DETECTION OF PREGNANCY ASSOCIATED MALARIA AMONG PREGNANT WOMEN AS A STRATEGY TO IMPROVE MOTHER CHILD HEALTH OUTCOMES IN BUNGOMA COUNTY, KENYA

JOSEPH MUKALA NKONGOLO

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DECLARATION AND APPROVAL

Declaration by the student

I the undersigned, declare that this thesis is my own original work and that it has not been presented in any other University or institution for academic purpose

Signature..... Date 03./.04./..2023
JOSEPH MUKALA NKONGOLO
PHDPH/2019/48569

Approval by the supervisors

We, the undersigned being the university supervisors for the above student confirm that this thesis was carried out and submitted by the candidate for examination with our approval.

Signature..... Date 03./.04./..2023
Dr. Dominic Mogere, PhD
Senior lecturer
School of Public Health
Mount Kenya University

Signature..... Date 03/04/2023
Dr. Peter Kirira, PhD
Senior lecturer
School of Pure & Applied Sciences
Mount Kenya University

Signature..... Date 3/4/23
Dr. Jesse Gitaka, PhD
Lecturer and Senior Research Officer
Directorate of Research and Development
Mount Kenya University

DEDICATION

This work is dedicated to you my wife Henriette Mitongo and my daughters: Josephine and Consolata for your support throughout this academic journey.

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ABSTRACT

Malaria is caused by a protozoa of genus Plasmodium and remains a major public health burden in the Sub-Saharan Africa. In Kenya the prevalence varies between 6.1 to 37% with harmful consequences to both the mother and her baby, including adverse pregnancy outcomes such low birth weight, high morbidity and mortality. However, effective antenatal strategies for improving maternal and child health outcomes through the prevention, early detection and treatment of malaria are still a challenge in resource-constrained settings. The objective of this study was to detect malaria and to determine its influence on the maternal and the child health outcomes. The response rate was 97%. Malaria test was conducted either via microscopy or rapid test before enrollment, then the cohort splits into malaria positive and negative. The sample size calculation was based on the prevalence of malaria in the unexposed group versus the prevalence of malaria in the exposed group according to the previous studies. Simple random sampling technique was used to enroll participants aged between 18-49 years and having about 16 weeks of gestation. The follow up period ranged from the first antenatal visit (March 2022) and delivery (December 2022). Permission was sought from relevant institutions and informed consent from the participants. Prerequisites on training, pre-testing of tools and standard operating procedures were satisfied. Categorical and outcomes data were analyzed using SPSS 27 and R plotting. Qualitative data were performed via Nvivo computer programs categorized under major themes and sub-themes. Chi-square, Fischer's exact and relative risk were used for bi-variate analysis at a p-value less or equal 0.05 (95%). The relative risk was 0.999, confidence level of 0.926-1.077. The prevalence of low birth weight was 4.6% with 6 cases of which 3 (4.5%) in the negative cohort and 3 (4.7%) in the positive cohort. Anaemic pregnant women were 41 (31.5%), HIV were 5 (3,8%), pre-eclampsia were 5 (3.8%), gestational diabetes were 2 (1.5%). Otherwise, majority of the participants were aged 18–25 years, were primigravida, were married, had secondary school level, earned between 20-30 thousand shillings, were resident in rural areas, and were in their second trimester. Marital status, gestational age and area of residence were associated with malaria but were not risk factors with a p-value less than 0.001, 0.001 and 0.028. A panel of sixteen proteins in malaria positive cohort, and six others in malaria negative cohort was identified after computing metadata sample using analysis of variance, t-test and adjusted Bonferroni with a relative influence of biomarkers varying from a mean difference of 2.856690795 to 0.217887462 in malaria positive cohort against -1.185322211 to 0.1622524175 in malaria negative cohort. There was difference in both cohorts with regard to knowledge of side effects p-value <0.01, different doses p-value <0.012 and prior information p-value < 0.003. The results revealed that birth cohort with malaria did not result in significant low birth weight. Therefore, this study recommends to conduct further research for a cost-effective test from the discovered novel biomarkers, which can be useful for low resource settings as an alternative option.

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LIST OF ABBREVIATIONS AND ACRONYMS

CDC	:	Centers for Disease Control and Prevention.
CIP	:	County Integrated Plan
DNA	:	Deoxyribonucleic Acid
FDG	:	Focus Discussion Group
FMS	:	Feline MC Donough Sarcoma
Hb	:	Hemoglobin
HIV	:	Human Immunodeficiency Virus
LLIN	:	Long Lasting Insecticide Treated Net
IPT-SP	:	Intermittent Preventive Treatment-Sulfadoxine Pyrimethamine
KDHS	:	Kenya Demographic Health Survey
KMIS	:	Kenya Malaria Indicator Survey
KMS	:	Kenya Malaria Strategy
MCH	:	Mother Child Health
MDG	:	Millennium Development Goals
MICS	:	Multiple Indicator Cluster Survey
PAM	:	Pregnancy Associated Malaria
PCR	:	Polymerase Chain Reaction
PEA/PLA	:	Proximity Extension and Ligation Assay
PFEMP-1	:	*Plasmodium falciparum* Erythrocyte Membrane Protein-1
PFHRP	:	*Plasmodium falciparum* Histidine Rich Protein
PMI	:	President Malaria Initiative
RDT	:	Rapid Diagnostic Test
SFLT1	:	Soluble FMS Like Tyrosine Kinase 1

SPSS	:	Statistical Package for Social Science
UNFPA	:	United Nations Population Fund
UNICEF	:	United Nation International Children's Emergency Fund
UNSDG	:	United Nations Sustainable Development Goals
WHO	:	Word Health Organization

CHAPTER ONE

INTRODUCTION

1.0 Introduction

This chapter discusses the general consideration and overview on background, problem statement, purpose, research objectives, hypotheses, significance/justification, scope, limitation, delimitation, assumption and operational definition of terms.

1.1 Background to the Study

Globally, malaria infection remains a serious communicable infectious disease. Malaria threatens the life of the half of world population and infected approximately 515 million people in Latin America, Asia and Sub-Saharan Africa region with one to three million deaths each year (WHO, 2021). Recently, malaria has affected 228 million people worldwide, with approximately 213 million in the Sub-Saharan Africa representing 93% of the total population. It is transmitted via female mosquito bites called anopheles, which are vectors of the widespread of the two causal agents namely Plasmodium falciparum and Plasmodium vivax. The most vulnerable persons being children and expectant women with consequences ranging from deadly complications such as anaemia, abortion, intrauterine fetal retardation, small gestational for age, prematurity and low birth weight. Indeed, the last decade was characterized by the effort to reduce malaria incidence from 71 to 57 over 1000 cases in the high risk zones (WHO, 2019). Worldwide low birth weight represents 15% to 24 % of burden. South-Asia and Sub-Saharan Africa are the most hit with 96% of cases, while a systematic review conducted in 2019 showed that 20.5 million newborns were having low birthweight representing 14.6% of all birth (WHO, 2014; WHO, 2019). Among as many as eleven million expectant women who were infected with malaria in 2018, the consequences translated to an estimated 872,000 low birth weights newborns being the highest record of 16% in

the Western Africa zone as compare to the central and Eastern Africa (Imboumy, 2020). The projections based on the global technical strategy 2016-2030 constitute a backbone of a new engagement that will excite many countries to accelerate malaria elimination programme, and contribute significantly to the reduction of malaria to 40%. Interestingly, countries that recorded less than hundred cases of malaria among autochthones population increased from seventeen in 2010, to twenty-five in 2017, and finally twenty-seven in 2018. Moreover, Algeria and Malaysia have not so far reported cases of malaria while China has been awarded a certificate of malaria elimination by WHO (Alonso *et al*., 2015; Zhou, 2021). The emerging issues of resistance of malaria to antimalarial drugs illustrated a lesson learnt from the misuse of septrin. Septrin is a medicine used to prevent pneumocystis carinii in immuno-compromised subjects.His misuse had produced collateral effect and led to resistance of Plasmodium falciparum in pregnancy, making it impossible for preventing mother-to-child transmission of HIV and malaria at the same time. The Global malaria control strategy also relies on insecticides and long-lasting mosquito nets to alter malaria consequences by increasing the rate of the total population coverage at 95% by 2025 as compared to 2006, where the essential of this strategy targeted the segment of population made with children and parturient (WHO, 2016-2030). The coverage of prevention method still is very low in the Sub-Saharan Africa according to relevant data available stressing that 25% of antenatal women were given only 2 times intermittent preventive treatment, and a one-third had used insecticide treated nets. From 2009 up to 2011 a survey done in Sub-Saharan Africa on the mosquito nets the previous night showed that only 35% either 5.2% up to 75% used the net, while Fansidar two doses was 24.5% compared to ANC second visit estimated at 84%. In 2015, the Fansidar and the net use were the less covered interventions from the package of all activities provided to pregnant women during

antenatal clinic (WHO, 2012). Roll Back Malaria partnership emphasizes that the antenatal care should be an entry point where every pregnant woman will be served with at least one dose of Fansidar and at least 80% will receive insecticide treated nets with ambition to reach 100% by 2025 (Pell *et al*., 2015). Sulfadoxine-pyrimethamine and mosquito treated nets remain highly cost-effective interventions proved to mitigate malaria burden among expectant women in developing countries. Consequently, the uptake and utilization still very low due to poor maternal knowledge and complicated guidelines and policy not facilitating easy task to healthcare providers to delivery promptly and efficiently this service during routine antenatal care (Hill *et al*., 2013). On the diagnostic efforts concerning malaria diagnosis, WHO issued a guideline which prioritizes malaria diagnostic before treatment to avoid therapeutic errors and minimizing unnecessary use of malaria drugs will have contributed to prevent resistance. Deletions genes consecutive to Plasmodium falciparum histidine rich protein 2 and 3 (pfhrp2, pfhrp3) which cannot be detected with rapid test in the proportion of 80% is a limitation of the rapid diagnostic tests in the area of endemic malaria. This limitation was found in Peru and Eritrea. Therefore, when the threshold of false negative goes beyond five percent it is recommended to conduct a survey to adopt the change of such method (Nsobya *et al*., 2021). However, biomarkers are essential to support the diagnostic effort. Likewise at Heriot-Watt University in the United Kingdom, this method was used to separate the plasma in view of discovering causes of diseases such as cancer, Zika virus, sepsis and malaria via nucleic acid and antigen. From these perspectives, new knowledge acquired on genomic markers sequence has highlighted the optimizing molecular diagnostic assay as matter of fact targeting a cytochrome oxidase sub-unit gene in a dried blood spot to detect malaria DNA and distinguish Plasmodium falciparum with Plasmodium vivax (Mielczarek *et al*., 2016). In 2019, WHO launched a vaccine called

RST, S/AS01 against malaria in Malawi, Ghana and Kenya to boost the immunity against Plasmodium falciparum in children with four backup doses. The doses offer a lifetime protection of four years that will prevent four out of ten cases of diagnosed malaria (WHO, 2019). In the effort to establish malaria diagnosis strategy, a study examined the real test effort to diagnose alteration of placenta in pregnancy associated malaria in the tropical zone, found that malaria and preeclampsia in pregnancy share common characteristics as the pathogenesis in both commences with the process of inflammatory response due to the erythrocyte damage. In malaria, there is sequestration of meroizoite/sporozoite in the placenta, with consequences reduced placental perfusion and release of free radicals such as soluble endogline, cytokine, soluble kinase tyrosine leading to fetal growth restriction, stillbirth, low birth weight, prematurity as well as increased morbidity and mortality. This study suggested that biomarkers to identify placental suffering may be combined to increase their specificity (Gueneuc *et al*., 2017). In line with different perspective to understand factors interacting with malaria, a study was carried in India as a cross-sectional survey focused on determinants of malaria associated with anemia in pregnancy. The survey established that during antenatal clinic, the complete history, vital signs and examination of expectant women were done simultaneously with blood sampling among parturient, in view of investigating the presence of Plasmodium. When results were out, pregnant women who were anemic were examined by medical doctors, benefited curative treatment with artemisinin combined therapy, and further advised to sleep in the insecticide treated nets. However, the result showed the vast majority of malaria cases in pregnancy were asymptomatic, while a few minorities exhibited signs like fever, nausea, dizziness and vomiting (Sohail *et al*., 2015). In Guinea on determinants of malaria in pregnant women, found that low immunity level exposed pregnancy to severe form of malaria, abortion, anaemia.

Moreover, this study found that women who resisted to malaria infection were those who lived more than 6 months in the same area, and they developed adaptability. It was highlighted a relationship absence between malaria versus occupation and education level, as well as the mean of transportation of the pregnant women while it is established that low socio-economic level, may prevent realization of full potential to comply with the malaria prevention guideline (Toure *et al*., 2019). Another research survey carried out in Gabon in 2020 focused on perspectives about awareness of malaria and use of accurate and efficacious methods or intervention tools targeting the improvement of the health status of expectant women and infants. This study highlighted that after use of intervention measures such as insecticide nets, 95 % of respondent knew that use of ITN prevented malaria transmission. Also, the study found that the communication channel using media constituted an important and reliable mean for information sharing in the community. The researchers recommended the establishment of collaboration between healthcare professional and media to increase the coverage (Imboumy-Limoukou, *et al*., 2020). In 2016, a study carried out in Nigeria on anemia associated with malaria revealed that 55.1% of expectant women were affected. However, malaria was a serious burden which led to high level of mother child negative outcomes (Okube *et al*., 2016). A research study conducted in Benin in 2017 revealed that there was absence of reliable information concerning malaria during the first gestation term due to the delay of antenatal visit, as well as the shortage of LLNI within various hospitals. However, there was an increased exposure to malaria as a result of the IPT/SP contra-indication during the first gestation term. These findings are a wake-up call for early antenatal care that can be beneficial to assist the pregnant women with necessary actions taken early to alter malaria complications as common leading cause of mother child morbidity and mortality (Accrombessi *et al*., 2018). In 2020, a research was carried out in Nigeria, Osun state

using a quasi-experimental design on the education effect of long-lasting nets during pregnancy. This study highlighted that policymakers should advocate for permanent educative effort aimed at improving the general knowledge among expectant women on the LLNI. The researchers found a positive relationship between the increase on the score of knowledge after the use of long lasting net (Sonibare *et al*., 2020). Addressing the matter of mother child health is sustainable development goal number three related to maintaining health and wellbeing. However, the millennium development goals 4, 5, 6 did not achieve its goals to reduce maternal and child mortality by two-third (WHO, 2017). Antenatal care visit is mostly started late in the middle of gestation instead of starting it immediately during the first term (Joyce, *et al*., 2012). Pregnant women do not proceed to antenatal visit in many areas after positive pregnancy tests (Gitimu *et al*., 2015). Kenya government malaria strategy plan 2019-2023 targeted to reduce significantly the death rate at 75% in 2023, it was stressed that 16% of outpatient visits realized were due to malaria episodes, and 75% in the high-risk group of people who can suffer from malaria any time. Factors such as weather, geographical location, rainfall and altitude determine malaria transmission in Kenya. Statistics show that 9.6 million people representing 19% were at risk of malaria in 2019 in the high altitude or highland zones. Bungoma, Kakamega and Baringo counties lie in this region, and they are also endemic zones along the Lake Victoria. The coastal region had 13.7 million representing 27% of population at risk of malaria. The transmission of seasonal malaria covers the northern and central part of the Country; the number of exposed persons were 11 million representing 23% of population at risk of malaria. Nairobi and its environs have 15 million of population representing 35% were at low risk (KMS, 2019-2023). Nonetheless, the strategies put in place to combat malaria, depend on one another and should be integrated in a form of a multisectoral relationship, whether it is about the

management of case, or about the prevention using sulfadoxine-pyrimethamine, mosquito nets or indoor spraying in different areas of malaria transmission, are coordinated by a regulatory national organ. The Kenya National Malaria Program working closely in partnership with other supportive agencies to assist the districts and counties as the execution level of ensuring the smooth process in line with prevention, detection, management of malaria cases based on WHO recommendations (KMS, 2019-2023). In fact, the survey done in Kenya found that 59% of family were covered with one LLIN, while 34% of family were using one LLIN for two members. About 42% of interviewees had used their mosquitos net one day before, 67% of dwellers in a household with at least one ITN while 39% of attendees during antenatal clinic having received a single dose of IPT/SP. Deworming strategy among expectant women still low rated at 30.7%. Iron-folate supplementation was estimated only at 60% with low level of knowledge noted among pregnant women on iron importance corroborating with studies pointing out lack of awareness, low education level, inadequate information, and poor antenatal attendance in large segment of pregnant women as big barriers to achieve the fixed goals for both uptake and utilization of the intervention (KDHS, 2014). The Kenya National Malaria Programme uses six main strategies to cut off the chain of malaria transmission in view of achieving its elimination by 2030 with strategies such as; appropriate prevention, malaria treatment according to the guideline, increasing of utilization of interventions measures put in place, strengthening activities and engagement for elimination to improve surveillance and information and finally, and to provide leadership for correct implementation of activities (PMI, 2020). A research carried out in Kenya in 2016 on malaria detection using rapid test found that the sensitivity and specificity of results were moderately acceptable to guide clinical decision in this resource constraint settings, while polymerase chain reaction test showed added

value and better performance but it is inaccessible and expensive (Wanja *et al*., 2016). The quantitative transcriptase reverse PCR is another highly sensitive genius method that can detect very low level of parasites during early stage of malaria by amplifying 18s ribosome DNA and RNA. This technique is very far superior to microscopy, which remains for WHO a gold standard for malaria diagnosis in evaluation of clinical trial, epidemiological surveillance, as well as eradication process (Kamau *et al*., 2011). A research study carried out in Kenya in 2017 about prevalence and determinants of malaria among children stated that malaria affects pregnant women and children from poor constraint resource settings. Barriers to access care included low revenue and low knowledge that hinder timely preventive action (Marufa *et al*., 2017). A research conducted locally in 2015 on socio-demographic factors, highlighted determinants to understanding the reason why malaria is still a big challenge and public health threat in the remote villages as compare to the peripheral settings, however findings revealed that remote areas were associated with long bushes, stagnant water along houses, which provide good breeding ground for mosquitos (KMIS, 2015). In the earlier decades, most Sub-Saharan African countries including Kenya made notable advances in efforts to improve mother child survival. However, Kenya has faced numerous barriers to quality healthcare. Some of the barriers include the problematic of underperforming health system, the new Covid-19 pandemic due to coronavirus from December 2019, and the challenges encountered by implementing universal health coverage. Other challenges include the ramping population demographic growth and epidemiologic shifts that have aggravated vulnerability with effect to contribute to the increase of mother child death by 2030 (Esamai *et al*., 2017; MOH, 2019). There are many other multifactorial components that have been enumerated in poorest resource settings which have contribute negatively to maternal and child health by pulling down performances, and

indicators of mother child well-being during pregnancy and after delivery such as poor and inefficient quality services, inequity in resource allocation and insufficient funding. Evidence-based researches have showed that the difference between developed and developing world resides in the increased burden due to high mortality and morbidity of newborns and women who have limited access to quality care. Other challenges include late and few antenatal contacts, delivery that occurs outside healthcare facilities. These challenges are attributed not only to the absence of skilled personnel but also inadequate and insufficient materials that can provide lifesaving support to the vulnerable category of this population segment made with expectant women and their infants (Brizuela *et al.*, 2019; Goudar, *et al.*, 2012).

In Kenya, the free maternity strategy established in 2013 and Beyond Zero Campaign initiative launched in 2014 were government efforts concerning aspects for the enhancement of critical areas targeting expectant women and infants contributing to the reduction of HIV through prevention mother-to-child transmission. However, these efforts appear like non-significant contributor factor to maternal and infantile death in the Kenyan context (Victor, 2014). The County Government of Bungoma listed malaria and anaemia as the most frequent disease burden in its integrated development plan 2018-22. The county government targeted to bridge existing gap by inverting the low rate of maternal and infant indicators through the offer of quality antenatal care service to the vulnerable population made with expectant women and infants to be deserved. It was estimated that the half of pregnant women in the county had not achieved ANC four visits as recommended by the WHO guideline. The current WHO guideline has reviewed the recommended antenatal care schedule increasingly from the previous four visits to the eight visits. Government statistics show a low rate of antenatal service in rural areas with 46% against 56% of their counterparts in the urban areas. In contrast, the national

ANC four visit is estimated at 52%. The strategies adapted to control malaria in this endemic zone concentrate on malaria prevention tools such as the use of mosquito net barriers, insecticide spay in high transmission areas, killing mosquito larvae, conceiving and applying adapted methods that contribute locally, and efficiently to reduce malaria incidence among vulnerable women and their unborn infants, and at the same time improving the community case management to the acceptable level of healthcare standards (CIDP, 2018-2022; KDHS, 2014). Neonatal morbidity and mortality constitute a serious public health threat in Kenya. Bungoma County is one of the most vulnerable with 31 deaths over 1000 live births, against a national average of 22 deaths over 1000 live births. On the other hand, a child survival project led by a team of researchers of Mount Kenya University and other local, and national partners is a tangible effort toward reduction of 30% of the mortality. The program has been implemented to improve and offer a reliable and accessible service through community participation and healthcare providers' commitment to reverse the trend of neonatal death. However, it is an ongoing project that has not yet reached its expected improvement indicators (Gitaka, *et al*., 2018). A household survey study carried out in Kisumu County in 2015 rated Plasmodium falciparum at 28% prevalence in female participants. A high likelihood of 40% of these participants being exposed to suffer from both malaria and anaemia on the heart of an entity prone to higher co-infection HIV-malaria. These findings could explain the reason why parturient exposed to malaria had significant low immunity at the same time (Jenkins, *et al*., 2015). A previous research study which was done in Bungoma County on contributing factors that impact negatively expectant women affected by malaria infection, found a prevalence of 21.6% with high likelihood of infection during the beginning of pregnancy or within the first three months of gestation as compare to the other remaining time during which the fetus is under maturation up to the delivery.

The study found that Plasmodium falciparum was the commonest type rated at 83%, the density of the parasite in the thin blood film was higher for the same type. The study recommended continuous screening, case management and preventive measure, as well as continuous health education session to curb malaria infection (Wekesa *et al*., 2018).

1.2 Problem Statement

Globally, malaria affects 25 million pregnancies, 228 million people worldwide with approximately 213 million in the Sub-Saharan Africa representing 93% of the world population with a significant magnitude among children and expectant women leading to all kind of complications, the commonest being low birth weight which represents 15% of all babies in the world (WHO, 2019; WHO, 2021). The relationship between malaria and low birth weight has been controversial, some research found that malaria was 11 times risk factor associated with low birth weight, or associated with preterm births or the use of intermittent preventive treatment-sulfadoxine pyrimethamine third dose was associated with high birth weight, while other studies did not conclude on the association (Dombrowski *et al*., 2018; Chua *et al*., 2021; Kayentao *et al*., 2013; Mikomangwa *et al*., 2019; Kalinjuma *et al*., 2020; Kipisi *et al*., 2017). In Africa, there has been a tendency to the decrease of malaria prevalence from 40% to 24% since the year 1900-1929 and 2010-2015 (Smith C, Whittaker M, 2014). In Nigeria, the prevalence of malaria was estimated from 58% to 66% (Dawakis, S et al., 2016). In Uganda,malaria accounts for 30-50% in outpatient visits (MOH, 2020). In Kenya, malaria range between 6.1% to 37% (DHIS, 2019).In the Sub-Saharan Africa region, data showed that malaria was responsible of high infant mortality rate, and that among ten children who died during the same period, nine of them died due to malaria (WHO, 2015). There were 2.6 million newborns deaths recorded in 2016, the majority could have been prevented through the use of quality care. In Kenya, maternal mortality is estimated at 362/100,000

live births nationally against 129/100,000 in Bungoma County. Sustainable Development Goals target to reduce it to below 70/100,000 live births by 2030 (Hug *et al*., 2017; KDHS, 2014; Say *et al*., 2014). Malaria infection still is a high burden health problem explained partly by socio-demographic determinants, poor health systems response, geographical location, favorable weather and environmental conditions like poor hygiene, long bushes, stagnant water along houses, which provide good breeding ground for mosquitos (Oladeinde *et al*., 2012; KMIS, 2015). In Kenya, there is an estimated 3.5 million new clinical cases of malaria and 10,700 deaths due to malaria, and those living in western Kenya where Bungoma belongs as a prone geographical area pay a huge tribute as compare to the other areas in the country (CDC Kenya, 2018). Different studies estimate prevalence of malaria during pregnancy in the country from 6.1%, 9% to 18%, 21% and 28%. These two high prevalence were recorded in the endemic lake areas and in Bungoma where expectant women of age group 18-27 years residing in the rural areas were more likely to be infected (Kenya DHIS2, 2019; Nyamu *et al*., 2020; Wanyonyi et al., 2019; Jenkins *et al*. 2015). Pregnant women and children are high-risk groups for malaria as they are typically affected most severely by the disease because of their low immunity, low level of preventive measures with sulfadoxine-pyrimethamine and long lasting insecticide treated nets recorded as the less covered activities during antenatal clinic (WHO, 2012). Malaria in 2018 was listed with anaemia as main causes of disease burden in the County; albeit half of pregnant women did not achieve antenatal four visits corroborating of low rate of antenatal service in rural areas of 46% against 56% in urban areas (CIDP 2018-2022). The utilization and uptake of highly cost-effective interventions such as sulfadoxine-pyrimethamine and mosquito treated nets are associated with poor maternal knowledge, complicated guidelines and policies issues baring healthcare workers to deliver efficient routine antenatal care (Hill *et al*., 2013).

Multiple indicator cluster survey highlighted that out of 57% of population who slept under the mosquito nets, 70% of them were pregnant, 22% only had received 2 doses and plus of intermittent preventive treatment. The County recorded 31 deaths of less than five years over 1000 live births against an estimated national average of 22 deaths over 1000 live births (Ndwiga *et al*., 2014; Gitaka *et al*., 2018; MICS, 2013-2014). Barriers to access these interventions remain poorly investigated in the current context (Hill *et al*., 2013). Innovation in terms of malaria identification, eradication and diagnostic is crucial for control and therapeutic rationality. WHO insists on testing before treatment to minimize resistance of malaria and gene deletion cases which are well documented in Sub-Sahara Africa and in Kenya. Gold standard methods based on microscopy and rapid test are confronted to the incapacity and limitation in diagnostic. Other methods are amplification of nucleic-acids based assay like polymerase chain reaction and loop-mediated isothermal amplification. In the low constrained settings prone malaria zone, these methods are often inaccessible and expensive. Therefore, malaria community skills should be oriented to continuously improve diagnostic and healthcare providers capabilities towards malaria control (Madkhali *et al*., 2022; Rajasekariah *et al*., 2023; WHO, 2015-2016, WHO, 2022). There is a critical shortage of credible studies in Bungoma County which can highlight the added value to detect, estimate and measure the occurrence of low birth weight as the one of main outcomes associated with malaria during pregnancy. These findings can contribute to improve malaria eradication and Sustainable Development Goal 3 by 2030. Addressing mother child health is a noble and crucial task insightful for changing current trends on malaria prognosis and resistance. Thus, resolving issues of deletion genes and false negative with contribution of biomarkers discovery can make difference for malaria eradication. Through existing potential proteins, abundance, diversity, interaction and association will malaria

detection in mother and child be improved and achieved? This is a question to be demonstrated to back the existing gap in the literature and informing audience through the new findings and perspectives. This is the reason as to why this study was conducted on detection of pregnancy associated malaria among pregnant women as a strategy to improve mother child health outcomes in Bungoma County.

1.3 Purpose of the Study

This study aimed at detecting pregnancy associated malaria among pregnant women in Bungoma County and to determine its influence of on the maternal and child health outcomes. This study was designed a nested prospective cohort, tasked with testing, enrolling and follow-up of respondents over time until delivery to determine if malaria constituted a serious major risk factor associated with low birth weight in Bungoma County. Malaria arena requires new perspectives to improve pregnancy detection, maternal and child outcomes with the contribution of Olink Proteomics discovery. Healthcare providers who are at the heart of preventive and cost-effective methods in the facility, their knowledge are key for the quality care service provision and elucidate the contribution of this service to attain maternal and child well-being. This interaction is crucial to evaluate the overall awareness on the side of pregnant women and those in charge of care in view of reducing high burden of malaria in pregnancy. A such ground can support and improve the health of both mother and child by tackling malaria as a deadly infectious diseases towards the Sustainable Development Goals Three.

1.4 Objectives of the Study

1.4.1 Broad Objective

To detect and determine the influence of malaria on low birth weight, assess the awareness, attitude and practices of pregnant women, as well as the knowledge of

healthcare workers on the intermittent preventive treatment and long lasting insecticide treated nets.

1.4.2 Specific Objectives

The study will be guided by the following objectives;

i. To establish socio-demographic factors associated with malaria among pregnant women.

ii. To determine the association between low birth weight and malaria among pregnant women.

iii. To ascertain the key proteomic biomarkers associated with malaria among pregnant women.

iv. To assess the awareness among pregnant women on the intermittent preventive treatment and the insecticide treated nets.

v. To assess the level of knowledge of healthcare workers on the intermittent preventive treatment and the insecticide treated nets.

vi. To assess the practices of pregnant women on the intermittent preventive treatment and insecticide treated nets.

1.5 Hypotheses

i. There is no significant association between socio-demographic factors and pregnancy associated malaria among pregnant women.

ii. There is no significant association between low birth and malaria among pregnancy women.

iii. There is no significant association between the proteomics biomarkers and pregnancy associated malaria.

iv. There is no significant association between pregnant women with awareness and without on the intermittent preventive treatment and the insecticide treated nets among pregnant women.

v. There is no significant association between the knowledge of healthcare workers on the intermittent preventive treatment and the insecticide treated nets.

vi. There is no significant association between pregnant women with good practices and without on the intermittent preventive treatment and insecticide treated nets.

1.6 Justification of the Study

Addressing maternal child mortality and morbidity has remained a global priority and a public health concern with emphasis on the current trends of malaria and its consequences on the general population in the Sub-Sahara Africa region, where Kenya and the selected County for this study "Bungoma" exhibit strong evidences that despite existing efforts, collective work and policies put in place, malaria in pregnancy still is a topic of high magnitude due to its high prevalence, morbidity and adverse pregnancy outcomes (Wekesa *et al*., 2018). Sustainable Development Goal 3 targets by 2030 to reduce maternal mortality at 70/100.000, child mortality at 25/1000 and neonatal mortality at 12/1000 live births while in Bungoma the current child recorded mortality still is above 30/1000 live births (Alkema *et al*., 2016). Therefore, in a health system with a mixed-up of multiple challenges and the resurgence of epidemics such as SARS-Covid 19 pandemic, lack of knowledge and ignorance, diagnostic and prevention measures gap heightened the curve of non-performance, and need to be addressed under innovative vision. Tackling malaria as one of main infectious diseases through a strong decision-making with target to improve insight on diagnostic, therapeutic, immunologic and prognosis can thereby contribute to better solutions. The novel Olink Proteomics technique can reduce a such gap between developed and developing countries with new

knowledge for healthcare workers once the results are available. This kind of cost-effective interventions are preferably needed to reach out to the entire community and the country at large to reduce adverse pregnancy outcomes.

1.7 Scope of the study

This was a prospective hospital-based cohort mixed methods qualitative-quantitative. The work was centered on malaria infection during pregnancy at Webuye hospital. Participants were enrolled and followed up to the delivery to determine the outcome of interest. Healthcare workers were questioned. The duration was from (March 2022) to (December 2022). Blood sample was collected for malaria test and the surplus used for proteomic analysis at Uppsala University. Questionnaire were used for collection of data as well as focus group discussion with pregnant women. Inferential and descriptive statistics were used for data analysis.

1.8 Limitation of the Study

This work was a nested cohort study anchored in the university study titled "Interdisciplinary research for an integrated community directed strategy to eliminate malaria in tropical Africa". The site chosen was Webuye hospital in Bungoma County, which one of malaria prone zone in Country. The study was tasked with objective to recruit pregnant women after testing for malaria which was considered as exposure factor, collect consecutive blood samples to identify predictive biomarkers, approach the use of some standards healthcare activities likewise; intermittent preventive treatment/SP and long lasting treating nets, and follow up pregnant women until the delivery. The strength of the cohort study is when there is a large sample size, population recruitment which was not the case of the current study where the respondents were selected at the clinic. Although cohort design can generate meaningful epidemiological data, it lacks randomization, therefore the relationship between different variables can be

generated only as association. Cohort study stands for observational study with no formal randomization for the assignment to the exposure or non exposure. Relative risk determine the risk of the event in the exposed versus the risk of the event in the unexposed.

1.9 Delimitation of the Study

Pregnant women socio-demographic factors were used as independent variables and analyzed by the means of Chi-square and Fisher' exact to determine the association. To determine the outcome of interest which was the low birth weight, the study enrolled pregnant women from 16 weeks gestation subdivided in both cohorts (malaria positive and negative) and followed up until delivery to determine the low birth weight as an outcome of interest in this study. The relative risk was used to measure the outcome of interest in this study.

1.10 Assumptions of the Study

There was a reflect of representative with the sample size for this study in both cohorts, and the exposure in both groups of participants was well defined. Malaria test was conducted before enrollment. A positive test was corresponding to the exposure and a negative test was non-exposure. The comparability of the outcome of interest in both group.

1.11 Operational Definition of Terms

Antenatal care: implies or defines healthcare standards deserved to pregnant woman by a skilled and trained medical professional, in the way that she must be healthy in achieving good outcome for both mother and baby throughout the period from conception to the delivery and post-delivery (WHO, 2016).

Detection: is defined as a process of checking a well-being or asymptomatic person in view of discovering a hidden condition by the means of tests and examination in a rapid and easily manner to the concerned population, and people who will be having the disease will be subjected to the treatment (WHO, 2016).

