNA1 (*P= 0.0057* and *0.0035*, respectively). Anti-MV, AMA1, MSP1, ZEBRA and EBNA1 were however comparable at birth and among the infants. Spearman correlation analysis showed a positive correlation between maternal and neonatal antibody levels. Multivariate linear regression analysis showed no influence of the confounding variables investigated on transplacental transfer of antibodies. These findings suggest that malaria chemoprophylaxis with either DP or SP does not affect the levels and subsequent transfer of MV as well as EBV and malaria specific antibodies. This study also demonstrates that more than 20% of the infants by six weeks postpartum are highly susceptible to measles infection thereby pointing to the need for a booster dose of measles vaccine in women of child bearing age before conception to increase the duration of protection in infants before vaccination. In addition, this data confirms the safety of IPTP- DP/SP which makes them suitable for use in malaria endemic settings after considering their efficacy in malaria management.

TABLE OF CONTENTS

DECLARATION ii
ACKNOWLEDGEMENT iii
DEDICATION iv
ABSTRACT v
TABLE OF CONTENTS vi
LIST OF ABBREVIATIONS AND ACRONYMS ix
DEFINITION OF TERMS x
LIST OF TABLES xi
LIST OF FIGURES xii
CHAPTER ONE: INTRODUCTION 1
1.1Background Information 1
1.2 Statement of the Problem 4
1.3 General Objective 5
1.3.1 Specific Objectives 5
1.3.2 Null Hypotheses 6
1.4 Significance of the Study 6
CHAPTER TWO: LITERATURE REVIEW 8
2.1 Measles Disease and its Etiology 8
2.2 Measles Virus and Measles Pathogenicity 8
2.3 Measles Immunity 10
2.4 Measles Vaccine and Prevention 12
2.5 Placental Transfer of Antibodies 13
2.6 Malaria and Measles Serological Responses 15
2.7 Malaria Chemoprevention, Antibody Production, and Transfer 16
CHAPTER THREE: MATERIALS AND METHODS 18
3.1 Study Area 18
3.2 Study Design 20
3.3 Study Population 20
3.4 Inclusion and Exclusion Criteria 21
3.4.1 Inclusion Criteria 21

3.4.2 Exclusion Criteria 21
3.5 Sample Size Calculation 21
3.6 Methods of Data Collection 22
3.6.1 Collection of Clinical Demographic Data 22
3.6.2 Laboratory Procedures 23
3.6.2.1 Sample Collection and Processing 23
3.6.2.2 IgG Multiplex Bead Assay 23
3.6.2.3 Total IgG ELISA 24
3.6.2.4 Placental Malaria Diagnosis 25
3.7 Data Management and Statistical Analysis 25
3.8 Ethical Considerations 26
CHAPTER FOUR: RESULTS 27
4.1 Demographic, Clinical and Laboratory Characteristics of the Study Participants 27
4.2 Levels of measles virus, EBV and Malaria Specific Antibodies in Maternal and their Infants from Nyando Sub-County Western Kenya 28
4.3 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Noenates Treated with IPTP-SP 29
4.4 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Infants Treated with IPTP-DP 31
4.5 Comparison of Levels of Antibodies between the DP and SP Treated Mothers, Neonates, and Infants 33
4.6 Vertical Transfer of Maternal Anti-measles, EBV, and Malaria Specific Antibodies 39
CHAPTER FIVE: DISCUSSION 43
5.1 General Introduction 43
5.2 Levels of Anti-measles Specific Immunoglobulin-G Antibodies in Mother-infant Pairs from Ahero Sub-County Hosital in Kisumu County, Western Kenya 44
5.3 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Infants treated with IPTP-SP and IPT-DP 45
5.4 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Infants Treated with IPTP-SP and IPT-DP 46
5.5 Vertical Transfer of Maternal Anti-EBV, Measles, and Malaria-specific Antibodies 47

5.6 Study Limitations 48
CHAPTER SIX: SUMMARY OF THE FINDINGS, CONCLUSIONS AND RECOMMENDATIONS 49
6.1 Summary of Study Findings 49
6.2 Conclusions 49
6.3 Recommendation from this Study 50
6.4 Recommendation for Future Studies 50
REFERENCES 52
APPENDICES 63

LIST OF ABBREVIATIONS AND ACRONYMS

AMA1	Apical Membrane Antigen 1
BSA	Bovine Serum Albumin
CMR	Cord Maternal Ratio
EBNA	Epstein Barr Virus Nuclear Antigen
DP	Dyhidroartemisinin Piperaquine
IPTP	Intermittent Preventive Treatment in Pregnancy
hFcRn	IgG specific Fc Receptor
Fc	Crystallizable Fragment
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
ELISA	Enzyme Linked Immunosorbent Assay
MV	Measles Virus
MSP1	Merozoite Surface Protein 1
PBS	Phosphate Buffer Saline
ZEBRA	BamHI Z Epstein - Barr virus Replication Activator

DEFINITION OF TERMS

Cord to maternal ratio	The ratio of the level of specific antibody in cord blood to that in the maternal blood of the mother.
Syncytiotrophoblast	Is the epithelial covering of the highly vascular embryonic Placental villi which invades the wall of the uterus to establish nutrient circulation between the embryo and the mother.
Hyperimmunoglobulinemia	Abnormally high levels of immunoglobulin in serum/plasma.

LIST OF TABLES

Table 4.1 Demographic and Clinical Characteristics of the Study Participants……..…….28

Table 4.2 Percentage reduction in Placental Transfer of Antibodies……41

Table 4.3 Unltivariate Regression for Confounding Variables….……42

LIST OF FIGURES

Figure 4.1 Levels of Measles, EBV and Malaria Antibodies ……..…….....29
Figure 4.2a Levels of antibodies in SP treated mothers and neonates ……..….30
Figure 4.2b Levels of antibodies in SP treated neonates and infants …….……...31
Figure 4.3a Levels of antibodies in DP treated mothers and neonates ……..…32
Figure 4.3b Levels of antibodies in DP treated neonates and infants ……...…….…...33
Figure 4.4a Levels of antibodies in SP vs DP treated mothers at enrollment ……..……....34
Figure 4.4b Levels of antibodies in SP vs DP treated mothers at lastvist ……....35
Figure 4.4c Levels of antibodies in SP vs DP treated neonates in cord …….……..36
Figure 4.4d Levels of antibodies in SP vs DP treated infants week1 ……...……..…..37
Figure4. 4e Levels of antibodies in SP vs DP treated infants week6 ……...…….……...38
Figure 4.5 Correlation between Cord and maternal antibody levels …….……...39
Figure 4.6 Vertical transfer of antibodies in DP vs SP treated mothers ……..…….....40

CHAPTER ONE

INTRODUCTION

1.1Background Information

Measles is one of the leading causes of vaccine-preventable deaths in children under the age of 5 years globally (Dabbagh *et al*., 2018). Measles virus (MV), the causative agent for measles, is an RNA virus of the genus *Morbillivirus* and paramyxovirus family (Furuse *et al*., 2010). Measles infection is associated with transient immune suppression characterized by the diminishing of antibodies against other pathogens as well as memory lymphocytes (Mina *et al.*, 2019) resulting in an increased risk of childhood morbidity and mortality for a period of more than 2 years (Griffin, 2010; Mina *et al*., 2015). The introduction of safe effective vaccines in 1963 led to the global reduction of measles-related deaths from 21.1 million to 140,000 deaths by 2018 (WHO, 2018).Even with the availability of Measles, Mumps, and Rubella (MMR) vaccine, 60% of measles cases were reported between 2013 and 2016 in sub-Saharan Africa among children aged 9-59 months, 79% of whom were unvaccinated or with unknown vaccination status (Masresha *et al.*, 2017). In Kenya, annual measles incidence ranges from 2-65 cases per million persons with about 76% of these being reported in infants aged less than a year (Kisangau *et al*., 2018). Measles, therefore, remains a major challenge in Kenya irrespective of the high vaccine coverage and concerted efforts to eliminate the virus.

Infants are protected early in life against infections by maternal antibodies which are either as a result of natural exposure or through vaccination. The MMR vaccine is administered at 9 and 18 months for the first and second doses respectively to minimize the interference from maternal antibodies and provide maximum protection as part of the Expanded Immunization Program in Kenya (Kasidet, 2017). Prior to vaccination, maternal antibodies provide primary protection

against the measles virus and the levels in infants correlate with maternal antibody levels (van den Berg *et al.,* 2011). However, the maternal antibodies wane with time and this increases susceptibility to measles infection, a situation that is controlled by the optimization of the first vaccine dose to coincide with the absence of the antibodies. Studies have also reported a decreased level of antibody titers against measles in pregnant women compared to non-pregnant women in other parts of the world including Iran, Guinea, Germany and the Netherlands (Baboonian & Griffiths, 1983; Honarvar *et al*., 2013; Miller, 2009). However, few studies have described measles antibody seroprevalence and transfer in mother-infant pairs within the malaria-endemic areas of sub-Saharan Africa, Kisumu County, western Kenya included.

Measles-specific maternal antibody concentration and vertical transfer is influenced by multiple factors including measles vaccination, natural measles infection history, malaria infection during pregnancy, maternal age, parity, gestational age and HIV infection (Kizito *et al.*, 2013). A known determinant of the maternal measles virus antibody concentration is the vaccination status of the mother. Mothers who received MMR vaccine tend to have a lower concentration of measles–specific antibodies compared to mothers who suffered from natural measles infection (Leuridan *et al*., 2010; Leuridan& Van Damme, 2007).

Therefore, infants born to measles-vaccinated mothers are more likely to have lower maternal antibody levels at birth, faster rate of decay of measles specific antibodies, and a shorter period of protection than infants of mothers who acquired measles naturally (Muscat *et al*., 2009; Van den Hof *et al.*, 1999; Van den Holf *et al*., 2002). The second dose of the measles vaccine was approved and adopted in 2016 by the Ministry of Health, Kenya, meaning that mothers of childbearing age in the country only received a single dose of MMR vaccine.Longitudinal

studies among mother-infant pairs with different measles infection/vaccination histories are therefore necessary to understand the risk of the disease within this population.

To reduce the adverse effects of malaria in pregnancy in malaria-endemic regions like Western Kenya, WHO recommends the use of Intermittent Preventive Treatment in pregnancy (IPTp) with at least two doses of SP (WHO, 2004). IPTp-SP has been shown to reduce malaria-related adverse events in pregnancy (Parise *et al*., 1998; Rogerson *et al*., 2000). It has been suggested that the reduction in exposure to *Plasmodium falciparum* brought about by malaria prevention strategies, such as chemoprophylaxis, might delay the development of malaria-specific immunity in children and adults (Collins *et al*., 1987; Quelhas *et al*., 2008). The rationale behind the use of IPTp for malaria prevention should be to reduce the clinical consequences of malaria without preventing the development or maintenance of immune responses. Previous studies have reported that the use of IPTp-SP, reduces malaria-specific antibody levels as well as subsequent transfer to the fetus (Staalsoe *et al*., 2004; Stephens *et al*., 2017; Teo *et al*., 2015). The effect of these anti-malarial interventions (IPTp-SP) on maternal antibody transfer and levels during pregnancy could be global or specific to various infectious agents, though this has not been described in the context of other diseases.

The emergence of malaria parasite resistance to SP-IPTp treatments in Africa and other malaria endemic regions like western Kenya has led to a number trials with Dihydroartemisinin Piperaquine (DP) as a new intervention for malaria prophylaxis during pregnancy (Desai *et al.*, 2016). The IPTp-DP for malaria prophylaxis during pregnancy could affect anti-malaria specific antibody levels and vertical transfer to the fetus, potentially in the same manner as IPTp-SP on malaria antibodies.Though this has not been explored. Artemisinins and its derivatives (DP included) together with sulfadoxine pyrimethamine have been shown to have

immunomodulatory effects on diverse components of the immune system including B and T cell responses. These drugs have been reported to suppress the secretion of cytokines and related signaling pathways, decreased T helper cells, upregulation of regulatory T cells (Tregs) and suppression of B cells and autoantibody production(Yao *et al.*, 2016).Through these immunomodulation and immunosuppression properties, these drugs could potentially reduce the levels and subsequent transfer of antibodies from mothers to neonates. This study, therefore determined the levels and efficiency of transfer of anti-measles specific antibodies as well as malaria (AMA1, MSP1) and EBV (ZEBRA and EBNA1) specific IgG as a positive control in pregnant women undergoing IPTp-SP and DP from Ahero Sub-County Hospital in Kisumu County, western Kenya.

1.2 Statement of the Problem

Measles is associated with transient immune suppression and increased risk of childhood morbidity and mortality for a period of more than 2 years with 140, 000 deaths reported globally in 2018. The World Health Organization's Expanded Immunization Program targets global eradication of measles by 2020, a goal that is increasingly becoming untenable as measles cases and incidents have been on the rise since 2016 with multiple cases reported in 2019, as a result of immunity gaps and geographic variation in vaccine coverage. For the eventual eradication of measles, it is necessary to stop measles transmission by establishing herd immunity through successful vaccination. Prior to vaccination, infants are protected by maternally derived antibodies which the levels in infants always correlate with the levels in mothers. The majority of women in Kenya today of child-bearing age only received a single MMR vaccine dose, translating to potential low maternal antibody levels and transfer. Malaria infection during pregnancy is known to influence immunity to measles in infants by suppression of transfer of

maternal antibodies. In malaria holoendemic areas like Kisumu County, western Kenya, IPTp-SP has been recommended for malaria chemoprophylaxis during pregnancy. Malaria chemoprophylaxis with SP has been shown to reduce the levels and transfer of malaria specific antibodies. IPTp- with SP/DP may influence antibody transfer that is protective against other diseases like measles, this however, remains to be studied. It was therefore, critical to evaluate the measles seroprevalence and transfer among mother-child pairs from this region and the possible effects of malaria chemoprophylaxis in pregnancy with DP and SP on antibody transfer.

1.3 General Objective

To investigate the levels and vertical transfer of anti-measles Immunoglobulin-G to infants in expectant women taking IPTp, from Ahero Sub-County Hospital in Kisumu County, western Kenya.

1.3.1 Specific Objectives

i. To compare the levels of anti-measles specific IgG in mother-infant pairs undertaking IPTp-SP at Ahero Sub-County Hospital in Kisumu County, western Kenya.

ii. To compare the levels of anti-measles specific IgG in mother-infant pairs undertaking IPTp-DP at Ahero Sub-County Hospital in Kisumu County, western Kenya.

iii. To compare the levels of anti-measles specific IgG in mother-infant pairs undertaking IPTp-DP and SP at Ahero Sub-County Hospital in Kisumu County, western Kenya.

iv. To determine the influence of confounding variables (placental malaria, maternal age, hyper/po-gammaglobulinemia, gravidity, and gestational age) on the vertical transfer of anti-measles antibodies of women from Ahero Sub-County Hospital in Kisumu County, western Kenya.

1.3.2 Null Hypotheses

i. There is no difference in anti-measles antibody levels between mother-infant pairs undertaking IPTp-SP.

ii. There is no difference in measles antibody levels between mother-infant pairs undertaking IPTp-DP.

iii. There is no difference in antibody levels in mother-infant pairs undertaking IPTp-SP and IPTP-DP.

iv. Confounding variables (placental malaria, maternal age, gravidity, hyper/po-gammaglobulinemia and gestational age) do not have an influence on anti-measles antibody transfer from mothers to infants.

1.4 Significance of the Study

Measles infections and related mortality due to measles induced immune suppression continues to be a major public health concern within this region. The reemergence or resurgence of measles outbreaks in highly immunized populations has shifted attention on the efficacy and durability of vaccine-induced immunity with some studies reporting low antibody levels to measles in the vaccination era compared to the unvaccinated population. Understanding the level of protection especially among women of child bearing age is key in designing policies that ensure measles protection among pregnant women and their respective infants. While about 70% of pregnant women from his region had high antibody levels against measles, more than 20% of the mothers and infants had low antibody levels against measles which is likely to increase the susceptibility of infections to measles early in life before vaccination. This therefore means that a vaccine booster dose is necessary among women of child bearing age or just before pregnancy as the vaccine cannot be given to pregnant women due to safety concerns. To reduce the adverse effects

of malaria in pregnancy which includes reduced placental transfer of measles specific antibodies from mothers to neonates, malaria chemoprophylaxis during pregnancy is recommended. While this treatment has been shown to affect the levels of malaria specific antibodies, elucidation of the global effect of these drugs is key especially on the safety concerns of DP that is currently under trial as a possible replacement for SP. Results from this study indicate that malaria chemoprophylactic treatment with SP and DP does not influence the levels and subsequent transfer of measles specific antibodies. This therefore confirms that IPTP-SP/DP is safe and lacks detrimental effects of levels and transfer of antibodies against other infections.

CHAPTER TWO

LITERATURE REVIEW

2.1 Measles Disease and its Etiology

Measles is a highly infectious and contagious disease caused by the measles virus (MV) that is spread via infectious aerosols (Ota *et al.*, 2005). Upon infection with MV, the initial target cells are alveolar macrophages and dendritic cells in the lungs. The virus is transported to the draining lymphoid organs and tissues, seeding a systemic infection with preferential tropism to B and T lymphocytes (Yanagi *et al*., 2006). The initial symptoms usually appear 10-14 days post-infection and are characterized by high fever, runny nose, bloodshot eyes, and tiny white spots on the inside of the mouth. Days later, rashes develop starting on the face and upper neck and gradually spreading downwards to the rest of the body (Laksono *et al.*, 2016). It can also cause serious complications including blindness, encephalitis, severe diarrhea, ear infection, bacterial pneumonia, and death; making measles to be one of the leading causes of childhood deaths worldwide with approximately 140,000 deaths reported in 2018 (Dabbagh *et al.*, 2018). The majority of measles-related deaths are associated with measles' transient immunosuppression characterized by the diminishing of antibodies against other pathogens (Mina *et al.*, 2019), reduction in memory lymphocytes, and increased risk of childhood morbidity and mortality for a period of more than 2 years (Griffin, 2010; Mina *et al.*, 2015). Malnourished children and people with reduced immunity are at high risk of measles (Laksono *et al*., 2016).

2.2 Measles Virus and Measles Pathogenicity

Measles virus (MV), belongs to the family Paramyxoviridae, genus Morbillivirus and of the order Mononegavirales(Griffin *et al.*, 2012; Ota *et al.*, 2005) . Measles virus is an enveloped virus with a single strand non-segmented negative-sense RNA genome (Griffin & Bellini, 2001). The virus was first isolated from the blood of measles infected persons in the 1950s (Enders &

Peebles, 1954). It is believed that the measles virus evolved from the rinderpest virus family (RPV), which is a pathogen of cattle (Furuse *et al.,* 2010). Measles virus is antigenically stable with few genetic differences between vaccine strains; however, the wild-types are more variable, and several different genotypes of wild measles virus are circulating worldwide (Bellini & Rota, 1998).

Like all morbilliviruses, MV is highly contagious and transmitted via the respiratory route. Once the virus is inhaled and a primary target cell infected, systemic spread ensues and clinical signs appear after 10-14 days (Griffin *et al*., 2012). In the virus particle, two surface glycoproteins, the fusion (F) and hemagglutinin (H) form a multimeric complex that mediates viral entry. The H protein binds to specific molecules (receptors) on target cells, while the F protein mediates membrane fusion between the virus envelope and the host cell plasma membrane (Kato *et al*., 2012; Riddell *et al*., 2007).Signaling lymphocyte activation molecule family member 1 (SLAMF1, also known as CD150) and Nectin cell adhesion molecule 4 (nectin-4) which is expressed by subsets of thymocytes, dendritic cells (DCs), macrophages, T- and B-cells, has been identified as a cellular receptor for MV (Dhiman *et al.*, 2004; Yanagi *et al.*, 2006). Vaccine and laboratory-adapted MV strains can utilize CD46 (Membrane Cofactor Protein) as an additional cellular receptor *in vitro*, but this receptor does not seem to play a major role during infection with these viruses *in vivo* (Dörig *et al.*, 1993).

Measles virus initiates its infectious cycle by attaching the H protein on the virus envelope to a cellular receptor on a target cell. Attachment of the H protein to a receptor triggers membrane fusion between the virus envelope and the plasma membrane of the target cell-mediated by the F protein (Lamb & Parks, 2007; Takeuchi *et al.*, 2012). Infection of memory lymphocytes by measles virus leads to depletion of immunologic memory resulting in immune-suppression. In

addition, systemic infection of DCs results in an impairment of antigen presentation functions. Measles virus replicates mainly in lymphoid organs throughout the body and produces syncytia causing damage to the immune system of infected individuals (Griffin, 2010; Mina *et al*., 2015). Massive replication in CD150+ T- and B-lymphocytes then results in the dissemination of the virus throughout the body. In addition, at the peak of virus replication, there is a high virus load, which may cause the spillover of viruses to other cell types with low affinity to the virus receptors (Griffin, 2010), leading to a disease state. Measles induced immune suppression results in increased morbidity and mortality for a period of more than two years especially among unvaccinated children under the age of five (Mina *et al.,* 2015). Prior to vaccination, infants are primarily protected against measles by maternal antibodies that are vertically transferred. The levels and success of transfer is depended on a number of factors in including malaria and HIV exposure as well as the levels of measles specific antibodies in mothers. With the surge in measles related morbidity and mortality since 2016, there is a need to access the levels as well as the efficiency of transfer of measles specific antibodies in malaria endemic regions like Kisumu County. The study aimed to compare the levels as well as the efficiency of transfer of anti-measles specific antibodies among women o IPTp- treatment attending to Ahero Sub-County Hospital in Kisumu County, western Kenya.

2.3 Measles Immunity

Once the virus enters the respiratory tract of the infected individual, the virus is picked by pulmonary dendritic cells and alveolar macrophages which then transports the virus to lymph nodes where immune responses are initiated (Ludlow *et al.,* 2013). Protection against measles is mediated by both antibody and T cell immunity. Cellular immune response against measles virus involves both CD4+ and CD8+ T cells that are directed against the Hemagglutinin protein (H)

and Fusion protein (F),(Bouche *et al.*, 2002).CD8+ lymphocytes exhibit measles specific cytotoxicity while CD4+ T cells provides T helper 1 response through the production of cytokines such interferon gamma that mediates viral clearance, providing T cell help required for isotype switching and affinity maturation of antibody-secreting B cells (Bouche *et al.*, 2002; Griffin *et al.*, 2012). Protective immunity to wild type measles virus is mediated by neutralizing antibodies targeting the H and F proteins thus blocking viral attachment and fusion (Bouche *et al.*, 2002; Lech *et al.*, 2014). Antibodies block virus infectivity by preventing the interaction between the H protein and its receptors CD150 (SLAM) and CD46. Some antibodies can also neutralize the viral infectivity by blocking and preventing the fusion of the virus to the host cell membranes mediated by the F proteins (Griffin *et al.*, 2012; Harvala *et al.*, 2016; Lech *et al.*, 2014). Infants are primarily protected against measles by maternally derived antibodies with antibody levels in infants correlating with maternal levels. Maternal measles specific antibodies in infants' wanes to undetectable levels by six months of age thereby increasing the susceptibility to measles infection prior to first vaccination at nine months (Leuridan *et al*., 2010). Additional studies into the levels of maternal antibodies in infants demonstrate that strong regional differences exist that affect the duration of protection in infants with some becoming susceptible by 4.5 months while others have detectable levels of antibodies by nine months thereby affecting vaccine uptake(Gagneur *et al*., 2008; Oyedele *et al*., 2005). Regional differences can be due to a number of factors such as exposure to wild type virus by the mother, which results in high antibody titres and higher rates of transfer in children than in those of vaccinated mothers. Maternal antibody levels are lower in children of vaccinated mothers and decay much earlier than in children of naturally infected mothers (Szenborn *et al*., 2003). Immunization should be successful when maternal antibodies have declined below the threshold of detection. In practice,

it is not possible to accurately determine this time point as it depends on the amount of maternal antibody transferred, region, gender, nutritional status, and exposure to measles virus. Therefore, the measurement of antibody levels would be required before immunization, which is not feasible in a clinical setting. With reports indicating early decline of measles vaccine in highly vaccinated populations, high maternal antibodies titers are therefore key in offering prolonged protection in infants from such settings. The levels/ degree of protection against measles in the setting of Ahero Sub-County Hospital where malaria is prevalent remains unknown.

2.4 Measles Vaccine and Prevention

There is no therapeutic treatment for measles, however, Measles Mumps and Rubella (MMR) vaccine is used for measles prevention. The World Health Organization (WHO) recommends a two-dosage vaccination for measles prevention (Jefferson *et al*., 2003).In Kenya, the MMR vaccine is administered at 9 and 18 months for the first and second doses respectivelywhich results in the production of protective neutralizing antibodies (KasidetManakongtreecheep, 2017). This vaccination regime/ program was designed to minimize the interference of maternal antibodies that has been shown to decline to undetectable levels 9 months of age and booster dose at 18 months to provide maximum protection(Niewiesk, 2014). Prior to vaccination, vertically transferred maternal antibodies are expected to provide protection against measles virus infections in infants and the antibody levels transferred to infants correlate with maternal antibody levels (Berg *et al.,* 2011).The global measles mortality rate has significantly declined over the past 18 years with a about 73% reduction decrease recorded between 2000 and 2018, with only 140,000 deaths occurring in 2018 from 23.2 million deaths (WHO,2019). This significant decline in measles related deaths is attributed to high vaccination coverage globally with the current rate standing at 85% and 71% vaccination coverage for one and two doses of

MMR vaccine respectively (WHO,2020). In Kenya, measles vaccination coverage is estimated to be at 89% and 54% for the first and second doses respectively by 2019 (MoH, 2020). For eventual elimination of measles as set by the WHO African Region (AFR) by 2020, impairing measles transmission is key through establishment of herd immunity by achieving a vaccination coverage of more than 95% (Masresha *et al* 2018), a goal that Kenya is yet to meet as measles outbreaks and measles related deaths are still being reported. The reemergence or resurgence of measles outbreaks in highly immunized populations has shifted attention on the efficacy and durability of vaccine- induced immunity with some studies reporting low antibody levels to measles in the vaccination era compared to the unvaccinated population (Leuridan *et al*., 2010).High vaccination coverage with a single-dose regimen has been unsuccessful in eliminating measles, necessitating the introduction of the second dose to provide protection for those with primary vaccine failure. The second dose of the measles vaccine was approved and adopted in 2016 by the Ministry of Health, Kenya, meaning that mothers of childbearing age in the country only received a single dose of MMR vaccine. Studies involving mother-infant pairs with different measles infection/vaccination histories are therefore necessary to understand the risk of the disease within this population. The study therefore aimed to determine the levels of measles specific objectives attending to Ahero Sub-County Hospital in Kisumu County, Western Kenya.

2.5 Placental Transfer of Antibodies

Infants are protected early in life by maternal antibodies that are transplacentally acquired predominantly through active transport across the placenta. This mechanism involves Fc receptors at the syncytiotrophoblast membrane that binds the IgG molecules, actively transported across the cell, and released into the fetal bloodstream (Pinto & Hart, 1997; Palmeira

et al., 2012; Saji *et al*., 1999; van den Berg *et al*., 2011). Immunoglobulin G (IgG) transport from mother to fetus begins as early as 13 weeks with the highest amount of transfer occurring in the third trimester (Saji *et al*., 1999). Immunoglobulin G may become saturated thus the amount of IgG transmitted from mother to fetus is dependent on the level of cell surface receptors since unbound IgG are destroyed by lysosomes. Maternal total IgG correlates with neonate IgG antibodies. Levels of measles antibodies in newborns are therefore dependent on both the level in their mothers' serum and the extent of placental transfer (Berg *et al*., 2011).