Low birth weight: is the weight of a newborn at any given gestation being less than 2500 g at birth time (Huo *et al*., 2015).

Malaria: is a parasitic and an infectious communicable illness transmitted through the bites of an Anopheles that inoculate a pathogen namely "plasmodium" responsible for the infection spreading in the body of a human being (WHO, 2015).

Mother child health outcome: comprises indicators of well-being during and after delivery such as mode of delivery, birth weight, pregnancy associated complications, stillbirth, prematurity among others (Moller *et al*., 2019).

Nested study: is a study which is conceived with subjects from the parent study to fit inside the same methodology (Turner, J.R, 2013).

Outcome: means endpoint or event, is a specific or oriented result or an effect trigged by use or no of anticipative action that can be measured. It is in addition a variable that is monitored during a study and susceptible to influence positively or negatively the final result under effect of external exposure or intervention over

the health of a given population. For example, normal delivery with both alive mother and child (Juliana, 2014).

Proteome profiling: is a technique or a new method for protein analysis in which protein complex is analyzed by translation of the signal to amplifiable DNA ligation or extension (Fredrik, 2011).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter focused on the research conclusions of others scientific publications with insightful views to inform the current research topic, including the development of the empirical literature model, the theoretical model, the operational framework, the research gap and the literature review summary.

2.2 Empirical Literature

Globally, malaria infection remains a serious communicable infectious disease. Malaria threatens the life of the half of world population and infected approximately 515 million people in Latin America, Asia and Sub-Saharan Africa region with one to three million deaths each year (WHO, 2021). Recently, malaria has affected 228 million people worldwide, with approximately 213 million in the Sub-Saharan Africa representing 93% of the total population (KMIS, 2019-2023). In Africa, there has been a tendency to the decrease of malaria prevalence from 40% to 24% since the year 1900-1929 and 2010-2015 (Smith C. & Whittaker M, 2014). In Nigeria, the prevalence of malaria was estimated from 58% to 66% (Dawakis, S et al., 2016). In Uganda,malaria accounts for 30-50% in outpatient visits (MOH, 2020). In Kenya, malaria range between 6.1% to 37% (DHIS, 2019). The literature review is broadly used to highlight historical perspectives about malaria among pregnant and understand the implications of the different variables developed in this study. Malaria infection in pregnancy prevalence is estimated globally between 6.1%at the global of the specific objectives and demographic factors n the perspective to search the essence of interaction and ideas grounded in the current study, there was substantial need to explore scientific papers related to the topic being developed. Each objective was guided by current and accredited body of knowledge in

the area of malaria associated pregnancy as to develop the arguments and critical findings before taking own view. Different objectives which were formulated in respect to the research aims, were equally developed in a scientific way to establish and address the controversial relationship between malaria and variables under study. The aims of the current research study were to establish socio-demographic predictors, which can explain malaria infection progress in pregnancy, to determine the association between low birth weight and malaria among pregnant women, to ascertain the key proteomic biomarkers associated with malaria among pregnant women, to assess the awareness among pregnant women on the intermittent preventive treatment and the insecticide treated net, to assess the level of knowledge of healthcare workers on the intermittent preventive treatment and the long lasting insecticide treated nets, and to assess the practices of pregnant women on the intermittent preventive treatment and insecticide treated nets. The literature review was assigned specific task to deepen with a critique focus lying on the insights of various publications in the malaria arena. These publications were mainly pertaining to malaria during pregnancy for a timeline of 10 years to find out author's conclusions and the findings or nature of statistical association, which could explain the relationship between the dependent, independent and outcome variables under the study (strong association, weak association or no association at all), strength of association, research design, sampling methods and other statistical model that have been used. Thereafter, these facts were looked upon to determine, if they could have had the same significance as per the current research study, how do they interrelate or exclude each another before arriving at the same or different conclusions. Therefore, this judgmental aspect could have significantly contributed to the logical comprehension straightforward to guide the scientific perspectives on the current topic focused on malaria in pregnancy.

2.2.1 Socio-Demographic Factors of Pregnant Women with Malaria

This objective aimed at establishing socio-demographic factors associated with malaria among pregnant women through review of previous research findings. Malaria and its consequences among pregnant women with focus on the relationship with the socio-demographic variables such as age of the expectant woman, marital status, the school attainment level, income level, gestational age, occupation and distance to the health facility will inform different strategies developed to control, combat and reduce malaria adverse effects. The current and past considerations of the wide scientific knowledge on the enumerated factors was very paramount to understand the kind of interrelation between malaria and the enumerated variables. A cross-sectional research study conducted in Sabatia Kenya found that the age median for participants was around 26.3 years, the majority were married, attained secondary education, unemployed, who were above 13 weeks' gestation and residing in the rural (Mutanyi *et al*., 2021). A hospital based case-control research study found that most of the participants were aged 25 – 34 years of age either 38%, multigravida (44%). Furthermore, the gestational age yielded a statistical relationship with malaria with p-value equal 0.002 (Afrifa *et al*., 2017). A research study examined socio-demographic characteristics of expectant women under prospective cohort design and showed that the majority of respondents were married and between 25-29 years of age (Agyem *et al*., 2020).A prospective research design was conducted in Moshi Municipal, Northeast Tanzania, Rufiji District and Coastal Area between July and October 2012 found that most participants were having a mean age of 25.2 years, with a gestational week of 37.2 weeks while in second/third trimester of pregnancy (Mosha *et al*., 2014). A retrospective research design was conducted in Ghana and showed that most of pregnant women were aged 20 to 29 years and rural dwellers. However, a significant number of these women were not married, and had no formal

education over a period of 5 years (Oppong, *et al*., 2019). A descriptive cross-sectional research was done in Mali targeting 10 health facilities, found that pregnant women mean age was between 20 to 29 years, the majority lived together with a husband with school attainment status equivalent to secondary were between 4 – 6 months of gestational age (Webster, *et al.,* 2013).A research design carried out in western Kenya in 2020 using cross-sectional analytic design investigated factors influencing malaria in pregnant women. The study concluded that socio-demographic factors significantly associated with malaria were age less than 25 years either 67% as compare to those above 25 years either 33.7%, or the place of residence with odds ratio 5.7, confidence level of 95% (Bally *et al*., 2020). A research design conducted in a community within Mwene-Ditu Township in the Congo-Kinshasa having a cross-sectional design on the prevalence and the risk factors of malaria among expectant women. The findings concluded that women who were married were yielding a statistical relationship with malaria, being single woman was a risk factor associated with increase of likelihood to suffer from malaria as compare to married women. Their mean age was 27 ± 6 years with low limit age at 17 years and higher limit at 43 years (Jean-claude *et al.*, 2018). A research design based cross-sectional was done in India on determinants of risk factors of malaria infection and anemia among expectant women found that the medium age for the respondents was between 18 to 38 years. This research finding showed that most respondents (94%) had acceptable status of formal education, those living in own house were 75.4%, household were 76%, only 12.3% were involved in farming, primigravida represented only 33.3%, (Sohail, *et al.*, 2015). In 2016 a descriptive research was carried out in Nigeria concerning what expectant mothers know concerning strategies which can prevent malaria and the use of IPT/SP. This study highlighted that women average age was 29 years ranging between 16 to 45 years. The majority were in the business (traders) representing 160

(36%), followed by students with 98 (22%), housewives with 53 (12%). Those who completed secondary education were the majority 158 (41%), primary education 68 (22, 6%) and no education 37 (8%). Christians were the majority with 76%, muslims 24%. Married 95.8% and single 4.2%. Multigravida 185 (41%), primigravidae 162 (36%) and secondgravida 103 (22.8%). The average gestation age 28 weeks ranging 8 to 38 weeks (Chukwurah, *et al*., 2016). A descriptive research design carried out in West Ghana in 2018 about the price of malaria treatment and maternal behavioral factors towards children care, a sample size of 574 women was obtained. The characteristics of participants were as follows; 433 women representing 76% were aged between 20 to34 years, 347 women representing 61% were illiterate, indeed only 3 women either 1% had post-secondary education level, their occupation was majorly related to farming with 81% of attendees (Dalaba *et al.*, 2018). A research design conducted in Malawi in 2015 using the malaria indicators survey about Malawian expectant women knowledge on when to bring the child with fever for treatment and preferences for healthcare, the women characteristics were described as follows; the average mean age of participants was 27.9 years, male counterparts were representing the majority of household 20 heads with 79%, education level was low, the majority of expectant women living in the rural area. This study concluded that the factor age and the residence were significantly associated with seeking healthcare in the public facility (Oyekale, 2015). In 2019 a descriptive research study was conducted in Guinea on determinants of malaria among expectant women. Furthermore, the finding showed that expectant women living in urban had low likelihood to develop the disease as compare to those living in the rural settings. Furthermore, profession, mean of transport to the health facility were not significantly associated with malaria infection as well as education level. However, it was clearly demonstrated that low socio-economic level influenced in a negative way a person to

achieve malaria prevention activities. Therefore, exposing pregnancy to the complications and risks that could have been avoided (Toure *et al.*, 2019). In 2017 a cross-sectional study was conducted in Senegal focused on determinants of uptake of intervention strategies in pregnant women with malaria. The average mean age of participants was between 20 to 34 years, while those beneath 20 years were only estimated at 10%, the majority with 65% never attended school, 12% only achieved high school level. The majority resided in the rural. 20% reported attending first antenatal contact in the first trimester of gestation either at six weeks (Mbengue *et al.*, 2017). In 2019 descriptive research examined the risk factors of malaria in slums with high prevalence. The study concluded that there were participants with low school attainment representing the majority with 72%; indeed 4% only had achieved high education level. Respondents who had malaria were 48.9%; the majority of them were unaware that in the area there was neither the existence of a midwife, nor a facility such as a health center. Participants who were also living in a sustainable house were unexposed to malaria compare to those living in a house made with adobe bricks (Hasyim, *et al.*, 2019). A cohort research conducted in Benin in 2018 looked at the determinants of malaria among parturient. It found that the average mean age of parturient was 26.8 years, the vast majority were multigravida either 92%, the mean number of antenatal visit was 2 for both planned and unplanned visits. Those with education level were 27% while 73% illiterate. Besides, 63% were monogamous, 64% had a body mass index between 18-25 kg/m2 with gestational age at first ANC corresponding to 6.7 weeks, 2% were HIV positive. Finally, 34% of the respondents were anemic with mean hemoglobin of 11g per deciliter (Accrombessi, *et al.*, 2018).

A research survey was carried out in Western Nigeria under cross-sectional design on the determinants of malaria among parturient in 2015. In that study the respondents mean

age was 26 years, 36% either the one-third of the sample size were illiterate, while 25% were at the primary level, and 21% were secondary level, and finally 17% were post-secondary. The study later concluded that the age was not having a strong relationship with malaria prevalence (Fana, *et al.*, 2015). In 2020, a research survey was done in Eastern Tanzania Moshi under cross-sectional design on determinants of malaria parasite in the placenta and its effect on the infant weight. The study highlighted that the majority of attendees either 72% were aged between 20-35%, while 34,6% were illiterate, only 10% had primary education level, 64% were married, 58% were multigravida, and 85% had gestational age above 37 weeks and 94% had used nets 48 hours prior to the study (Mariki and Chilongola, 2020). A research survey was done in 2020 in Eastern Uganda using a cross-sectional design on factors associated with malaria prevention practices and neonatal outcomes highlighted that parturient mean age was 24.63 years ranging between 14 to 43. The vast majority of the parturient either 81% had a life earning below 300,000 Shillings each month. Moreover 51.9 % attended secondary and post-secondary school level, and 59% of 124 participants were primipara or paucipara, 88.5% of 186 attendees were married, only approximately half of them attended at least more than four antenatal visits (Nekaka, *et al.*, 2020).

In 2019, a study done in Bungoma County under a cross-sectional design on determinants of malaria and analysis of risk affecting expectant mothers found that the majority were aged 18 to 27 years either 128 (79 %), they had elevated risk to be infected with malaria, and were residing in the rural 129 (79.6%) as compare to those living in the urban area with 33 (20.4%). Additionally, muddy house exposed to malaria with 110 (67.9%) as compare to cement house 52 (32%), primary school level was more prone to malaria with 79 (49%) compare to post-secondary and higher education with 22 (13%), unemployed 7 (53.7%) were more prone to malaria infection as compare to those employed, second

trimester was more prone to malaria as compare to others (Wekesa, *et al*., 2019). A research survey done in Kwale County in 2020 under cross-sectional was designed to evaluate risk factors of asymptomatic malaria infection and anaemia. 308 interviewees aged 26.6 years with the interval of 20 to 32 years old. The average gestational age was 21.8 weeks with the interval of 15.8 to 27.8, and 173 expectants women represented more than the half of attendees either 56.2% while in their second trimester of pregnancy, and 40 (12.9 %) had positive malaria against 62.7 % who had asymptomatic malaria. In fact, women below 20 years old had thrice the likelihood of having malaria in pregnancy as compare to those > 20 years. Moreover, the asymptomatic malaria likelihood was higher in pregnancy gestation above 16 weeks (Nyamu *et al*., 2020). A research survey was carried out in Rwanda in 2021 comprising a cohort design on the evaluation of the outcomes parameters among expectant mothers having complicated and non-complicated plasmodium. The number of respondents was 446 participants. The majority had simple malaria (80.9%), the predominant age cohort 18 years and above (16-44), the majority were in the rural (85.4%). There was no strong relationship among parturient having complicated malaria in the urban versus rural, neither the age, nor the gravidity were statistically significant as compare to the severity. The gestational age correlated to the severity where third trimester was more severe. In fact, complicated malaria had 85 either 57.6% in third term of gestation, while the second term could only account for 29.4% (Ntasumumunyange *et al*., 2021). A research survey carried out in Congo-Kinshasa in 2014 under cross-sectional design on the diagnostic method of malaria detection in pregnancy. The average age was 27 years (22 to 33 years). Primigravidae were 26%, the average gravidity was 3 pregnancies, the vast majority were married with 81% while 66% of them had not finished secondary school, 37% attended clinic at 12-24weeks. of gestation. Young women were more vulnerable to malaria as compare to old

women in that they do not have natural immunity. Single women were likely exposed as compare to married. Finally, the gravidity was not found statistically associated with Plasmodium falciparum (Matangila *et al*., 2014). A research survey carried out in 2017 in Nigeria Abeokuta Ogun state on determinants of malaria in pregnancy and delivery with a sample size of 211 women. Age group (28-33 years) were more prone, and placental infection was also common with 18 women either 19%, against only 8 cases either 8.5% of malaria infection in the umbilical cord. Furthermore, multigravida were less exposed than primigravida as well as for placental and cord blood malaria (Babalola *et al*., 2017). A research survey carried out in Nyando District, Nyanza in 2016 on Effectiveness observed during delivery of antenatal care interventions in pregnant women. Mean age was 23 years (11 to 46), 31% were primigravida, 43.7 % in the first visit, 23.4% second visit and only 26.3% in third visit. Gestational age 30 weeks within a range of 10 to 42. Notably, first visit attended during the first trimester was only 1%. Luo 84.0 %, Luyha only 7 % and Kalenjin 4.2%. The study did not conclude on strong relationship concerning socio-demographic factors and mother child interventions (Dellicour *et al*., 2016). A research survey carried out in Mwene Ditu town in Kinshasa-Congo under community cross-sectional design revealed no relationship concerning plasmodium and location of respondent. Those living in rural were 11%, peri-urban 16% and urban 14%. Moreover, living in peri-urban had high prevalence of malaria as compare to the rural hence no relationship between groups (Jean-claude *et al*., 2018). A research survey carried out in Kakamega County under cohort design found that marital status and gestational age were observed to have no significant association with rate of malaria infections, p-value of 0.683 and 0.419 respectively (Waiswa *et al*., 2022). A research survey done in Bumula division of Bungoma County under a facility longitudinal design found that village of residence and marital status (married 92.5%)

provided to have a non-significant association to malaria infections, p-value 0.188 and 0.848. In a study done in Kenya, there was no relationship reported concerning gestational age and malaria; p-value greater than 0.05 (Makokha, 2014). A randomized controlled trial research study was conducted in Mali looking at the harmlessness of IPT/SP among expectant women and found that not married was statistically significant among expectant women with malaria with p-value less than 0.001 (Kayentao, 2014). A hospital based descriptive research was carried out in Ebonyi state Nigeria and found that the first and the second trimester of pregnancy was statistically significant among expectant women with p-value less than 0.001 (Iyke *et al*., 2013). A cross-sectional research conducted in a community of expectant women living in the Sherkole District (West Ethiopia) between July and August 2018 found that malaria cases among expectant women yielded statistical association among women in their second trimester with odd ratio of 7, 95%, confidence interval of 2.8 to 20.2 (Gontie *et al*., 2020). A prospective research study was carried in Uganda and found that peripheral malaria infection among expectant women was higher and largely associated with their place of residence in rural areas (DeBeaudrap *et al*., 2013). Strikingly, the following studies found that there was no significant relationship with socio-demographic characteristics of pregnant women. A research survey conducted in Ethiopia concluded concluded to the absence of strong relationship between marital status, residence and malaria prevalence in expectant mother p-value= 0.078 and 0.176 respectively. Hence malaria-HIV co-infection was dominantly higher in second trimester as compare to other stages of gestational age (Asmamaw *et al*., 2013).

A research survey was carried out in Nigeria under facility-based design.The research showed no relationship concerning gestational age, residence, and marital status at p-value >0.05. However, the study findings revealed that third and second term pregnancy

had elevated high risk of malaria (Bishop and Aliyu., 2017). A research design was carried out in Cameroon under prospective cross-sectional design concluded to the absence of relationship between marital status and rate of malaria infection at p-value= 0.230 (Anchang-Kimbi *et al*., 2015). A research survey conducted in Abakaliki, Southern Nigeria under experimental design found that most pregnant women were found to be within gestational age corresponding to second and third term. However, the gestational age did not have strong relationship with malaria among the pregnant women, the p-value was superior to 0.05 (Odikamnoro *et al*., 2014). A research survey conducted in a community Gilge Gibe Dam area, Southwest of Addis Ababa under cross sectional design concluded that gestational age and residence of participants were not significantly associated with malaria at p-value of 0.675 (Getachew *et al*., 2013). A research survey done in Cameroon under prospective cross-sectional design revealed the absence of a relationship concerning plasmodium infection and married at a p-value 0.230 (Anchang-Kimbi *et al*., 2015). A research survey done in Jawi Northwest Ethiopia under facility cross-sectional design showed that marital status and all levels of gestational age had non-significant association to malaria outcome, p-value of 0.526 and 0.57 respectively (Tilahun *et al*., 2020). A research survey conducted in Northwest part of Ethiopia under cross-sectional design found that place of residence and gestational age (above 2 trimesters) had no significant association to malaria infection, p-value 0.442 and 0.497 respectively (Almaw *et al*., 2022). In the above sub-section, socio-demographic factors like respondent age, education level, residence area, mean of transportation, profession, marital status, gestation, parity and religion were looked at in the various enumerated studies which were cited in the different contexts. Some studies found that socio-demographic characteristics of respondents were statistically significant or strongly associated with malaria status among pregnant women. Other studies showed the absence

of relationship between socio-demographic variables and malaria status among pregnant women, while other studies were just descriptive and inconclusive. Furthermore, some studies demonstrated even that some socio-demographic variables were significant risk factors, and in relationship with high probability of malaria occurrence in expectant mothers. In fact, these studies employed different research study designs, methodologies, sampling size and different statistical model to arrive to their final findings, knowing also the fact that they were conducted in various geographical areas with different environmental exposure. There was statistical relationship at p-value equal 0.000 but also 0.05 in the extreme cases. The risk factor is well understood when it comes to show strength effect or the result of an association direction calculated on the basis of Adjusted Odd Ratio (AOR) or the relative risk or by computing regression analysis with a significant sampling method. Cross-sectional study designs were among the majority, they are snapshot studies carried out during a short period, and do not consume a lot of resources like the case for other type of research study designs like longitudinal, randomized controlled trial, which were very scanty and are susceptible to emerge the reality associated with the true findings. Therefore, there can be a kind of limitation come to snapshot studies and not to capture the essence shared through the existing findings that make a better understanding of the different parameters under study. Generally, this characteristic is recognized to most research conducted in the Sub-Saharan Africa region. These research studies were significant in establishing the association but not the risk understood as a causal relation between the socio-demographic factors and malaria status.

2.2.2 Association between Low Birth Weight and Malaria in Pregnancy.

Low birth weight is defined as the weight at birth for any newborn below 2500 g despite pregnancy gestation (Huo *et al*., 2015). This objective highlighted association between

low birth weight and malaria in pregnancy. Globally, Low birth weight prevalence is estimated around 15% to 24% (WHO, 2020; Bakken, L. and Iversen, P. O, 2021). In the Sub-Saharan Africa, plasmodium parasite constituted a major risk factor associated with newborn weight decrease. Studies are silent about the prevalence of low birth weight due to the specific cause in a given population, such as malaria at the regional, national, County and community level. The following were research studies conducted previously with highlight about association of plasmodium parasite and reduced newborn weight. A study conducted in the Africa region in 2021, focused on malaria effect in infants at the delivery highlighted that the first born child in a context of decreased malaria prevalence was to be protected against the low birth weight only through utilization of LLIN (Heng *et al.*, 2021). A descriptive research was done in Dar-es-Salaam, Tanzania under cross-sectional observational facility-based and concluded that malaria positive pregnant women were 11 times fold to induce reduced newborn weight as compare to malaria negative (p-value<0.05). The lower birth weight prevalence was recorded at 6.5 % (Mikomangwa *et al*., 2019). A research conducted in Cruzeiro do Sul Brazil under population observational design found that plasmodium falciparum infection among pregnant women was relatively associated with preterm births (p-value< 0.016), late preterm births and low birth weight p-value less than 0.011 (Dombrowski *et al.*, 2018). A research survey conducted in Tanzania under longitudinal design concluded that there was significant reduced fetal growth rate in pregnant women exposed to malaria as compared to non-exposed. Thus, parasite exposure principally affected primigravida as compare to secondigravida women (Schmiegelow *et al*., 2017). A research survey conducted in Sudan on the relation between low birth weight and sub-microscopic malaria found pregnant women with malaria in their placenta was associated with a ligand CXCL9 that upgrades the secretion, production and induction of cytokine,

chemotaxis and leucocytes (Mohammed *et al*., 2013). A research survey conducted in China concluded that malaria in pregnancy was statistically associated with preterm neonates. The newborns were small with higher risk of mortality due to complications of brain immaturity, hemorrhage and acute respiratory infections (Chua *et al*., 2021). A research study done in 2014 focused on newborn reduced weight as effect of plasmodium parasite presence. It established that newborn sex, high blood pressure during pregnancy, cigarette and primigravida had strong association with newborn reduced weight, except the mother age. The presence of malaria infection was associated with low birth weight (Rijken *et al*., 2014). A research survey was carried out in 2016 in Uganda under the cohort design to enroll 1218 pregnant women for timing consequences of malaria during pregnancy. This study highlighted that maternal gravidity and the age of pregnancy were key factors for the determination of malaria risk in the child. It was found that malaria and newborn reduced weight were strongly related, as well as other negative consequences more pronounced during 12 weeks preceding the delivery (Beaudrap *et al*., 2016). A research survey was carried out in Tanzania in 2017 under a longitudinal design concluded that early malaria infection contributes to the reduction in weight of the fetus. Furthermore, the low primary education was associated with exposed to malaria with 89% as compare to non-exposed with 70% with p-value equal 0.05. The invasion of plasmodium parasite when LLIN and IPT/SP were initiated late, contributed to the fetus weight loss of approximately 200 g (Schmiegelow *et al*., 2017).

In a study conducted in Malawi on malaria intervention effects and low birth weight found that the use of independently and combined interventions: long lasting insecticide treated nets and intermittent preventive treatment was associated with 20% low birth weight decrease and advocated for the promotion and continuation of two preventive strategies among pregnant women (Nkoka *et al*., 2020). A research survey conducted in

Ghana reported that among multiple benefits of using LLIN and noted a net decrease of miscarriage and stillbirths concomitantly reported also in the previous studies, which considered the insecticide treated net use as a paramount and significant success. Respondents in the respective focus group discussion which were taking place, identified a female participant in a village namely Framposo, who affirmed that not sleeping in the net can favor mosquito bites, therefore exposing the fetus who is in the uterus to get malaria and not develop properly (Manu *et al*., 2017). A study conducted in 2019 on determinants of newborn weight reduction in Ethiopia stressed that increased use of IPT among pregnant women had strong relationship with dropping of newborn weight reduction. Thus, one dose of IPT did not show statistical association with reduction of newborn weight, while two doses and above showed strong association (Wachamo *et al*., 2019). A research survey was carried out in Tanzania focusing on sub-microscopic placental malaria and reduced infant weight in HIV negative mothers. It concluded that pregnant women who were exposed to placental malaria had high likelihood of not having used long lasting mosquito nets. The odds ratio was 1.75%, confidence level 95%, p-value 0.03, anemic odds ratio 1.59% at confidence level of 95%, p-value 0,001 (Kalinjuma *et al*., 2020). A research survey carried out on risk factors of plasmodium versus reduced infant weight in the East-Africa region in 2021 highlighted that expectant mothers receiving IPT/SP more than thrice had low birth weight as compare to those who had used less than 2 doses, while another research study carried out in Uganda did not found the relationship between administration of IPT/SP and reduced infant weight (Bakken and Iversen, 2021; Braun *et al*., 2015). A randomized control research survey carried out in 2014 comparing the use of daily Cotrimoxazole or Sulfadoxine-pyrimethamine in pregnancy associated HIV highlighted that protective effect of Cotrimoxazole was beneficial also to prevent malaria, anemia, malnutrition and HIV.

The study stressed that the use of this medicine has a beneficial effect of reducing low birth weight which was more pronounced in multigravida as compare to primigravidae (Manyando *et al*., 2014). A systematic randomized and quasi-randomized study carried out in 2015 to test the preventive effect of antimalarial on the birth weight showed that use of antimalarial drugs had an impact on the reduction of reduced infant weight of 27%, confidence level of 0.56 to 0.97 and a risk ratio estimated at 0.73 as compare to parturient with no prevention. Furthermore, the study highlighted that gravidity had an effect on birth weight reduction among expectant with first pregnancy experience and second time as compare to multigravida with a risk ratio of 0.59, at 95%, interval between 0.39 to 0.90. However, other studies did not conclude to the statistical significance or relationship (Muanda *et al*., 2015; Kayentao *et al*., 2013). However, the following researches found that malaria was not a predictor of low birth weight. A cohort research design conducted in Dar-es-Salaam, Tanzania concluded that placental malaria among pregnant and HIV negative women were not having strong relationship with newborn weight drop with p value above 0.05 (Kalinjuma *et al*., 2020). A nested cohort observational study carried out in Uganda found that expectant mothers who did not have signs of placental malaria were more likely to have non-significant trend towards an increased risk of adverse pregnancy outcome, such as low birth weight, preterm or small gestational age (Kipisi *et al*., 2017). In Sudan, a case-control research survey was carried out in Medani Hospital. The finding of this study showed that placental malaria infection among pregnant women was not associated with low birth weight with p-value > 0.05 (Mohammed *et al*., 2013). In the literature, other causes of low birth weight rather than malaria which should be known for scientific curiosity were cited in the following research studies. A research survey was carried out in China in 2016 as a case- control design on the effect of lifestyle in newborns weight drop. It found that expectant mothers

who were exposed to passive smoking had increased risk of reduced newborn weight with OR 1.404 at the confidence level of 95%, and women above 35 years had increased risk of newborn weight drop with OR 1.713 at 95% confidence level (Xi *et al*., 2020). A research survey study conducted in Ethiopia examined data and found out a prevalence of low birth weight of 21%, a confidence level of 95%, interval between 17.5 to 26. The commonly cited causes of low birth weight were due to the obstetric factors; 40 cases were preeclampsia representing 11%, 18 cases either 5.45 % were premature rupture of membranes, antepartum hemorrhage and anemia. Pregnancy age was a predictor of low birth weight in less than 37 weeks' pregnancy as compare to pregnancy of more than 37 weeks (Alebel, 2017).

This sub-section demonstrates that low birth weight was a result of the positive relationship between the interaction of plasmodium and pregnancy. Various research survey were conducted in different geographical regions and used different study designs, different sampling methods to test if the presence of plasmodium among expectant women increased risk for low birth weight. The majority of studies were longitudinal studies likewise cohort, case-control and randomized controlled trials. In spite, the cross sectional research study designs were only few. It has been found that reduced infant weight had strong relationship with the presence of malaria infection during pregnancy, being therefore the key predictor. However, other research demonstrated that despite the presence of malaria alone or associated with other conditions it did not lead to low birth weight. Other factors which were cited in various studies as having direct effect or impact on low birth weight were utilization of intermittent preventive treatment-sulfadoxine-pyrimethamine, antimalarial medicine, parity of the mother, lifestyle (smoking), high blood pressure, sex of the child, maternal age, other obstetric conditions such as age of pregnancy (below 37 weeks), premature

rupture of membranes, antepartum hemorrhage and anemia. Some research highlighted that the early use of intermittent preventive treatment had positive relationship with reduced low birth weight, while late use had association with high rate of reduced birth weight as well as IPT/SP 1, 2 and 3. The existence of plasmodium in the body of parturient had affinity for placenta with consequence increased inflammation, placental hypoperfusion and reduced fetal grow with reduced infant weight. These results yield meaningful relationship between malaria infection and reduced infant weight. Therefore, these findings give insight to discuss own research findings once computed to determine, if there was a significant association (strength) using the longitudinal cohort design among pregnant women who had parasite of plasmodium versus those who did not have.