Vertical transfer of maternal antibodies to the fetus is affected by multiplenon-infectious factors such as placental abnormalities, maternal plasma IgG levels, maternal age, parity and gestational age (Palmeira *et al*., 2012). Moreover, maternal infections such as human immunodeficiency virus (HIV) and malaria can also affect the efficient vertical transfer of antibodies in a pathogen-dependent manner (Okoko *et al*., 2001; Palmeira *et al*., 2012).

Falciparum malaria infection during pregnancy as well as placental malaria has been shown to influence the levels and transfer of measles specific antibodies by causing pathological changes in the placenta, such as basement membrane thickening which may result in reduced expression and damage of FcRn antibody receptors (Galbraith *et al.*, 1980; Okoko *et al*., 2001; Scott *et al.*, 2005). Maternal antibody concentration is a known determinant of the measles vertical antibody transfer which is naturally increased by vaccination and natural measles exposure. Mothers who received MMR vaccine during childhood tend to have a lower concentration of measles–specific antibodies compared to mothers who suffered from natural measles infection (Leuridan *et al.*, 2010; Leuridan& Van Damme, 2007). Therefore, infants born to measles-vaccinated mothers are more likely to have lower maternal antibody levels at birth and thus a shorter period of protection than infants of mothers who acquired measles naturally (Hof *et al.*, 1999; Hof *et al.*,

2002). While multiple studies have described impaired maternal antibody transfer among pregnant women exposed to HIV and placental malaria in this region, the impact of factors such as malaria endemicity, single-dose measles vaccination and malaria chemoprophylaxis on maternal antibody transfer efficiency remains unknown. In this regard, this study compared the levels of measles specific antibodies in mother infant pairs from Ahero Sub-County in Kisumu County, western Kenya undertaking malaria chemoprophylactic treatment with either DP or SP as well as determine the impact of the confounding variables such as placental malaria, maternal age, hyper/po-gammaglobulinemia, gravidity, and gestational age on measles antibody transfer.

2.6 Malaria and Measles Serological Responses

The WHO's Expanded Program on Immunization (EPI) is targeted at malarious areas such as western Kenya, emphasizing the need to understand the effect of malaria and antimalarial drug use on vaccine immunogenicity and efficacy during infancy. Previous studies investigating the effect of Intermittent Preventive treatment for malaria during infancy (IPTPi) on serological responses to measles and other EPI vaccines, found no evidence suggesting that treatment of clinical malaria with SP or other antimalarial drugs at or around the time of vaccination does not compromise vaccine responsiveness(Crawley *et al*., 2012). Further studies confirmed that malaria chemoprophylaxis prior to vaccination in malaria endemic settings did not impair immunogenicity to measles vaccines (Bradley-Moore *et al*., 1985; Cénac *et al.*, 1988; Schellenberg *et al.*, 2001). Antimalarial drugs and malaria infections are known to induce malaria immunomodulation and immunosuppression through impaired macrophage function, altered cytokine production, a depletion of T or B cells, impaired dendritic cells, elevated nitric oxide production, and elevated prostaglandin E during febrile malaria (Ocaña-Morgner *et al*., 2003; Rockett *et al*., 1994; Snyder *et al.*, 1982; Urban *et al.*, 1999). Possibly through the above

mechanisms, malaria infection and antimalarial drugs might affect antibody levels to measles during pregnancy and subsequent transfer to infants, this however remains to be determined. This study there for aimed to determine the levels of measles specific objectives attending to Ahero Sub-County Hospital in Kisumu County, Western Kenya.

2.7 Malaria Chemoprevention, Antibody Production, and Transfer

To reduce the adverse effects of malaria during pregnancy in malaria-endemic regions like western Kenya, WHO recommends the use of IPTp with at least two doses of Sulfadoxine Pyrimethamine (WHO,2004.). Intermittent Preventive Treatment in pregnancy with SP has been shown to reduce malaria-related adverse events in pregnancy (Parise *et al*., 1998; Rogerson *et al.*, 2000). With the emergence of malaria parasite resistance against IPTp-Sp, DP is currently under trial as a possible replacement for SP (Desai *et al*., 2016). Previous studies reported IPTp-SP reduces anti-malaria antibody levels and transfer to infants thus increasing their risk of malaria infections early in life (Staalsoe *et al.,* 2004). It has been suggested that the reduction in exposure to *P. falciparum* brought about by malaria prevention strategies, such as chemoprophylaxis, delays the development of malaria-specific immunity in children and adults (Collins *et al.*, 1987; Quelhas *et al.*, 2008). Artemisinins and its derivatives (Dihydroartemisinin included) together with Sulfadoxine Pyrimethamine have been shown to have immunomodulatory effects on diverse components of the immune system including B and T cell responses. These drugs have been reported to suppress the secretion of cytokines and related signaling pathways, decreased T helper cells, upregulation of regulatory T cells (Tregs) and suppression of B cells and autoantibody production (Yao *et al*., 2016). The use of IPTp for malaria prevention aims to reduce the clinical consequences of malaria without preventing the development or maintenance of immunity. The effect of these anti-malarial interventions (IPTp-

SP and DP) on maternal antibody transfer and levels during pregnancy could be specific to *P. falciparum* or have off-target effects that impact antibody transfer and protection from other infections. In this regard, this study compared the levels of measles specific antibodies in mother infant pairs from Ahero Sub-County in Kisumu County, western Kenya undertaking malaria chemoprophylactic treatment with either DP or SP.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted at the antenatal clinic (ANC) and maternity ward of Ahero Sub-County Hospital in Kisumu County, western Kenya (Latitude: Lo 0°10'28.63" N, Longitude: 34°54'58.68" E) as a nested study from a parent study titled “IMPROVE study” comparing the superiority of Dihydroartemisinin Piperaquine (DP) over Sulfadoxine Pyrimethamine (SP) for malaria Intermittent Preventive treatment in pregnancy (IPTp). Kisumu County, a lake bordering region, is of high malaria burden with malaria prevalence of 28% of the total global malaria incidences (WHO,2017). In addition, this region experiences high malaria parasite resistance to Sulfadoxine Pyrimethamine (SP) (WHO, 2017). Furthermore, the majority of the population living within the study site live within the rural set ups of low socioeconomic status and are highly exposed to infectious diseases like measles. Ahero Sub County Hospital offers prenatal, delivery and postnatal care services to mothers and infants, thus serving as a suitable site for enrolling participants for this study. Figure 3.1 shows the map of the study area.

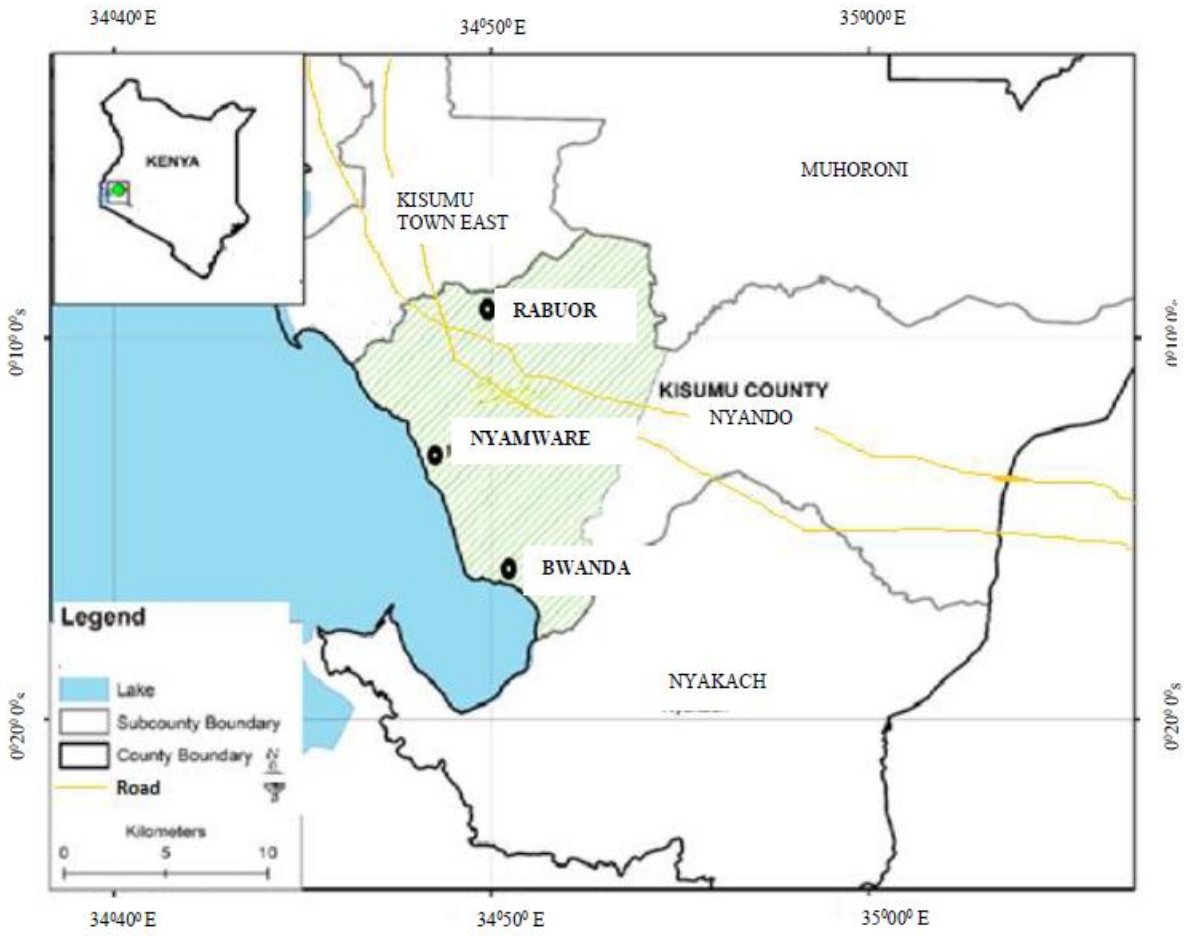

Figure 3.1: Map of Kisumu County showing the study site. The blue arrow points to the location of Ahero Sub-County Hospital within Nyando sub county. Source; Abuta *et al.*, 2018.

3.2 Study Design

To determine the influence of Intermittent Preventive Treatment in pregnancy with SP or DP on measles specific antibodies, this study adopted a retrospective-prospective study design in order to elucidate the influence of malaria intervention on antibody levels and transfer. Expectant women of gestational age 16-28 weeks were enrolled in this. Prior to initiation of IPTp treatment, blood samples were collected and this was termed the enrollment visit. Monthly IPTP treatment was administered to the enrolled participants in the subsequent antenatal clinical visits till delivery with blood samples being collected on every visit. Infants from the respective mothers were followed for six weeks postpartum. Since this study was stemming from a parent study, the sample time points used here were dictated by the sample time points collected by the mother study.

3.3 Study Population

Samples used in this study were obtained from participants enrolled between April 2018-December 2019 in a multinational, individually-randomized, 3-arm, partially-placebo controlled superiority trial comparing the efficacy, safety and tolerance of monthly IPTp-SP (control) versus monthly IPTp-DP, alone or combined with a single course of azithromycin at enrolment (1gr/daily for 2 days) to reduce the adverse effects of malaria and curable STIs/RTIs in 4,680 women in 10 sites in high SP resistance areas in Kenya, Malawi, and Tanzania. Kenyan sites included Ahero, Rabuor and Homabay with each site enrolling a total of 600 participants.

To investigate whether IPTp induces global or antigen-specific immune suppression and a transfer of anti-measles IgG antibodies to the fetus in expectant women taking IPTp from Ahero Sub-County Hospital in Kisumu County, western Kenya, a retro-prospective study design was adopted in which a total of 132 mother-infant samples obtained by quesi-convenience sampling

from Aherosite were used. Participants included in the study were from mothers who had deliveries during the week (between Monday to Friday). Sample cohorts were those collected from the mothers at enrollment(baseline), at last visit before birth, and cord blood (representative of the neonate blood) taking either IPTp-SP or IPTp-DP. Infant samples were collected at week one and six. The rationale for collecting and and including this infant sample was to help determine the impact of IPTp exposure as well as the rate of decay of measles specific antibodies over within the study population. Plasma obtained from the blood samples was used to determine IgG antibody levels against anti-measles, anti-EBV (ZEBRA (BZLF1) and EBNA1) and anti-Malaria (AMA1 and MSP1) using Luminex (XMap technology).

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

i. Expectant women, gestation week 16-28 in their current pregnancy
ii. HIV negative
iii. All gravidae
iv. Women residents of the study area and who consent to participate in the study and deliver in the study hospital
v. Women should not have started using any of the study drugs in their current pregnancy

3.4.2 Exclusion Criteria

i. Women who move outside of the study area before delivery
ii. Women who become HIV positive during this pregnancy
iii. Women who delivered outside the study hospital

3.5 Sample Size Calculation

In this study, the samples were already collected and therefore made use of archival samples. Convenience samples from 132 pregnant women attending Ahero sub-county hospital for their

ANC between April 2018 to December 2019 were used. Respective infants' samples from 66 mothers who had completed a set of mother-infant pairs after delivery were also used. Sample size was calculated using the R calculator that makes use of the formula described in Zhang *et al* 2017. Since the study was interested in comparing two groups with continuous outcomes, adopting the formula by Zhang was the most appropriate sample size calculation formula taking into account the treatment interventions. With the formula bellow to determine **n;**

$= \frac{Z_{1-a/2}^2 P(1-P)}{d^2}$,using the function;

pwr.t.test (**n**=NULL, **d** = 0.5, **sig.level**= 0.05, **power** = 0.8, type = "Paired")

Where: -

d= desired effect >0.5

Sig. Level=Type 1 error probability > 0.05

p= power (1-Type 2 error probability) > 0.8

$Z_{1-a/2} Z_{1-a/2}$ = Standard normal variate (1.96)

Passing the parameter n as NULL, the formula uses the determined parameters i.e., d, sig. level & p to compute the missing parameter. This gave a sample size of 34 samples for each group giving a total of 68.

3.6 Methods of Data Collection

3.6.1 Collection of Clinical Demographic Data

A questionnaire was administered to the pregnant women who consented to the study by the parent study. The information obtained included: age, number of children they have (parity), history of using Intermittent Preventive Treatment in their current pregnancy, date of last

menstrual cycle. Gestational age was calculated based on the date of the last menstrual period as provided by the mothers and by ultrasound dating. Variables such as malaria status during each visit, placental malaria diagnosis, age, parity and gestational ages were extracted from the IMPROVE database matched to the study participants included in this sub-study for analysis.

3.6.2 Laboratory Procedures

3.6.2.1 Sample Collection and Processing

Maternal venous blood was obtained during antenatal clinic visits and at birth. Approximately 1mL of blood by finger prick was collected in the EDTA microtainer tube (Becton Dickinson, USA) by trained clinicians at enrollment and during their last ANC visit. About 3ml of cord blood at delivery was collected from cord veins in a lithium heparinized microtainer tube. Infants at week one and six weeks after birth were heel pricked and 300ul of blood collected in EDTA microtainer tubes (Becton Dickinson, USA).Collected samples were transported in ice packs to UMMS/KEMRI lab for plasma isolation. Plasma fractionation from the whole blood samples was done by centrifugation at 300g (gravity) for 10 minutes and the plasma supernatants were stored in labeled tubes at -20^0C awaiting subsequent antibody measurement using Magpix Luminex (Luminex Corporation, Austin, Texas).

3.6.2.2 IgG Multiplex Bead Assay

Measurement of antibody specific IgG was done using plasma samples stored at -20^0C based on Magpix XMAP technology (Luminex Corporation, Austin, Texas, USA) using Luminex protocol. Measles vaccine virus (Edmonston strain) extract (grown in Vero cells), two recombinant EBV antigens as controls; EBV nuclear antigen-1 (EBNA1), Epstein-Barr virus BZLF1-encoded replication activator (ZEBRA/Zta), and two recombinant malaria proteins; apical membrane antigen-1 (AMA-1) and Merozoite surface protein-1 (MSP-1) were tested in this study. All recombinant proteins were covalently attached to Bio-Plex COOH carboxylated

magnetic beads (Bio-Rad Laboratories, Hercules, CA), via their carboxyl and amine groups based on manufacturer's protocol. Bio-plex COOH carboxylated magnetic beads (1.25×10^7 microspheres/ml) were coupled to the corresponding antigens at 100µg/500µl and anti-IgG measurements for measles, EBV and malaria antigens done in XMAP 200 Multianalyte Analyzer (Bio-Rad Laboratories, Inc.) as previously described(Cham *et al.*, 2009) with subtle modifications. In brief, antigen-coupled bead sets were combined and incubated with human plasma in the multiplex format. After the primary incubation, the beads were washed and incubated with the secondary antibody; biotinylated IgG conjugated with streptavidin Phycoerythrin (PE). The beads were then washed and simultaneously analyzed on a BioPlex reader set to read a minimum of 50 beads with unique fluorescent signature and the results expressed as median fluorescence intensity (MFI). These results were then exported in Microsoft Excel for data analysis. Healthy United States of America (USA)-based malaria-naive adult blood donors were used as negative assay controls for malaria antibodies. USA based infants yet to be immunized against measles and with no known previous measles aged 6-12 months were used as negative control for measles. Plasma from children 4-6 months of age shown to have maternal antibodies that had waned was used as negative controls for EBV. Kenyan adults who had antibodies against EBV, measles and malaria were used as positive controls.

3.6.2.3 Total IgG ELISA

Total IgG in maternal venous blood at the last scheduled visit was determined by ELISA using human IgG total ELISA kit from Thermo Fisher Scientific as per manufacturer's instructions. High protein binding ELISA (Thermo scientific) plates coated with monoclonal antibody to human total IgG, were washed twice with 400µl/well wash buffer (PBS- 1%Tween20). 100µl of the pre-diluted standards and samples were then added to the appropriate wells in

duplicates.100µl of Assay Buffer (PBS- 1%Tween 20-10% BSA) was added to blank wells. 50µl of diluted HRP-conjugate (Horseradish Peroxidase) antibody was added to all the wells, sealed, and incubated for 1hour at room temperature (18^0C to 25^0C) on a plate shaker. The plates were then washed four times with a wash buffer and 100µl/well of 5, 5, 3, 3, tetramethylbenzidine (TMB) substrate solution added. After 30 minutes of developing time, a 100µl stop solution was added to stop the reaction then plate read at 450nm on Imark microplate reader (Bio-Rad). Concentration in terms of optical densities (ODs) of total IgG in plasma was extrapolated from standard curves.

3.6.2.4 Placental Malaria Diagnosis

Malaria microscopy and placental malaria diagnosis were done on maternal samples before isolation of plasma for antibody detection from each participant recruited. Blood films were prepared from fresh blood obtained from finger-prick using a sterile lancet needle. Both thick and thin films were made to determine parasites per white blood cells and red blood cells respectively. The thin films were air-dried, fixed with methanol, and dried before staining with 7.5% Giemsa solution for 15 minutes and read at ×100 magnification using Nikon Eclipse E200 microscope. Parasite densities (parasite/ul of whole blood) were calculated by; (number of parasites counted/WBC counted) x 8000 WBC/ul (assumed count). To determine placental malaria, a 1x1 square centimeter piece of the placental biopsy was cut, fixed in methanol, and then screened for malaria parasites on a microscope by tough prep.

3.7 Data Management and Statistical Analysis

Data generated were entered into an MS Excel spreadsheet and analysis was performed in R version 3.5.1(Team & Others, 2013). Data were categorized as follows; case number, maternal antibody levels at enrollment(baseline/pretreatment), antibody levels at last ANC visit, antibody

levels in cord blood (neonate), antibody levels at week one and week six for the infants. Univariate descriptive statistics were calculated using Fisher's exact tests for categorical variables: The levels of anti-measles, EBV and malaria in mother infant pairs within the DP and SP arm of treatment was determined by Wilcoxon paired rank tests. Mann Whitney U test was used to compare median antibody levels between the DP and SP treated mothers and neonates. Cord to maternal ratio (CMR), the ratio of the level of specific antibodies in cord blood to that in the maternal venous blood was used as a measure of placental transfer of antibodies. Spearman correlation was used to determine correlation between antibody levels in maternal blood at last visit and cord. Linear regression analysis was used to determine the influence of confounding variables including; gestational age, arm of the study, malaria exposure, hypogammaglobulinemia and parity of the on the transplacental transfer of antibodies. All $P \leq 0.05$ were considered statistically significant.

3.8 Ethical Considerations

Permission to carry out this study was obtained from the School of Graduate Studies (SGS) of Maseno University, (Appendix 1). Ethical approval was obtained from the Scientific and Ethical Review Unit (SERU) at the Kenya Medical Research Institute (KEMRI), Kenya, (Appendix2) and from Liverpool School of Tropical Medicine London (Appendix 3). Clinical information for the parent study was obtained under the approval of KEMRI-SERU and written informed consent from the participants (Appendix 4). All participants were also identified on a unique ID that linked the samples with clinical and demographic information without revealing personal identifiers such as name.

CHAPTER FOUR

RESULTS

4.1 Demographic, Clinical and Laboratory Characteristics of the Study Participants

In total, plasma from 132 pregnant women were analyzed in this study. Out of which 63 were in the DP arm and 69 were in the SP arm, respectively. Out of the 63 in the DP arm, 39 mother-infant pairs were followed for six weeks after delivery and while 27 mother-infant pairs on the SP arm were followed for six weeks after delivery. A total of 66 infant samples were excluded from the analysis as they either reached the lab later than 3 hours from the time of collection or their visits coincided with weekends and therefore could not be processed on time. **Table 4.1** provides a summary of the demographic, clinical and laboratory characteristics of this study participants.

Table 4.1: Demographic, clinical and laboratory characteristics of the study population.

		DP	SP	*P*-value
	Mothers	n=63	n=69	
	Infants	n=39	n=27	
Age (median [25th -75th quartiles])				
Parity				
Primigravida		n=20	n=22	0.798
Secundigravida		n=21	n=26	0.503
Multigravida		n=22	n=21	0.896
Gestational age-weeks(median)				
Enrollment		21	22	0.471
Last visit		36	37	0.573
Delivery		38	39	0.514
Malaria exposure (positive)				
Enrollment		n=4	n=8	0.213
Last visit		n=3	n=7	0.673
Delivery		n=3	n=4	0.143
Birth weight (Median [25th -75th quartiles])		3.0 [2.7-3.4]	3.1 [2.4-3.02]	0.56

Data are presented as numbers unless stated otherwise. Mothers (n=132) were categorized based on the treatment arm; DP (n=63) and SP (69). Out of the 132, consisted of a complete set of mother-infant pairs (n=66) whose infants were followed up to six weeks with; n=39 in DP and n=27 in SP, respectively. Abbreviations; DP- Dihydroartemisinin Piperaquine and SP-Sulfadoxine Pyrimethamine. Differences in proportions between the groups were determined by the Fisher exact test. The groups were similar at baseline regardless of age, malaria exposure, parity and gestational age.

4.2 Levels of measles virus, EBV and Malaria Specific Antibodies in Maternal and their Infants from Nyando Sub-County Western Kenya

The levels of antibodies in the study population (132 mothers and 66 infants) for the antigens of interest were measured by Luminex Magpix technology and recorded as Median Fluorescent Intensity (MFI). This study first stratified anti-MV, EBV and malaria levels based on quartiles; MFI values below the 25th quartile was defined as low, between the 25th and 75th as medium, and those above the 75th quartile as high levels. The levels were then expressed as percentages as

shown in **Figure 4.1** About 25% of mothers and 26% of the infants had low levels of measles specific antibodies.

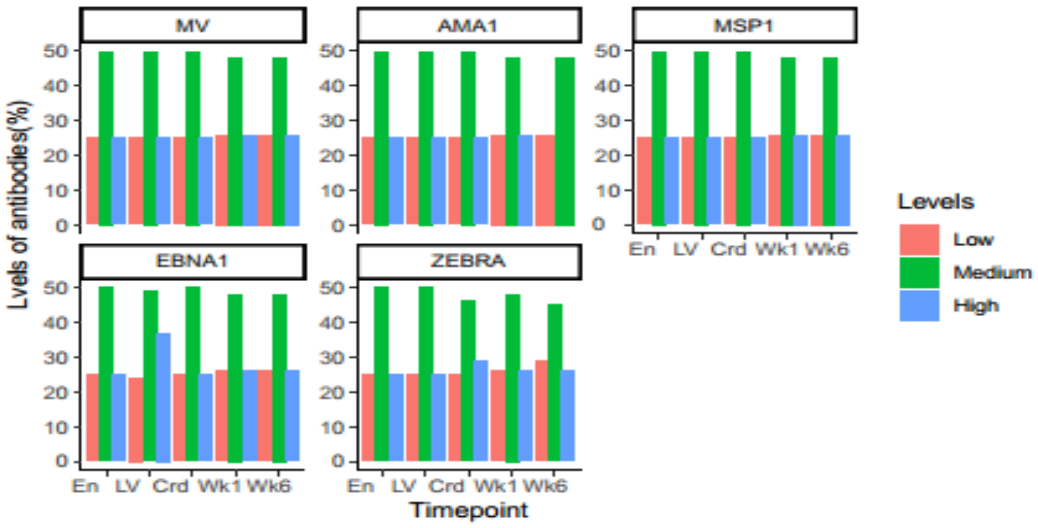

Figure 4.1: Data presented as percentage of counts of individual samples as stratified by the quartiles. Antibody levels to measles, malaria and EBV were measured by Luminex Technology and expressed as Median Fluorescent Intensity (MFI). MFI values below the 25th quartile were defined as low, between the 25th and 75th as medium, and those above the 75th quartile as high levels. Counts in each level were then expressed as percentage of the total sample size (Mothers-N=132, Infants-n=66). Key; En-enrollment, LV-last visit, Crd- cord, wk1-week1, wk6-week6.

4.3 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Noenates Treated with IPTP-SP

In order to answer the first objective, the study compared the levels of anti-measles, AMA1, MSP1, EBNA1 and ZEBRA antibodies in maternal, neonatal (cord) and their respective infant samples for the various time points undertaking IPTp-SP. This study reports a general trend of significant decrease in the median concentration of antibody levels specific for measles from enrollment (pre-treatment) to delivery. This trend in decline in antibody levels was also noted in MSP1, EBNA1, and ZEBRA antigens (**Figure 4.2a**). However, the median concentration of antibody levels for AMA1 was lower at enrollment (pre-treatment) compared to the other time points (last visit and in cord). Antibody levels during the last visit and cord (neonate) were

comparable for all the antigens on women undertaking IPTp-SP as illustrated in **Figure 4.2b**. Upon comparing antibody levels in neonates and infants' samples at one and six weeks respectively for SP treated mothers, measles specific antibody levels had significantly declined by six weeks post-partum (*P*. value **0.0052**).Antibody levels against AMA1, MSP1, ZEBRA and EBNA1 were comparable between the neonatal samples and infant samples at one and six weeks postpartum respectively. *P*-values are indicated on the figures with significant values at $P \leq 0.05$.