2.2.3 Proteome Extension Assay Methods and Pregnancy Associated Malaria

This objective meant to ascertain the proteomic biomarkers associated with malaria and to compare the findings of new technology called "Proteomics Olink" or proteome extension assay to the current existing commonly used tests (rapid diagnostic test, microscopy) but also polymerase chain reaction as confirmatory method commonly used in research to detect asymptomatic cases. Proteome extension assay or proximity ligation assay is a new technique developed at Uppsala University to analyze complex proteins in the blood sample using translation and signal via deoxyribonucleic acid extension, and for now little is known about this method. The process toward the realization of this technique consisted in the collection of blood sample within both cohorts (malaria positive and negative) at Webuye hospital. The proteome extension assay technique has been recently developed at Olink Proteomics in Uppsala, as a variant of the proximity ligation technique. The technique permits proteins measurements at the limits of detection and dynamic ranges similar to those of regular sandwich ELISA immunoassays, but while ELISA typically measure individual proteins in 50 microliter

of sample. Proximity ligation assay allows simultaneous analysis of 92 proteins and 4 controls in only 1 microliter aliquots of serum, plasma, CSF, tissue lysate. Therefore, one of the focus of this study was to test and ascertain potential proteomics biomarkers associated with malaria among pregnant women. In recent future, the reliability and validity of this technique will be put to task for early diagnosis of undetectable malaria, asymptomatic and resistant deletion gene cases. Should the technique be promising, the Olink proteome inception and findings can be translated into research development for new therapeutic protocols and malaria prognosis (Bjorkesten *et al*., 2017). A systematic review study was carried out in United Kingdom in 2015 to identify biomarkers in early detection of pregnancy associated malaria, with other diseases such as preeclampsia. This study highlighted that there were different biomarkers used such as vascular endothelial growth factor, C-reactive protein, metalloprotease 12, pregnancy associated plasma protein A, inhibin-A, placental protein 13, disintegrin, placental growth factor (PGF), Soluble fms-like tyrosine kinase-1, matrix metallopeptidase, Tumor-necrosis factor receptor 1, sex hormone-binding globulin, with a range of 10% of false positivity and a pooled sensitivity estimated at 0.40 and a confidence level of 95%, interval between 0.39 to 0.41. The importance of these multiple biomarkers use was to detect early diseases that can rise later in pregnancy and play a key role in early initiation of prevention and treatment. Biomarkers showed good efficiency in combination than when used individually due to low level of predictive value (Wu *et al*., 2015). A research study was conducted in 2020 on the usefulness of proteome profiling during early gestation in the diagnosis of preterm delivery in women with preterm labor, it was established that a number of maternal circulating proteins were dysregulated and reduced at 11 to 13 weeks of pregnancy. Therefore, highlighting the need of conforming early diagnosis through dosage of vitamin D binding protein and other such as alpha-one antitrypsin, alpha-one

beta (Fernando *et al*., 2020). In a cross-sectional study conducted in the Democratic Republic of Congo in 2014 on the diagnostic method of malaria detection in 332 pregnant women using rapid test with 81% sensitivity against 94% specificity. Concerning microscopy, the sensitivity was rated at 67% against 97% specificity. This study concluded to the effectiveness of rapid test in diagnosing Plasmodium falciparum in pregnant women with asymptomatic malaria (D'Silva *et al*., 2020). A cross-sectional research study was carried out in Nigeria in 184 pregnant women between 36 and 40 weeks gestation to ascertain anaemia, low birth weight as outcomes associated with malaria. The results showed a distribution of 40 expectant women either 70% had malaria through testing of either blood cord or blood test or both at the same time through microscopy and rapid test, 41 (76%) were anemic, and 37 (68%) delivered babies under the normal expected weight. The study highlighted that the likelihood of taking intermittent preventive treatment against malaria was a predictor associated with malaria parasitaemia in pregnancy (Muhammad *et al.*, 2016). A hospital based cross-sectional study was conducted in 2019 in Rwanda focused on comparison of malaria tests using histidine rich protein 2 and microscopy test among 264 women. The result of the tests showed that the specificity of microscopy was 100% against 59% of rapid diagnostic test. However, conducting both microscopy and rapid test showed that true negative results were 19%, while false positive result on histidine rich protein 2 which turned negative to microscopy was 13%, compare to the 32% of negativity by conducting both microscopy and rapid test and positivity after conducting microscopy which turned negative to rapid test was zero (Niyibizi and Gatera, 2020). In a randomized control trial research study conducted in Cameroon in 2019 about different diagnostic methods of testing malaria in a group of 320 males and females. There were four methods: temperature, microscopy, RDT and PCR. Temperature results showed that 65% either

208 over 320 patients presented with fever above 37.50C, whereby among those who were having fever at the beginning of the study being 156 patients either 76.44% were positive to PCR malaria test, 49% either 55 over 122 patients without fever tested positive to malaria during PCR. This study highlighted that the nested PCR results of 214 over 320 representing 66.87% were malaria positive. In fact, some results that were tested negative to microscopy and SD bioline turned positive to nested PCR (Moyeh *et al*., 2019). A research study carried out in Nigeria on efficiency of malaria diagnostic test in low constraint settings in 2020, showed that rapid test to diagnose malaria constitute the pillar backbone in achieving reliable sensitivity and specificity and it was cost-effective method, but with inherent challenges of not detecting other malaria parasites causal agent other than Plasmodium falciparum. Hence molecular biomarker being advanced detection method has subjected to further challenges such as lack of equipment, personnel and inaccessibility in resource constraint settings (Makanjuola and Taylor, 2020). A cross-sectional research design was conducted in 2021 in Ghana on polymorphism of various types of Plasmodium with no apparent malaria signs with a total sample size of 374 subjects. Microscopy and PCR were the reference tests done with results as follows; PCR and microscopy had identical results in detection of Plasmodium falciparum with a Chi-square of 0.405 against 0.452 respectively. On view of this results and finding the study encouraged the use of deoxyribonucleic acid for amplifying 18 S of ribo-nucleic acid reliable in detection of four malaria species compare to microscopy that was able to detect only Plasmodium falciparum with 129 subjects over 210 representing 61.4% against 95 subjects over 164 representing 54% of PCR. The study concluded that there was no statistically difference in both group of subjects in regard to the species of Plasmodium falciparum (Bredu *et al*., 2021). In 2017 a research study was done in Sub-Saharan Africa to streamline the role of diagnostic method for

placental malaria with anticipation using a key biological biomarker. This study found prevention and treatment of pregnancy associated malaria not making possibility of reversing falciparum placental malaria. The condition was associated with collateral damages and total absence to eliminate the stress because of the circulating inflammatory cytokines, chemokines, immunoglobulin, free radicals that impair the placental functioning contributing to intrauterine growth retardation, poor intravillous space blood flow leading to stillbirth and other poor feto-maternal outcomes (Gueneuc *et al.,* 2017). A cross-sectional study in 2021 about what healthcare workers know and practice to diagnose malaria in the public institutions in Guinea to align to the standards and requirements. This research highlighted that there was a significant gap in knowledge and practice towards acceptable level of compliance to the guidelines. The study at the hospital level found that 100% of patients were checked through microscopy for diagnosis of malaria, while in the peripheral centers the diagnosis was made based on symptomatology. Finally, this study concluded to low knowledge regarding guidelines with significant difference between workers of the hospital exhibiting acceptable level of knowledge compare to the health centers (Berrocal *et al.,* 2021). A cross-sectional survey conducted in India in 2015 centered on determinants of malaria associated with anemia in pregnancy. The survey established that during antenatal clinic, the complete history, vital signs and examination of pregnant women were done at the same time with sampling of the blood of the parturient, in view of investigating the presence of Plasmodium. When results were out, pregnant women who were anemic were examined by medical doctors, benefited curative treatment with Artemisinin combined therapy, and further advised to sleep in the insecticide treated nets. However, the result showed the vast majority of malaria cases in pregnancy were asymptomatic, while a few minorities exhibited signs like fever, nausea, dizziness and vomiting (Sohail *et al.,* 2015). In a

research study conducted in 2016 at Heriot-Watt University in the United Kingdom on process to separate the plasma in view of discovering causes of diseases such as cancer, Zika virus, sepsis and malaria via nucleic acid and antigen. From these perspectives, new knowledge acquired on genomic marker sequence has highlighted the optimizing molecular diagnostic assay as matter of fact targeting a cytochrome oxidase sub-unit gene in a dried blood spot to detect malaria DNA and distinguish Plasmodium falciparum with Plasmodium vivax (Mielczarek *et al*., 2016). In this sub-section, the sensitivity and specificity of common tests for testing malaria such as microscopy and rapid test were found to be either or no associated with pregnancy associated malaria and vary according to the study design. The PCR method showed added value for testing asymptomatic malaria or negative to the two cited methods.

Importantly, proximity ligation assay is compatible with dried spot blood bio-banking where a punch of 1.2 mm from a disc could be used to analyze a panel of 92 proteins. Whereby the proximity ligation assay being of superior value to detect early biological biomarkers associated with placental malaria has not been widely tested in pregnancy associated malaria among pregnant women. In the current studies there is no association between biomarkers and for example sociodemographic factors. This was the reason as to why this method was being tested in the local set up to identify relevant and potential biomarkers that might predict sub-optimal fetal growth and serve in the future as an alternative to the local point-of-care cost-effective test adapted for low constrained settings.

2.2.4 Awareness of Pregnant Women on IPT and ITN

This objective meant to assess the level of awareness of the intermittent preventive treatment and long lasting insecticide treated nets in pregnancy associated malaria among pregnant women. Awareness of long lasting insecticide treated net and intermittent

preventive treatment is key to achieve prevention of malaria during pregnancy. The following were studies which addressed the awareness and its implication in malaria prevention. In Ghana a cross-sectional descriptive research study carried out in 2011 on the use of SP in pregnancy as a game changer of life during pregnancy found that 64% of respondents were aware and acknowledged that after taking the medicine, they did not get sick like before (Dalaba, *et al*., 2018). In Afghanistan a case-control study conducted in 2015 on determinants, analysis of clinical factors and perceptions of pregnant women with anaemia associated with malaria found that pregnant women who named the transmission of malaria as linked to bites of mosquitos were 97 %, and those who cited that using the long-lasting mosquito nets was the best prevention method were 81 %. Furthermore, 90% of women knew the risk posed by malaria (Howard *et al*., 2015). A study conducted in Osun State, South-West Nigeria using a quasi-experimental design on the education effect of long-lasting nets use among pregnant women concluded that long lasting insecticide mosquito nets was cited as best intervention. Therefore, advocating for a such strategy through a permanent educative effort will improve knowledge, attitude and practice to prevent malaria during pregnancy. There was a statistically significant association between the increase on the score of knowledge after the use of long lasting insecticide treated nets (Sonibare *et al*., 2020). A study conducted in Sub-Saharan Africa from 2009 up to 2011on the coverage of prevention method revealed that IPT-SP was very low. A quarter of parturient received only two doses of IPT-SP and a one-third had used long lasting insecticide treated nets. Moreover, mosquito net used during the previous night ahead of survey was estimated at 35%, Fansidar two doses were 24.5%, while ANC second visit was estimated at 84%. Furthermore, come to 2015, IPT/SP and the net use were among the less covered interventions from the package of all activities provided to pregnant women during ANC

(WHO, 2012; WHO, 2015). In India a qualitative research study conducted in 2018 on preventing and treating malaria in pregnancy yielding 83 expectant women showed that 88% were aware that mosquito bites can transmit malaria, and half reported that the use of mosquito nets during sleeping prevents mosquito's bites. In fact, the majority of subjects had good attitude and knowledge. However, the recommendation was to strengthen health education by teaching intermittent preventive treatment/sulfadoxine-pyrimethamine at every routine clinic (Sabin *et al*., 2018). A descriptive cross-sectional study was carried out in 2013 in Ghana and it was focused on the perceptions of community members vis-à-vis of malaria as well as the way they were behaving. 92% believed that one of efficacious way to overcome malaria is through prevention, 85.8% knew that sleeping under long lasting insecticide treated nets protects against malaria, cost was mentioned by 10% of participants as a barrier to access the net, as well as feeling uncomfortable when sleeping under the net by 4.2%. Some reported that they go for traditional medication because the hospital is expensive (Alexander *et al*., 2013). A descriptive cross-sectional study conducted in Nigeria in 2016 about what pregnant women know about malaria prevention and the use of sulfadoxine-pyrimethamine. This study yielded a sample size of 450 subjects. The majority 355 (78.8%) perceived that malaria was a threat for health. In conclusion, knowledge was found statistically associated with education level because mother with good knowledge cited sulfadoxine-pyrimethamine as medicine used to prevent malaria during pregnancy (Chukwurah *et al.*, 2016). A descriptive cross-sectional survey was conducted in Indonesia in 2019 on determinants of malaria infection in parturient dwelling in malaria prone areas. The knowledge of malaria preventive measures is very important. Therefore, it can be used to achieve prevention and elimination of malaria and improve individual and collective behaviors. Furthermore, not knowing preventive measures was a mean of not acting

timely against malaria, and no having knowledge on the availability of a health facility had 1.2 times likelihood to suffer from malaria as compare to knowing. (Hasyim *et al*., 2019). A cross-sectional research study carried out in 2020 in Gabon about factors associated with knowledge and prevalence of malaria in reproductive age women and children used a sample size of 1307 participants. It found that almost the majority of respondents either 97.7% heard about malaria, and they know that it was due to the mosquito bites, only 73% said that they know that mosquito nets were preventing malaria. Furthermore, this study had put an accent on the implementation of other methods on the fight against malaria. In spite, high knowledge level about malaria still is a major concern with regard to his high prevalence in the rural area (Imboumy *et al*., 2020). A cross-sectional survey was conducted in 2017 in Kenya on determinants of Plasmodium falciparum in children. Only 1% of women did not know the importance of using the nets. In fact, there was a noted gap between knowing that nets protect against mosquito bites and the practice to use the nets. However, it was found that those who said that the use of nets was very important had high prevalence of malaria as compare to those who said just that it was important. The higher was the knowledge, the higher malaria prevalence. For those who said that people shall be using insecticide treated nets all the time, they represented 67%, against those who disagreed and represented 32%, there was a correlation between low malaria prevalence and disagreement, translating therefore to the need to focus on awareness programs and behavior change communication (Sultana *et al*., 2017). A cross-sectional study carried out in Sabatia Kenya found that there was a good number of pregnant women who had good knowledge of intermittent preventive treatment sulfadoxine-pyrimethamine benefits, but did not know the exact time for the beginning of intermittent preventive treatment sulfadoxine-pyrimethamine and never experienced sulfadoxine-pyrimethamine side effects. The

marital status, knowledge of benefits of sulfadoxine-pyrimethamine and gestation age were significantly associated with uptake of sulfadoxine- pyrimethamine, with women who had good knowledge of benefits having higher likelihood of receiving the third dose than those with poor knowledge (Mutanyi *et al*., 2021). A prospective cohort research study conducted at the Coastal region of Kenya indicated that majority of pregnant women who had received different doses of intermittent preventive treatment sulfadoxine-pyrimethamine were more prone to malaria due to the association attributed with moderate and severe anemia (McClure *et al*., 2014). A mixed-method research design found that majority of the respondents had fair knowledge about intermittent preventive treatment sulfadoxine-pyrimethamine (69.2%), with 7.3% and 23.7% having poor knowledge. A significant 95.5% were motivated to using intermittent preventive treatment sulfadoxine-pyrimethamine. Furthermore, the study showed that respondents were well informed that malaria can cause death and other effects such as anemia, abortion, low birth weight and prematurity (Peters and Naidoo, 2022). A prospective cross-sectional study concluded that the uptake of intermittent preventive treatment/ sulfadoxine-pyrimethamine among pregnant women was positively associated with malaria at p-value = 0.008 (Anchang-Kimbi *et al*., 2015). A research study design carried out in Malawi found that the difference in intermittent preventive treatment/sulfadoxine-pyrimethamine dose among pregnant women residing in Southern Region of Malawi had a significant impact on malaria test outcome at p-value< 0.001 (Gutman *et at*., 2013). A prospective cohort research design found that the majority of women were observed to have used two or more doses of intermittent preventive treatment/sulfadoxine-pyrimethamine. There was a statistically significant association between intermittent preventive treatment/ sulfadoxine-pyrimethamine dosage and malaria test at p-value <0 .001 (Agyeman *et al.,* 2020). A qualitative research study design using focus group

discussion concluded to inconsistent findings in that in Kenya and Mali pregnant women despite having knowledge of correct dose and intake intervals, they felt that the intermittent preventive treatment sulfadoxine-pyrimethamine was very powerful and should not be used during pregnancy as it could cause harm to the baby or lead to miscarriage. Furthermore, intermittent preventive treatment sulfadoxine-pyrimethamine was still being to treat malaria proving to be both effective and ineffective at preventing malaria (Hill *et al*., 2015). A cross-sectional study carried out in Western Uganda revealed that an approximated more than 50% of pregnant women had correctly taken at least two doses of intermittent preventive treatment sulfadoxine-pyrimethamine while more than 80 % had taken at least one dose of intermittent preventive treatment sulfadoxine-pyrimethamine during their pregnancy timeline (Braun *et al*., 2015). A prospective study conducted in Moshi Municipal in Northeast Tanzania, Rufiji District and Coastal Area between July and October 2012 revealed that approximately 91% of the women were aware and had used more than one dose of intermittent preventive treatment sulfadoxine-pyrimethamine. It was found to be significantly associated with malaria test versus outcome at p-value < 0.015 (Mosha *et al*., 2014). A cross-sectional research study revealed that approximately all respondents had heard about intermittent preventive treatment sulfadoxine-pyrimethamine, with 57% stating that intermittent preventive treatment sulfadoxine-pyrimethamine was convenient with malaria prevention in both mothers and unborn children, and 15.4% felt that it was used to treat malaria. However, nausea, vomiting, body weakness, headache, dizziness, abdominal pain and diarrhea were reported as unwanted effects of intermittent preventive treatment/ sulfadoxine-pyrimethamine use (Odongo *et al*., 2014). Ango *et al*., (2018), carried out a descriptive research design examining awareness, perception and utilization of IPT/SP and LLIN, majority of respondents were aware of the medicine used to prevent malaria

while expectant and they were 60.6%. Almost half (47.9%) of women believing that tablet to prevent malaria was safe when the baby is in the mother's womb and it offers the necessary protection. Majority of expectant women had used IPT/SP just once either 72.4%. No statistical difference was observed between the two variables (malaria status and IPT/SP use) at p-value > 0.05. Peter and Naidoo (2022), in a mixed-design study qualitative-quantitative conducted in Nigeria found that 68.5% had good knowledge on the benefits associated with IPT/SP during pregnancy, while about 58% could not tell the schedule for IPT/SP. Waiswa *et al*., (2022), in a prospective cohort research design carried out in Kakamega County showed that majority of the respondents had an advanced knowledge and constantly used LLIN method to control and combat malaria. Furthermore, there was statistical difference between the three variables (ownership, use of LLIN and rate of malaria) at p-value of 0.001. A descriptive cross-sectional study found that using none dose or just one dose of the intermittent preventive treatment/sulfadoxine-pyrimethamine was significantly associated with clinical malaria as compare to women who had received two or more intermittent preventive treatment sulfadoxine-pyrimethamine doses. Approximately 75% of pregnant women attending antenatal clinic had access to intermittent preventive treatment/sulfadoxine-pyrimethamine with more than 78% having received at least two doses (Peter, 2013). A descriptive cross-sectional hospital based study found that majority of pregnant women had advance knowledge on malaria prevention using intermittent preventive treatment sulfadoxine-pyrimethamine (65%). An important proportion representing 42% of these women were getting their awareness from healthcare providers and a relatively 57% of them had complied with intermittent preventive treatment/sulfadoxine-pyrimethamine doses as compare to 43% who had not taken intermittent preventive treatment/sulfadoxine-pyrimethamine doses in the current pregnancy (Mohamoud *et al.*,

2022). In the above sub sub-section, some studies showed that the level of awareness of intermittent preventive treatment and insecticide treated net among pregnant women was good while others stressed that it was poor. Lower or higher was the knowledge, poor or good was malaria response, hence the goal of preventive measures can be grounded on continuous effort towards health education and training. In fact, other studies concluded low level of awareness was a result of low exposure to the existing preventive measure among pregnant women.

2.2.5 Knowledge of Healthcare Workers on IPT and ITN

This objective meant to assess the level of knowledge among healthcare workers concerning intermittent preventive treatment and long lasting insecticide treated nets in pregnancy associated malaria among pregnant women. Knowledge for service providers is crucial for quality care provision. Good knowledge can translate in good service while poor knowledge is detrimental to the adequacy of quality care. A qualitative research study was carried out in 2018 in India on management and preventive measures against malaria in pregnancy yielding 202 healthcare providers during the in-depth-interview. It was found that the knowledge score was good, healthcare care workers knew that malaria was a serious threat in pregnancy, fears were expressed by 70% with regard to the refusal of the treatment judging it harmful to the fetus. The study pointed out a key aspect of lack of awareness as a barrier to achieve malaria prevention. A gap between knowledge, attitude and practice was noted, therefore calling for advocating training programs to empower healthcare service providers with communication skills during ANC sessions (Salin *et al*., 2018). A qualitative research study conducted in Malawi found that antenatal clinic knowledge was higher among the nurses. Hence nurses had a clear rationale of administering intermittent preventive treatment/sulfadoxine-pyrimethamine drugs of two doses during a pregnancy. However, all nurses didn’t agree on the timing

of the doses concerning the gestational age. No women were given intermittent preventive treatment/sulfadoxine-pyrimethamine doses to take home, all the doses where directly administered at the clinic. Delays in the first place, HIV positive patients and intermittent preventive treatment/sulfadoxine-pyrimethamine, written guidelines served as the reasons given by nurses and other caregivers for not succeed to having given the first or second dose of intermittent preventive treatment-sulfadoxine-pyrimethamine the way it could be (Yoder *et al.*, 2015). A cross-sectional study found that among the healthcare providers, the majority either 57.1% were providing basic obstetrics care and 77% were practicing in tertiary health facilities. More than half, 62.2% were aware of the current recommended intermittent preventive treatment/sulfadoxine-pyrimethamine as per the World Health Organization recommendations, while 39.1% had a correct intermittent preventive treatment/sulfadoxine-pyrimethamine knowledge. Furthermore, the study established that 72.2% of healthcare workers were able to correctly prescribed intermittent preventive treatment/sulfadoxine-pyrimethamine to pregnant women during he second and the third trimester of pregnancy (Oluwasomidoyin *et al.*, 2020).

A cross-sectional research study design found that majority of the healthcare providers were between the age of 23 to 37 years, and reported that most women 81.3% and 66.4% owned an insecticide treated nets and used them regularly. According to 97.8% healthcare providers, the insecticide treated nets were very effective malaria prevention method. 33.6% of healthcare providers were able to deliver health education related to insecticide treated nets, and 98.7% of the patients and caregivers were very much aware of the insecticide treated nets. Majority of both pregnant women and caregivers knew the signs and symptoms associated with malaria, and 82.2% knew cause and complications of malaria infection, and 90% knew who were high risk subjects (Nyavor *et al.*, 2017). A meta-analysis approach research study conducted in Africa in 2013 on determinants

of malaria preventive interventions related to the use, accessibility and delivery of service among expectant women. The study highlighted that there was an element of lack of knowledge concerning administration and the time for intermittent preventive intervention on the side of nurses, doctors and traditional birth attendants calling therefore for supervision and retraining towards knowledge improvement (Hill *et al*., 2013). A case-control study conducted in Nigeria found that due to lack of knowledge, respondents were not able to name the benefits of preventive interventions (long lasting insecticide treated mosquito nets). The unavailability of this method was a hindrance to the performance among both healthcare workers and pregnant women (Ugboaja and Oguejiofor, 2017). A cross-sectional research study design found that healthcare providers interviewed were aware of long lasting insecticide treated mosquito nets, but only 76.1% had advance or good knowledge of long lasting insecticide treated mosquito nets, and they were able to give the various reasons cited as hindrance to the use of mosquito nets by pregnant women (Israel *et al*., 2018). A health based cross-sectional study done in Ghana found that healthcare workers correctly administered intermittent preventive treatment with sulfadoxine-pyrimethamine (76.3%), while only 23.3% had good knowledge, awareness and eligibility of guidelines. Those who did not offer the drug explained that there was shortage (De Gaulle *et al*., 2021). A cross-sectional study carried out in Tanzania targeting post-delivery women and health care workers found that 36.1% of respondent were nurses or midwifes. Approximately 80.6% of the healthcare providers had adequate knowledge about intermittent preventive treatment/sulfadoxine-pyrimethamine. In the majority of the health facilities, 87.5% had intermittent preventive treatment/sulfadoxine-pyrimethamine stock well updated and 75% of Direct Observed Therapy strategy fulfilled (Mchwampaka *et al*., 2019). A cross-sectional research study was carried out in 2012 in Nigeria on assessment of healthcare

workers knowledge of malaria management using chemotherapy methods in expectant women attending private versus government hospitals. This study targeted 52 workers who had mandate to conduct mother child health service. In the private hospital 100% of interviewees knew that malaria was a health threat for mother and child health against 84% in the general hospitals. Furthermore, 87% and 70% of interviewees respectively in general and private hospitals knew that reduced total blood compartment was a direct effect of malaria. Furthermore, 65% against 25% respectively in general and in private hospitals pointed out to small for gestation as a direct effect of malaria, as well as they knew that one of severe complication was death in 59% against 15% respectively in general and in private hospitals (Onwujekwe *et al*., 2012). A cross-sectional descriptive design study carried out in Nigeria in 2012 about what healthcare workers know and practice to prevent malaria in expectant women. This study found that 75% of workers knew the role of IPT/SP. 75% of respondents named sulfadoxine-pyrimethamine, 78% knew that it was to start in the second term of gestation while 63% knew the timing for administration. Besides, a good number of interviewees knew the existence of policy guideline and a few of them could state correctly other aspects related to the directly observed treatment (Arulogun and Okereke, 2012). A qualitative research study was carried out in 2011 in Tanzania focused on the approach to evaluate the effect of determinants of chemoprophylaxis in pregnancy associated malaria. It was worthy noted that training targeting workers to improve intermittent preventive treatment skills, revision of guidelines can contribute to increase the coverage messages. Should they be considered as key antenatal activities to be undertaken by service providers (Gross *et al*., 2011). A qualitative research study was carried out in Ghana in 2011 on what and how healthcare providers screen and prevent malaria among pregnant women. The study showed that 88% of workers had knowledge of the theme related to preventive

intermittent treatment of malaria. The subsequent training was a big predictor of knowledge. Furthermore, subjects cited that prevention was better than curative management of malaria, and workload as a barrier to the implementation of this strategy. In conclusion, it was found that improving health of women attending antenatal care was a key predictor of healthcare provider's practices (Paintain *et al*., 2011). A qualitative research study was conducted in Tanzania in 2012 focused on motivation of antenatal healthcare workers rendering service to expectant women. They knew that administration of IPT was a key preventive measure to prevent malaria, they were not happy concerning overworking, insufficient supervision and poor communication of county management team. In fact, evidence showed that poor working conditions affected negatively motivation for antenatal care workers to delivery well their job. Those in private facility were comfortable with equipment like presence of water, electricity and infrastructure allowing them to better conduct directly observed treatment of sulfadoxine-pyrimethamine as compare to their counterparts in the public facilities (Mubyazi *et al*., 2012). However, the following studies argued that there was a gap underlying healthcare worker's skills, which could contribute to poor service delivery regarding intermittent preventive treatment/sulfadoxine-pyrimethamine and long lasting insecticide treated mosquito nets. A mixed research design conducted in Ghana among healthcare providers and pregnant women found that intermittent preventive treatment/sulfadoxine-pyrimethamine intake was a challenge due to missed antenatal clinic, intermittent preventive treatment/sulfadoxine-pyrimethamine knowledge deficiencies and stock shortage in health facilities. Healthcare providers' negligence, absenteeism, and drugs side effects posed as a major challenge for intermittent preventive treatment/sulfadoxine-pyrimethamine strategy (Dun-Dery *et al*., 2021). A qualitative research study found a relatively poor healthcare workers' knowledge of intermittent preventive

treatment/sulfadoxine-pyrimethamine, mode of administration and reasons behind the use of the intermittent preventive treatment/sulfadoxine-pyrimethamine drugs. The healthcare workers found a non-existent practices and agreed that intermittent preventive treatment/sulfadoxine-pyrimethamine doses should be administered as a directly observed therapy to ensure that pregnant women take it during the hospital visit in their presence (Peters and Naidoo., 2020). A qualitative research study design was conducted and found that there was a lack of knowledge of intermittent preventive treatment/sulfadoxine-pyrimethamine, guidelines and limited certainty about the drug's safety or efficiency. Antenatal clinic for pregnant women was inconsistently offered in health facilities and intermittent preventive treatment/sulfadoxine-pyrimethamine guidelines provision in Uganda were reported as inconsistent and could not reflect recommended World Health Organization policy at the time of the research (Rassi *et al.*, 2016). A mixed method research study design done in Nigeria found that only 14.2% of the healthcare providers were well trained on intermittent preventive treatment/sulfadoxine-pyrimethamine and were able to supervise provision of the intermittent preventive treatment-sulfadoxine-pyrimethamine doses. However, all the sampled health facilities were well equipped with resources for conducting intermittent preventive treatment-sulfadoxine-pyrimethamine activities. Furthermore, the research showed that there was no health facility which reported shortage or out of stock of sulfadoxine- pyrimethamine. Indeed, the study concluded that knowledge of guidelines on preventive strategies promote fair service delivery (Liman, 2019). In this sub-section, some studies showed that healthcare provider's knowledge of intermittent preventive treatment and insecticide treated nets was good, while other studies found that it was poor. Adequate knowledge of healthcare providers can translate into good service

delivery to the beneficiaries. Therefore, training was a major predictor of knowledge improvement.

2.2.6 Practices of Pregnant Women on IPT and ITN

This objective means to assess the practices of intermittent preventive treatment and insecticide treated nets in pregnancy women. Practices are different from knowledge for example you can know the existence of a preventive measure but refuse to accomplish it due to some reason or barriers. The following were studies that described the end product of achieving a desired preventive measure as planned whether it was important or no. A research study conducted in Malawi in 2018 examined the barriers and enablers of insecticide treated net and sulfadoxine-pyrimethamine uptake among nursing women. It found that taking this medicine at home was associated with low uptake and among reasons that were given to take sulfadoxine at home were shortage of cups and clean water in the health facility (Azizi *et al*., 2018). There was a designed prospective descriptive research study, which was done in Lagos, Nigeria in the year 2012 grounded on the use of sulfadoxine-pyrimethamine and its administration to expectant women. This research reported that participants who acknowledged that they did not use IPT given by healthcare workers were entrusted that once they arrive at their home will be able to use the medicine which was not the case (Onoka *et al*., 2012). There was a descriptive research study carried out among expectant women living in the Sub-Saharan Africa region in 2018 about evidence based of the sulfadoxine-pyrimethamine use and mosquito net barriers during pregnancy. In fact, the study come to the conclusion that the third dose of intermittent preventive treatment was still low far from achieving universal coverage in this part of the world due partially to the poor status of household members, weak health system and lack of enforcement of existing policy put in place (Yaya *et al*., 2018). A randomized controlled trial research comparing safety of

intermittent preventive in pregnancy in Mali concluded that malaria prevention with insecticide treated mosquito nets was significantly associated with a 18% decreased risk of neonatal mortality and rate of malaria infections (Kayentao, 2014). A qualitative research study conducted on malaria exhibiting opinion of a health specialist, reported that some pregnant women have accessed only one dose of intermittent preventive treatment while on their third trimester due to the delay. That is why they are being taught by nurses and midwives to prevent malaria. In fact, the compliance of such efficacious prevention methods is connected to the satisfaction of the user's reason to why long lasting insecticide treated mosquito nets were well tolerated as compare to the intermittent preventive treatment (Muhammad *et al.*, 2021).

In a research study conducted in Mozambique in 2017 on the coverage of intermittent preventive treatment versus insecticide treated nets among antenatal women suggested that insecticide treated nets and intermittent preventive treatment out of stock constituted barriers to performance on the coverage (Salomao *et al.*, 2017). A descriptive hospital-based research design study was done within Accra-Ghana in 2019. The goal was to determine the uptake of the sulfadoxine-pyrimethamine molecule used as "intermittent preventive treatment" during pregnancy to determine its association with the birth weight outcomes. This research study highlighted that the third dose sulfadoxine-pyrimethamine "intermittent preventive treatment" molecule was associated to the normal birth weight in the cohorts of expectant women (Quakyi *et al.*, 2019). Concerning the practices of long lasting treated nets, there was a meta-analysis research done to deepen the reasons for not using nets by expectant mother living in low constrained setting of the African regions. The study concluded that owning a mosquito nets was largely different as compare to its utilization. Furthermore, it was found that despite the availability of nets, pregnant women had other reasons for not using nets, they reported that they were

uncomfortable or they did not have risk to malaria exposure or they had forgotten (Pulford *et al*., 2011). A research study was conducted on the long lasting treated nets and found that ownership and sleeping under nets differed. Furthermore, the study recommended that to strengthen the current long lasting mosquito nets strategy, other preventive measures should be also simultaneously used, among respondents who were using other preventive methods, the ownership of long lasting mosquito nets was higher and presented as good practices of prevention strategies as effective and efficacious methods to rely on when it comes to combat malaria among pregnant women (Duut and Alhasan, 2022). A qualitative study conducted in Peru reported that 7 participants who had to say that prevention of malaria should be a matter of high priority used at the same time mosquito nets and believed in it as an efficacious preventive mean (Iyer *et al*., 2019). A qualitative study conducted through different focus group discussions in both Kenya and Mali on the effectiveness of using treated mosquito nets found that pregnant women impregnated their nets once arrived to the expiration time, and were boosting the insecticide contained product by using a powder of the name Powertab for those living in Kenya, and those who were living in Mali, they were using a method for soaking nets commonly known as bloc. The participants in this study talked about a precision, saying that they were oftenly using long lasting mosquito nets to avoid mosquito nuisance and prevent malaria (Hill *et al*., 2015). A retrospective descriptive review of MOH data 2012 to 2015 and WHO on the use of long lasting insecticide treated nets in the prone malaria zones found that the use of long lasting treated net was higher in the coastal as compared to the lakes areas but with a decrease of intermittent preventive treatment-sulfadoxine-pyrimethamine 1 and 2, as well as long lasting mosquito nets in the year 2012 to 2015 despite the improvement of number of pregnant women attending antenatal clinic who received long lasting mosquito nets and the increase of the distribution of mosquito nets

(Karoki *et al.*, 2016). There was a prospective cohort research study design which was carried out, an approximated 65% owned insecticide treated nets and 44% often used them at home and found that the majority of participants in the study were close to the last period preceding the full maturation either second gestational term (53.9%) and multigravida (47.6%). (Agyeman *et al.*, 2020). A research study carried out in Ethiopia found that owning more than one mosquito nets was two times fold associated to the mosquito net barriers use as preventive strategy against malaria infection during pregnancy (Kuse *et al.*, 2022). A descriptive research design was conducted within Uganda in 2017 to determine the behavioral aspects of expectant mothers with regard to the use of insecticide barriers used during night. The study showed that some respondents were satisfied on reasons of how and why they were cleaning and hanging their nets outside under the sunlight. Afterward the nets were suspended over the corners of the bed and sleeping inside by covering fully the all edges of the net under the bed to ensure that no mosquitos penetrate inside (Taremwa *et al.*, 2017). In 2018 a qualitative research method carried out on preventive strategy to control malaria in expectant women. The study showed that respondents expressed their views and attitude towards such illness and its effects on the neonatal outcomes. Furthermore, these expressions were clarified to give meaningful explanation to what could have been considered as key, the study revealed that despite the existence of a modern method that was mosquito barrier nets, the irregularity in use was observed by the respondents, who were more likely close to use any other traditional methods such as tree leaves and rubbing oils to protect themselves against malaria in the absence of reassurance and lack of education (Sabin *et al.*, 2018). A research study design conducted in Zanzibar on the reasons and risks of use of long lasting treated mosquito nets, a pregnant woman during a focus group discussion had to say “Every day we sleep under mosquito nets and if we get sick we should access

care to a dispensary" (Female, Bukoba Rural (Braun *et al.,* 2015). A descriptive research design carried out in Kampala-Uganda in 2020 based on translating knowledge into practices as relevant method for expectant mothers towards improve the use of the insecticide treated nets at home. The study highlighted that the health worker after educating pregnant women followed them at home to counter check how they translated the knowledge acquired to install the net. A pregnant woman narrated that she was told to expose the net one night outside and one day over sunlight before hanging to the bed. At the same time this exposure reduced the smell (Taremwa *et al*., 2020). The World Health Organization in 2014 gave insight for controlling malaria during pregnancy, based on a package of continuous distribution of LLNI and IPT-SP at each antenatal clinic contact to every pregnant woman, from the second trimester either 20 to 36 weeks pregnancy. This study highlighted that despite taking 2 doses of SP placental malaria was still deleterious as compare to those with less than two doses. There is a strong recommendation to give this medicine in the presence of the nurses preferentially after meals. The strategy is called the directly observed therapy (DOT) consisting in swallowing three tablets in the presence of the healthcare worker. Each tablet was dozed at 500 milligrams containing fansidar and pyrimethamine dozed at 25 milligrams mg (WHO, 2014). There was a meta-analysis design research carried out on guidelines used in different WHO regions-based chloroquine resistant treatment during pregnancy in the year 2021. The study stated that the insecticide barrier nets use at the beginning of conception, and sulfadoxine-pyrimethamine "intermittent preventive treatment" at the beginning of the second term of pregnancy were cost-effective strategies for preventing adverse pregnancy outcomes. Therefore, the accent was put on the directly observed treatment. When pregnant women is HIV positive using Cotrimoxazole, there is no need to continue using intermittent preventive treatment-sulfadoxine pyrimethamine. In

addition, DOT was the practical capability of observing a pregnant women swallowing her IPT-SP in the presence of a healthcare worker (Khalid and Reginald, 2021). A qualitative research study conducted in India in 2018 researched on the prevention and treatment of malaria in pregnancy. Researchers used a sample size of 202 healthcare providers who was subjected to focus group and in-depth- interview concerning the attitude, some worried that the use of some drugs may create abortion, and the most reliable way of them getting information is through the doctors and nurses. Further, they trust their prescription as opposed to message heard from the radio which are not more reliable because of advertising to sell their products, some confirmed that the eating of clean and well prepared food, drinking clean water can prevent malaria to enter into the body. However, concerning practice, some consulted a witchdoctor, drank rice water, paracetamol to treat fever (Sabin *et al.*, 2018). A cross-sectional study carried out in Western Uganda involving 915 pregnant women, two-thirds reported to have used a bed net in the previous night had owned a mosquito net (Koenker *et al.*, 2013). In fact, the following were studies which indicated inconsistency in the use of intermittent preventive treatment-sulfadoxine-pyrimethamine of 2 doses and plus and long lasting mosquito nets looked as a failure to completeness or compliance. A health facility based cross-sectional research study carried out done in Jawi District Northwest part of Ethiopia found that majority of pregnant women were not using long lasting mosquito nets, revealing that it is felt like suffocation and generated a lot of heat at night (Tilahun *et al*., 2020). A retrospective study carried out in the endemic malaria zones in Kenya found that about four people given long lasting mosquito nets do not use then consistently exposing them to malaria risk, despite a high long lasting mosquito nets coverage malaria cases increased over time. Therefore, calling to look into patient leveled studies to assess if long lasting mosquito nets are used appropriately. In fact, emphasize should be put on

other complementary malaria prevention strategies to address this gap concluded the study (Machini *et al*., 2016).