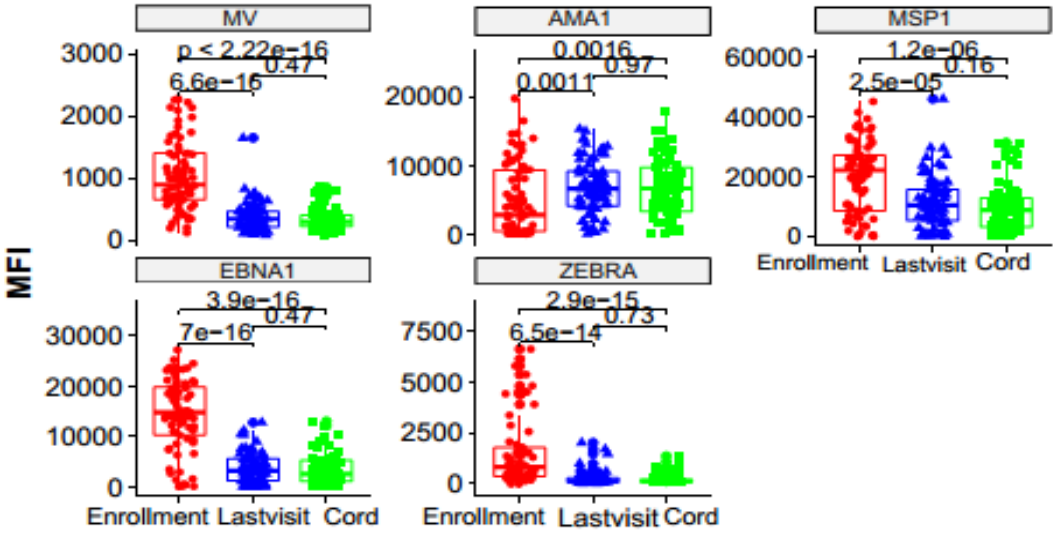

Figure 4.2a Data presented as Median Fluorescent Intensity (MFI). Levels of anti-measles, EBV, and malaria specific IgG antibodies in women and their respective neonates (Cord) treated with SP (N=132) were determined by Luminex. Mann Whitney U test was used to analyze the median difference between the three time points with the p values indicated on the figures. Centre lines represent median with lower and upper boundaries representing first and third quartiles respectively.

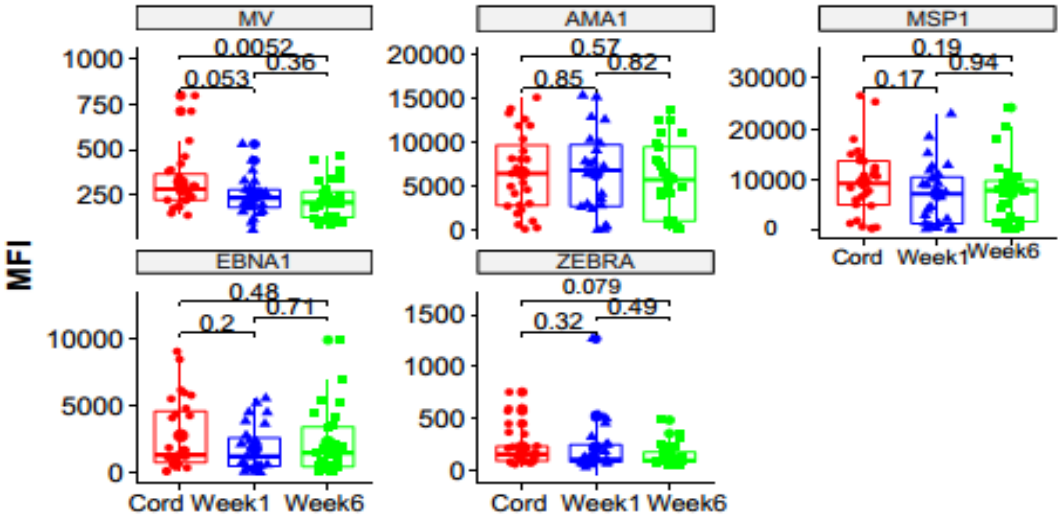

Figure 4.2b. Raw Median Fluorescence Intensity (MFI) levels of measles, EBV, and malaria specific IgG antibodies in neonates and infants from women treated with SP (n=27). Differences between the individual time points were determined by Mann Whitney U test. Centre lines represent median with lower and upper boundaries representing first and third quartiles, respectively.

4.4 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Infants Treated with IPTP-DP

Similarly, this study compared the levels of anti-measles, AMA1, MSP1, EBNA1 and ZEBRA antibodies in maternal, neonatal (cord) and their respective infant samples for the various time points undertaking IPTp-DP in order to answer the second objective. As seen in the SP standard care group, there was a significant decrease in the median concentration of antibody levels specific for measles from enrollment to delivery. This trend in decline in antibody levels was also noted in MSP1, EBNA1, and ZEBRA antigens (**Figure 4.3a**). The median concentration of antibody levels for AMA1 was however lower at enrollment (pre-treatment) compared to the other time points (last visit and in cord). Antibody levels during the last visit and cord (neonate) were comparable for all the antigens on women undertaking IPTp-DP as illustrated in (**Figure 4.3a**). When the study compared the levels of antibodies to measles, AMA1, MSP1, ZEBRA,

EBNA1 in neonates and infants' samples at one and six weeks, respectively, for DP treated mothers, the antibody levels were comparable for the three time points for all the antigens tested (**figure 4.3b**). *P*-values are indicated on the figures with significant values at $P \leq 0.05$.

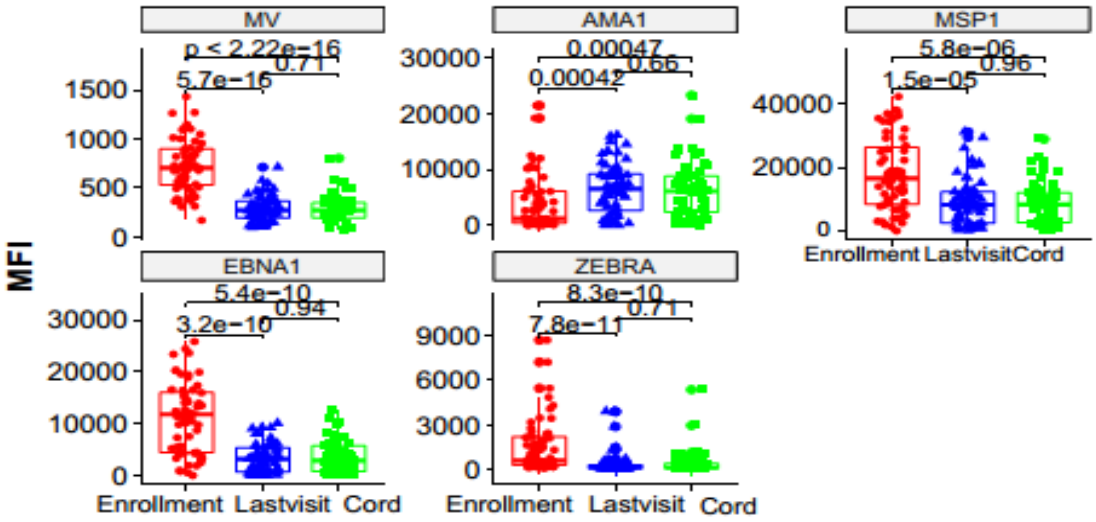

Figure 4.3a. Data presented as raw Median Fluorescent Intensity (MFI). Levels of anti-measles, EBV, and malaria specific IgG antibodies in women and their respective neonates (Cord) treated with SP (N=132) were determined by Luminex. Mann Whitney U test was used to analyze the median difference between the three time points with the p values indicated on the figures. Centre lines represent median with lower and upper boundaries representing first and third quartiles, respectively.

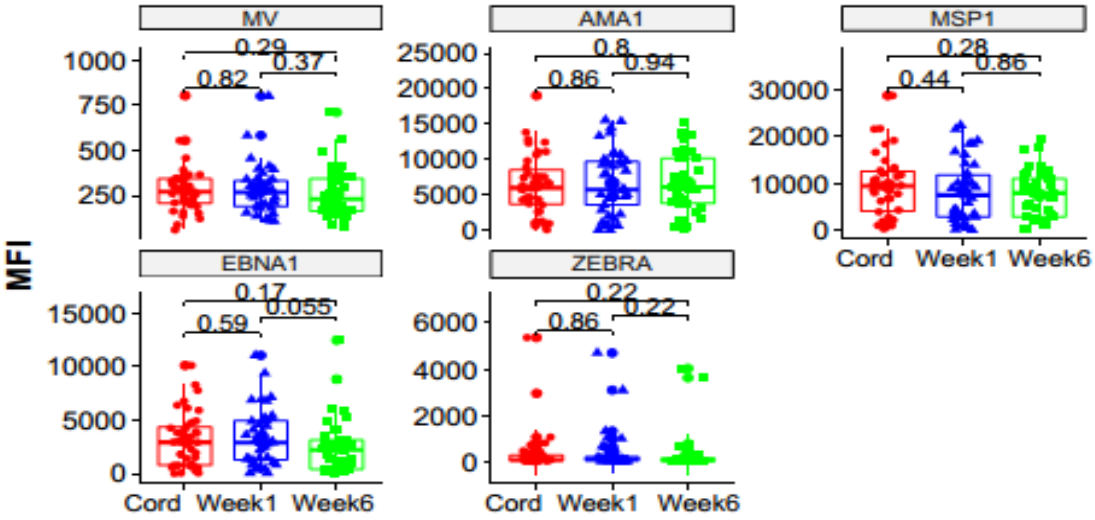

Figure 4.3b. Row Mean Fluorescent Intensity (MFI) levels of measles, EBV, and malaria specific IgG antibodies in neonates and infants from women treated with DP (n=39). Differences between the individual time points was determined by Wilcoxon rank test Centre lines represent median with lower and upper boundaries representing first and third quartiles, respectively.

4.5 Comparison of Levels of Antibodies between the DP and SP Treated Mothers, Neonates, and Infants

To investigate if exposure to any of the study drugs during pregnancy affected the antibody levels in the mothers or their respective infants, this study compared antibody levels between the two groups for the various time points. The antibody levels for the antigens tested were comparable between the two groups for the various visits for AMA1, MSP1, and ZEBRA antigens. However, antibody levels against EBNA1 and measles significantly differed at enrollment with a *P*-value of 0.0035 and 0.00057, respectively. Also, at week1, EBNA1 antibodies significantly differed between the groups with *P*-value of 0.011 as illustrated in **Figures4.4a-4.4e.**

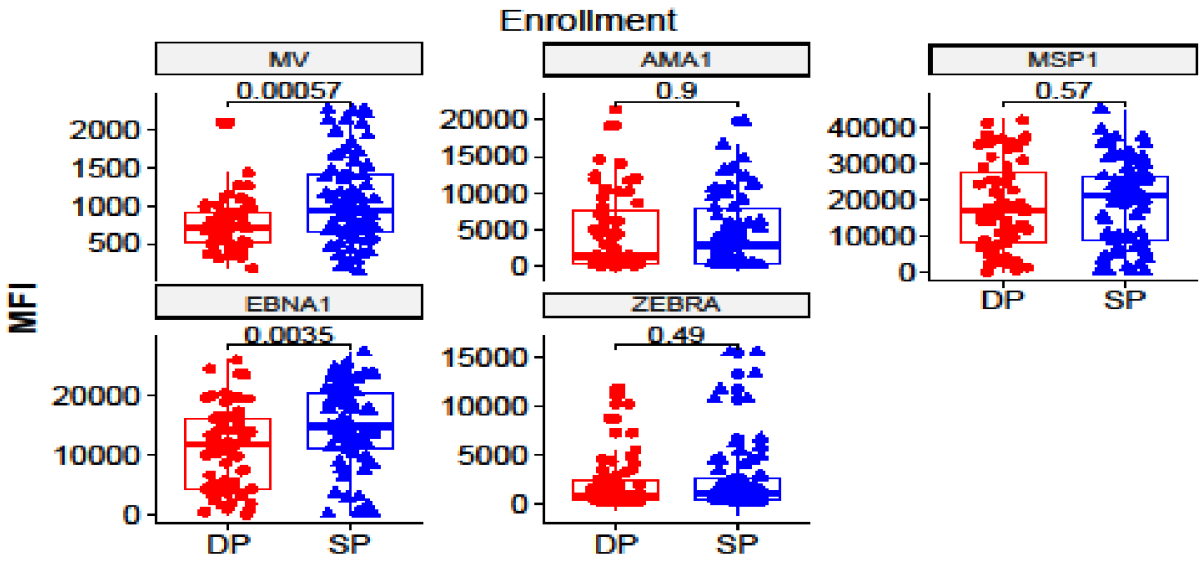

Fig 4.4a. Data presented as Median Fluorescent Intensity (MFI). N=132(DP=63 & SP=69). Median MFI levels of measles, EBV, and malaria specific IgG antibodies in mothers, from DP and SP arm of treatment were compared by Wilcoxon rank test. *P*. values on the figures represent the difference in median values between the two groups at enrollment. Centre lines represent medians with lower and upper boundaries representing first and third quartiles, respectively.

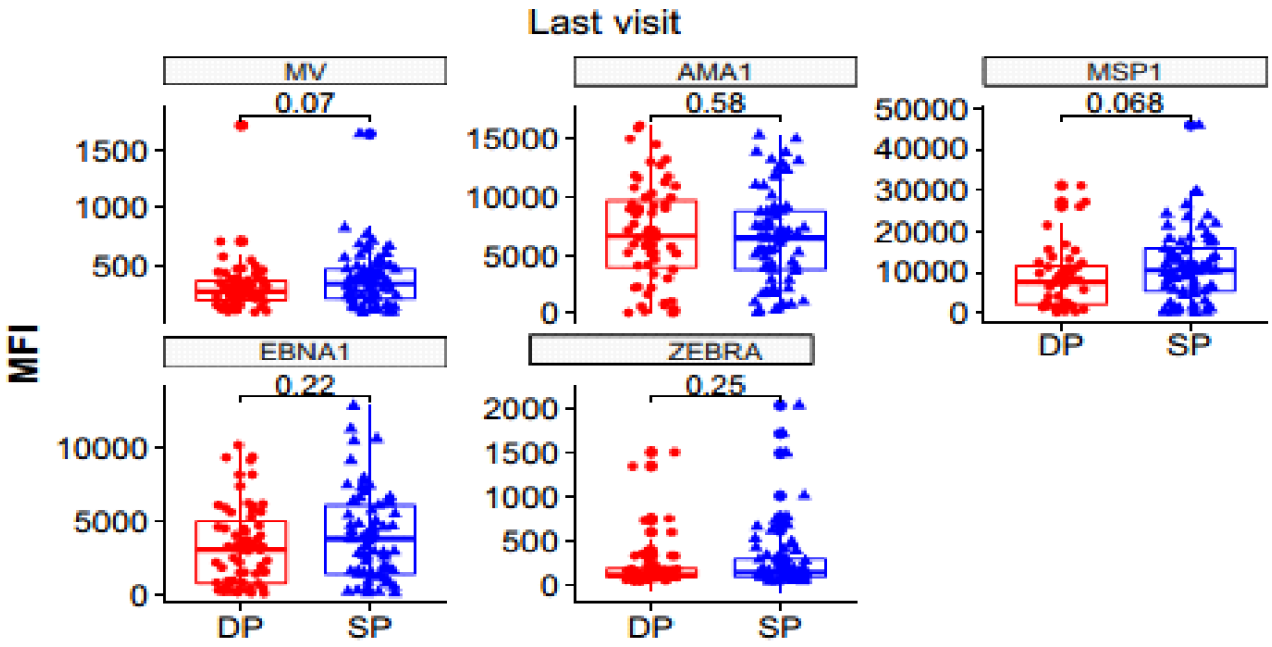

Figure 4.4b. Data presented as Median Fluorescent Intensity (MFI). N=132(DP=63 & SP=69). Median MFI levels of measles, EBV, and malaria specific IgG antibodies in mothers, from DP and SP arm of treatment were compared by Wilcoxon rank test. *P*-values on the figures represent the difference in median values between the two groups at last visit. Centre lines represent medians with lower and upper boundaries representing first and third quartiles, respectively.

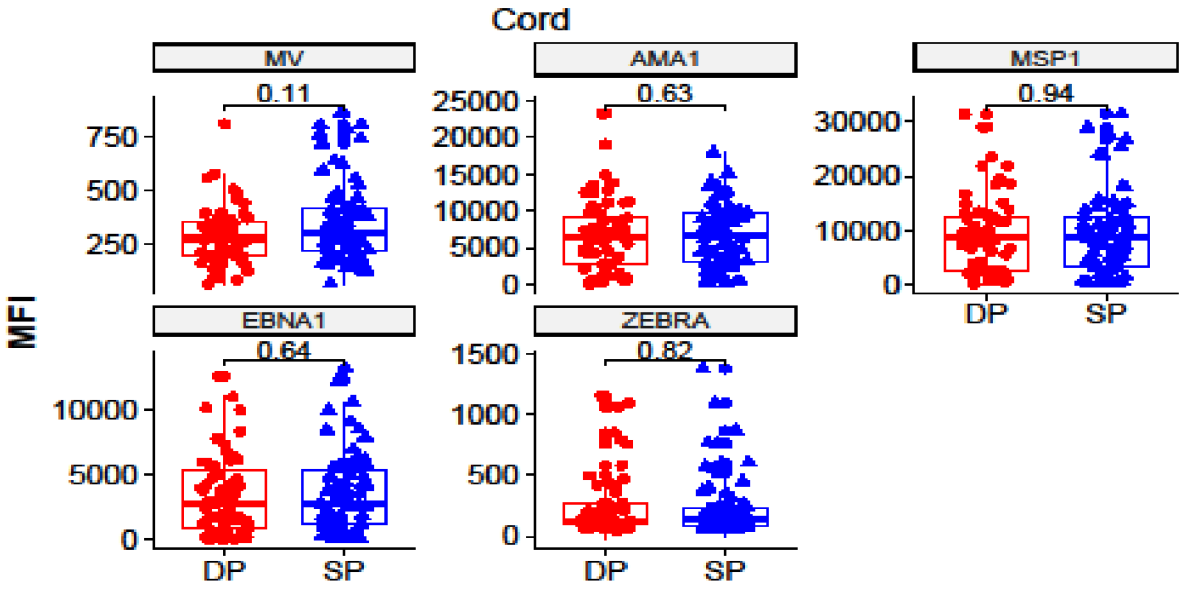

Figure 4.4c. Data presented as Median Fluorescent Intensity (MFI). N=132(DP=63 & SP=69). Median MFI levels of measles, EBV, and malaria specific IgG antibodies in mothers, from DP and SP arm of treatment were compared by Wilcoxon rank test. *P*. values on the figures represent the difference in median values between the two groups in neonates (cord). Centre lines represent medians with lower and upper boundaries representing first and third quartiles, respectively.

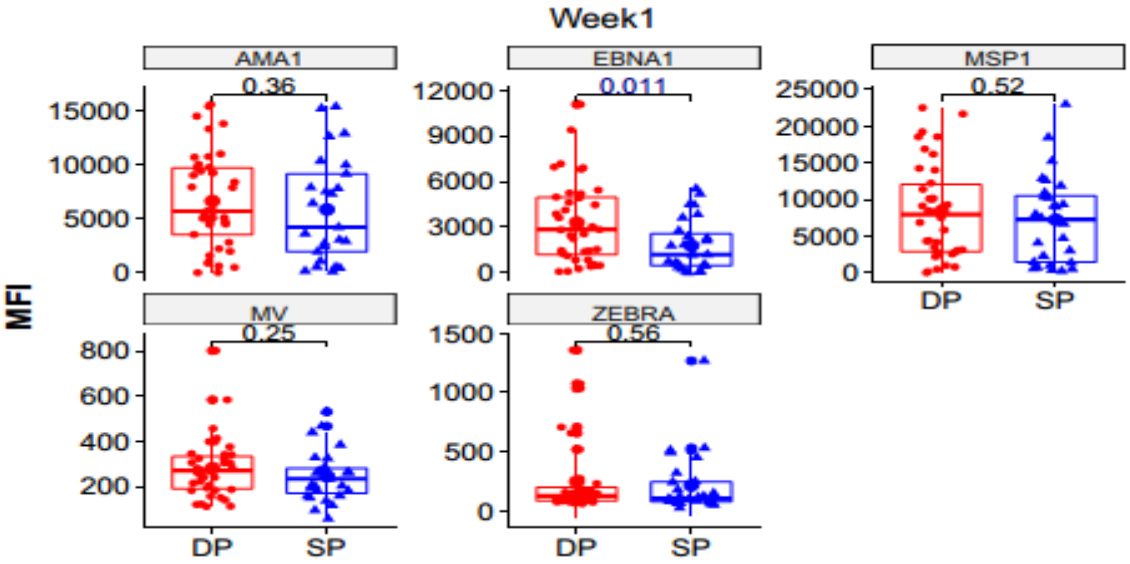

Figure 4.4d. Data presented as Median Fluorescent Intensity (MFI). N=66 (DP=39 & SP=27). Median MFI levels of measles, EBV, and malaria specific IgG antibodies in mothers, from DP and SP arm of treatment were compared by Wilcoxon rank test. *P*. values on the figures represent the difference in median values between the two groups at one week. Centre lines represent medians with lower and upper boundaries representing first and third quartiles, respectively.

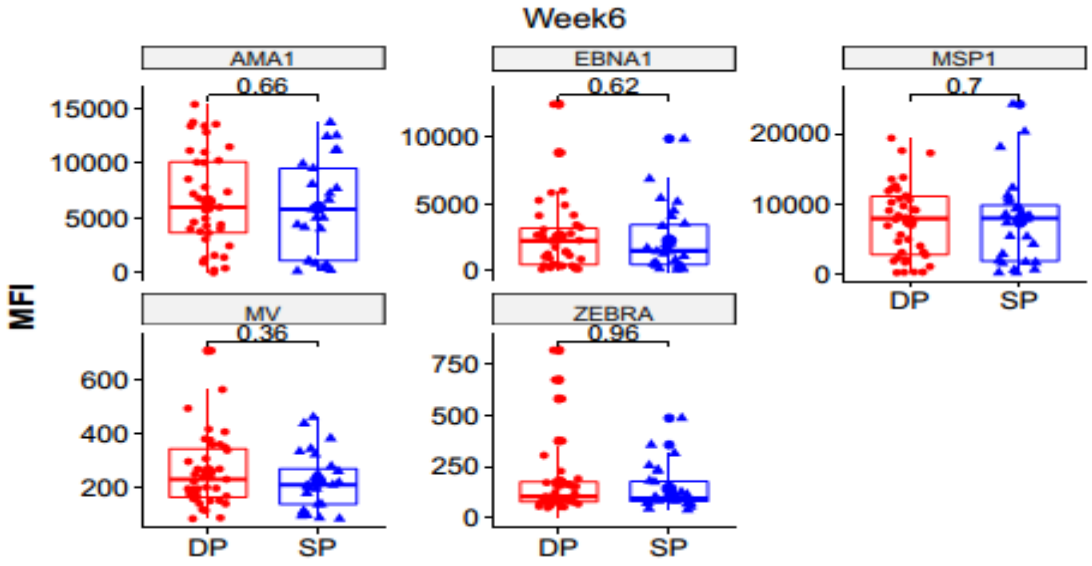

Figure 4.4e. Data presented as Median Fluorescent Intensity (MFI). N=66 (DP=39 & SP=27). Median MFI levels of measles, EBV, and malaria specific IgG antibodies in mothers, from DP and SP arm of treatment were compared by Wilcoxon rank test. *P*. values on the figures represent the difference in median values between the two groups in infants at six weeks. Centre lines represent medians with lower and upper boundaries representing first and third quartiles, respectively.

Since the study observed similar levels of anti-measles, EBV and malaria antibodies in mothers (last visit) and their neonates (cord), this study then correlated the specific antibody levels in the maternal venous blood to blood levels in their neonates to determine if the maternal level antibody level is a predictor of neonatal antibody levels. The study observed a significant positive correlation between antibody levels in maternal and neonatal levels for the specific antibody levels. **Figure 4.5** provides the summary for the significance levels and R statistics for the correlation.

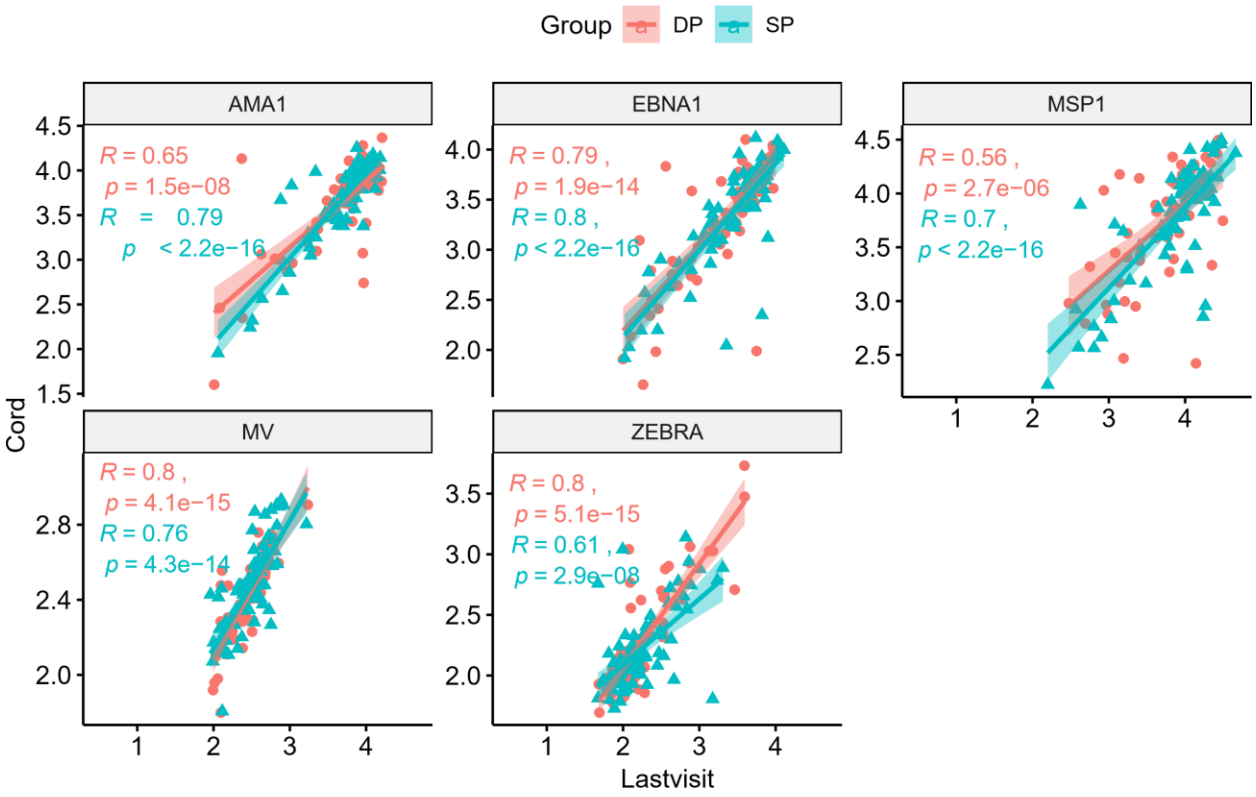

Figure 4.5 Correlation between Log-MFI of maternal and neonates (n=63; DP & n=69; SP) anti-EBV, malaria and measles in SP and DP treated groups. The correlation between maternal and neonatal antibody levels was assessed by Spearman correlation, where P<0.05 was considered significant.