A retrospective study found that the majority of the women were observed to have taken at least 1 dose of intermittent preventive treatment-sulfadoxine-pyrimethamine as compare to the required intermittent preventive treatment dose of three or more. The poor intermittent preventive treatment intake coverage was highly attributed to factors such as transportation challenges and delays in allocation of malaria commodities in Ghana's healthcare facilities (Oppong *et al.*, 2019). A study conducted in Zanzibar argued on the reasons and risks of use of long lasting treated mosquito nets that a pregnant woman during a focus group discussion argued that "*the danger was there as we had no knowledge and few of us were using the nets during night, we were mainly relying on local herbals"* female from Zanzibar (Koenker *et al.*, 2013). A qualitative study carried out in Mozambique reported the word of a pregnant women going to antenatal clinic who recognized sulfadoxine-pyrimethamine tablet as the white tablets given to pregnant women, and took in the presence of healthcare worker. "*I was not told more but I was given three tablets which I took"*. Strikingly, she had this to add "*some pregnant women even when advised and counselled do not complete the doses of sulfadoxine-pyrimethamine*" maternal and child nurse (Arnaldo *et al*., 2019).

In the above sub-section, the intermittent preventive treatment and the insecticide treated nets during pregnancy were the two cost-effective strategies recognized to date by WHO for malaria prevention and improving maternal newborns outcomes. The use of sulfadoxine-pyrimethamine at home as well as the use of insecticide treated nets pose some serious controversy. Despite WHO recommendations towards preventive use of the two products, the utilization and the uptake remain low supported by the data in the published literature attributing partially the causes of no performances to the existence

of unclear policies, less skilled and motivated healthcare workers. This gap highlights disparities between different practices of insecticide treated nets and intermittent preventive treatment which are in line with the malaria prevention or related to the behavioral context.

2.3 Theoretical Model

The theoretical model used in this study belongs to Andersen model that was developed in 1973 for accessibility and use of healthcare services. This model posited that societal factors, health service system factors, individual factors immensely influence the use of health services. Its constructs include: need, enabling and predisposing.

Need: comprise individual perception and evaluation of functional capacity, symptoms and general state of health. Enabling: is a set of family beliefs and available community resources and accessibility to use those resources. Predisposing: comprise education, knowledge, religion, ethnicity, occupation and set of beliefs. The users, the knowledge about disease and values limited to the attention of psychosocial factor make briefly predisposing with socio-demographic factors, which according to Andersen and Newman model establish the relationship between the attitude and consideration of health service.

1976

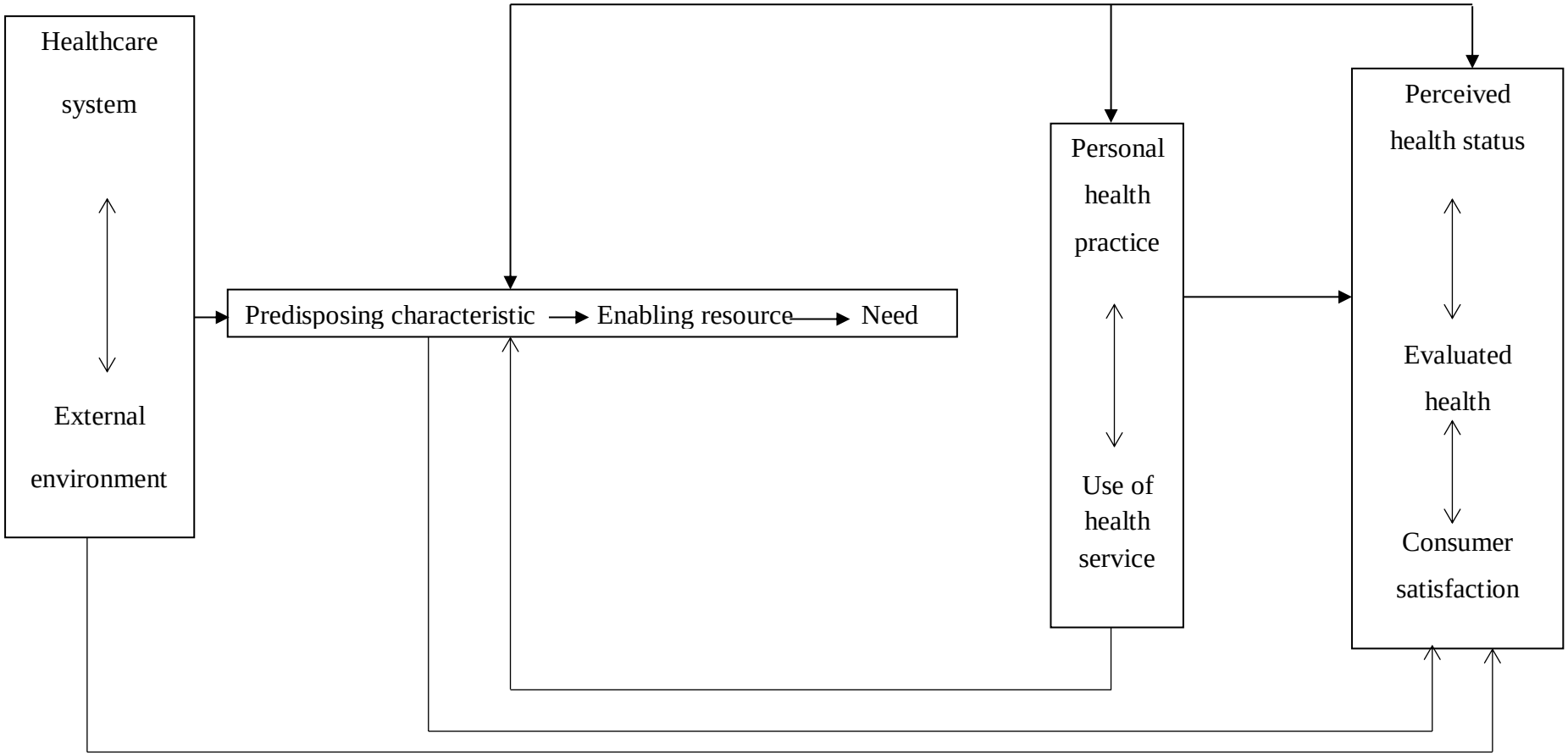


Figure 2. 1: Theoretical Model of Andersen & Newman (1976)

Source: Tesfaye *et al.* (2018)

2.4 Conceptual Framework

The operational framework has at the left side of the page independent variables: socio-demographic characteristics, laboratory aspects, predictors of low birth weight, the awareness and the practices of pregnant women on the insecticide treated nets and the intermittent preventive treatment, as well as knowledge of healthcare workers on the intermittent preventive treatment and the long-lasting insecticide treated nets. The independent variables affect positively or negatively pregnancy status and when it either malaria positive or negative, this will culminate either in the likelihood of good or bad outcome (low birth weight or normal weight). In routine antenatal clinic pregnant women benefit intermittent preventive treatment sulfadoxine-pyrimethamine and long-lasting insecticide treated nets besides other supplements such as folic acid, deworming. In the case of confirmed malaria, the standard of care “treatment” is administered as per the World Health Organization and Ministry of health guidelines.

2.4 Conceptual Framework

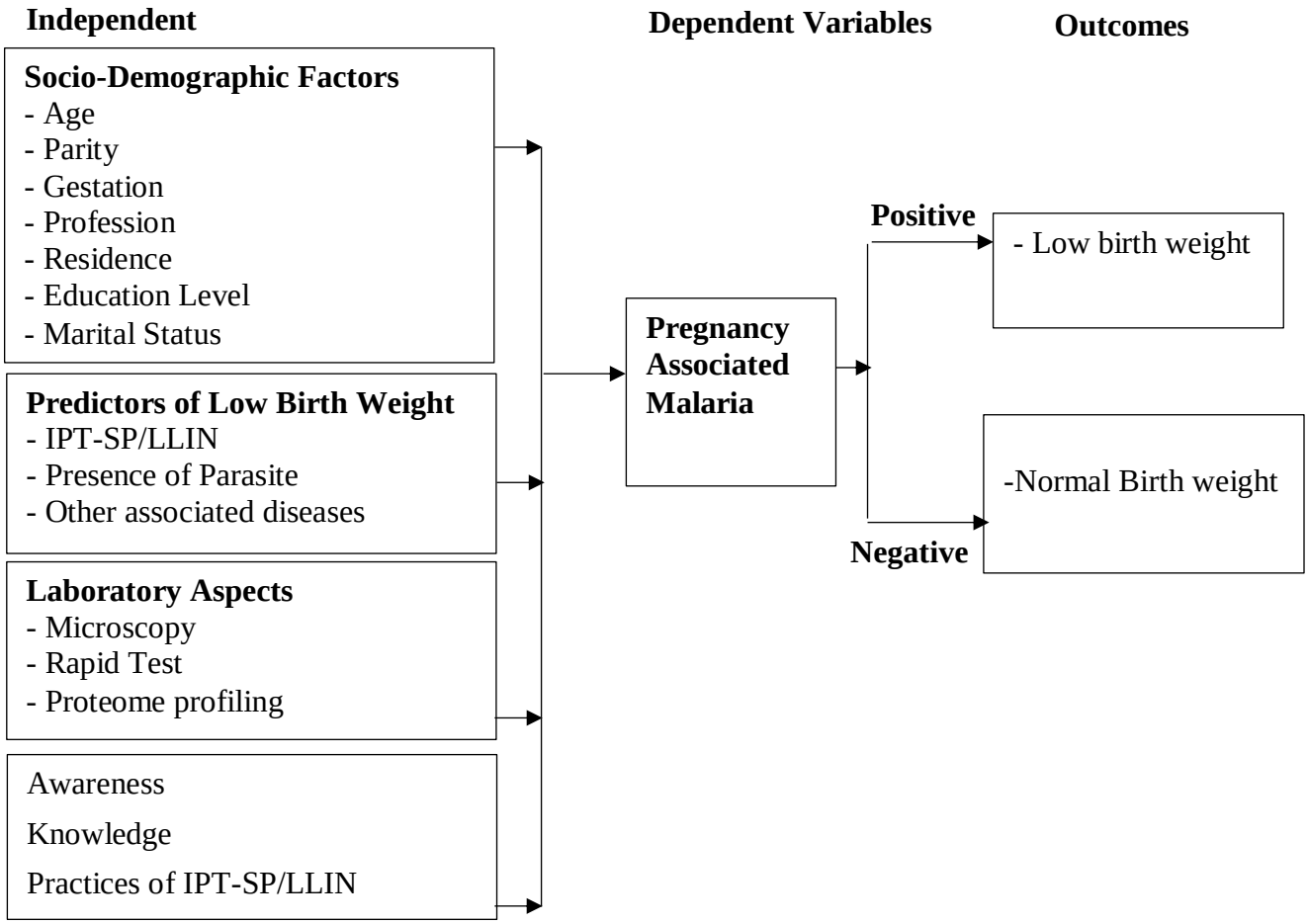


Figure 2. 2: Conceptual Framework

2.5 Research Gaps

It has been clearly noted that there are very few studies conducted in Kenya about cost-effective double strategy towards malaria detection and prevention in pregnant women during the last ten years. The patent case is one of Bungoma County a one location in prone malaria zone in the Western Kenya, where there was one study conducted on prevalence and species of Plasmodium. Globally, what is lacking in the literature review is a critical shortage of strong study designs that can describe the full account of pregnancy associated malaria and adverse maternal and fetal outcomes and at the same time looking at the different cost-effective strategies to detect and prevent malaria and this is the wake-up call of WHO to scientists and researchers to fill the gap for sustainable solutions and overcoming critical issues and gap on the field of malaria resistance and laboratory diagnostics.

Various studies looked at malaria on a single aspect whether it is detection, or prevention with intermittent preventive treatment alone or even use of insecticide treated nets alone, or no mention at all to the healthcare service provider's challenges. This underlined the importance of conducting this current research study with at the same time the goal to determine and estimate the relationship between malaria and low birth weight, and at the same time the association to the critical knowledge and practices gap that exist in the service provision and can constitute a hindrance to achieve preventive activities (IPT-SP, LLIN) which at the same time are subject of controversy. For instance, determining the contributive role of new Olink Proteomics in early malaria detection will be impactful task which can contribute to control, reduce, eliminate and eradicate malaria in endemic zones like the case of Bungoma County.

Many studies were looking to the single and isolated factors such as resistance to treatment or preventive methods used, prevalence or species of plasmodium, knowledge,

attitude and practices, asymptomatic versus symptomatic malaria and socio-demographic characteristics. No tool until this day has been proved to detect early malaria in pregnancy and placental malaria with accuracy giving space to appreciate in real time the limitations, advantages and disadvantages of each conventional laboratory technique in low constrained resources. Therefore, the proximity extension ligation method as one of recently developed technique at Olink Proteomics at Uppsala University, a variant of proximity assay technique that permits protein measurement at the limits of detection and dynamics ranges similar to those of regular sandwich ELISA immunoassays allows simultaneous analysis of 92 proteins and 4 controls in only 1 microlitre aliquots of serum and plasma can show a significant advantage as compare to the existing methods. This method is not available in the local settings and it is a very costly technology. It described to enroll each protein using a pair of oligonucleotide-modified antibodies, when such a reagent pair binds its target protein, a DNA polymerase extends one of the oligonucleotides, copying that on the other antibody brought in proximity by binding the same protein molecule.

The DNA extension products, each representing a detected protein, is then measured by real-time PCR, in an instrument, where 96 samples can be interrogated with respect to 96 analytes in parallel, compatible with dried spot blood bio banking having easy conservative method, efficiently and reliably resulting in early detection of malaria in pregnancy than microscopy and rapid diagnostic test. At the difference of other studies, this research study will determine whether this new technique can establish high quality sensitivity and specificity that will contribute to follow the patterns of biomarkers that may predict sub-optimal fetal growth in early gestational term pregnancy associated with malaria as a unique proteomic signature to detect sub-microscopic malaria and shed mechanistic insight for future therapeutic development.

2.6 Summary of Literature Review

Concerning socio-demographic factors published papers and scientific articles were scrutinized, majority were cross-sectional descriptive and very few cohort studies, case control and RCT. There were studies that concluded that there was no statistically significance and others found that there was strong statistical relationship owing the fact that each of this study used statistics and methodologies in a different way. The vast majority of papers unanimously concluded that pregnant women attended antenatal care late with low rate of attendance in the first trimester with consequence that there was late diagnosis that could interfere with normal mother child health wellbeing, failure to establish early prevention strategies. Therefore, there is need to implement research that address multiple factors looking at the detection, prevention and service provision gap to improve maternal and child health. Concerning age group majority was situated between 20 to 36 years as statistically associated with care seeking behavior among pregnant women in public institutions, likewise the paper published by Oyekale *et al.* (2015) in Ghana. Age of gestation in expectant women was found statistically significant with the severity of malaria in the third trimester for certain authors, while others pointed out the two first trimesters.

Moreover, the residence was statistically associated with malaria in pregnancy for attendees living in rural zones having high likelihood of malaria as compared to those in urban areas (Touré *et al.*, 2019). Concerning education level, some authors found that majority of expectant women had low school attainment. The majority of expectant women who attended antenatal clinic were multigravida as compared to primigravida, but young women and first trimester of pregnancy were susceptible to develop malaria as compared to old women while contradictory findings were found by other scholars. Generally, factors like income of wife, religion, smoking, age at first pregnancy, age at

the marriage, and number of children per woman were amongst the rarely studied factors. In regard to the diagnostic aspects, majority were cross-sectional, few were meta-analysis (systematic review) or randomized control trial. These papers did not link the aspect of diagnostic strategy as a major component of early disease detection as per the current challenge to determine undetected malaria. The results presented vary with different study locations and technique used (microscopy, rapid test or PCR). Nonetheless, microscopy and rapid test mentioned still are backbone diagnostic for malaria, since they are inexpensive and affordable.

WHO recommends microscopy as a gold standard test for malaria testing. PCR has high sensitivity and specificity but not accessible in most low-income countries. It was established that few available papers have mentioned the new method of proximity ligation or extension assay as a promising technology. But due to scanty data it becomes practically difficult to appreciate its superiority as compared to the most common used methods. Its advantage is that a specific protein is analyzed by translation of the signal to amplifiable DNA sequence via DNA ligation or extension essay. The key to the success of PLA is that target molecules are recognized by some cases up to seven probes consisting of affinity binders. It collects, conserves and analyzes easily 92 proteins in a dried blood spot of 1.2 millimeter using proximity extension essay. Furthermore, this technique relies on improved sensitivity associated with blood proteins analysis yet to be confirmed in our study area.

The application of the new technique in developing countries with limited access to 8 C storage would clearly facilitate inexpensive bio-banking for large and consecutive future sample and invaluable insights for genetic wide association studies. Concerning objective three, four, five and six 37 published papers were explored whereby majority were cross-sectional, cohorts, case-controls, randomized control trials, qualitative and

prospective descriptive. Low birth weight predictors are well elucidated beside the presence of parasite that interfere with placental perfusion preventing the fetal growth. The current scientific knowledge of these factors do not necessarily translate into knowledge and practices in both pregnant women and healthcare to improve immediate malaria situation. The aspect of the awareness, knowledge, and practice of intermittent preventive treatment and use of long-lasting mosquito nets reveals a serious problematic trend of disparity and lack of consistency between knowledge, attitude and practice relying mainly on patient self-reported information since this study was conducted in the hospital set up.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

This chapter focused on discussing research methodology, research design, location of the study, target population, sampling procedures and techniques, sample population, construction of research instruments, validity and reliability, data collection methods and procedures, data analysis techniques and procedures, as well as ethical considerations.

3.2 Research Methods

cohort designs are longitudinal studies where a group of people sharing the common features are followed for a time in view of establishing the occurrence of an outcome of interest given the fact that there is a certain exposure at the beginning of the study. The ultimate goal is to determine whether there is an association between the exposure and the outcome insinuating the idea that there was no no outcome of interest at the beginning. Retrospective cohorts trace back into the cohort information to identify the possible cause of the outcome which has already occurred (The University of North Carolina, 2009- 2013). An example of a prospective cohort study, expectant can be enrolled for a certain number of years so that later their children can be followed to determine early maternal influence on the post natal characteristics; retrospective cohort design are bigger than prospective cohorts (Andrade, C, 2022). Research design: cohort studies. *Indian Journal of Psychological Medicine*, *44*(2), 189-191). This was a nested prospective cohort research study with both quantitative qualitative data collected in Webuye hospital from March 2022 to December 2023. Therefore, this research study used at the same time the Ethical approval certificates for the main University study: MKU/ERC/2027: Interdisciplinary research for an integrated community directed strategy to eliminate malaria in tropical Africa. The study was tasked with objective to

recruit pregnant women and collect consecutive blood samples to identify predictive protein profiles. The candidate approved documents were MKU/ERC/2100, license No. NACOSTI/P/22/16233, County authorizations as far as the human blood sample is concerned. Therefore, the study procedures respected all relevant ethical standards and regulations governing research involving human sample requirements and compliance supported by the material transfer document and certificates of analysis for completion of proteomics work. Research assistants were recruited and trained before the study and the tools elaborated were duly pretested. The sample size was calculated on basis of prevalence of malaria in the non-exposed versus exposed. Pregnant women with 16 weeks' gestation were randomly enrolled (simple random) in the study after consenting. They were selected from triage or antenatal service during routine clinic and administered open-ended questionnaire to collect key information. Furthermore, other necessary information during blood sampling, subsequent visits, delivery time, organized sessions were collected.

Prior to the enrollment malaria test was conducted via rapid test and microscopy. These tests for malaria diagnosis were provided by KEMSA for hospital routine activities. But during the study period to ensure the quality assurance was to be maintained, the results reading were confirmed by a second lab technologist. Participants were enrolled in two cohorts (positive and negative malaria), the follow up was eased throughout during routine clinic using registers (MOH 405: ANC, MOH 333: Maternity, MOH 204: Laboratory) or contacted via phone number in the case of absenteeism. Participants were enrolled in the study as exposed if malaria test turned positive and unexposed if malaria test turned negative, and both groups were followed up until delivery.

Two focus group discussions were conducted in the hospital set up to collect qualitative data from pregnant women. Focus group discussion were organized to gain the deep

understanding on the awareness and practices on the intermittent preventive treatment and the long lasting insecticide treated nets among pregnant women, whereas open-ended questionnaire were administered to healthcare workers working in the mother child health service to get their insights about the knowledge they have on the intermittent preventive treatment and long lasting insecticide treated nets. Data were collected and analyzed using SPSS 27 version and R plotting. Categorical data were analyzed by the means of Chi-square, Fisher's Exact to compare different variable in the two cohorts. Outcome of interest was analyzed by the mean of relative risk. Proteomic data were analyzed using multidimensional scaling, Simpson and Chao indexes (non- parametric tests), multiple ANOVA, T-test, Bonferroni adjusted test and gene differentiation. Knowledge and practices were analyzed via measure of tendency (frequency, percentage) and MAXQDA software computer programs. The blood sample for malaria test and proteome profiling were collected among both cohorts of pregnant women, centrifuged and conserved at the temperature of +2 to +8 C. Samples were carried to the University laboratory in dry ice before the shipping to Uppsala University.

3.3 Study Design

This was a prospective cohort study design conducted in Webuye hospital. The design flow through a matrix showing the mode of selection of participants which was based on exposure to malaria or no. Those exposed were malaria positive case and non-exposed were negative. Thereafter, follow up conducted up to the delivery to determine the outcome of interest.

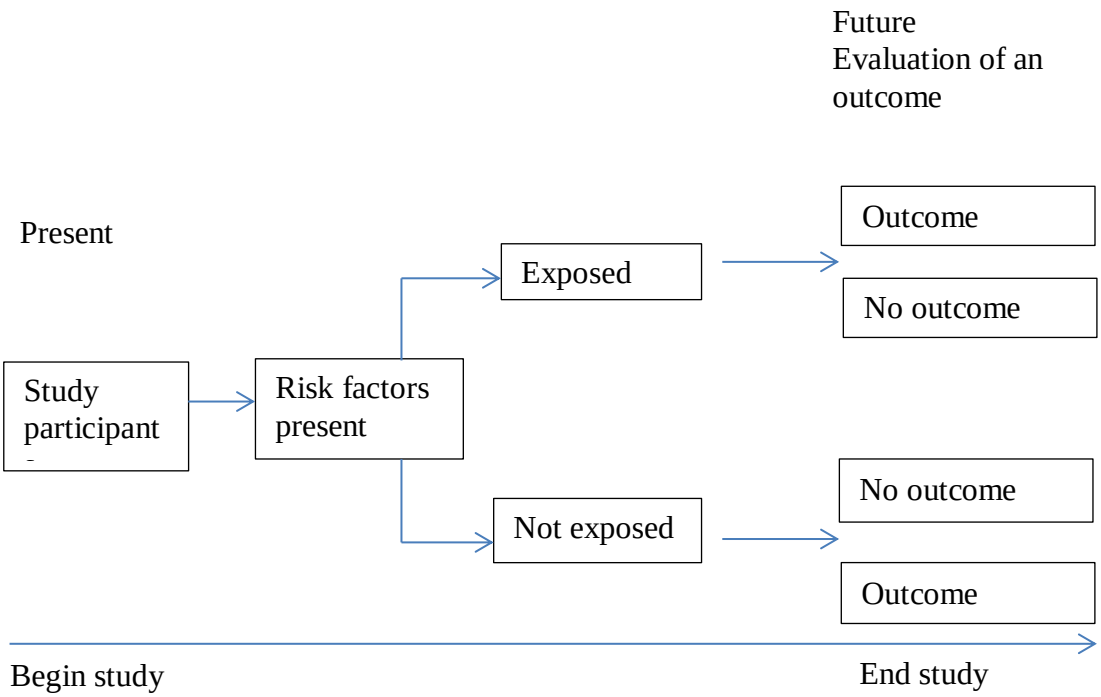


Figure 3. 1: Study Design

3.4 Location of the Study

This study was conducted in Bungoma County, Webuye hospital. The County has a population estimated at 1,919,490 with 939,105 males and 980,385 females, 429,762 women of childbearing age 15-49 years. There is 12 sub-Counties, 45 wards and 149 Sub-locations. The County has a total of 197 health facilities, 1 County referral hospital, 1 County hospital, 8 sub-County hospitals, 19 health centres, 89 dispensaries, 60 private clinics and 19 faith based facilities as indicated. The top diseases were malaria, anaemia, pneumonia, diarrhoea and peptic ulcers The County covers an area of 3032 km2 and lie between a latitude 00 28'and latitude 10 30' North of the Equator, and longitudinal 340 20'East and 350 15'East of the Greenwich meridian. It borders the Republic of Uganda to the Northwest, Trans-Nzoia County to North-East, Kakamega County to the East and South-East, and Busia County to the West and Southwest. It is characterized by two rainy seasons, the long rain season goes from March to July, and a short season from August

to October with an annual rainfall ranging between 400 mm to 1,800 mm. The temperature varies between 00c and 320c (KNBS, 2019; CIDP 2018-2022).

3.5 Target Population

The study targeted pregnant women aged between 18-49 years from 16 weeks of gestation. At this gestational age, pregnant women are safe to be receive intermittent preventive treatment and starting routine antenatal clinic in the rural set up.

3.5.1 Inclusion Criteria

Pregnant women or participants were selected based on acceptance to participate and deliver in the facility by giving verbal or written informed consent. The selected participants were within their sixteenth weeks of pregnancy and above.

3.5.2 Exclusion Criteria

Pregnant women or participants having mental disease or retardation and not leaving in the County for the last six months were excluded from the study.

3.6 Sampling Procedures and Techniques

The sampling technique for the hospital was purposive due to the fact that the hospital chosen was one of the Counties referral facility located in a high-risk malaria zone. The sample size calculation formula took into account the prevalence of malaria in the non-exposed group estimated at 28% according to the study of Nyamu *et al.* (2020) and the prevalence of malaria in the exposed estimated at 6.1% according to the DHIS2 (2019).

3.7 Sample Population Calculation

The sample population calculation formula was as follows:

$$n = \frac{\left\{ z_{\frac{\alpha}{2}} \sqrt{2(p(1-p)} + z_{1-\beta} \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right\}^2}{(p_1 - p_2)^2}$$

Where:

P_1 is the prevalence of outcome in unexposed group is 28%.

P_2 is the prevalence of outcome of exposed group that is 6.1 %.

Beta (10%),

Alpha (5%),

Confidence level of 95%,

Z alpha (1.96), Z beta value (1.28),

Sample size for group-1(n1=60),

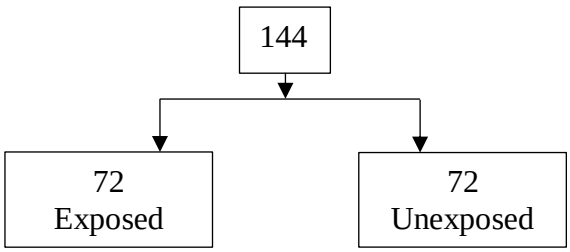
Sample size for group-2 (n2=60),

Sample size for both group (n1+n2=120),

Attrition (%=20%), Total sample size with attrition=144.

$$n = \frac{\left\{1.96\sqrt{2(0.28(1-0.28)} + 1.28\sqrt{0.28(1-0.28) + 0.6(1-0.6)}\right\}^2}{(0.28-0.6)^2}$$

n = 144 that is the total number of the participants in the study for both exposed and non-exposed groups. Therefore, the flow for the study design was as follows:



3.8 Construction of Research Instruments

3.8.1 Data Collection Instrument

Data collection process started with conception of questionnaire and the task was conducted with the help of supervisors and improved with the inputs from research assistants in the hospital set up. Questionnaire questions were prepared from model of from World Health Organization and United Nations International Children Emergency Funds as per objective to maintain idea's flow. Questions were identified, formulated appropriately, selected and provided with options for answers, then scored and validated. The questions were conceived for each objective but adapted to the real time information

from the field and meaningful for further analysis. Concerning objective one, the questionnaire was focused on the date of last period or menses for respondent targeting determination of the gestational age and approximate the date of delivery, as well as the age of pregnancy by date, if the obstetric ultrasound was not performed. The question on the age of the respondent comprised different age groups from 18 to 49 years. The parity of participant comprised following subdivisions: primigravida, secondgravida, multigravida, grandmultigravida. The gestational age was subdivided in different pregnancy trimesters either from first trimester falling between the limit of 1 to 12 weeks, second trimester within the limit of 13 to 26 weeks, and third trimester from 27-38 weeks. The school attainment was categorized under primary education, secondary education and college or university. The occupation of the respondent was categorized as housewife, employed and self-employed. The income of the participant was coded in low income, middle income and high income. The distance from home to the health facility was coded in less than one hour working time to reach the health facility or more than two hours to reach the health set up.

Concerning the second objective pertaining to predictors of low birth weight the following were the questions to be asked to the respondent in regard to the intermittent preventive treatment/sulfadoxine-pyrimethamine received and number of doses, the previous history of diabetes mellitus, history of preeclampsia, history of HIV, overweight, smoking, drinking. The objective three being related to the laboratory analysis of samples collected did not have questionnaire but sheets containing code and uniprot values. The following objective had questions related to the knowledge, attitude and practices, as well as close and open-ended questionnaire, in-depth views and opinions collected during focus group discussion comprising; training of malaria received in the last five years, the linkage between the training and the career, the

availability of a soft copy or a harcopy on malaria guideline in the service, the general knowledge on the long lasting insecticide treated nets and the intermittent preventive treatment/sulfadoxine-pyrimethamine, the composition, doses, side effects, the timing or schedule, advantages, contra-indications, the directly observed treatment, distribution, the stock and shortage, efficacy of each methods and barriers. The pre-testing of structured questionnaire tool for simulation and training of assistant researchers were perquisites before data collection. The summary of the different tools used during this research study was as follows;

Closed and open-ended questionnaire to collect key information on socio-demographics for the objective one.

Outcome tool for collecting data pertaining to objective two.

Laboratory sheets for collecting data pertaining to objective three.

Focus group discussion guide to collect data on awareness and practices of pregnant women on the long lasting insecticide treated nets and the intermittent preventive treatment/sulfadoxine-pyrimethamine pertaining to objective four and six.

Key informant interview guide to collect informations from healthcare workers in antenatal service pertaining to objective five.

Desk review check list to collect data on policy documents and guidelines used in antenatal service.

3.9 Validity and Reliability/Trustworthiness

Judging quality of research and performance is very crucial for the scientific approach leading to the reliability, which was achieved through statistical tests used and study design. The validity in the study is achieved by minimizing systematic errors in sample selection, pretesting tools before data collection. Thus, the process of thorough verification, correction and validation of tools before field work needed insights of

experts while fundamentally linked to validity aspect. Therefore, any change, correction and adjustment of idea, criteria and field work contextualization should be envisaged timely as a matter of consensus and team work for determination of quality data and trustworthy results. Sample representation and external performance of any findings are crucial for descriptive and analytic research. Quality assurance and control applied thoroughly to the specimen manipulation and blood sample(Joubert *et al.,* 2007).

3.9.1 Variables and measurement level

Independent variables were socio-demographics: age groups (18-25, 26-33, 34-41, 42-49), gestation (first, second, third), education level (none, primary, secondary, college/university), income level (low, middle, high), marital status (married, divorced, single, widowed, monogamous, polygamous), residence (rural, urban), awareness (no, yes), Knowledge (above 70%, 50 to 69%, less than 50%), practices (good, bad), microscopy (negative, positive), rapid test (negative, positive).

Dependent variable: Pregnancy associated malaria.

Outcome variable: low birth weight (<2500 g), normal birth weight (>2500), normal delivery (alive newborn).

3.9.2 Pretesting of questionnaire

Questionnaire was conceived and adapted from WHO and UNICEF antenatal care model, then corrected by three supervisors prior to the field work. Research assistants were trained and given handbooks. Session was organized at Kanduyi hospital and conducted with help of assistant researchers who were informed to select ten pregnant women present the day of antenatal clinic session for simulation. English and Kiswahili versions of questionnaire were availed. Before starting, the aspect of ethics and consent form were clearly explained and taken care of. Only participants who agreed were allowed to continue to the next step. Each question was read for the participant,

explanation also given by the assistant researcher (nurse). Answers were immediately circled with pencils. During the session the time was recorded, the inputs were collected, reformulation of the correct idea was noted to effect the change after discussion with supervisors. Finally, the modified and corrected version was adopted and validated, hardcopies were printed, and ready to use during enrollment process in mother child health service, outpatient and maternity

3.10 Data Collection Methods and Procedures

Data were collected by trained assistant researchers. Before data collection starts in the hospitals, assistants were trained to familiarize with the tools such hospital registers from mother child service, maternity, laboratories and antenatal booklets as well as the questionnaire. Pregnant women who visited the hospital with signs of fever or headache or without any signs were also included and oriented to the triage service in out-patient and referred to the antenatal service, where clear explanation on consent was given and the test performed in the laboratory. Samples were collected and immediately tested for malaria, the surplus of blood was conserved at Mount Kenya University laboratory, thereafter shipped for the test to be carried out at Uppsala University where the available equipment to undertake proteomic processes.. If malaria turned positive to microscopy or rapid test participant was to benefit medical attention in the service as per the guideline and the enrollment in the study was ticked as exposed. If malaria test was negative participant was enrolled as non-exposed. Questions were administered to each participant but those very sick or in critical condition were recorded and attended later when the treatment was initiated and participant able to express clearly her opinion on whether to participate or no. An appointment for subsequent antenatal contact was given. A malaria test for pre-selected group of women was repeated later and samples collected for three consecutive months. Therefore, the two cohorts: without malaria and with

malaria were followed up to the delivery (The sampling of blood for proteome profiling were conducted in a ratio of 50 exposed and 100 non-exposed). The serum sample was obtained after centrifugation and stored in a refrigerator at +8 C with correct labelling of sample ID, code and date of collection. The sample was carried to Mount Kenya laboratory before shipping to Uppsala University where new technology Olink Proteomics is performed.

The focus group discussion was prepared and conducted for two group each having 6 participants. Participants were randomly selected from a group of 74 pregnant women and informed timely by the nurse in charge of mother child health service with prior consent of the hospital management about the venue and the time of the meeting. Before the session, all requirements were satisfied including compensation under form of transport as well as the informed consent obtained before proceeding specifically for the recording of voice and taking pictures. The session of sixty minutes was successfully conducted, recording performed and open questions were asked and responses noted by two assistants. The purpose was to collect data on IPT-SP and LLIN awareness and practices using in-depth interview and discussion. Collection of data of healthcare worker's knowledge on the intermittent preventive treatment and the long lasting insecticide treated nets was done via the administration of a closed and open-ended questionnaire.

Proximity Ligation Assay

Informed written consent is obtained before blood sampling. A pipette of 25 ml EDTA used onto a Whatman DMPK-C, the drop of blood on the tip of the pipette is only the one to touch the paper that is dried for three hours at the ambient temperature, the EDTA blood is centrifuged within 15 minutes at 2000 revolutions per minute, the plasma is collected at 1.2 millimeter of diameter disks punched out from dried blood spot and dried

plasma spot with a uni-core of 1.2 millimeter micro puncher in the healthcare card. proteins measured from 1.2 ml disk in 1ml aliquot of wet blood and wet plasma are analyzed.

Microscopy Test for Malaria

This method is the standard rule recommended for parasitological diagnosis of malaria. A written informed consent is obtained from the patient. Blood to be obtained from the pregnant women, the films made in mixing the drop and methanol and 3% Giemsa staining within 30 minutes. Rinsing of the slide with distilled water then exposed for drying at the temperature of the room, reading via the microscope using 100x objective immersion oil. When 100 fields are examined and no malaria parasites observed the test is negative. Thick smear is used to quantifying and detecting the parasite, whereas thin smear for species identification. The presence of asexual parasites was determined via thick film and quantification done by using every 200 white blood cells. Parasites density calculated by microliter of blood with WBC count of 8×109.

Rapid Diagnostic Test for Malaria

It is immunochromatographic test to detect specific parasite antigens. The histidine-rich protein 2 (HRP2) is specific for Plasmodium falciparum. There is lactate dehydrogenase (LDH) or aldolase having the ability to differentiate between Plasmodium falciparum and non- Plasmodium falciparum such ovale, vivax and malaria. According to the national guideline the use of Rapid diagnostic test is not recommended for follow up and cannot determine the density of parasite.

Quality Control and Assurance

Quality control and assurance applied to every step for achieving validity and consistency of the sample. The consent was be obtained prior to sample the participant blood. Laboratory supplies for microscopic and rapid tests used were for the hospital. The

researcher together with the lab technician regularly read the date of expiration for reagents and tests, and accompanying delivery documents from KEMSA to ensure that quality is maintained. The conservation of the supplies were checked at regularly during the period when the study was undertaken. The blood sampling technique was done by a qualified technician. The sharp materials were immediately disposed as contaminated materials in safety boxes to promote and observe infection prevention and control measures. The sample collected was conserved in the refrigerator under the temperature of +2 to +8 C. The lab technicians performed procedures and re-reading of slides for confirmation of positivity or negativity of the results. In the case of doubt, a second reading was done by a another parasitologist to confirm the results for ensuring quality control throughout the entire process from the collection, local test, conservation and shipping of blood samples to Uppsala University.

3.11 Data Analysis Techniques and Procedures

The collected data were scrutinized and cleaned from field errors. Thereafter, screened to ensure set standards were achieved before validation. Afterward, data were fed into the computerized system for quantitative qualitative statistical analysis with meaningful variables (categorical: nominal and ordinal). The editing and coding were effected into the SPSS 27 version and R plotting for readiness, analysis and interpretation. During the data analysis, Chi-square and Fisher's Exact were used for categorical data analysis pertaining to objective one. Objective two was calculated by the mean of relative risk to measure the occurrence of outcome. Objective three was computed by the means of gene differentiation methods (Uniprot) using mean, multiple ANOVA, T-test and Bonferroni adjusted test. Objective four was analyzed through Chi-square of association and in-depth views and opinions collected via focus group discussion and analyzed by the means of MAXQDA software. Objective five was analyzed through descriptive statistics

(frequency and percentage). Finally, the objective six was analyzed by the means of frequency, percentage, as well as in-depth views and opinions collected during focus group discussion via recording, transcribing, coding and analyzing using MAXQDA software.

The results for each objective was computed and displayed under the form of tables, figures (pie-chart and graphics). The final findings were discussed, thereafter elaborated under the form of conclusion, recommendations, policy research and practices, as well as new knowledge in the malaria arena, to determine the particular contribution to improve mother child health as a major concern of the Sustainable Development Goal three. The dissemination was captured through journal publications of each objective, meetings, conferences, submission of thesis hard and softcopy at the university library, NACOSTI and County level.

3.12 Ethical Considerations

Permission before data collection was sought and obtained from the Ethical Reviews Committee of Mount Kenya University, NACOSTI, County and hospital authority. Pregnant women as well as healthcare workers were informed on the free voluntary participation in the study, respects, rights during and after, as well as withdrawal at any stage of the study. The benefits, confidentiality, risks and mitigation, harms and compensation (payment) if the case were clearly explained to the participants before data collection and those who agreed were enrolled. Thereafter came blood sample collection preceded by the explanation of the procedure including minimal side effects and explanation in regard to the transfer of samples outside the country, a careful document as a memorandum of understanding “material transfer agreement” was duly signed between both institutions.

3.12.1 Informed Consent

The respondents were provided with a consent form before data collection, and informed about the benefits and their rights so that when they decide to participate or not, it is a free decision. The laboratory test sampling was also conducted after explanation about temporary effect that may occur after pricking. Participants were explained about privacy of video and image recording during focus group. The sample collected, video and image recorded were guaranteed for use of only one purpose which study and were discarded immediately with the ending of the study not for any other utilization.

3.12.2 Confidentiality

Participants were explained about respect and privacy of the informations, the results of different tests and no disclosure to the third part (the name was replaced by a code), protection of individual data and information only for the purpose of the study was guaranteed between the two parties.

3.12.3 Potential Risks and Psychological Harm

This study was not in interest of conflict with any other party, and therefore cannot generate any harm. Participants were handled in a professional and comfortable way and environment and assured to ask any question about procedure, malaria test and results. All used materials for injection were disposed safely according to the standards. Only qualified healthcare workers performed procedures according to the guidelines.

3.12.4 Confidence or Significance Level

The confidence level Alpha or error term used in this study will be 0.05 (95%). Therefore, the α value is the probability of rejecting the null hypothesis when it true (Type I error). The significance level is a measure of the strength of the evidence that must be present in the underlying sampled data before the null hypothesis is rejected or conclude that the effect is statistically significant.

CHAPTER FOUR

RESEARCH FINDINGS AND DISCUSSIONS

4.0 Introduction

This chapter comprises main research findings related to each of the six objectives, data analysis and statistics were computed to achieve meaningful relationship between independent, dependent variables and the outcomes. The research findings were presented under the forms of tables, figures and text boxes. The discussion regarding different data sets whether quantitative or qualitative was conducted in depth to explain the relationship of variables on view of the statistical significance or no.

4.1 Response rate

The tool response rate was at 97% whereas 140 respondents out of 144 returned the questionnaire with only 3% of loss to the follow up.

4.2 To establish socio-demographic factors associated with malaria.

4.2.1 Socio-Demographic Characteristics of the Respondents

There was strict observance in provision of consent by the respondents participating in the study, and being tested for malaria after taking their blood samples. The results were obtained by conducting both rapid and blood slide tests to confirm the diagnosis. A total of 140 (100%) were tested for malaria of which 70 (50%) were negative. After computing for Chi-square and Fisher's exact to test for the socio-demographic characteristics association in the malaria positive cohorts versus malaria negative at p-value less than 0.05 (95%) confidence level, the findings showed that the majority were in the 18-25 years' age-group, primigravida, married, with secondary level of education, middle income level, self-employed, residents of rural areas and in the second trimester. There was significant association between marital status (p-value <0.001), gestational age in

weeks (p-value <0.001) and area of residence (p-value < 0.028) and malaria test. See table 1 below.

Table 4. 1: Socio-Demographics Characteristics of the Respondents

Variables	Overall N = 140	Malaria test Negative n = 70	Malaria test Positive n = 70	p-value
Age in years, n (%)				0.220
18-25	89 (63.6)	40 (57.1)	49 (70.0)	
26-33	36 (25.7)	20 (28.6)	16 (22.9)	
34-41	15 (10.7)	10 (14.3)	5 (7.1)	
Parity, n (%)				0.190
Primigravida	53 (37.9)	22 (31.4)	31 (44.3)	
Secondgravida	40 (28.6)	20 (28.6)	20 (28.6)	
Multigravida	32 (22.9)	21 (30.0)	11 (15.7)	
Grand multigravida	15 (10.7)	7 (10.0)	8 (11.4)	
Marital status, n (%)				<0.001
Married	117 (83.6)	67 (95.7)	50 (71.4)	
Single	23 (16.4)	3 (4.3)	20 (28.6)	
Level of education, n (%)				0.21
None/Primary	38 (27.1)	15 (21.4)	23 (32.9)	
Secondary	67 (47.9)	34 (48.6)	33 (47.1)	
College/University	35 (25.0)	21 (30.0)	14 (20.0)	
Occupation, n (%)				0.369
Employed	20 (14.3)	11 (15.7)	9 (12.9)	
Unemployed	50 (35.7)	21 (30.0)	29 (41.4)	
Self-employed	70 (50.0)	38 (54.3)	32 (45/7)	
Income level, n (%)				0.056
Low	49 (35.0)	18 (25.7)	31 (44.3)	
Middle	55 (39.3)	33 (47.1)	22 (31.4)	
High	36 (25.7)	19 (27.1)	17 (24.3)	
Area of residence, n (%)				0.028
Rural	73 (52.1)	30 (42.9)	43 (61.4)	
Urban	67 (47.9)	40 (57.1)	27 (38.6)	
Gestational age (%)				<0.001
First trimester	43 (30.7)	33 (47.1)	10 (14.3)	
Second trimester/Third trimester	97 (69.3)	37 (52.9)	60 (85.7)	

4.2.2 Characteristics of Newborns

Chi-square was used at p-value less than 0.05 (95%) confidence level to determine the association between characteristics of the newborns versus malaria test. The prevalence of low birth weight was 4.6% either 6 cases. There were 140 pregnancies of whom 129

(92.2%) live births, only 4 (2.29) were admitted, 1 (0.7%) stillbirth in malaria positive, 10 (7.1%) were miscarriages. 1 (0.8%) very low birth weight in malaria positive plus low birth weight with 5 (3.8%), normal delivery with 115 (88.5%), caesarian section 15 (11.5%). Females 78 (60%) against 52 (40%) male newborns. There was no statistical difference noted between the two groups with regard to the conception product outcome, birth weight, mode of delivery and child sex. Different variables under the study had a p-value ranging from 0.450 to 0.830. Therefore, newborns characteristics were not statistically significant in both malaria positive and negative groups. See table 2.0 below.

Table 4. 2: Characteristics of the Newborns

N = 140

Variables	Overall, N = 140	Malaria test		p-value
		Negative, n = 70	Positive, n = 70	
Conception product outcome, n (%)				0.820
Miscarriage	10 (7.1)	4 (5.7)	6 (8.6)	
Stillbirth	1 (0.70)	0 (0.00)	1 (1.4)	
Alive	125 (89.3)	64 (91.4)	61 (87.1)	
Admitted	4 (2.9)	2 (2.9)	2 (2.9)	
Birthweight for single baby, n (%)				0.790
Very low	1 (0.8)	1 (1.5)	0 (.00)	
Low	5 (3.8)	2 (3.0)	3 (4.7)	
Normal	119 (91.5)	61 (92.4)	58 (90.6)	
Macrosomia	5 (3.8)	2 (3.0)	3 (4.7)	
Mode of delivery, n (%)				0.450
Normal	115 (88.5)	57 (86.4)	58 (90.6)	
CS	15 (11.5)	9 (13.6)	6 (9.4)	
Sex for single child, n (%)				0.830
Males	52 (40.0)	27 (40.9)	25 (39.1)	
Females	78 (60.0)	39 (59.1)	3960.9)	

4.3 To determine predictors of low birth weight in pregnancy associated malaria.

4.3.1 Predictors of birth weight in pregnancy associated malaria.

Fisher' exact was used for value equal or less than 5 in the table while relative risk was calculated for malaria and non-malaria cases versus predictors of birthweight to determine the risk ratio. Overall, the majority of newborns who had normal birth weight were from non-anemic women 89 (68.5%, 95% CI: 0.917 – 1.081), were non-reactive 125 (96.2), absence of gestational hypertension 125 (96.2) and non-diabetic 128 (98.5). HIV was diagnosed in five of the mothers giving a prevalence rate of 3.8%. None of predictors enumerated including malaria test was not significantly associated with birth weight (Fisher Exact p-value > 0.9) and Relative Risk = 0.996 at 95% C.I:917-1.081. See table 3.0 below.

Table 4. 3: Predictors of Birth Weight in Pregnancy Associated Malaria
N = 140

Variables	Birth weight				
	Overall, N = 130	**Abnormal, n = 6**	**Normal, n = 124**	**Fisher's exact p-value**	**RR 95%CI**
Anemia, n (%)				0.923	
Normal	89 (68.5)	4 (66.7)	85 (68.5)		
Abnormal	41 (31.5)	2 (33.3)	39 (31.5)		0.996 (0.917-1.081)
HIV infection, n (%)				>0.99	
Non-reactive	125 (96.2)	6 (100.0)	119 (96.0)		
Reactive	5 (3.8)	0 (.00)	5 (4.0)		
Hypertension in pregnancy, n (%)				>0.99	
Normal	125 (96.2)	6 (100.0)	119 (96.0)		
High	5 (3.8)	0 (.00)	5 (4.0)		
Gestational diabetes, n (%)				>0.99	
Normal	128 (98.5)	6 (100.0)	122 (98.4)		
Gestational-diabetes	2 (1.5)	0 (.00)	2 (1.6)		
Malaria test results, n (%)				>0.969	0.999 (0.926-1.077)
Negative	66 (50.8)	3 (50.0)	63 (50.8)		
Positive	64 (49.2)	3 (50.0)	61 (49.2)		

Note: Abnormal (Very Low and Low Birth Weights); Normal (Normal Weight and Macrosomia)

After computing Chi-square, malaria test was significantly associated with anemia (p-value <0.001). HIV, hypertension, and gestational diabetes were not associated with malaria test (p-value >0.058). There were 2 (2.9%) malaria positive mothers who had severe malaria. The portion of mothers with HIV reactive results were higher among the malaria positive than in the negative with 4 (5.7%) versus 1 (1.4%) respectively. Consequently, there were 5 (7.1%) and 2 (2.9%) of the malaria negative mothers who were hypertensive and had gestational diabetes respectively. None of the malaria positive mothers was hypertensive nor had gestational diabetes. See table 4.0 below.

Table 4. 4: Associated conditions during pregnancy by malaria results

N = 140

Variables	Overall, N = 140	Malaria test		p-value
		Negative, n= 70	Positive, n = 70	
Anemia, n (%)				<0.001
Normal	96 (68.6)	61 (87.1)	35 (50.0)	
Mild	30 (21.4)	9 (12.9)	21 (30.0)	
Moderate	12 (8.6)	0 (0.0)	12 (17.1)	
Severe	2 (1.4)	0 (0.0)	2 (2.9)	
HIV infection, n (%)				0.370
Non-reactive	135 (96.4)	69 (98.6)	66 (94.3)	
Reactive	5 (3.6)	1 (1.4)	4 (5.7)	
Hypertension in pregnancy, n (%)				0.058
Normal	135 (96.4)	65 (92.9)	70 (100.0)	
High	5 (3.6)	5 (7.1)	0 (.00)	
Gestational diabetes, n (%)				0.500
Normal	138 (98.6)	68 (97.1)	70 (100.0)	
Gestational diabetes	2 (1.4)	2 (2.9)	0 (.00)	

4.4 To establish the key proteomic profiling biomarkers associated with malaria.

This objective was achieved through a pilot study which collected blood sample of 142 participants where 50 were malaria positive and 92 malaria negative, each cohort had two plates and the number of proteins extracted from each participant were 184 proteins.

Four internal control were added to each sample to monitor the quality of assay performance as well as the quality of individual samples. The sample type was serum and the quality control was performed in two steps: Each sample was evaluated on the standard deviation of the internal control whose value was between 0.2 normalized protein ratio (NPX), only data from the sample plate that passed this quality control were reported. Secondly, quality of each sample was assessed by evaluating the deviation from the median value of the controls for each individual sample, sample that passed less than 0.3 NPX from the median passed the quality control. Non metric multidimensional scaling was used to distinguish the similarity and dissimilarity between cohort proteins as well as the separation in positive and negative proteins. The diversity difference was assessed through Simpson and Chao indexes. Standard deviation was calculated for each protein mean for determining the protein group mean value difference and T-test to compare the protein mean of the two groups to determine whether protein mean groups are different from one another. The ideal was to determine if there was association between protein mean and the low birth weight

4.4.1 Plotting Rarefaction Curve

Rarefaction curves were an indicator of sampling adequacy by assessing whether the cumulative number of species in this case protein levels had reached an asymptote (Braukmann, *et al.,* 2019). See figure: 4.0 below.

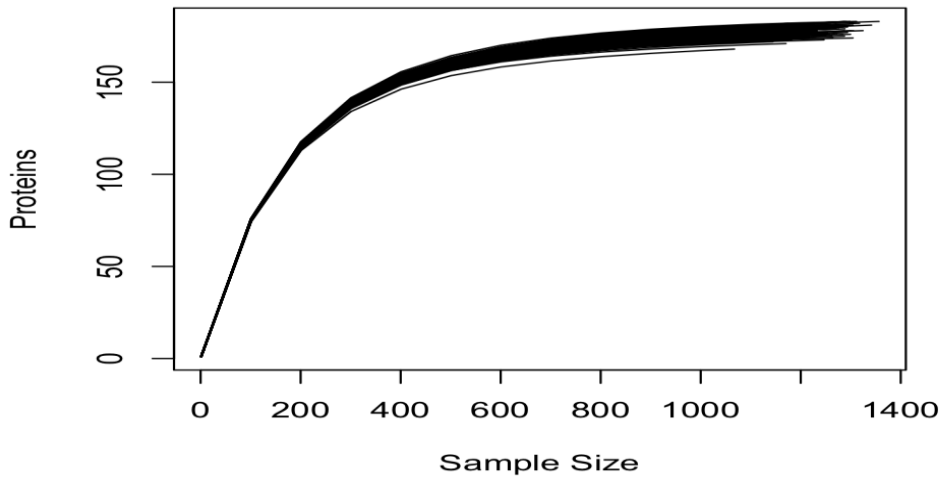


Figure 4. 1: Proteins Rarefaction Curve

4.4.2 Non-Metric Multidimensional Scaling

Nonmetric Multidimensional Scaling (NMDS) was run with stable solution from random starts, axis scaling and species scores. NMDS tries to find a stable solution using several random starts. In addition, it standardizes the scaling in the result, so that the configurations are easier to interpret, and adds species scores to the site ordination. Gower distance was used to compute dissimilarity indices. Gower, Bray–Curtis, Jaccard and Kulczynski indices are good in detecting underlying ecological gradients (Pane, *et al.*, 2017).

Gower distance was calculated as:

Gower $d_{jk} = (1/M)\sum_i \frac{|x_{ij}-x_{ik}|}{\max x_i - \min x_i}$

Binary: $(A+B-2J)/M(A+B-2J)/M$ where M is the number of columns (excluding missing values), x_{ij} and x_{ik} refer to the quantity on species (column) i and

sites (rows) j and k. A and B are the numbers of species on compared sites, and J is the number of species that occur on both compared sites (Protasov, 2017).

The spider diagram shows 1=positive test and 0=negative test. The shared proteins in the overlapping shape and unique proteins are sticking out. See figure 5 below.

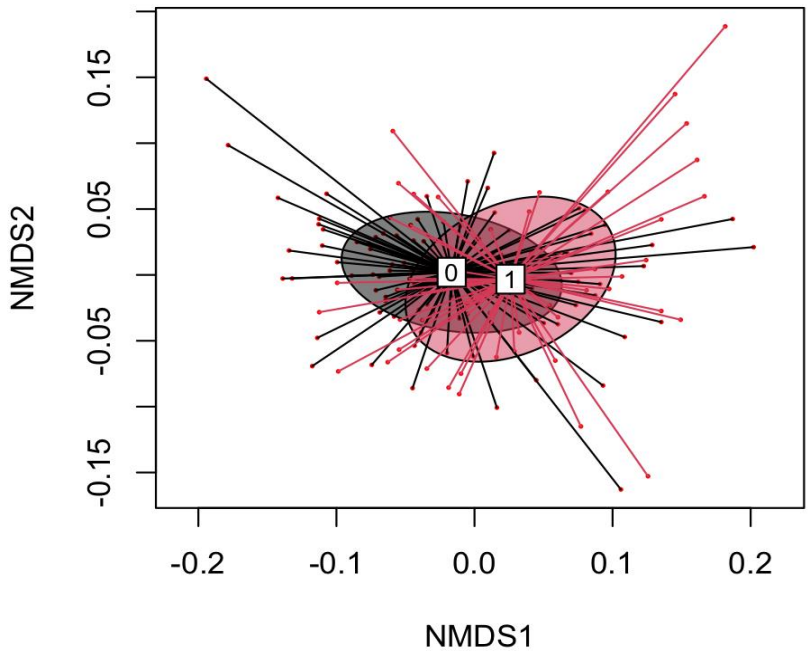


Figure 4. 2: NMDS – Malaria Outcome

(0 – Test Negative & 1 – Test Positive)

The mode of delivery showed that more likely delivery of the majority was uniform and newborns were alive. See figure 6.0 below.

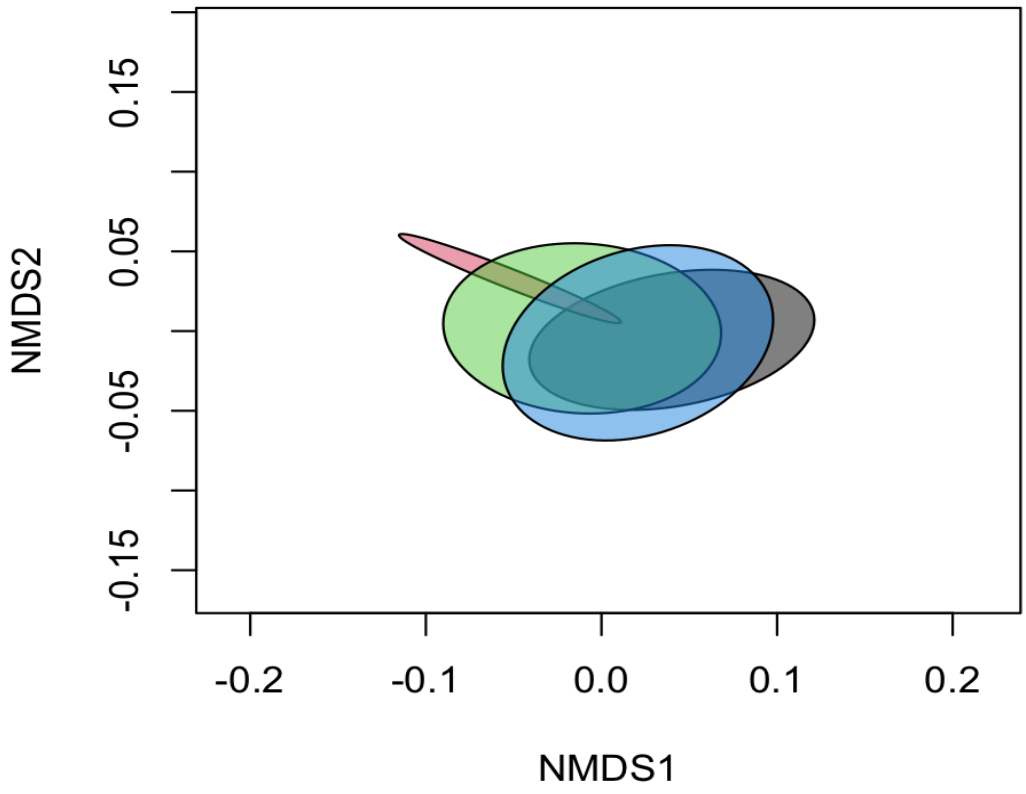


Figure 4. 3: NMDS – Mode of Delivery

4.4.3 Differences in Diversity: Simpson and Chao Indices

Simpson Index

Simpson's index was calculated as 1-D where D=∑p_i^2 and p_i is the proportional abundance of protein i. There was good overlap and high diversity in malaria positive. See figure 7.0 below.

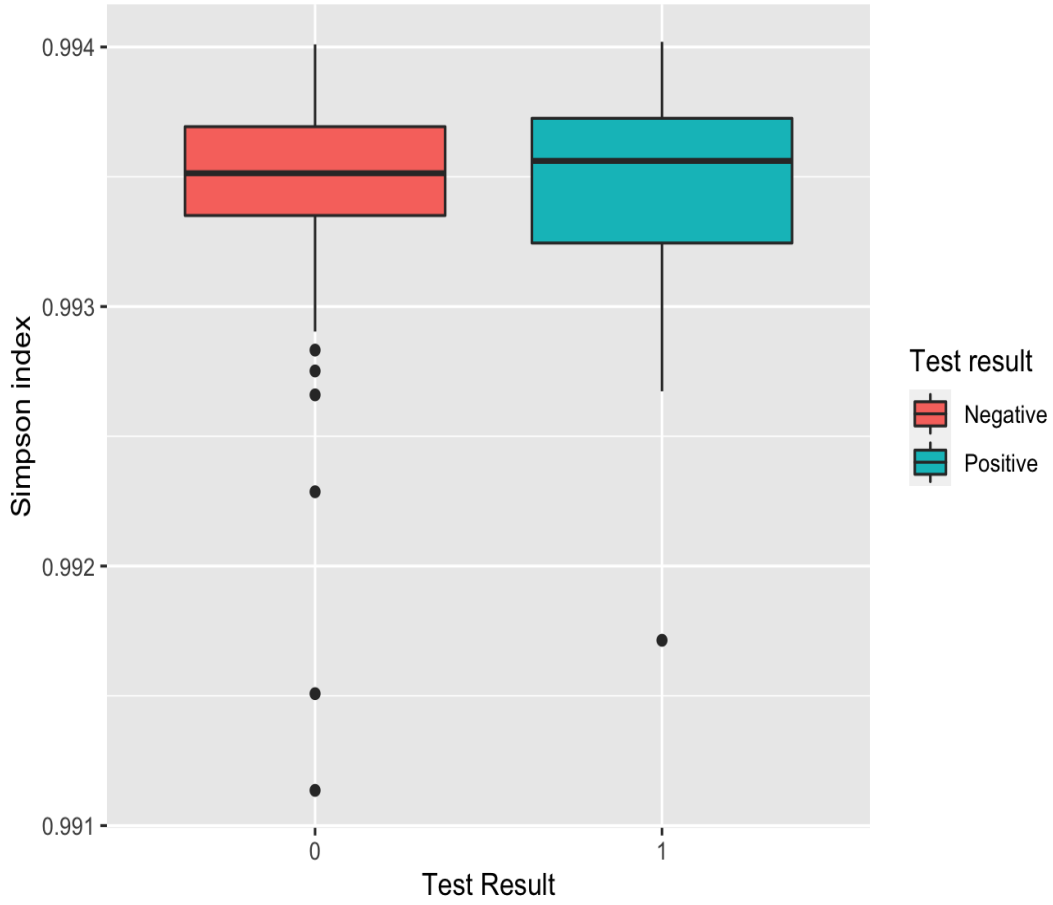


Figure 4. 4: Simpson Plot Index by Test Results

(Indicator: Color "Red" – Negative & "Blue" – Positive)

Proteins diversity was noted with regard to caesarian section and normal delivery captured through a perfect fitness model. See figure 8 below.

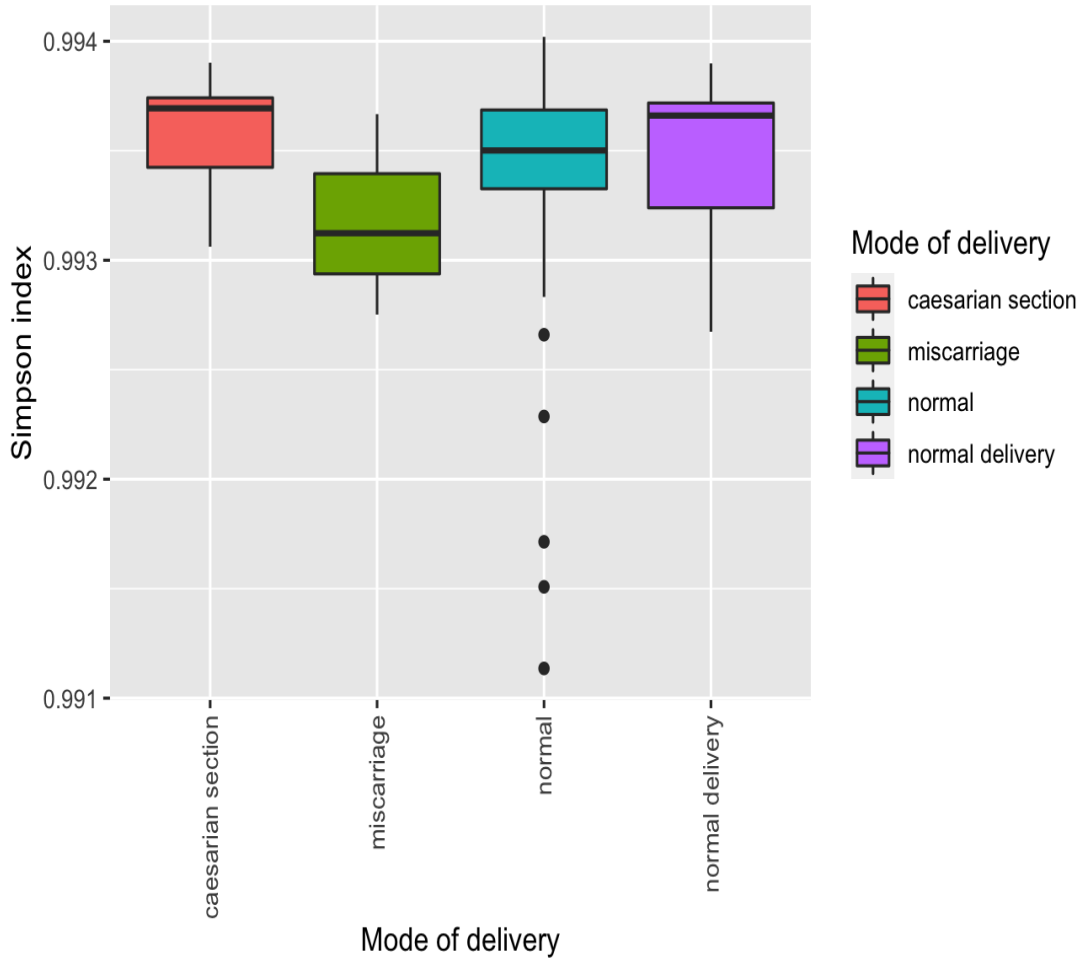


Figure 4. 5: Simpson Plot Index by Mode of Delivery

(Indicator: Colors "Red" – Caesarian Section, "Green" – Miscarriage, "Blue" Normal & "Purple" – Normal Delivery)

The diversity was perfectly observed with proteins in the secondary education but fitness model was perfectly observed with the university education. See figure 9.0 below.