4.6 Vertical Transfer of Maternal Anti-measles, EBV, and Malaria Specific Antibodies

This study next determined if there was difference in the placental transfer of antibodies between the SP and DP treated participants. Placental transfer was measured as the ratio of the level of antibody in cord blood to that of maternal blood at last visit, i.e., the cord blood/maternal blood ratio (CMR). There was no statistically significant difference in antibody transfer between the two SP and DP treated mothers **(Figure 4.6)**.This study further sought to determine if there was a reduction in measles, malaria, and EBV specific antibody transfer due to exposure to either of the study drugs. The percentage reduction in transfer of antibodies was determined by subtracting the median maternal antibody levels from last visit time point from that of cord and

the multiplied by 100. **Table 4.2** provides the summary of percentage reduction in transfer for the individual antigens stratified by arm of treatment. There was no significant difference in reduction in transplacental transfer of ant-malaria, EBV, and measles from the mothers to their neonates due to exposure to any of the study drugs as shown in **Table 4.2**.

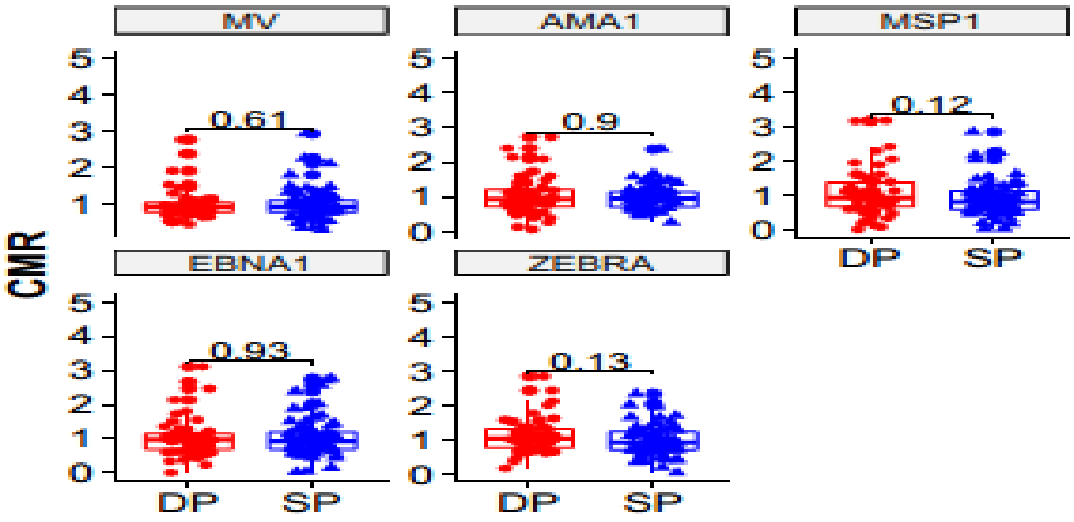

Figure 4.6. Data presented as the cord/maternal ratio (CMR). Difference in placental transfer was determined by Wilcoxon rank text.

Table 4 2: Reduction in Placental transfer of Antibodies

Antibody	Treatment	%Reduction	*P*-value
Anti-measles	DP-0.027(2.409-2.436)	-2.7	0.58
	SP-0.019(2.483-2.502)	-1.9	0.51
Anti-MSP1	DP-0.009(3.777-3.786)	-0.9	0.14
	SP-0.092(3.765-3.857)	-9.2	1
Anti-EBNA1	DP-0.007(3.276-3.283)	-0.7	0.93
	SP0.058(3.395-3.337)	5.8	0.44
Anti-ZEBRA	DP0.031(2.280-2.249)	3.1	0.7
	SP-0.032(2.230-2.262)	-3.2	0.7
Anti-AMA1	DP-0.03(3.640-3.670)	-3	0.49
	SP-0.007(3.668-3.675)	-0.7	0.8

Data represents the mean log10 MFI. Percentage reduction in transfer of antibodies was determined by the formula; (mean of cord -mean of last visit) x100. Statistical differences of $P \leq 0.05$ are considered significant as determined by paired t-test. (n=63; DP & n=69; SP).

When this study performed univariate linear analysis to determine the effect of maternal treatment on transplacental transfer of anti-EBV, malaria and measles antibodies using the SP arm of treatment as the reference group before adjusting for potential confounding factors such as maternal age, hyper/po-gammaglobulinemia status, parity and gestational at delivery with the SP treated group as the control group. It was observed that there was no significant association between malaria treatment with DP/SP on transplacental transfer of EBV, malaria and measles as shown in T**able 4.3.** The study went ahead to perform a linear multivariate regression while adjusting for the confounding variables mentioned, this too did not show any significant association

Table 4.3: **Univariate linear regression analysis for the influence of treatment on the transplacental transfer of EBV, malaria and measles**

Outcome	**OR (95%CI)**	***P*-value**
Anti-measles	0.84 (0.34-1.95)	0.539
Anti-AMA1	0.79 (0.55-1.14)	0.567
Anti-MSP1	0.99 (0.97-1.01)	0.842
Anti-EBNA1	0.77(0.56-1.21)	0.941
Anti-ZEBRA	1.03 (0.98-1.08)	0.126

Shows the univariate linear regression analysis for the CMR (Cord/Maternal Ratio) which was the measure of placental transfer. Using SP treated group as the reference group, the table shows the outcome for univariate analysis, (n=63; DP & n=69; SP).

CHAPTER FIVE

DISCUSSION

5.1 General Introduction

The global measles mortality rate has significantly declined over the past 18 years with a 73% reduction recorded between 2000 and 2018, with only 140,000 deaths occurring in 2018 from 23.2 million deaths (WHO, 2019). This significant decline in measles related deaths is attributed to high vaccination coverage globally with the current rate standing at 85% and 71% vaccination coverage for one and two doses of MMR vaccine, respectively (WHO,2020). In Kenya, measles vaccination coverage is estimated to be at 89% and 54% for the first and second doses respectively by 2019 (MoH, 2020). For eventual elimination of measles as set by the WHO African Region (AFR) by 2020, stopping measles transmission is key through establishment of herd immunity by achieving a vaccination coverage of more than 95% (Masresha *et al* 2018), a goal that Kenya is yet to meet as measles outbreaks and measles related deaths are still being reported.

The reemergence or resurgence of measles outbreaks in highly immunized populations has shifted attention on the efficacy and durability of vaccine-induced immunity with some studies reporting low antibody levels to measles in the vaccination era compared to the unvaccinated population (Leuridan *et al*., 2010).High vaccination coverage with a single-dose regimen has been unsuccessful in eliminating measles, necessitating the introduction of the second dose to provide protection for those with primary vaccine failure. IPTp treatment in pregnancy is recommended to reduce the adverse effects of malaria during pregnancy. These antimalarial drugs used have been shown to have immunomodulatory and immunosuppression effects on host immunity. The off target or other beneficial effects of these drugs in antibody transfer during

pregnancy remains unknown. This study reports the finding from prospective study of mother-infant pairs that received a single dose of MMR vaccine undergoing malaria chemoprophylaxis with either SP or DP.

5.2 Levels of Anti-measles Specific Immunoglobulin-G Antibodies in Mother-infant Pairs from Ahero Sub-County Hosital in Kisumu County, Western Kenya

In an attempt to understand the levels of measles specific antibodies in mother infant pairs from Ahero Sub-County Hospital in Kisumu County, western Kenya, this study stratified anti-measles antibody levels based on quartiles; MFI values below the 25^{th} quartile was defined as low, between the 25^{th} and 75^{th} as medium, and those above the 75^{th} quartile was stratified as high irrespective of the treatment arm. This study reports that more than 75% of the mothers and infants had high levels of measles specific antibodies. Previous studies looking at levels and seropositivity to measles among healthy Kenyan adults reported 96% seropositivity (Merke*l et al*., 2014). Other studies on pregnant women from other countries assessing levels and seropositivity to measles, reports seropositivity to measles to range from 70%- 90% (Dayan *et al*., 2005; Kennedy *et al*., 2006; Shoda *et al*., 2011). This study reports 75% of the mothers to have high antibody titers/seropositive to measles, a finding that is consistent than what is reported in other countries as reported by (Dayan *et al*., 2005; Kennedy *et al.*, 2006; Shoda *et al.*, 2011)and lower than what was reported in Kenya adults as noted previously (Merkel *et al.*, 2014). While all the mothers enrolled in the study received a single dose of measles vaccine, more than 20% of the mothers and neonates presented with low antibody levels to measles which translates to measles seronegative and increased susceptibility to measles inflectionally in life for the infants. This difference could possibly be due to variation in geographic vaccine coverage, genetic differences that affect vaccine uptake, interpersonal differences on the degree of wanning immunity as well as difference in exposure to measles virus.

5.3 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Infants treated with IPTP-SP and IPT-DP

When the study compared antibody levels in mother-infant pairs from the SP and DP arms of treatment, this study reports a trend in decline in anti-measles specific antibodies in mothers from enrollment (pre-treatment/baseline) to delivery. There was a significant decrease in antibody levels between the enrollment samples and the other samples at last visit and the cord (neonatal samples) at delivery. This trend in decline in antibody levels is attributed to increase in plasma volume during pregnancy that dilutes maternal antibodies as pregnancy progresses, a concept termed as hemodilution. Finding that is consistent with (Baboonian& Griffiths, 1983; Miller, 2009). This study also reports similar levels of anti-measles IgG in mothers at last visit and neonates at delivery as well as comparable levels of antibodies in neonates and infants at week1, infants at week 1 and week6. However, the levels of measles antibodies had significantly declined by six weeks postpartum in comparison with the neonates in the SP arm of treatment. Though not statistically significant, the median difference of antibody levels in neonates and infants at six weeks post postpartum in the DP arm had also declined. This therefore signifies an indication of early measles waning immunity in measles which is consistent with other studies that reported early waning immunity for measles antibodies (Leuridan *et al.*, 2010; Leuridan & Damme, 2007).

As seen with measles, a similar trend in decline of antibody levels during pregnancy was reported for MSP1, EBNA1 and ZEBRA antibodies throughout pregnancy in all the study groups due to hemodilution (Baboonian & Griffiths, 1983; Miller, 2009). However, antibody levels against AMA1 were low at enrollment but increased with uptake of IPTp and then plateaued at last visit and delivery, finding that is consistent with Stephens *et al*.., 2017 that reported a similar

trend for the GLURP malaria antigens. Also, antibody levels in neonates and infants were comparable for AMA1, MSP1, EBNA1, and ZEBRA antigens.

Antibody levels to measles are influenced by a number of factors including; age, vaccination status, natural exposure to measles, educational level, and socio-economic status (Bodilis*et al*., 2014). Data on level of education, socio-economic status, and disease history was not collected by the parent study and therefore were not assessed by this study. This study did not find any significant correlation between maternal age (range 15 to 40 years), malaria status, and measles antibody levels. This finding contrasts with the results of other surveys that found a significant correlation between measles seropositivity and age (Dayan *et al.*, 2005; Kennedy *et al*., 2006; Shoda *et al.*, 2011) but is consistent with other studies by (Honarvar*et al*., 2013). Collectively, these findings suggest that IPTp with SP or DP did not affect the levels of measles specific antibodies during pregnancy as the drugs possibly do not seem to exert their effects on plasma B lymphocytes that are responsible for antibody production.

5.4 Comparison of Antibody Levels Specific to MV, EBV, and Malaria in Mothers and their Infants Treated with IPTP-SP and IPT-DP

In order to answer objective three, the study compared measles specific antibody levels between the SP treatment which is the standard care and the DP treatment for the various time points investigated by the study. Antibody levels specific for measles, AMA1, MSP1, ZEBRA, and EBNA1 were comparable at last visit, cord and infants at week one and six weeks between the SP and DP treated groups. However, ant-measles and EBNA1 specific antibodies differed significantly at enrollment (pre-treatment). Since this difference was observed at enrollment just before the initiation of treatment, this difference could not be attributed to the effect of treatment, but it can rather possibly be due to small sample size. Previous studies addressing the effect of

malaria Intermittent Preventive Treatment in infants (IPTi) on vaccine serological responses during infancy reported no effect of malaria chemoprophylaxis on antibody serology against measles (Crawley *et al*.., 2012). Additionally, previous work on the effect of IPTP-SP reported decreased levels of malaria specific antibodies in women administered on SP which could possibly be due to reduced malaria incidences and thus low antibody production. This study reports no difference or effect of IPTP treatment with either DP or SP on anti-measles specific antibody levels. To the best of my knowledge this is the first study to investigate the difference and effect of IPTP with SP or DP on measles antibody levels.

5.5 Vertical Transfer of Maternal Anti-EBV, Measles, and Malaria-specific Antibodies

A number of conditions are known to affect the maternal-fetal transfer of IgG antibodies, including HIV infection, placental malaria, gestational age and maternal hypergammaglobulinemia (Okoko *et al.* 2001; Scott *et al*. 2005). In this study, only HIV negative mothers were enrolled, the study did adjust for total IgG antibody levels (hyper/hypogammaglobulinemia), maternal malaria status, parity and gestational age in the multivariate analysis, to evaluate the effect of IPTp on the transplacental transfer of antibodies. This study report no association between IPTp with either SP or DP for anti-malaria, measles, and EBV antibodies. Also, there was no statistically significant difference in the transfer of antibodies between women enrolled in the SP or DP arm of treatment. Previous work investigating the effect of IPTp with DP and SP on malaria antibodies did not find any of the study drugs to affect vertical transfer of maternal antibodies to their respective neonates (Desai *et al*., 2015; Stephens *et al*., 2017). Findings that are consistent with this studies' report on antimalaria antibodies.