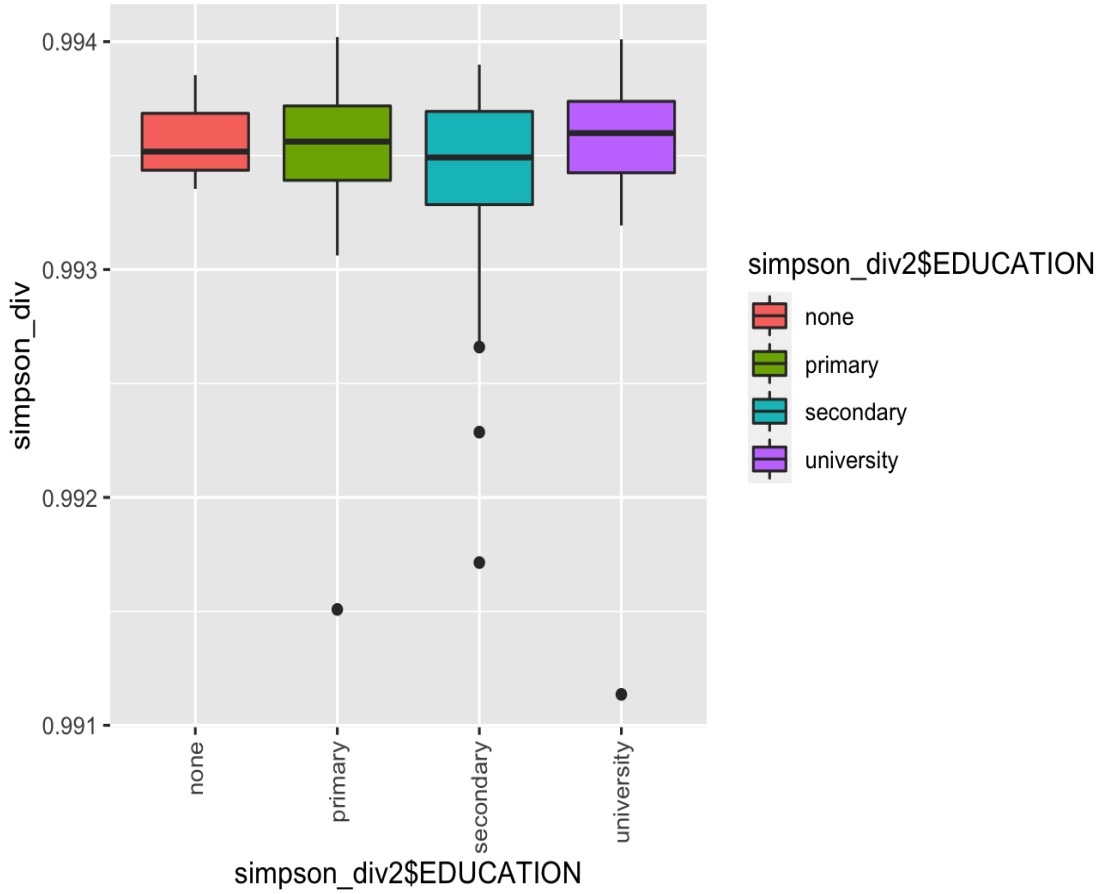


Figure 4. 6: Simpson Plot Index by Education

(Indicator: Colors "Red" – None, "Green" – Primary, "Blue" – Secondary & "Purple" – University)

The incidence-based estimate, Chao, uses the frequencies of species or in this case proteins in a collection of study subjects.

Chao $S_P = S_0 + \frac{a_1^2}{2a_2}\frac{N-1}{N}$

Where the following, S_P represents the extrapolated richness in a pool, S_0 the observed concentration of proteins in the collection, a_1 and a_2 are the concentration of proteins

occurring only in one or only in two sites in the collection, p_i is the frequency of proteins i, and N is the number of patients in the collection (Gotelli, N.J., Colwell, R.K, 2001). The richness of proteins was higher in malaria positive cohort as compare to malaria negative cohort. See figure 10.0 below.

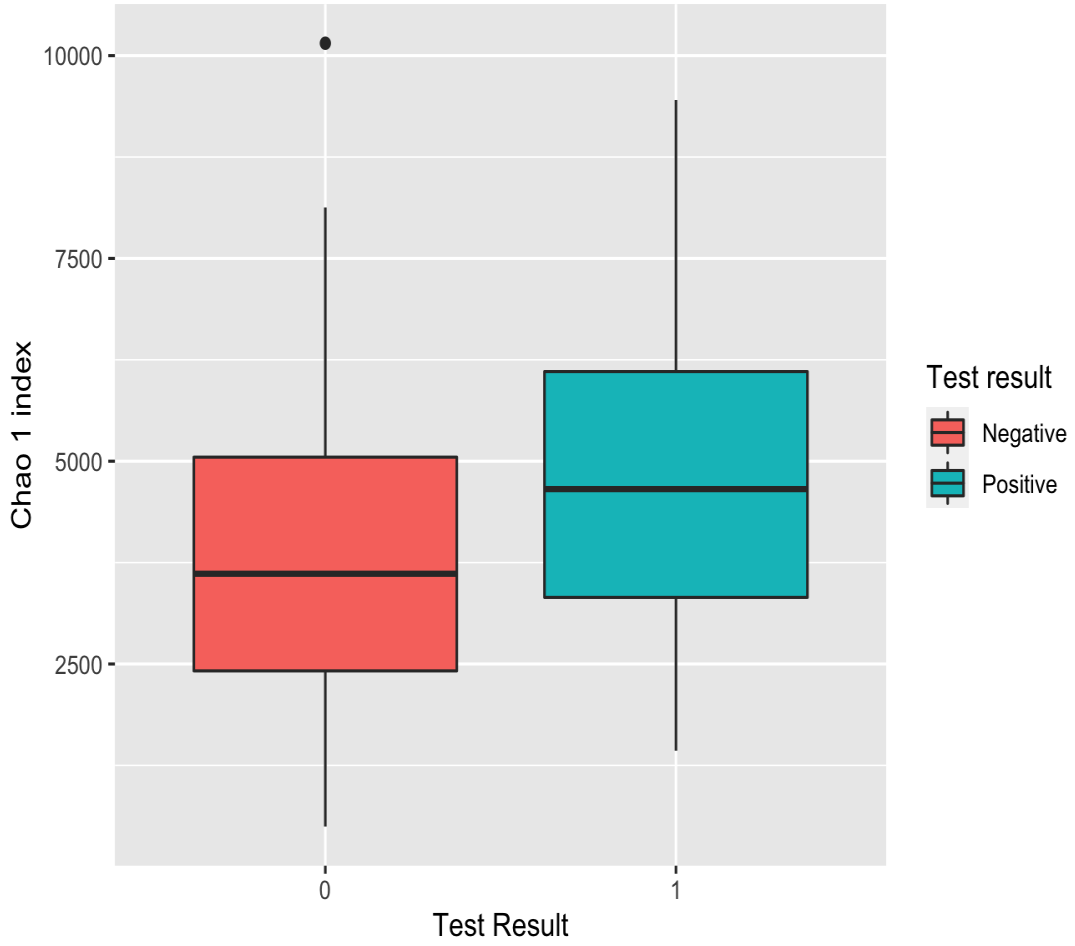


Figure 4. 7: Chao Plot Index by Test Results

(Indicator: Color "Red" – Negative & "Blue" – Positive)

The richness was observed with caesarian section and perfect with normal delivery. See figure 11 below.

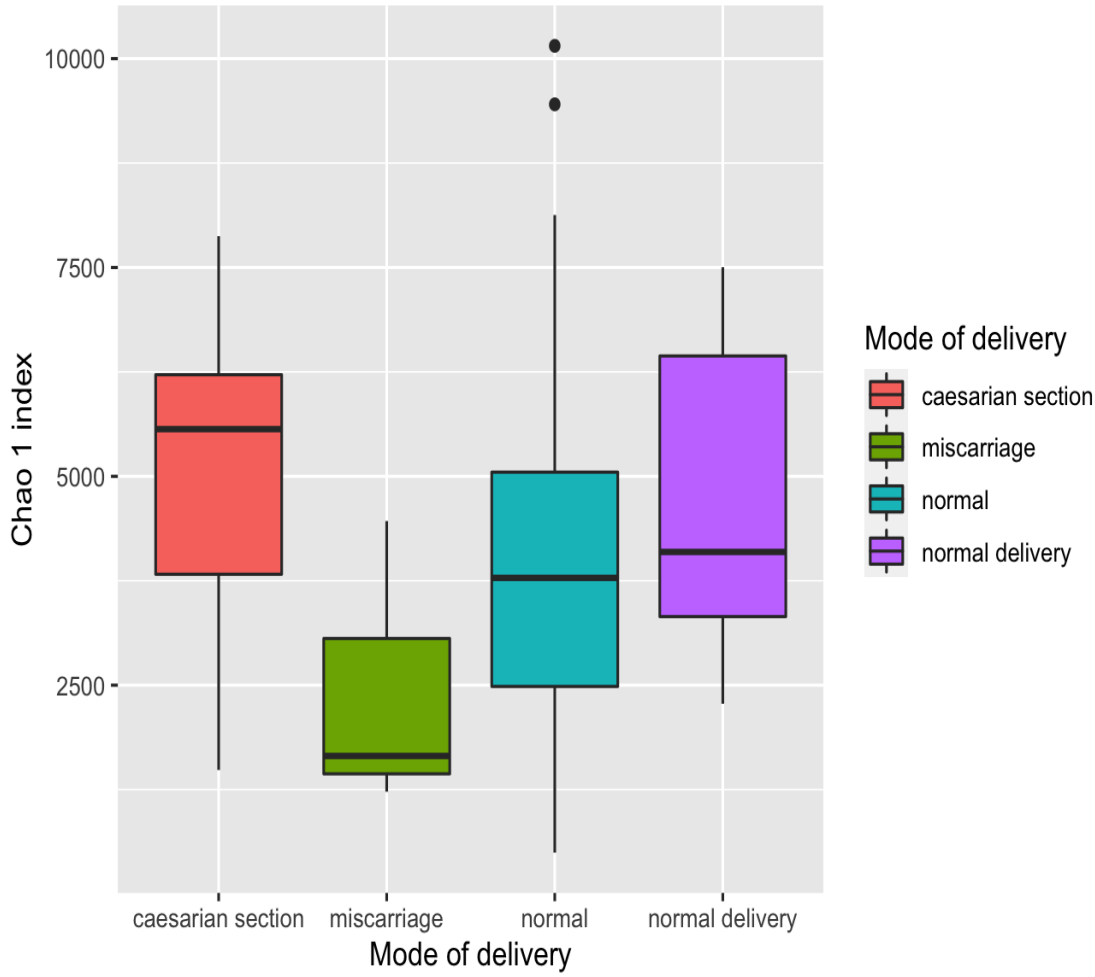


Figure 4. 8: Plot Index by Mode of Delivery

(Indicator: Colors "Red" – Caesarian Section, "Green" – Miscarriage, "Blue" – Normal & "Purple" – Normal Delivery)

Richness and abundance of proteins were high in malaria positive than malaria negative. See Figure 12 below.

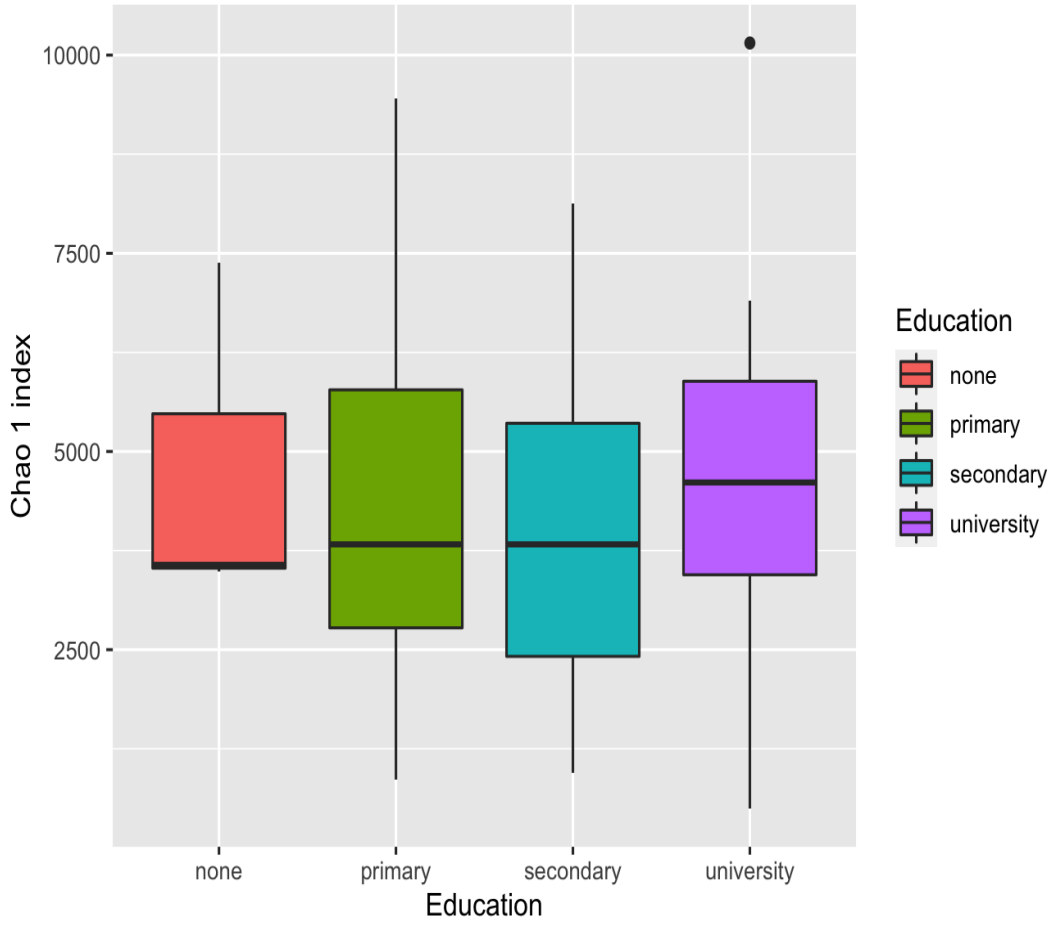


Figure 4. 9: Chao Plot Index by Education

(Indicator: Colors "Red" – None, "Green" – Primary, "Blue" – Secondary & "Purple" – University)

4.4.5 How different are the test results and other covariates in protein.

Permutation Multivariate Analysis of Variance Using Matrices to Assess Protein Composition Differences.

Permanova consists of analysis of variance using distance matrices for partitioning distance matrices among sources of variation and fitting linear models to distance

matrices; uses a permutation test with pseudo-FF ratios. Multiple ANOVA showed that the test result, gestation and parity were strongly associated with proteins (p-value<0.001) whereas mode of delivery and education were moderately associated with proteins (p-value<0.05) with 0.019 and 0.031 respectively. The ideal was to predict the potential proteins which could have been associated with the outcome of interest " low birth weight" but this model did not show that association. See table 5.0 below.

Table 4. 5: Multivariate Analysis of Variance Using Matrices.

	Degrees of freedom	Sum of squares	R squared	F-value	p-value
Test result	1	0.15	0.046	7.83	0.001
Mode of delivery	3	0.09	0.029	1.61	0.019
Education	3	0.09	0.028	1.58	0.031
Gestation	1	0.10	0.032	5.38	0.001
Age	1	0.021	0.007	1.15	0.262
Parity	9	0.31	0.1	1.86	0.001
Residual	128	2.38	0.76		
Total	146	3.14	1.00		

The mean difference of all 184 proteins were calculated but due to the variation for each individualized protein with regard to the maximum and minimum, analysis of variance and T-test were computed and the result corrected with adjusted Bonferroni correction test to avoid the type I error which is the probability of rejecting the null hypothesis when it is true. The relative influence was significant for biomarkers with high mean difference in both cohorts. The mean difference varied from 2.856690795-0.217887462 in malaria positive cohort and from -0.162524175 to -1.185322211 in malaria negative cohort. Strong signal expression or high mean protein difference was observed in 16 proteins in malaria positive cohort with the most expressed protein having a mean difference value of 2.856690795 and less expressed having 0.217887462, whereas the strong signal expression or high mean protein difference was observed in 6 proteins in malaria

negative cohort with the most expressed protein having a mean difference value of -0.162524175 and the less less expressed having -1.185322211. See table 4.6 below.

Table 4. 6: Relative Influence Biomarkers

The relative influence significant for biomarkers with high mean difference degree

Significantly enriched proteins malaria cohort	**Significantly enriched proteins no malaria cohort**
1. P80098 called C-C motif chemokine (C-C motif chemokine): Mean diff. 2.856690795	1. Q9NSA1 named FGF21_human (fibroblast growth factor 21) Mean diff. -1.185322211
2. P10145 named IL8_human (interleukin CXCL8): Mean diff. 1.683712561	2. P78423 named CX3CL1_human (Fractalkine) Mean diff. -0.450212433
3. Q13541 named 4EBP1_Human (eukaryotic translation factor 4E-binding protein 1): Mean diff. 1.434214386	3. Q99748 called NRTN (Neurturin protein) Mean diff. -0.392388749
4. P09874 named PARP1-human (polyADP-ribose polymerase): Mean diff.1.262194175	4. P 14784 named IL2RB (Interleukin-2 receptor) Mean diff. -0.311892626
5. Q16698 named DECR_human.(2,4-dienoylof-COA reductase 3E-enol-COA): Mean diff. 1.015867608	5. Q9NYY1 (IL20-human Interleukin-20) Mean diff. -0.257765889
6. Q99075 named HBEGF_human (proheparin binding epithelial growth factor) : Mean diff. 1.014586988	6. P01138 called NGF_human (Beta-nerve growth factor) Mean diff. -0.162524175
7. Q9Y6K9 called Nemo-human Mean diff. 0.885636649	
8.P22004 called BMP6_ human (Bone morphogenetic protein 6) Mean diff. 0.817531842	
9.P47992 called XCL1_ human (lymphotactin) Mean diff. 0.635413398	
10. Q13043 named STK4_human (Serine-threonine protein kinase) Mean 0.622472678	
11. P13726 called TF_human (Tissu factor) Mean diff. 0.368789368	

12. P31994 named FCG2B_human (low affinity immunoglobulin gamma)
Mean diff. 0.366971813

13. P01730 called CD4- human (T-cell surface glycoprotein CD4) Mean0.343758696

14. P25116 called PAR1_human (proteinase-activated receptor 1) Mean diff. 0.253762626

15. P07204 called TRBM_human (thrombomodulin) Mean diff. 0.243285094

16. Q9BWV1 called BOC_human (brother of CDO) Mean diff. 0.217887462

4.5 To assess the awareness among pregnant women on the IPT and LLIN.

4.5.1 Awareness of IPT/ SP

The questions were asked to assess pregnant women knowledge on the intermittent preventive. Chi-square was used to determine the knowledge in both malaria positive and negative cohorts. The findings showed that there was a significant difference in proportions between malaria negative and positive groups among mothers' knowledgeable on the side effects (p-value = 0.001), different doses (p-value = 0.012), and those who were informed about intermittent preventive treatment before administration (p-value = 0.003), The proportion of mothers knowledgeable about side effects and different doses were higher among the malaria positive group as compare to the negative group (52.9% versus 25.7% and 20.0% versus 5.7%) respectively. However, only half of the malaria positive group were informed about intermittent preventive treatment sulfadoxine-pyrimethamine before it was administered as compared to 74.3% in the negative group. Majority of pregnant women 59 (42.1%) reported that healthcare workers (HCW) had a very good attitude towards provision of healthcare. 124 (88.6%)

respondents trusted the information given by healthcare workers. Therefore, pregnant women awareness was observed with side effects and different doses of IPT/SP in the cohort of positive malaria, and prior information in the cohort of negative malaria. See table 7.

Table 4. 7: Intermittent Preventive Treatment

Variables	Overall, N = 140	Malaria test		p-value
		Negative, n = 70	Positive, n = 70	
Benefits of IPT, n (%)				0.610
No	63 (45.0)	33 (47.1)	30 (42.9)	
Yes	77 (55.0)	37 (52.9)	40 (57.1)	
Knowledge of side effects of IPT, n (%)	55 (39.3)	18 (25.7)	37 (52.9)	0.001
Different doses of IPT, n (%)	18 (12.9)	4 (5.7)	14 (20.0)	0.012
Safe drug during first trimester, n (%)	83 (59.3)	47 (67.1)	36 (51.4)	0.058
Ever refused to take IPT, n (%)	20 (14.3)	13 (18.6)	7 (10.0)	0.150
Have you been informed about IPT, n (%)	87 (62.1)	52 (74.3)	35 (50.0)	0.003
Trust information given by HCW, n (%)	124 (88.6)	65 (92.9)	59 (84.3)	0.110
Attitude of HCW towards provision, n (%)				0.300
Bad	24 (17.1)	9 (12.9)	15 (21.4)	
Good	57 (40.7)	32 (45.7)	25 (35.7)	
Very good	59 (42.1)	29 (41.4)	30 (42.9)	
IPT Doses given, n (%)				<0.001
None	29 (20.7)	24 (34.3)	5 (7.1)	
Once	34 (24.3)	17 (24.3)	17 (24.3)	
Twice	46 (32.9)	11 (15.7)	35 (50.0)	
Thrice	31 (22.1)	18 (25.7)	13 (18.6)	

Variables	Overall, N = 140	Malaria test Negative, n = 70	Malaria test Positive, n = 70	p-value
Benefits of IPT, n (%)				0.610
No	63 (45.0)	33 (47.1)	30 (42.9)	
Yes	77 (55.0)	37 (52.9)	40 (57.1)	

The benefits of using IPT-SP during pregnancy among the 140 women interviewed, 75 (76.3%) reported that IPT-SP prevents malaria, followed by 24 (13.2%) who reported that it prevents malaria and protects baby and mother. Other reasons included protecting baby and mother only (6.6%), it makes the mother strong (2.6%), and it is used for deworming (1.3%). See figure 13.0 below.

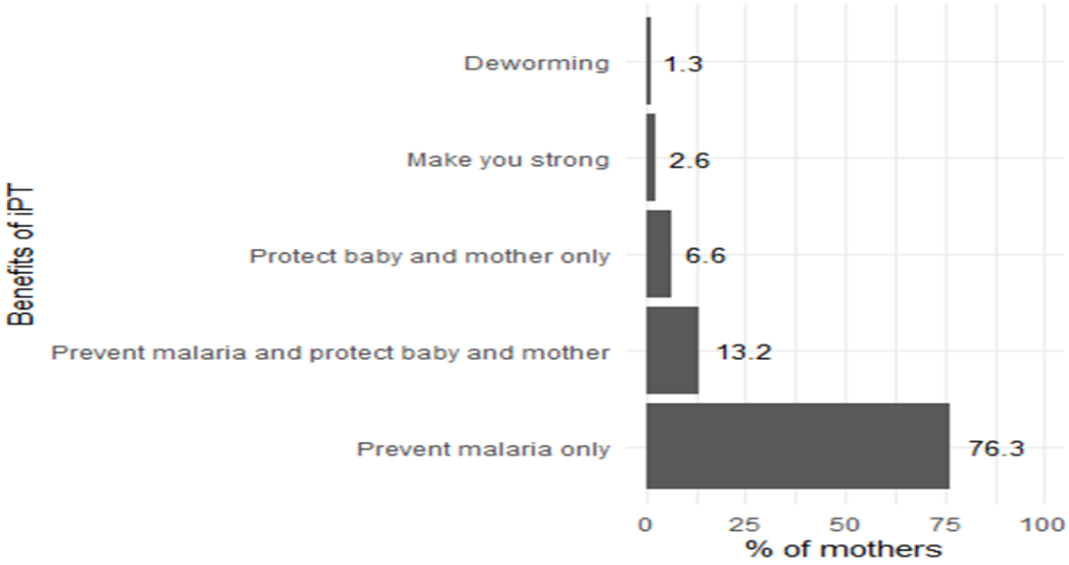


Figure 4. 10: Benefits of intermittent preventive treatment

The visualized benefits of IPT-SP in 75 (76%) of respondents against 24 (13%) and 3% cited deworming. See figure 14 below.

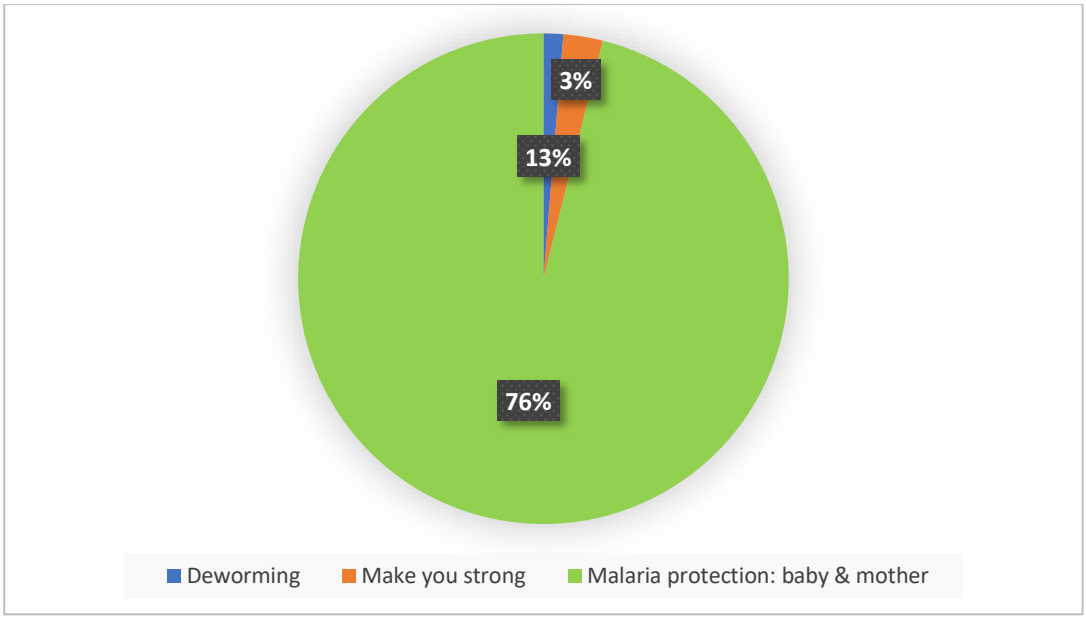


Figure 4. 11: Visualized Reported Benefits of IPT-SP

The study also assessed the reason why it is unsafe to use SP-IPT during the first trimester. Among the 140 participants interviewed, 30.9% had the opinion that it causes abortion, 10.9% said it causes vomiting, 91% premature labor and 7.3% said it causes fatigue as well as other reasons are shown in figure 15 below.

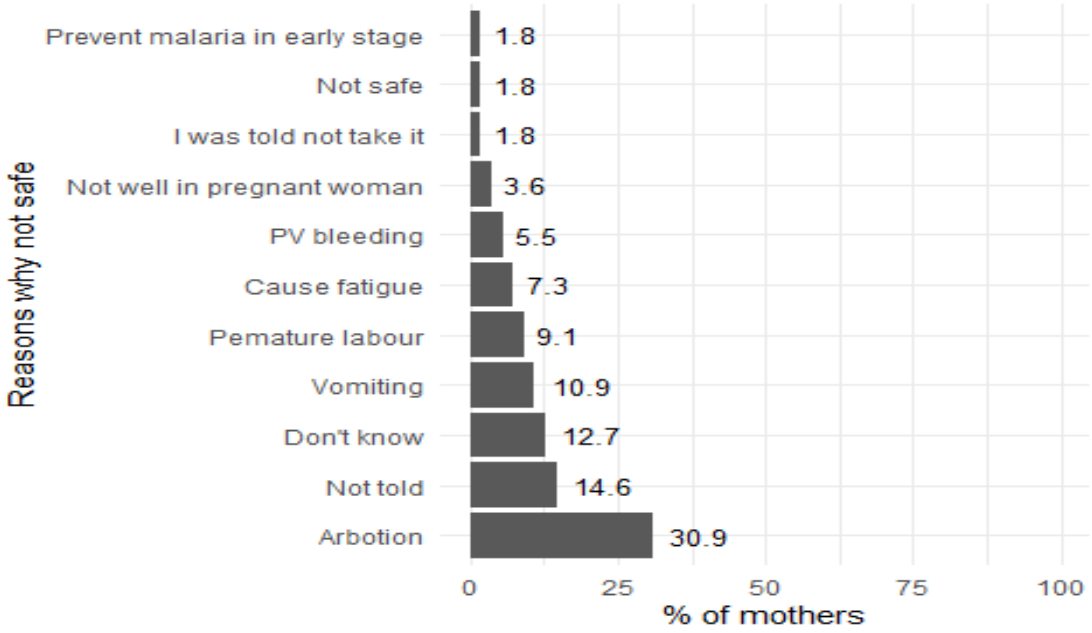


Figure 4. 12: Reported Reasons of Not Using IPT-SP during the First Trimester

The most common sources of information on IPT-SP and Insecticide treated mosquito nets mentioned were nurses (85.7%), followed by doctors (6.4%) as well as others. See figure 16 below.

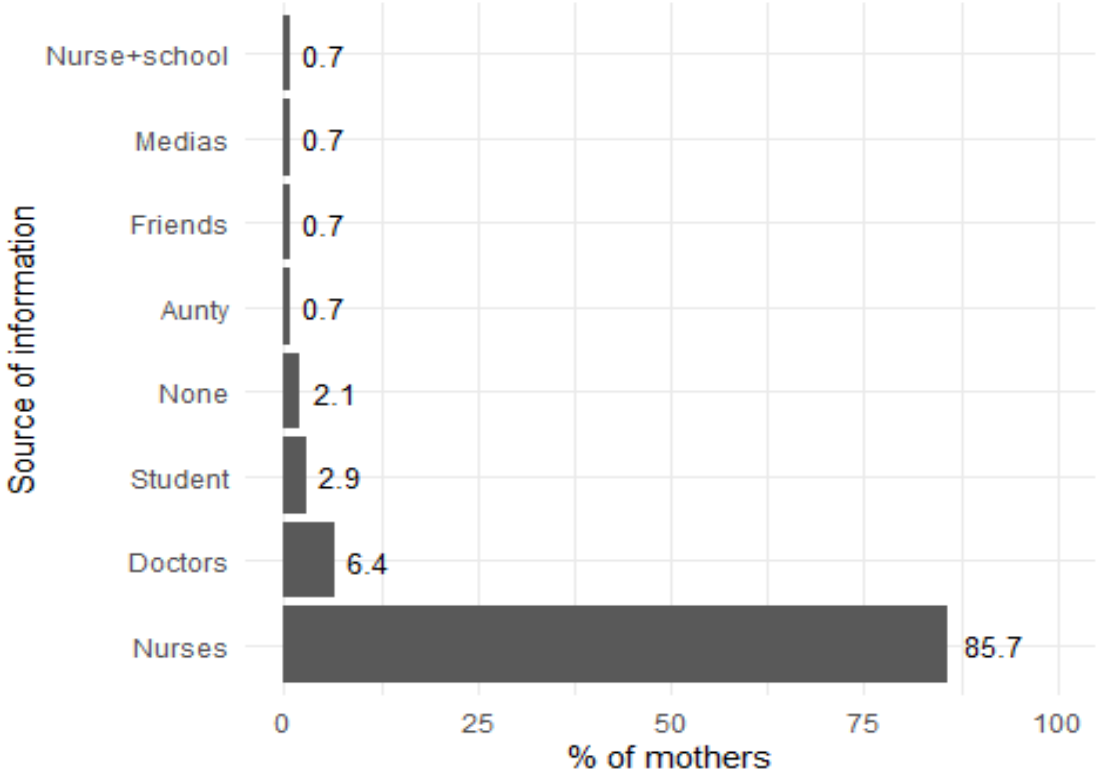


Figure 4. 13: Sources of Information about IPT-SP and Treated Mosquito Nets

A total of 133 (95.0%) of the mothers had ever used mosquito nets while 138 (98.6%) own the mosquito nets and used them a night before the survey. Though long lasting mosquito nets ownership was not statistically significantly. See table 8 below.

Table 4. 8: Insecticide Treated mosquito nets

N=140

Variables	Overall, N = 140	Malaria test		p-value
		Negative, n = 70	Positive, n = 70	
Ever received mosquito net, n (%)	133 (95.0)	68 (97.1)	65 (92.9)	0.440
Own Mosquito net, n (%)	138 (98.6)	68 (97.1)	70 (100.0)	0.500

Most of the respondents reported not being told the benefit of using a long lasting treated mosquito nets during the clinic (52.9%). However, 20.0%, 7.1%, 4.3% reported not using due to fear of toxicity, breathlessness and fear rashes respectively. See figure 17 below.

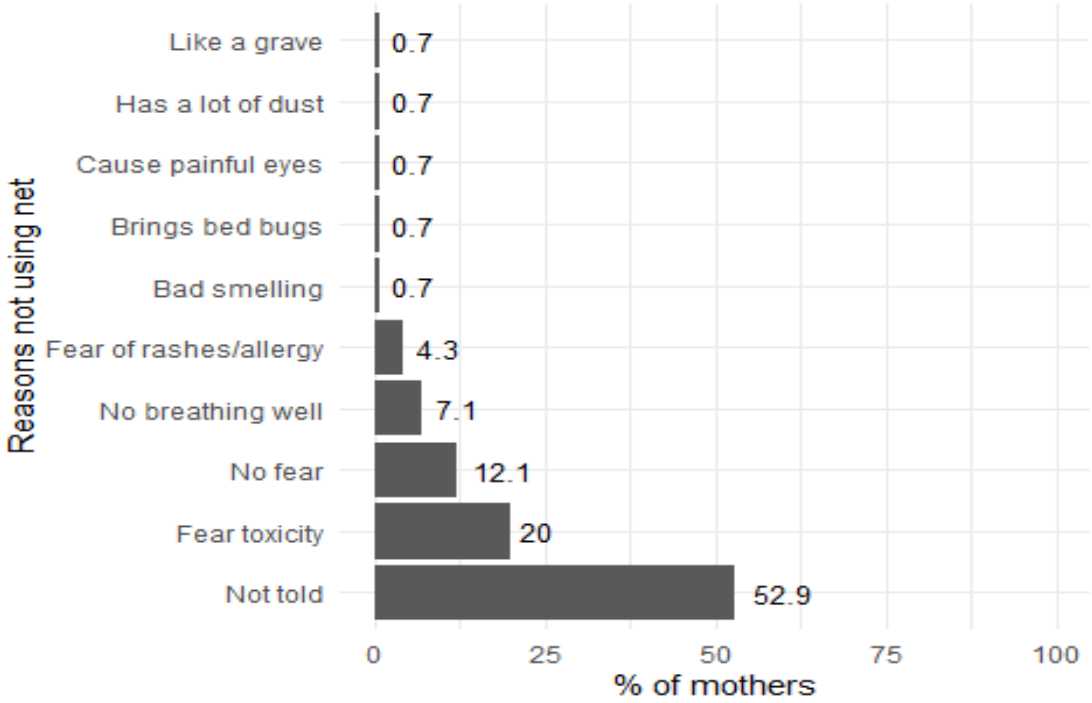


Figure 4. 14: Reported Reasons of Not Using Mosquito Nets

4.5.2 Intermittent preventive treatment and Long lasting treated nets

Two focus group discussion were organized with pregnant women having and not having history of malaria to discuss in depth and get their views and opinions about the awareness and the practices of the intermittent preventive treatment/sulfadoxine-pyrimethamine and the long lasting insecticide nets use during antenatal clinic. Each

group was made of six respondents with a total of 12 respondents. Their answers were recorded, translated and coded before in Vivo analysis then compared. From the table below more than half of respondents either 7(58%) were aware of the benefit of sulfadoxine-pyrimethamine against 5 (42%). Pregnant women expressed that Sulfadoxine-pyrimethamine prevents malaria and those aware were 7 (58%), see table 9 below.

Table 4. 9: Benefit of Sulfadoxine-pyrimethamine

Knowledge of the benefit of SP-IPT	Frequency	Percentage	Cumulative
Aware	7	58%	58%
Unaware	5	42%	100%
Total	12	100 %	100%

Pregnant women with malaria 7 (58%) were aware that IPT-SP is beneficial during pregnancy. They had this to say "This medicine prevents mother from getting malaria". See text box 1 below.

Text box 1. Theme: Intermittent preventive treatment/sulfadoxine-pyrimethamine among pregnant women with malaria, subtheme: benefit of Intermittent preventive treatment/sulfadoxine-pyrimethamine.

"This medicine helps to reduce the effects of malaria and prevents mother to contract malaria so that the baby can be protected". Mother three focus group discussion one.
"It prevents the mother from malaria and other illnesses so that the baby remains safe". Mother four focus group discussion one.
"It prevents the mother from miscarriages and at the same time prevent the foetus to get malaria". Mother five focus group discussion one.
"It prevents the mother from getting malaria and her foetus while strengthening mother immunity". Mother six focus group discussion one.
"Taking this medicine helps prevent malaria to the mother and unborn baby". Mother three focus group discussion two.
"The medicine prevents pregnancy from malaria". Mother five focus group discussion two.
"It prevents the mother from getting malaria and her foetus while strengthening mother immunity". Mother six focus group discussion two

Pregnant women 5 (42%) were unaware that IPT-SP prevents malaria during pregnancy had this to say "I do not know or I have never heard about the importance of this medicine

called sulfadoxine- pyrimethamine" 5 (42%) and this was enough a reason for concern to have an important number of participants not knowing the benefit of a key intervention during antenatal. See text box 2 below.

Text box 2. Theme: Intermittent preventive treatment among pregnant women without malaria, subtheme: benefit of sulfadoxine-pyrimethamine.

"I have never heard about it before although it helps to prevent malaria and keep the baby healthy and treat at the same time other diseases". Mother one focus group discussion one.
"I have never been told about something like that but it prevents diseases". Mother two focus group discussion one.
"I do not know the importance of taking this medicine and I was not told about it". Mother one focus group discussion two.
"I do not know the importance of taking it". Mother two focus group discussion two.
"I do not know because I was not told". Mother six focus group discussion two.

4.5.3 Schedule of the intermittent preventive treatment/sulfadoxine-pyrimethamine.

The study participants 7(58%) were aware of the schedule of the sulfadoxine-pyrimethamine against 5(42 %), see table 10 below.

Table 4. 10: Schedule of SP-IPT

SP-IPT SCHEDULE	Frequency	Percentage	Cumulative %
Aware	7	58 %	58%
Unaware	5	42%	100%
Total	12	100%	100%

The majority of pregnant women with malaria ether 7(58%) were aware of the schedule of intermittent preventive treatment/sulfadoxine-pyrimethamine had this to say "It is three doses given each month". See text box 3 below.

Text box 3. Theme: Intermittent preventive treatment among pregnant women with malaria, subtheme: Schedule of sulfadoxine-pyrimethamine.

"It is three doses given at each one-month interval and the side effects include vomiting". Mother three focus group discussion one.
"It is three doses with one-month interval". Mother four focus group discussion one.
"It is three doses after every 30 days". Mother six focus group discussion one.

"It is three doses but after taking I vomited it and never took it again". Mother two focus group discuss two.
"I know it is three doses during every antenatal visit with 30 days of interval, I took it and vomited but other mothers say that they had fever and got sick after taking it" Mother three focus group discuss two.

"It is three doses during every antenatal visit with one-month interval, I also vomited, had loss of appetite, strengths and dizziness after taking it". Mother four focus group discuss two
"I know it is given three doses during each antenatal, when I was going to Eldoret in a hospital I was given this medicine and I vomited". Mother five focus group discuss two.

Pregnant women without malaria 5 (42%) who did not know the schedule of IPT-SP had this to say "I do not know about it because I have never been given" This was a reason of concern of almost 42% of study respondents not aware about an important activity such as strategy focused on prevention of malaria among expectant women. Text box 4 below.

Text box 4. Theme: Intermittent preventive treatment among pregnant women without malaria, subtheme: schedule of sulfadoxine- pyrimethamine

"I know it is one dose and I have never been given this treatment during my first pregnancy when I was attending a district hospital in Eldoret no information was given to me". Mother one focus group discussion one.
"I don't know about it". Mother two focus group discussion one.
"I do not have a clue since I have never been given this medicine and I have never used it". Mother five focus group discussion one.
"I do not know the schedule of this medicine and side effects because during my first pregnancy I was not given". Mother one focus group discussion two.
"I have never used it before therefore do not know". Mother six focus group discussion two.

4.6 To assess the level of knowledge of healthcare workers on the IPT and LLIN.

This objective addressed the knowledge of healthcare workers on the intermittent preventive treatment as well as the long lasting mosquito nets. It is mainly captured through the descriptive statistics using frequency, mean and percentages.

4.6.1 Socio-Demographic Characteristics of the Health Workers

In total 39 healthcare workers (HCWs) participated in the study in order to address this objective. Females were 28 (71.8%), the majority were aged 23-37 years old (n = 23; 59.0%), having a tertiary level of education (n = 39; 100 %), nurses (n = 28; 71.8%). Of the HCWs, 33 (84.6%) have ever worked in MCH/ANC, of whom 15 (45.5%) had worked for less than one years, 14 (42.4%) for 2-5 years and only four had worked for more than five years in the MCH/ANC service. There were 13 (33.3%) of the HCWs that were working at the MCH at the time of the study. See table 11 below.

Table 4. 11: Socio-Demographic Characteristics of Healthcare Workers

Variables	N = 39
Gender, n (%)	
Female	28 (71.8)
Male	11 (28.2)
Age group in years, n (%)	
23-37	23 (59.0)
38-52	13 (33.3)
53-57	3 (7.7)
Level of education, n (%)	
Tertiary	39 (100)
Professional training, n (%)	
clinical officer	6 (15.4)
Doctor	2 (5.1)
Midwife	3 (7.7)
Nurse	28 (71.8)
Ever worked in MCH/ANC, n (%)	33 (84.6)
Currently working in MCH, n (%)	13 (33.3)
Years of experience in MCH/ANC, n (%)	
< 1 year	15 (45.5)
> 5 years	4 (12.1)
2-5 years	14 (42.4)

4.6.2 Training and Availability of Guidelines

Healthcare workers 20 (51.3%) reported that had ever received training on malaria, all of them agreed that the training received was beneficial to their work. 18 (46.2%) reported possessing new guidelines for antenatal clinics on IPT/ITN. See figure 18 below.

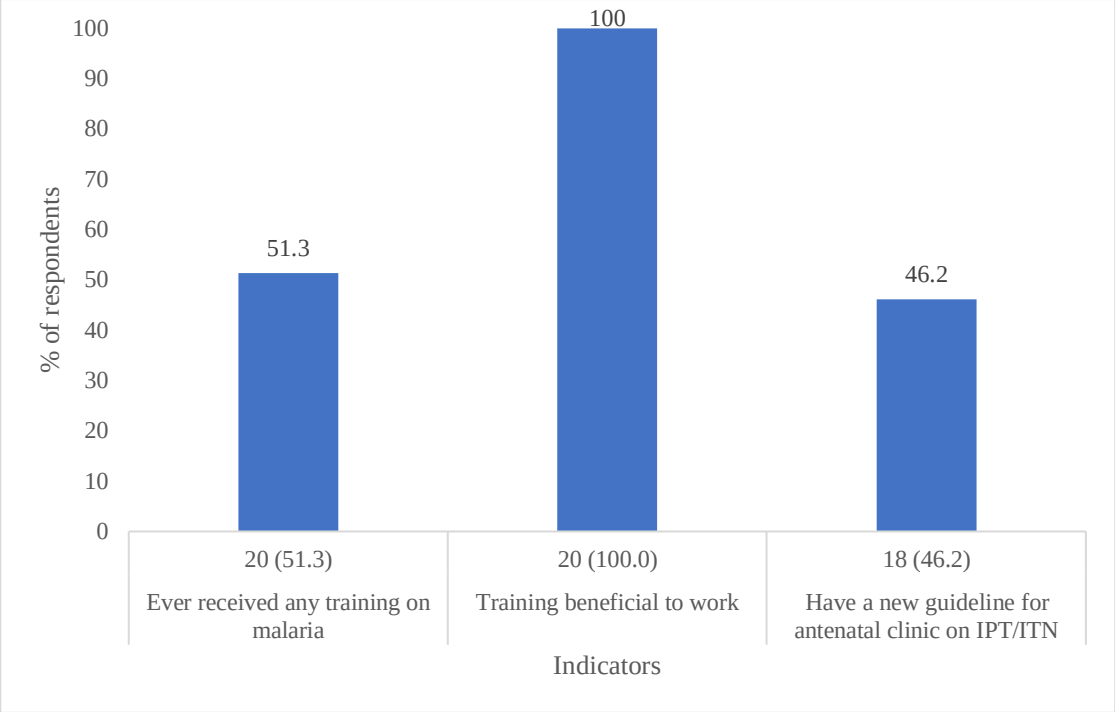


Figure 4. 15: Training and Availability of Guidelines

4.6.3 Healthcare Worker's Knowledge on the Intermittent Preventive Treatment

Different components of knowledge of IPT-SP among the HCWs were assessed using percentage. Four questions were asked to the respondents, each answer was allocated 2 points if correct and zero if incorrect. The total was multiplied by 100%, when the average was below 49% (bad), 50-69% (good) and above 70% (very good). The findings showed that the majority (n=28; 71.8%) reported that IPT should be started during the second trimester of pregnancy, the common known side effect of IPT/SP reported was nausea and vomiting (n = 28; 71.8%). Most HCWs knew the advantages of IPT as a preventive measure of malaria (n = 35; 89.7%). Majority also had the knowledge that PMTCT women should not take IPT-SP. Other known cases where IPT-SP should not be given during pregnancy includes those who were allergic to Sulphur as reported by 29 (74.4%) of the HCWS or those under other medications (n = 10; 25.6%). Respondents (n=32, 82.1%) knew that IPT-SP should be associated with low dose folic iron 0.4mg.

Therefore, The majority of HCW had very good knowledge toward IPT/SP. See table 12 below.

Table 4. 12: Healthcare Workers Knowledge on the IPT

Questions	N = 39
State when the IPT-SP should be started during pregnancy?, n (%)	
First trimester	11 (28.2)
Second trimester	28 (71.8)
What are the side effects of IPT-SP, n (%)	
Nausea, vomiting	28 (71.8)
Others	11 (28.2)
Do you know advantages of IPT-SP in pregnancy?, n (%)	
Prevent malaria	35 (89.7)
Others	4 (10.3)
Do you know that PMTCT women should not take IPT-SP?, n (%)	37 (94.9)
In which other case IPT-SP should not be given during pregnancy, n (%)	
Allergic to sulfur	29 (74.4)
Under other medication	10 (25.6)
Do you know that IPT-SP should be associated with folic iron 0.4 mg?, n (%)	32 (82.1)

Healthcare workers who knew that a pregnant woman can receive 4 doses of IPT-SP (n=7, 17.9%), others mentioned even more than 5 doses (n=5, 12.8%), 0 dose (n=6,15.4%) for those who did not know. See figure 19 below.

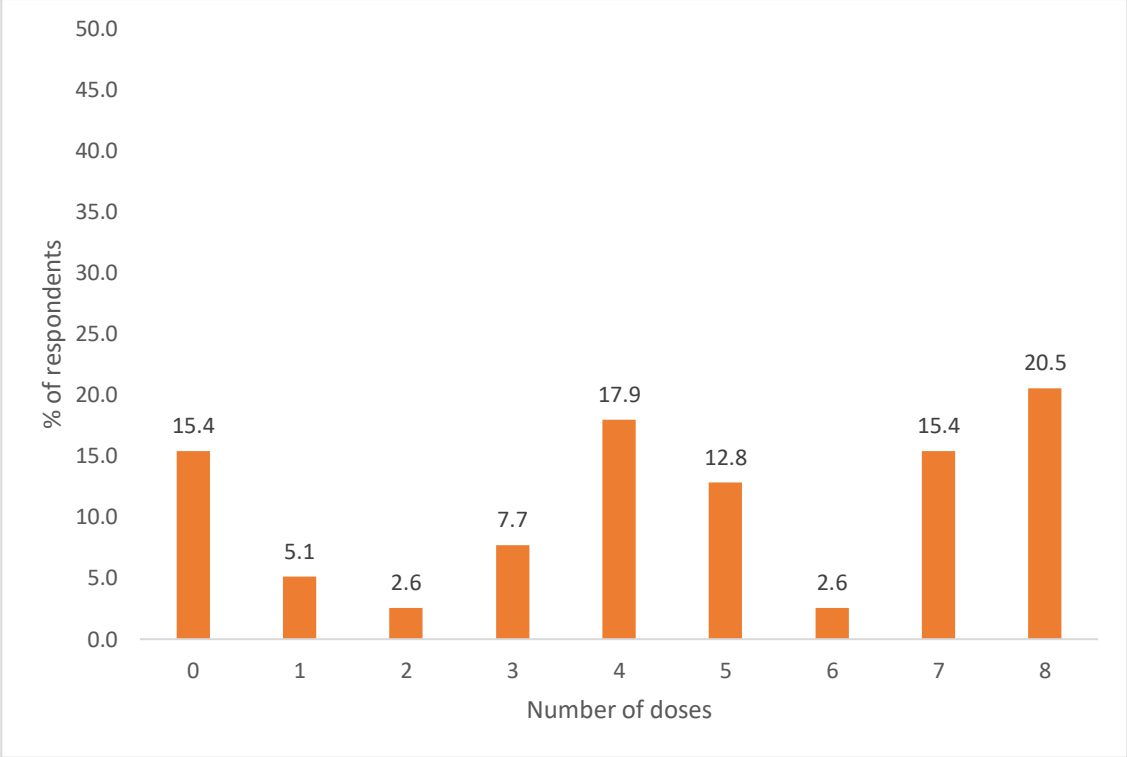


Figure 4. 16: Knowledge of Number of Doses of IPT-SP

4.6.4 Healthcare Workers' Knowledge on Long Lasting Insecticide Nets

The components of knowledge of ITN were assessed among healthcare workers where 39 (100%) approved the benefit of sleeping under ITN during pregnancy, while 37 (94.5%) knew that LLIN can last for three years, 32 (82.1%) reported that they distribute long-lasting nets during MCH activities and 23 (59.0%) reported they run out of stock in LLIN. See figure 20 below.

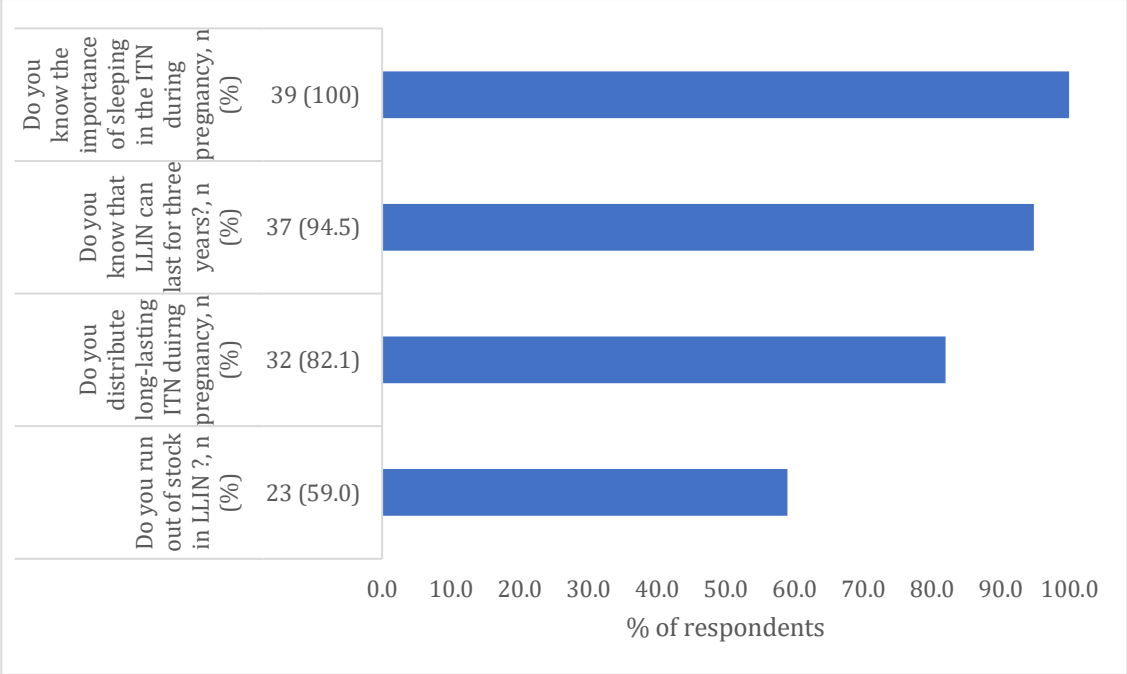


Figure 4. 17: Knowledge on Long Lasting Insecticide Treated Mosquito Nets

4.7 To assess the practices of pregnant women on the IPT and LLIN

The proportion of pregnant women who used long lasting treated nets the previous night varied. Those with malaria 70(100%) used their long-lasting treated nets as compare to their counterparts without malaria 68 (97.1%). The use of LLIN was not associated with malaria test (p-value >0.05). See table 13 below.

Table 4. 13: Long Lasting Insecticide Treated Nets Use by Pregnant Women

Variable	Overall, N = 140	Malaria test		p-value
		Negative, n = 70	Positive, n = 70	
Use mosquito net last night, n (%)	138 (98.6)	68 (97.1)	70 (100.0)	0.500

There was 65 (92%) coverage of IPT/SP +2 doses among positive malaria women against 46 (55.7%) among negative malaria. See figure 21 below.

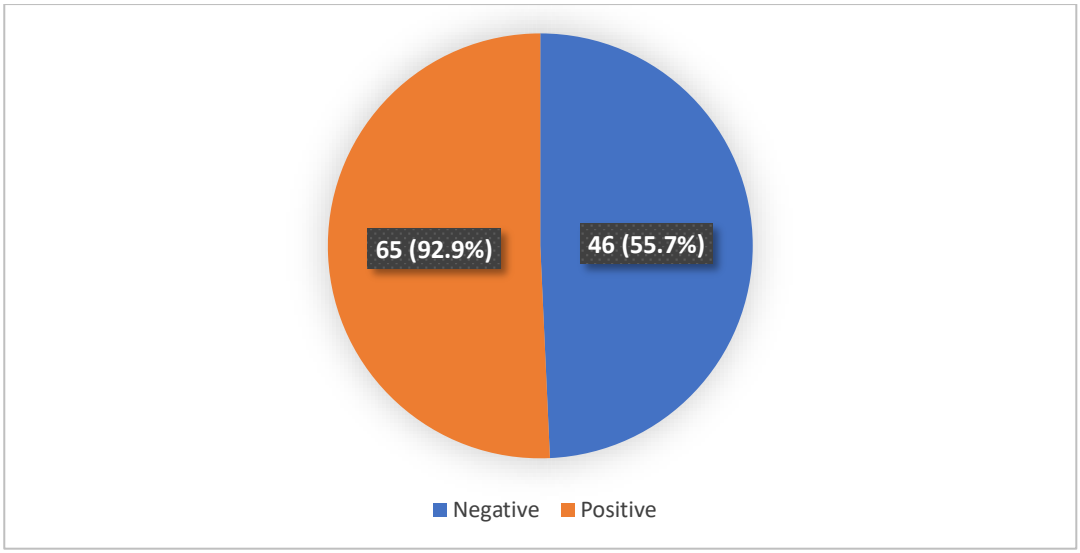


Figure 4. 18: IPT coverage and malaria test among pregnant women

There was statistical association between the number of IPT-SP doses and malaria test (p-value <0.001). Overall, most women received two doses of IPT (n = 46; 32.9%). Among the malaria negative women, 24 (34.3%) did not receive any dose while 11 (15.7%) received two doses. This was different with malaria positive women 35 (50.0%) who received two doses of IPT-SP and only 5 (7.1%) had received any dose. The total coverage of 2 doses and + was higher among the positive pregnant women 48 (68.6%) against negative women 29 (41.4%). See table 14 below.

Table 4. 14: Different Doses of IPT Received by Pregnant Women.

Variable	Overall, N = 140	Malaria test		P-value
		Negative, N = 70	Positive, N = 70	
IPT Doses given, n (%)				<.0001
None	29 (20.7)	24 (34.3)	5 (7.1)	
Once	34 (24.3)	17 (24.3)	17 (24.3)	
Twice	46 (32.9)	11 (15.7)	35 (50.0)	
Thrice	31 (22.1)	18 (25.7)	13 (18.6)	

The cohort of malaria positive respondents 35 (50%) received 2 doses of IPT-SP against 11 (15.7%) in the cohort of negative respondents. Those who received 3 doses were 18 (25.7%) in negative cohort versus 13 (18.6%) in positive cohort. See figure 22 below.

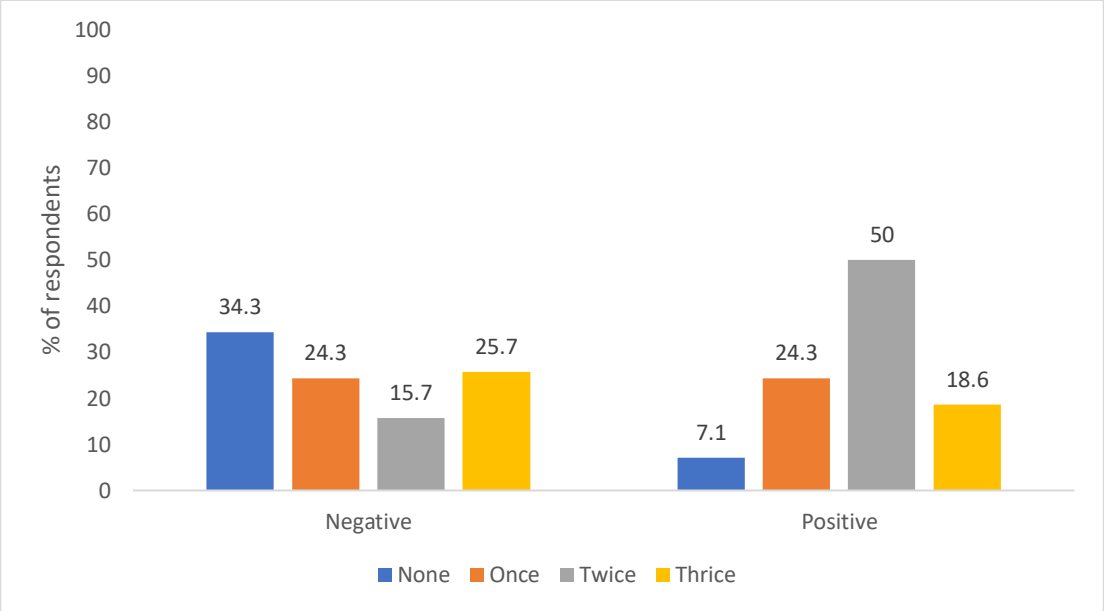


Figure 4. 19: Knowledge of Different Doses of IPT in Pregnancy

4.7.1 Practices of intermittent preventive treatment/ sulfadoxine-pyrimethamine.

A focus group discussion to collect pregnant women opinions was organized. Their views were captured, recorded, transcribed, then coded and analyzed by the mean of MAXQDA to identify practices with regard to the completeness of the sulfadoxine-pyrimethamine during pregnancy. 10 (83%) pregnant women had good completeness of IPT/SP as a key element toward prevention of malaria during pregnancy. See table 15 below.

Table 4. 15: Practices of intermittent preventive treatment

Sulfadoxine-pyrimethamine	Frequency	Percentage
Practicing/completing	10	83%
No practicing/refusing	2	17%
Total	**12**	**100%**

10(83%) of study participants who had taken and accepted the intermittent preventive treatment/sulfadoxine-pyrimethamine, had this to say; I had swallowed this medicine twice or thrice. See text box 5 below.

Text box 5. Theme: Practices of intermittent preventive treatment among pregnant women with malaria, Subtheme: Acceptance and completeness of sulfadoxine pyrimethamine.

"I am sure it is almost two time I have been given this medicine when going to the antenatal clinic, I took it with water after the nurse explained to us and I have never refused to swallow it". Mother two focus group discussion one.

"The nurse told me that it is very paramount to take each month until I will be close to deliver for me to stop malaria disturbance I am going regularly to clinic and I received one dose so far". Mother three focus group discussion one.

"I am confident that I took three times those tables somehow they were not friendly but the nurse explained it clearly that it was to curb malaria infection". Mother four focus group discussion one

"I swallowed three doses each time I went for clinic the nurse insisted for immediate swallowing in the hospital although there were some challenges". Mother five focus group discussion one.

"I had taken three times and fell nauseated, the nurse was very supportive saying it is a good way to tackle malaria". Mother six focus group discussion one.

"I swallowed one dose of three tablets and encouraged to be continuously coming to take, I did not find any difficulty since the explanation were clear". Mother one focus group discussion two.

"I got two doses and decided to end there due to the side effects". Mother two focus group discussion two.

"I can confirm that I took two doses but did not find any reason to be right since I also use folic acid". Mother five focus group discussion two.

"I cannot tell with exactitude how many doses so far but think had gotten twice. At every time "I go for antenatal visit I have been explained and swallowed on spot two consecutive times and I have never refused to take the medicine". Mother one focus group discussion one.

"I am sure to have used three doses when attending clinic, it is making me strong and my baby will be safe". Mother six focus group discussion two.

2(17%) of study participants who did not know the schedule of the intermittent preventive treatment/SP, had this to say; I refused it since I use another method for preventing malaria this is the reason to why even did not accept to complete the subsequent doses during antenatal clinic. See text box 6 below.

Text box 6. Theme: Refusing to complete sulfadoxine pyrimethamine among pregnant women without malaria. Subtheme: Reason of not completing sulfadoxine pyrimethamine.

"*I did not take it and did not find that it is a problem since I use mosquito nets, and use some leaves that chase mosquito in the house*". Mother three, focus group discussion two.

"*I have not yet taken it and refused just like that*". Mother four focus group discussion two

4.7.2 Practices of insecticide treated nets

During the focus group discussion, answers of pregnant women with and without malaria were captured, recorded, transcribed and coded to analyze the awareness and the practices of long lasting insecticide treated nets. 12 (100%) pregnant women with malaria acknowledged that they were aware that long lasting insecticide treated net is key to prevent malaria during pregnancy. See table 16 below.

Table 4. 16: Awareness of long lasting treated nets.

Awareness of ITN	Frequency	Percentage
YES	12	100%
No	0	0%
Total	12	100%

12 (100%) of respondents reported that they owned a long lasting insecticide treated net which prevents them against the bites of mosquitos. See text box 7 below.

Text Box 7. Theme: Long lasting insecticide treated nets, Subtheme: Own insecticide

"I own a long lasting net, and it prevents malaria, boost the immunity and prevent the bites of mosquitos which contribute to contaminate the mother and her unborn baby with malaria infection" (mother one, two, three, four, five and six: focus group discussion one. Mother one, two, three, four, five, six: focus group discuss two).

12 (100%) of study participants reported that they were sleeping under the long lasting treated nets. See table 17 below.

Table 4. 17: Practice of long lasting insecticide treated nets

Practices	Frequency	Percentage
Sleeping under nets	12	100%
Not sleeping	0	0%
	12	100%

Respondents who sleep under long lasting treated nets, had this to say; it is good to sleep under mosquito nets. See text box 8 below.

Text Box 8. Theme: Practices of long lasting insecticide treated nets, Subtheme: Sleeping under the long lasting insecticide treated nets.

"I Sleep under the net and it is good and when I get sick immediately I visit the doctor" Mother 1: Focus group discussion one

"It is advisable to sleep under the net and I do it frequently, I visit the doctor earlier, clear stagnant water around my house". Mother 2: Focus group discussion one

"It is good to sleep under the net, clear bushes, visit the hospital whenever she suffers from malaria". Mother 3, Focus group discussion one.

"I sleep under the net, avoid dirty things and drain stagnant water to maintain safe environment" Mother 4: Focus group discussion one.

"I sleep under the net, practice hand-washing and keep cleanliness". Mother 5: Focus group discussion one.

"I sleep under the net, keep good hygiene by cleaning bushes around my house and draining stagnant water". Mother 6: Focus group discussion one

"I sleep under insecticide treated nets" Mother 1: focus group discussion two

Among the malaria positive cases, use of artemether-lumefantrine was the most common treatment given (n = 58; 82.9%), followed by use of artesunate 10 (14.3%) and quinine with 2 (2.9%). See figure 23 below.

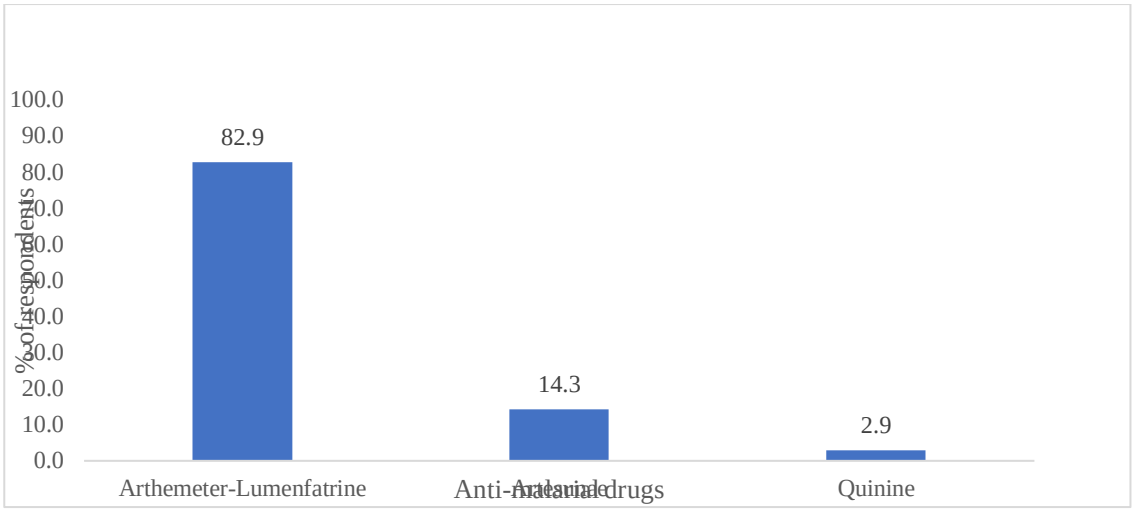


Figure 4. 20: Malaria Treatment Administered.

4.8 Discussion of Results

4.8.1: Socio-demographic factors associated with malaria among pregnant women.