5.6 Study Limitations

Exposure to wild type measles virus is a key determinant of anti-measles specific Immunoglobulin-G antibody levels. Women who are exposed to the wild type virus have been reported to have high antibody titers and longer duration of protection for their infants as compared to vaccinated mothers. This study, however, could not determine whether the measles specific antibody levels reported in the mothers here, were solely due to vaccination or some had natural exposure to the wild type virus. Additionally, this study did not have the opportunity to have a control group of women that were not enrolled for IPTp and therefore could not assess the influence of each individual drug on vertical transfer. Additionally, other factors such as level of education and socio-economic status were not picked up by the parent study and these were never evaluated.

CHAPTER SIX

SUMMARY OF THE FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Study Findings

This study determined the levels of anti-measles antibodies and the antigen specific effects of Intermittent Preventive Treatment in pregnancy with either SP or DP on mother infant pairs. The findings indicate that more than 70% of pregnant women from Ahero Sub County Hospital in Kisumu County, western Kenya have high antibody levels to measles which signifies immunity. We however note that more than that 20% of infants have low levels of measles specific antibodies at six weeks postpartum, therefore increasing their susceptibility to measles infection. The antibody levels to measles, EBV and malaria antigens tested were comparable between the two IPTp study groups for both maternal and infant samples. Neither of the study drugs induced immune suppression during pregnancy or influenced vertical transfer of maternal IgG to measles, EBV, and malaria.

6.2 Conclusions

i. IPTp with SP does not reduce the level of measles specific antibodies among women from Ahero Sub-County Hospital in Kisumu County, western Kenya.

ii. IPTp with DP does not reduce the level of measles specific antibodies among women from Ahero Sub-County Hospital in Kisumu County, western Kenya.

iii. There was no difference in antibody levels between mother-infant pairs between DP and SP treated participants from Ahero Sub-County Hospital in Kisumu County, western Kenya.

iv. Confounding variables (placental malaria, maternal age, parity and hypo/per-gammaglobulinemia) do not have an influence on anti-measles antibody transfer from

mothers to infants in women from Ahero Sub-County Hospital in Kisumu County, western Kenya.

6.3 Recommendation from this Study

i. IPTP treatment with SP is safe as it does not reduce the levels and subsequent transfer of measles specific antibodies in mother-infant pairs.

ii. IPTP treatment with DP is safe as it does not reduce the levels and subsequent transfer of measles specific antibodies in mother-infant pairs.

iii. Either SP or DP can be used for malaria chemoprophylaxis after considering their efficacy in malaria management as both did not have any influence on measles immunity.

iv. Though this study did not find any impact of the confounding variables on measles antibody levels and subsequent transfer, this could probably be due to small sample size, these factors therefore should be investigated further taking into account the natural exposure to measles virus as well as number vaccines doses given.

6.4 Recommendation for Future Studies

i. Future studies to assess the levels of measles specific antibodies prior to the initial measles vaccination at different ages on a larger scale, so as to address the would-be risk of vulnerability to measles before vaccination.

ii. The impact of IPTP-DP and SP on the IgG isotypes specific for measles should be assessed to have a complete picture of the effect of these drugs on measles antibody levels and transfer.

iii. Supplementary Immunization Activities (SIA) for measles should be continued in order to help acquire herd immunity and stop measles transmission.

iv. Since measles vaccine is immunogenic and measles outbreaks still occur there is a need to do a genetic profile of circulating measles virus, to determine whether there are re-emerging strains that are not covered in the MMR vaccine.

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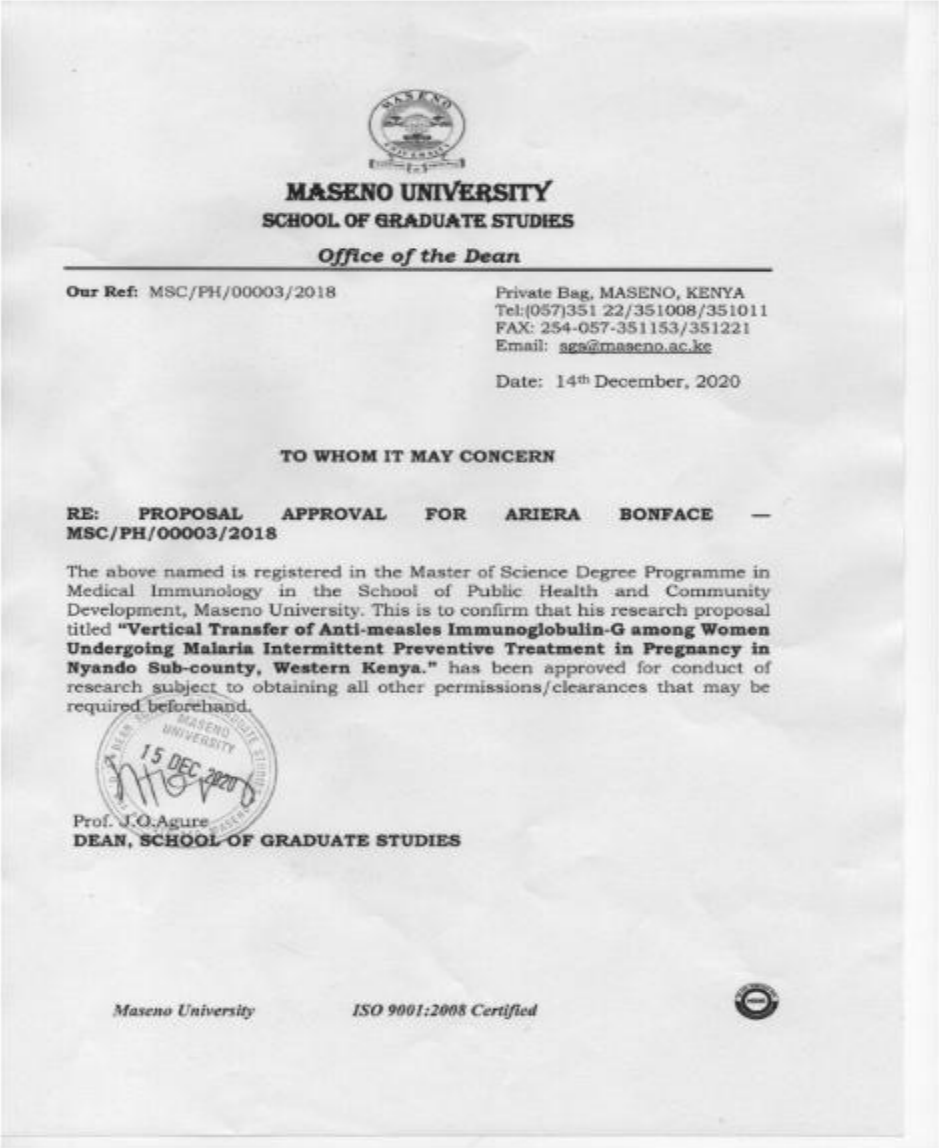
APPENDICES

APPENDIX 1: MASENO SGS APPROVAL



MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MSC/PH/00003/2018

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Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 14th December, 2020

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR ARIERA BONFACE — MSC/PH/00003/2018

The above named is registered in the Master of Science Degree Programme in Medical Immunology in the School of Public Health and Community Development, Maseno University. This is to confirm that his research proposal titled **"Vertical Transfer of Anti-measles Immunoglobulin-G among Women Undergoing Malaria Intermittent Preventive Treatment in Pregnancy in Nyando Sub-county, Western Kenya."** has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

15 DEC 2020

Prof. J.O.Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

APPENDIX 2: KEMRI-SERU APPROVAL

KENYA MEDICAL RESEARCH INSTITUTE

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Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **January 18, 2018**

TO: **DR. SIMON KARIUKI/PROF. FEIKO TER KUILE, PRINCIPAL INVESTIGATORS.**

THROUGH: **THE DIRECTOR, CGHR, KISUMU.**

24/1/2018

Dear Sirs,

RE: **SERU PROTOCOL No. 3421 *(RESUBMITTED REQUEST FOR AMENDMENT 1)*: IPTP WITH DIHYDROARTEMISININ-PIPERAQUINE AND AZITHROMYCIN FOR MALARIA, SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS IN PREGNANCY IN HIGH SULPHADOXINE-PYRIMETHAMINE RESISTANCE AREAS IN KENYA, MALAWI AND TANZANIA: AN INTERNATIONAL MULTI-CENTRE 3-ARM PLACEBO-CONTROLLED TRIAL (V2.2 28Nov17) (IMPROVE TRIA-2015-1076).**

Reference is made to your letter dated 28th November 2017. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledge receipt of the following revised study documents on 4th December 2017;

1. SERU amendment submission form.
2. Protocol v2.2 28 Nov 17 clean
3. Protocol v2.2 28 Nov 17 tracked

This is to inform you that the issues raised at the 268th Joint Committee B and C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **18th October, 2017** have been adequately addressed.

You are therefore **authorized** to implement the following amendments accordingly:

1. Administrative updates
   a. Addition of reference numbers issued by NatHREC and KPPB and TFDA.
   b. Addition of clinicaltrials.gov numbers.
   c. Addition of Dr Eva Maria Hodel from LSTM to replace Dr Christine Bachman.
   d. Addition of Dr Hellen Barsosio, a medical officer, to site specific addendum.
2. Removal of reference to the economic components from short and long summaries.
3. Update of the text for the nutritional assessment in new-borns.
4. Addition of heelprick sample from new-borns at day 7 and at 6-8 weeks follow up visit for diagnosis of malaria and antibody responses pg 11,35.
5. Addition of measurement of drug levels in cord and placental blood for safety (Table 1), umblical cord sampling pg 35, plasma piperaquine levels, full blood count, renal function and liver function pg 41.

APPENDIX 3: LIVERPOOL SCHOOL OF TROPICAL MEDICAL SCHOOL IRB APPROVAL

Professor Feiko ter Kuile
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
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Tuesday, 13 June 2017

Dear Professor ter Kuile,

Research Protocol (16-049) 'IPTp with dihydroartemisinin-piperaquine and azithromycin for malaria, sexually transmitted and reproductive tract infections in pregnancy in high sulphadoxine-pyrimethamine resistance areas in Kenya, Malawi and Tanzania: an international multi-centre 3-arm placebo-controlled trial'. IMPROVE (Improving PRegnancy Outcomes with intermittent PReVEntive treatment in Africa) (IMPROVE TRIA-2015-1076)

Protocol Number	Date	Date Received
v1.1	01May2017	18 May 2017

Thank you for your correspondence of 5 June 2017 providing the necessary Kenya in-country approval for this project. I can confirm that the protocol now has formal ethical approval from the LSTM Research Ethics Committee for Kenya based research activity.

The approval is for a fixed period of three years and will therefore expire on 12 June 2020. The Committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Continued adherence to all in-country ethical requirements.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee.
  Failure to do so could result in suspension of the study without further notice.
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements is a breach of the LSTM Research Code of Conduct and will result in withdrawal of approval and may lead to disciplinary action. The Committee would also like to receive copies of the final report once the study is completed. Please quote your Ethics Reference number with all correspondence.

Yours sincerely

Dr Angela Obasi
Chair
LSTM Research Ethics Committee

APPENDIX 4: IMRROVE CONSENT PARTICIPATION FORM

IMPROVEConsent forms-All-in-one(v2.2-28Nov17)
PIS and CS: English

2.1.2. Consentstatementforscreeningandparticipationinthetrial(allwomen) (English)

IPTpwithdihydroartemisinin-piperaquineand azithromycinformalaria,sexuallytransmittedand reproductivetractinfectionsin pregnancyinhigh sulphadoxine-pyrimethamineresistanceareasin Kenya,MalawiandTanzania

IMPROVESTUDY

ConsentStatementforScreeningandParticipationin theMainTrial

Yoursignaturebelowmeans thatyouvoluntarilyagreeforyouandyourchildtoparticipateinthis research:

TheabovestudyhasbeenexplainedtomeandIhavebeengiventheopportunitytoask questions.IagreetobescreenedtoseeifIamsuitabletobeinthestudy.IunderstandthatI am free tochoose to beinthis studyandthat saying "NO"will have no effect onme.	Please circle your response below
Iunderstandthatmyfurtherparticipationinthemaintrialwilldependonthetestresults.I also understandthatmyagreeingtobescreeneddoesnotmeanthatIhavetoagreeto beinthe study.Iunderstand thatIcan changemymindat any timefrom participatingwithouthavingto give a reason andwithoutanyconsequences to my health care. IfIameligibletoparticipateinthetrial,Iagreeformeandmychildtotakepartinthestudya nd understand that I am freetochooseto beinthis studyandthat saying "NO"will haveno effect onme or my child. Iagreeformy,ormychild'sbloodtobetestedforroutinetestsconducted forroutineante natal care(HIV, syphilis,lowblood)andforinfectionssuchasmalaria,forfactorsthatprotectagainst theseinfections,andfor drug levels.Ialso understandandagree that my ormychild's samples maybesent tolaboratoriesoutside the countryforteststhat cannot be done here.Ialso understand andagree forme,ormy child, tobe examinedandweighted.Iunderstandthat relevantparts ofmy,or mychild's healthrecords andfactscollectedduringthestudy maybe lookedatbyresearchstaff.I givepermissionfor these personstohaveaccessto my,ormy child'srecords.Ialsogivepermissiontosharethefactscollectedthroughthisstudy,with	**YES**(I do provide consent) **NO**(I do not provide consent)

outmy name andaddress or other informationthat may identify me.			
IDnumber:_._.___	Name	Signatureorthumbprint*	Today's date
Adultproviding consentforself			
Witness*			
Studystaffconsenting participant			

*Awitnessisonlyneedediftheparticipantcannotread.Thewitnessmustbeapersonindependentfromthe study.Theparticipantcanprovideathumbprintandverballystatehis/herconsentinthepresenceofa witness who will then sign.