This objective was to establish socio-demographic factors associated with malaria among pregnant women. The discussion concerning this objective was developed with regard to the factors which were statistically significant like marital status (p-value<0.001), area of residence (p-value<0.028) and gestational age in weeks (p-value<0.001) to determine whether other previous studies findings were in support or in contradiction of the current findings. Thereafter, opening a window for drawing own conclusion. Contrary, the variables such as age groups in years, parity, level of education, occupation level, income level had a p-value above 0.05, therefore, not statistically significant. Interestingly, the association which was found in our study was not a risk factor but a simple association. The following studies found that there was a statistical association with regard to marital status, area of residence or gestational age. Previous research looking at marital status found that majority of pregnant women were married (Mutanyi *et al*., 2021; Agyem *et al.*, 2020; Nyamu *et al.*, 2020), contrasting with Oppong *et al*., 2019 who found that the majority were not married. Concerning the gestational age, studies found that it was the

second trimester of pregnancy (Mosha *et al.,* 2014), while others pointed out the second/third trimester of pregnancy, and even further stressed that the gestational age of participants was statistically associated with malaria (Afrifa *et al*., 2017; dikamnoro *et al.,* 2014; Iyke *et al*., 2013; Gontie *et al*., 2020). Other studies pointed out the second trimester only (Agyem *et al.,* 2020; Nyamu, 2020; Webster *et al*., 2013; Asmamaw *et al.,* 2013). Concerning the residence, majority of respondent were rural residents (Agyem *et al.*, 2020; Oppong *et al*., 2019), while Debeaudrap *et al*., (2013), found that peripheral malaria infection among pregnant women was higher and largely associated with their place of residence (rural areas). Strikingly, different studies conducted in India, Nigeria, Cameroon, Kenya found that there was no statistical association between marital status and gestational age (Makokha, 2014; Waiswa *et al*., 2022; Tilahun *et al*., 2020; Anchang-Kimbi *et al*., 2015; Getachew *et al*., 2013; Asmamaw *et al.*, 2013; Bishop & Aliyu, 2017; Almaw *et al*., 2022). Marital status was found to be a very important variable in the context of malaria in the previous research showing that not married for example was associated with increased risk to contract malaria, while married was associated with some health benefits (Jean-Claude, M et al., 2018). Kayentao (2014), carried out a randomized controlled trial study in Mali focused on safety of IPT-SP in pregnancy and found that not married was significantly associated with malaria in pregnancy (p-value< 0.001). To support the credence on the above findings, a study conducted in the Sub-Saharan Africa region focused on malaria effect in infants at the delivery highlighted that the first-born child in a context of decreased malaria prevalence was protected against the low birth weight with the use of long-lasting insecticide treated nets (Heng et al., 2021). Gestational age as well as area of residence were mentioned as important variables when it comes to consider malaria approach. For i.e malaria was coexisting with long bushes and stagnant water while pregnant women in the first trimester was more exposed

than other trimesters. These variables should come first during the planning of malaria interventions.

4.8.2: Association Low Birth Weight and Malaria in Pregnancy

This objective was to determine the association between low birth weight and malaria in pregnant women. Malaria test was significantly associated with anemia at a p-value less than 0.001. Prevalence of low birth weight was estimated at 4.6%. The relative risk of malaria was 0.999, showing that in both cohorts (malaria positive and negative) the presence of malaria did not result in significant low birth weight. malaria was not a risk factor for low birth weight (RR=0.999). Whether malaria was associated with HIV, hypertension, and gestational diabetes no influence on birth weight was observed. Malaria causes low birth weight via a well-known mechanism triggering the placental expression to VAR2CSA, the unique surface antigen responsible of sequestration of plasmodium falciparum evolving in the process of inflammation with vasogenesis, angiogenesis and nutrient transportation dysregulation which affect the fetal development. There is also the severity of malaria effects depending on the level of pre-acquired antimalarial immunity, previous exposure to the infection and the acquired immunity depend on the level of transmission in the residence area (Chua, C. L, 2021). Notably, studies revealed strong evidence during the last two decades on the decline of malaria concerning its prevalence, clinical cases and disease burden at the community level. This research showed that malaria declined to 40% from 2000-2015 in the Sub-Saharan Africa region. The current result could be attributable majorly to the preventive measures put in place in the healthcare system consisting in the improvement of quality antenatal care through appropriate case management, behaviors and attitudes shaped by social and cultural factors (Bhatt, 2015). Studies highlighted that quality interventions were offered to pregnant women during the antenatal clinic, likewise maternal folic acid

supplementation in low- and middle-income countries, which was found associated with an increased mean birthweight and decreases in the incidence of low birthweight and small for gestational age. Exposure to more than 4 antenatal visits had decreased incidence of low birth weight (Jonker et al., 2020). Another study showed that Artemether-lumefantrine was associated with trend towards decreased low birth weight and pregnancy loss (Munhlenbacks et al, 2020). A study carried out in Guinea, malaria immunity for pregnant women was acquired after 6 months of living in the endemic area. Moreover, low immunity induced severe malaria, leading to anemia, abortion and low birth weight (Toure et al., 2019), In our study we found that 89% of participants with malaria were treated with Artemether-lumefantrine. Although, current evidence suggesting that the efficacy of antimalarial drugs in preventing low birth weight may decrease with *Plasmodium* resistance, antimalarial medications were used for prevention during pregnancy and showed a significant low birth weight reduction of 27% in the cohort that used the drug when compared with the control group (Muanda et al., 2015). Malnutrition and malaria share the same geographical area and it contributes to increased disease burden in pregnancy. However, both appear important contributors to low birth weight, and nutrient supplementation during pregnancy appear to be an attractive and feasible intervention to minimize the risk of low birth weight (Manu et al., 2017). In a study that analyzed 23 systematic reviews on nutritional interventions during pregnancy, a few factors including provision of vitamin A, low-dose calcium, zinc, and multiple micro-nutrients were associated with reduced risk of low birth weight (da Silva et al., 2017). Similarly, several earlier micro-nutrient supplementation studies in malarious regions such as Sub-Saharan Africa provided evidence of improved birthweight, increased gestational length and reduced odds of LBW (Unger et al., 2016). Evidence suggested that maternal undernutrition is positively associated with low birth weight. In

Kenya and Congo Democratic Republic, it was established that the association between malaria infection and reduced fetal growth was greatest among malnourished women (Cates et al., 2017). However, in Benin, the effect of malaria infection on fetal growth velocity was greatest among women with low anthropometric status There was no association found between malaria and low birth weight in studies carried out in Uganda, Sudan, Tanzania (Mohammed *et al*., 2013; Kalinjuma *et al*., 2020). While in Malawi, India, Kenya, and Brasilia, it was found that malaria was associated with low birth weight (Kipisi *et al.*, 2017; Patel *et al*., 2017; Nyamu *et al*., 2020; entongo *et al*., 2020; Mikomangwa *et al*., 2019; Haider et al., 2013; Thompson, *et al*., 2020. Chua *et al*., 2021). Nkoka *et al*., (2020), in Malawi was able to demonstrate malaria intervention effects and low birth weight and found that whether combined or no LLIN and IPT/SP both showed a decrease of 20% in low birth weight. Manu *et al*., (2017), reported that among multiple benefits of using LLIN, it was noted a significant decrease of miscarriage and stillbirths concomitantly reported also in the previous studies, which considered the insecticide treated net use with a lot of obtained benefits at a cheaper cost. Even from the viewpoints of respondents during the realization of focus group discussion held for the purpose, a woman in a village namely Framposo affirmed that not sleeping in the net can favor mosquito bites, therefore exposing the fetus who is in the uterus to get malaria and not develop properly. Mosha *et al*., (2014), carried out a prospective observational research design in Northeastern Tanzania, Coastal regions and Rufiji District among pregnant women and concluded that the use of sulfadoxine-pyrimethamine based intermittent preventive did not influence minimal risk of parasites that hide in maternal placenta. However, the corresponding effect to lower birth weight did not have statistical effect at p-value > 0.05. Ndeserua *et al*., (2015), carried out a descriptive research design evaluating the risks of specific malaria parasites that hide in maternal placenta versus

infants negative effects and concluded that IPT/SP second doses didn't have sufficient effect to prevent the occurrence of adverse birth outcomes such as anemia among expectant women in Tanzania. Ndeserua *et al.,* (2015), in Tanzania got right to demonstrate that malaria and it's corresponding IPT/SP treatment doses (at least 2 doses) among pregnant women was non-significantly attributed to any risk of lower birth weight. Sangare (2014), found that iron deficiency (anemia) related to ferritin or C-reactive protein are largely associated with malaria infection on a minimal scale within pregnant women. Kayentao *et al*., (2013), found that among pregnant women suffering from malaria, intermittent preventive treatment with at least 3 doses of IPT-SP was significantly associated with a higher birth weight and minimal risk of low birth weight as compare to 2 or less doses. Rahmati *et al*., (2017), addressed the relationship between maternal anemia, malaria and low birth weight found a significant association. Maternal anemia, malaria and babies having low birth weight in first trimester were relatively significant (p-value < 0.05). Sohail *et al*., (2015), in India discovered non-significant relationship between anemia and malaria test (negative or positive) among pregnant women.

4.8.3: Key Proteomic Profiling Patterns Associated with Malaria

This objective was to establish the key proteomic profiling patterns associated with malaria. Proteins diversity, interaction and separation were observed in multidimensional scaling (Simpson index), whereas richness and abundance of proteins was observed with Chao index. The key proteomic biomarkers were obtained through computation of standard deviation, T-test and the mean difference of 184 proteins included in the data set. The overall mean for malaria positive cohort was 4.0 and a standard deviation of 0.73023756, whereas the mean for malaria negative cohort was 3.1 and a standard deviation of 1.04367181, and an overall mean of 4,0 with a standard deviation of

0.73023756 obtained from the maximum of 4.69705107and a minimum of 3.23657595 in malaria positive cohort against a maximum of 4.19295347 and minimum 2.10560986 in the malaria negative cohort. The mean difference of proteins was calculated from the T-test and corrected with Bonferroni adjusted alpha at p-value of 0.05 for excluding type I error. The mean difference varied from 2.856690795-0.217887462 in the cohort of malaria positive and from -0.162524175 to -1.185322211 in the cohort of malaria negative. The following were the six significantly enriched proteins which were found after statistical computation, as well listed herein according to their relative influence order as per the cohort of malaria negative: P01138, Q9NYY1, P14784, Q99748, P78423, Q9NSA1. The following were the 18 significantly enriched proteins as per the cohort of malaria positive listed herein according to the degree of their relative influence: P80098, P10145, Q13541, P09874, Q16698, Q99075, Q9Y6K9, P22004, P47992, Q13043, O00182, P13726, P31994, P01730, P25116, P07204, Q9BWV1 (O00182, O00220 were not registered Uniprot). Indeed, biomarkers diversity with Simpson test, and good richness (abundance) of proteins according to Chao test were perfectly demonstrated, as well as a good interaction and separation of proteins in the multidimensional scaling visualization. Furthermore, the highly statistical association between proteins, test result, gestation age in weeks and parity in multivariate ANOVA was established besides the expression of 18 potential biomarkers among the positive malaria cohort and 6 potential proteins in the negative malaria cohort. Interestingly, these findings confirm an undoubted progress towards new biomarkers discovery and an originality which has not been found or challenged in the current literature. Hammond, *et al*., (2014), confirmed that the superiority of the recently developed proximity ligation assay by Olink/proteomics to process at once a huge number of proteins in each individual plate with its internal control to obtain highly quality performance either in

blood or plasma without need for separation, or a solid support where the protein is recognized by the intermediate of an antibody or prion protein. In a study by Mathema, V. B & Na-Bangchang, K. (2015), in a research approach for biomarkers and proteomics raised challenges for the need of new biomarkers discovery, he stressed that this time of malaria eradication, the parasite and its drug resistance mechanism should be understood with deep knowledge such plasmodium falciparum lactate hydrogenase and aldolase. However, due to cross-resistance and multiple resistant strains of malaria the emergence of new therapeutic options should be envisaged, and highly expression biomarkers discovery and validation are needed for good clinical value of concurrent infections (malaria and covid-19 pneumonia). Foko *et al.*, (2022), in a research study targeting biomarkers for severe malaria raised importance of prognostic biomarker which can be based on biological or clinical characteristic to reach an objective measurement on the outcome. Hence biomarkers are not specifics to pathogen but to the response of the host, and therefore to be regarded for prognosis, and prediction of diseases as complementary but not for pathogen diagnostic. Al-Amrani *et al.*, (2021), revealed that millions of proteins and about 26000 to 31000 human genome codes exist and there is need to support their role in medicine. Nevertheless, few enumerated biomarkers were discovered previously: CX3CL1, CXCL4, CXCL9, CXCL10 were associated with uncomplicated case of Falciparum malaria, while our study finding displayed CX3CL1 as a biomarker associated with negative malaria. However, our study showed superiority concerning high number of expressed biomarkers as compare to the previous studies. In the current research study, sixteen proteins signaled as key biomarkers associated with positive malaria, whereas six biomarkers only were associated with negative malaria. The attempt to correlate the biomarkers to the low birth weight has not been demonstrated by the current study, rather the association between the subject's characteristics and

potential biomarkers such as parity, mode of delivery, test result and gestation age. Therefore, there is need to conduct further research. Harald Mischak *et al*., (2015), elucidated the key role of successful discovery of biomarkers in longitudinal cohorts on high risk subjects was to contribute to the advancement of molecular disease diagnostic and prognostic, and envisaged diseases management. Biomarkers hindrance still a lack of specifications, and finally he suggested that well defined signatures markers needed subsequent validation in study population. In his findings the author mentioned a limited list of biomarkers which have been identified in cancers diseases such as ovarian cancer, colon cancer. However, these biomarkers were totally different in regard to which that have been found in this study to confirm disease and biomarker relation.Gnidehou and Yanow (2021), research about relevance of a central protein called VAR2CSA present in pregnant women, and having a protective role in plasmodium falciparum placental malaria form. The results demonstrated the existence of VAR2CSA protein in men and children in Brazil, and in South Saharan African countries such as Kenya, Malawi, Benin, and Tanzania where malaria is endemic. However, the findings for the current sixteen proteins versus six proteins among pregnant women at Webuye hospital have not been challenged in the previous researches for any possible existence in the non-pregnant population. Reason to why there need to amplify the research with high expectations to cover the horizon of the unknown in biomarkers arena. Common biomarkers which were cited in previous research studies were tumor necrosis factor (TNF), C reactive protein (CRP), thus Sangare (2014), in a non-randomized trial study design found that iron deficiency (anemia) related to ferritin or C-reactive protein are largely associated with malaria infection on a minimal scale within pregnant women. Vascular endothelial growth factor (VEGF), tyrosine kinase (TK) but they were not among the current cited

biomarkers which can confirm the originality of the Olink Proteomics on research of highly prognostic biomarkers as compared to conventional protein detection methods.

4.8.4: Awareness among Pregnant Women on the IPT and LLIN

This objective was to assess the awareness among pregnant women on the intermittent preventive treatment and the insecticide treated nets. This study confirmed that there was a significant difference in proportions between malaria negative and positive groups among mothers' knowledgeable on the side effects (p-value = 0.001), different doses (p-value = 0.012), and those who were informed about intermittent preventive treatment sulfadoxine-pyrimethamine before administration (p-value = 0.003). The proportion of mothers knowledgeable about side effects and different doses was higher among the malaria positive group as compare to malaria negative with 52.9% versus 25.7% and 20.0% versus 5.7% respectively, intermittent preventive treatment doses were statistically significant. Mutanyi *et al*., (2021), in a cross-sectional study carried out in Sabatia Kenya found that a good number of pregnant women had a good knowledge of intermittent preventive treatment sulfadoxine-pyrimethamine benefits, but did not know the exact time for the beginning of intermittent preventive treatment sulfadoxine-pyrimethamine and never experienced sulfadoxine-pyrimethamine side effects. The marital status, knowledge of benefits of sulfadoxine-pyrimethamine and gestation age were significantly associated with uptake of sulfadoxine-pyrimethamine, with women who had good knowledge of benefits having higher likelihood of receiving the third dose than those with poor knowledge. McClure *et al*., (2014), indicated that the group who got different doses of IPT/SP was more prone to malaria due to the association attributed with moderate and severe anemia. Peters and Naidoo (2022), conferred that good number among participants had fair knowledge towards intermittent preventive treatment sulfadoxine-pyrimethamine versus those having poor knowledge. Gutman *et at*., (2013),

in Malawi concluded that the IPT/SP difference within the various research participants consisted in residing in Southern Region of Malawi. This cohort had a significant impact on malaria test outcome at p-value less than 0.001. Agyeman *et al.,* (2020), in a prospective cohort research design found that the majority of women were observed to have used two and above IPT/SP doses. The study concluded that between dosage, malaria test and IPT/SP there was a statistical significance. Hill *et al*., (2015), carried out a qualitative research design in two countries; Mali and Kenya using focus group discussion. Further, he found that having correct knowledge of doses and IPT/SP intake intervals, expectant women felt that the method was still very powerful to be avoided during pregnancy due to possible induction of miscarriage. Furthermore, intermittent preventive treatment sulfadoxine-pyrimethamine was still being to treat malaria proving to be both effective and ineffective at preventing malaria. Braun *et al*., (2015), carried out a descriptive research design within Western Uganda and revealed that an approximated more than 50 % of respondents had correctly used one dose and above of IPT/SP, whereas more than 80 % had taken one dose only during their gestation timeline. Mosha *et al*., (2014), carried out a prospective research design within Moshi Municipal in Northeast Tanzania, Rufiji District and Coastal Area between July and October 2012 revealed that approximately 91% of the women were aware and had used more than 1 dose of IPT/SP. Furthermore, the study concluded that there was statistical significance with birth outcome (p-value < 0.015). Odongo *et al*., (2014), carried out a descriptive research design and found that approximately all respondents had heard about IPT/SP and 57% who had stated that medicine stated that it was convenient with malaria prevention in both mothers and unborn children, and 15.4% felt that it was used to treat malaria. However, nausea, vomiting, body weakness, headache, dizziness, abdominal pain and diarrhea were reported as unwanted effects of the molecule used to prevent

malaria in expectant women. Peter (2013), in a descriptive cross-sectional study found that using none dose or just one was significant associated with clinical malaria as compare to women who had received two or more doses of medicine used to prevent malaria in expectant women. Approximately 75% of pregnant women attending antenatal clinic had access to IPT/SP with more than 78% having received at least two doses. Mohamoud *et al*., (2022), in a descriptive cross-sectional research design conducted in the hospital found that majority of female expectants had advanced awareness on disease prevention employing commonly used medicine (65%). 42% of these women were getting their awareness from healthcare providers and a relatively 57% of them had consumed IPT/SP doses as compare to 43% who had not used IPT/SP doses in the current pregnancy. The following studies raised controversy on the association between knowledge and intermittent preventive treatment sulfadoxine-pyrimethamine doses.

Ango *et al*., (2018), carried out a descriptive research design examining awareness, perception and utilization of IPT/SP and LLIN, majority of respondents were aware of the medicine used to prevent malaria while expectant and they were 60.6%. Almost half (47.9%) of women believing that tablet to prevent malaria was safe when the baby is in the mother's womb and it offers the necessary protection. Majority of expectant women had used IPT/SP just once either 72.4%. No statistical difference was observed between the two variables (malaria status and IPT/SP use) at p-value > 0.05. Peter and Naidoo (2022), in a mixed-design study qualitative-quantitative conducted in Nigeria found that 68.5% had good knowledge on the benefits associated with IPT/SP during pregnancy, while about 58% could not tell the schedule for IPT/SP. Waiswa *et al*., (2022), in a prospective cohort research design carried out in Kakamega County showed that majority of the respondents had an advanced knowledge and constantly used LLIN method to control and combat malaria. Furthermore, there was statistical difference between the

three variables (ownership, use of LLIN and rate of malaria) at p-value of 0.001. Nega *et al*., (2015), carried out descriptive research design within Ethiopia areas and concluded that majority of pregnant women frequently used long lasting insecticide treated nets (54.8%) and 5.6% used long lasting insecticide treated nets "sometimes". Participants who failed to use the insecticide treated nets either 39.3% had a high likelihood of falling sick due malaria infection at p-value less than 0.001. Makokha (2014), in a hospital-based longitudinal research study conducted in Bumula division of Bungoma County found that approximately 60.0% of pregnant women owned nets (where 87.8 were long-lasting, while 11.6% were conventional nets). Further, the study concluded to a statistical difference with regard to the net ownership and rate of malaria infection at p-value less than 0.05. Nyamu *et al.*, (2020), carried out a descriptive cross-sectional research design and found that 80.5% of respondents owned a treated or untreated mosquito nets, where 93.2% used the mosquito net in their previous night, and 82.7% have always used a mosquito net while sleeping. The following were research that argued that participants did not have adequate knowledge on the topic (insecticide treated nets "LLIN"). Lack of information on preventive method such as LLIN of course can be associated with increase of malaria rate. Koenker and Rweyemamu (2013), conducted a research study in Zanzibar on the reasons and risks of use of LLIN and argued that a pregnant woman in a focus group discussion said "the danger was there as we had no knowledge and few of us were using the nets during night, we were mainly relying on local herbals (Female, Zanzibar). Gontie *et al*., (2020), carried out a descriptive community research design in Sherkole District situated in West Ethiopia, majority of pregnant women didn't own or use mosquito barrier net. Lack of this barrier mosquito net as well as lack of information on services related to malaria was highly associated to malaria infection rate. Ango *et al*., (2018), carried out a descriptive research design examining awareness, perception

and utilization of IPT/SP and LLIN. The study revealed that about 86.0% did not have knowledge about the mosquito net. However, 52.8% owned an insecticide treated nets, with 26.0% using LLlN on a regular basis. Majority of women not using LLIN felt that it increases temperatures at night and their homes could not accommodate this barrier method. No significant association was found between LLIN and malaria results (p-value> 0.05). Sohail *et al*., (2015), carried out a descriptive cross-sectional research design and found that LLIN ownership was uncommon, expectant representing the majority were using none pyrethroid -impregnated bed nets at their homes a night. Ugboaja and Oguejiofor, (2017), carried out a case-control research design in Nigeria, which was interested to demonstrate benefits of preventive interventions during pregnancy. The study listed lack of knowledge on the benefits of use of LLIN and unavailability as hindrance to the performance among both healthcare workers and pregnant women. Good level of knowledge of IPT/SP and LLIN contributed to achieve malaria preventive measures, while poor knowledge led to the non-use of preventive methods.

4.8.5: Level of Knowledge of Healthcare Workers on the IPT and LLIN

This objective meant to assess the level of knowledge of healthcare workers on the IPT/SP and LLIN. It was performed by computing descriptive statistics on both opinions and answers collected from healthcare workers in the hospital set up. The knowledge of the guidelines on malaria prevention strategies based on IPT/SP and LLIN by the healthcare providers is key to success of quality care delivery. The ideal is that healthcare workers should be well trained and supervised prior to conduct such medical activities during antenatal clinic, which some time constitute a challenge. Following were previous studies supporting findings consistent with good knowledge among healthcare workers who are the frontline workers offering preventive service. Therefore, their knowledge

were key towards successful conduct of IPT/SP and LLIN strategies. Bello and Oni., (2020), found that having worked in the antenatal care service and practising obstetric care in tertiary health facilities was associated with good knowledge on the side effects of IPT/SP. Israel *et al*., (2018), found that healthcare providers knowledge on long lasting insecticide treated nets were good due to the fact that they were able to identify they were able to give the various reasons cited as hindrance to the use of mosquito nets by pregnant women. Yoder *et al*., (2015), reported that nurses had clear rationale of administering intermittent preventive treatment-sulfadoxine-pyrimethamine drugs of two doses during a pregnancy. However, all of them did not agree on the timing of the doses concerning the gestational period of women. De Gaulle *et al*., (2021) in Ghana revealed that healthcare workers had good knowledge used guidelines and explained the advantage of medicine. Mchwampaka *et al*., (2019), connected adequate knowledge of IPT/SP to the management of stock which were well updated direct observed therapy strategy fulfilled. However, the following authors argued that there was a gap underlying healthcare worker's skills which could contribute to poor service delivery regarding intermittent preventive treatment-sulfadoxine-pyrimethamine and long lasting treated nets. Dun-Dery *et al*., (2021), in a mixed research design conducted in Ghana among healthcare providers and pregnant women found that intermittent preventive treatment-sulfadoxine-pyrimethamine intake was a challenge due to antenatal clinic missed, intermittent preventive treatment-sulfadoxine-pyrimethamine knowledge deficiencies and stock shortage in health facilities. Health providers' negligence, absenteeism, and drugs side effects posed as a major challenge for intermittent preventive treatment-sulfadoxine-pyrimethamine strategy. Peters and Naidoo (2020), attached good use of intermittent preventive treatment-sulfadoxine-pyrimethamine to the close follow up when medicine is administered in the facility. Rassi *et al*., (2016), pointed out the lack of knowledge of

intermittent preventive treatment-sulfadoxine-pyrimethamine as a matter of policy inconsistency in a national context whereby in Uganda the guidelines were not giving clear instructions about the drug's safety or efficiency. Liman (2019), could really evoke the training and supervision of healthcare workers as key factors in the delivery of intermittent preventive treatment-sulfadoxine-pyrimethamine Mohammed *et al*., (2022), carried out a descriptive research design and found that most of the healthcare workers were between 26-34 years of age, with majority 47.4% were midwives and 56.2% were effectively adhering to hospital guidelines. Approximately 73.3% of the healthcare workers documented intermittent preventive treatment-sulfadoxine-pyrimethamine services administered to pregnant patients attending antenatal clinic. A total of 75% of the healthcare workers in the facilities provided health education programs associated with malaria. All healthcare workers who received intermittent preventive treatment-sulfadoxine-pyrimethamine training had 3.6 times likelihood to compile and execute information as compare to their colleagues who did not get trained.

Maheu-Giroux and Castro., (2014), in a quantitative research study conducted in Sub-Saharan Africa found that most facilities were providing antenatal clinic services, having intermittent preventive treatment-sulfadoxine-pyrimethamine guidelines implemented as a normal pregnant women routine check-up, and having good record and update stock-out of IPT/SP. Most healthcare providers (26%) had received IPT/SP training (Relative Risk = 1.21; 95% CI:1.09 – 1.35) and majority of health providers were enrolled as nurses or midwives (36%). A proportion of 22% of the 124 healthcare providers were more likely to provide anti-malaria prophylaxis to their ANC patients.

Oluwasomidoyin *et al.*, (2020), carried out a descriptive research design and found that majority of healthcare givers 57.1% were providing obstetrics with 77% practicing in tertiary health facilities. More than half, 62.2% were aware of the current recommended

IPT/SP by the WHO, while 39.1% had a correct IPT/SP knowledge. Among the caregivers, 72.2% correctly prescribed IPT/SP to expectant women during three-quarter of their gestational period. Nyavor *et al*., (2017), in a cross-sectional study found that majority of the healthcare providers were between the age of 23 to 37 years, and reported that most mothers 81.3% and 66.4% owned an insecticide treated nets and used them regularly. According to the health care providers, the insecticide treated nets were very effective in prevention of malaria 97.8%. A proportion of 33.6%, most healthcare providers provided education related to insecticide treated nets, and 98.7% of the patients and caregivers were very much aware of the insecticide treated nets. Majority of both pregnant women and caregivers knew the signs and symptoms associated with malaria, and 82.2% knew what causes malaria and lastly 90% knew who was at a higher risk malaria infection. However, all the sampled health facilities were well equipped with facilities for conducting intermittent preventive treatment-sulfadoxine-pyrimethamine. No health facility reported shortage or stock-out of Sulfadoxine-pyrimethamine. Indeed, knowledge of guidelines on preventive strategies promote fair service delivery.

4.8.6: Practices of IPT and LLIN among Pregnant Women

This objective was meant to assess the practices of intermittent preventive treatment and long lasting mosquito nets among pregnant women. The idea behind the formulation of this objective was to hear from pregnant women during focus group discussion and open-ended questionnaire what they do exactly at the household level to prevent malaria as opposed to what they know, which has already been covered in the knowledge of pregnant women. However, in this exercise which was very different from the knowledge, and was a doing side of something to ensure that malaria will be prevented through the use of a device, a medicine or a method. It is clearly stated that this research study was not conducted on the field or at the household level to merge practical

knowledge and practices of exposing or hanging long lasting insecticide treated nets, but in the hospital set up. In the current study, it has been noted as practices, the strong statement of how pregnant women did it. This firm declaration, confirmation and description of using any of the enumerated methods and how often the method was used was exactly considered as practices. For example, pregnant women practicing long lasting insecticide treated nets were those who consistently slept every night under the net and those who reached at least more than 2 doses of intermittent preventive treatment. The consistently use of long lasting mosquito nets is strongly recommended and advised during pregnancy in moderate to high malaria transmission zone. Every pregnant woman to prevent malaria in prone areas should sleep every night under a long lasting insecticide treated nets, whereas intermittent preventive sulfadoxine-pyrimethamine should be taken under the directly observed treatment after the quickening or the second trimester of pregnancy. It comprises use of three tablets each one month interval until 36 weeks of gestation either before delivery. Conferring to the study of Duut and Alhasan, (2022), ownership and sleeping under LLIN were two different things and to strengthen the current long lasting mosquito nets strategy, there is need to use other preventive method simultaneously, using other methods was seen as a boost of sleeping under LLIN. Hence, Iyer *et al.,* (2019), in Peru demonstrated that prevention of malaria should be a matter of high priority using mosquito nets. Altogether Hill *et al*., (2015), brought up the experience of pregnant women refreshing the insecticide with Powertab (Kenya) or re-impregnated it with Bloc (Mali) as a effective method to mosquito nuisance and prevent malaria. Kayentao (2014), comparing the safety of IPT/SP in pregnancy revealed at the same time that it was able to contribute around 18% decreased risk of neonatal mortality and rate of malaria infections. In fact, Karoki *et al*., (2016), stressed that despite increasing number of pregnant women attending ANC visits there was disparity in the

utilization and the uptake of IPT/SP and LLIN. Agyeman *et al*., (2020), attributed the often use of LLNI to the fact that is was owned at home. Kuse *et al*., (2022), Ahorlu *et al.*, (2019), reported that suffering complications of malaria first raised the motivation of LLNI use. *"I and my small daughter were not sleeping under mosquito nets but when she got sick, I took her to the hospital and she was very pale and died. Since that time we have to sleep inside mosquito nets even if it is too hot" (Female caretaker of a child under five, Central Region, FGD).* Koenker and Rweyemamu *et al*., (2013), reported that pregnant women argued that"Every day we sleep under mosquito nets and if we get sick we should access care to a dispensary" (Female, Bukoba Rural) while for Braun *et al*., (2015), those who used previous mosquito owned it. In fact, the following were studies which indicated inconsistency in the use of intermittent preventive treatment-sulfadoxine-pyrimethamine of 2 doses and above and long lasting mosquito nets likely as a failure vis-a-vis completeness or compliance due to different barriers and lack of support and information. Tilahun *et al*., (2020) in India, reported that that not using mosquito nets for pregnant women was justified through the feeling of suffocation and a lot of heat at night. Machini *et al*., (2016), share thoughts about risks and consequences of malaria which stimulates with time the demand in the use of LLIN and insist on the appropriate use. Oppong *et al*., (2019), in a retrospective study found that the majority of women were observed to have taken at least 1 dose of intermittent preventive treatment-sulfadoxine-pyrimethamine as compare to the required intermittent preventive treatment dose of three or more. The poor intermittent preventive treatment intake coverage was highly attributed to factors such as transportation challenges and delays in allocation of malaria commodities in Ghana's healthcare facilities. Koenker and Rweyemamu, (2013), in Zanzibar argued on the reasons and risks of use of long lasting treated mosquito nets reporting that a pregnant woman argued that *"the danger was there*

as we had no knowledge and few of us were using the nets during night, we were mainly relying on local herbals (Female, Zanzibar). Arnaldo *et al*., (2019), in Mozambique reported the word of a pregnant women going to antenatal clinic who recognized sulfadoxine-pyrimethamine tablet as the white tablets and argued that *"I was not told more but I was given three tablets which I took". Strikingly, she argued that "Some pregnant women even when advised and counselled do not complete the doses of sulfadoxine pyrimethamine" (maternal and child nurse).* Muhammad *et al*., (2021), brought in the aspect of the delay as critical barrier to achieve the recommended dose of intermittent preventive treatment. That is why they were being taught by nurses and midwives to know how to prevent malaria. In fact, the compliance of such efficacious prevention methods could have been correlated to the satisfaction of the user's. However, in this case it was not. This was the reason to why long lasting insecticide treated mosquito nets were well tolerated as compare to the intermittent preventive treatment according to this study findings. On my opinion the intermittent preventive treatment/sulfadoxine-pyrimethamine is essentially practiced through DOT (directly observed treatment) and it is as well a cost-effective strategy to prevent malaria among pregnant women until these days. It has subsequent intakes and should be started in the second trimester due to its effects on the fetus during the organogenesis, swallowing of three tablets each month presents challenge for some pregnant women who have been reporting unwanted effects (nausea, vomiting) and some beliefs or perceptions like it provokes abortion. During coronavirus time, and even nowadays, this method encounters other challenges such as bringing own cup, the facility to avail clean and potable water, and running out of this medicine in the facility due to the shortage, which are true issues to consider and address if one wanted to succeed this strategy. Therefore, due to these enumerated limitations this method is regarded as a stringent as compare to the use of

long lasting mosquito nets, which once after hanging it in the house, it is ready for use and also can be used at any period of pregnancy without contra-indications. In future, the ideal will be to manufacture a preventive antimalarial drug intended to combat malaria during pregnancy that can be using a single tablet to replace sulfadoxine-pyrimethamine, and at the same time having minimal side effects such as nausea, vomiting and burning sensation, will be a friendly alternative option.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

This chapter presented main findings as well as the highlights related to chapter four. It comprised summary of findings, conclusions for each objective, recommendations and new knowledge as contribution of the current research study on policy formulations and practices.

5.1 Summary

This research study was titled “Detection of pregnancy associated malaria among pregnant women as a strategy to improve mother child health outcomes in Bungoma County, Kenya". The broad objective was to detect malaria and to determine its influence on the mother and the child health outcomes. The following were the specifics objectives: To establish socio-demographics factors associated with malaria, to ascertain the key proteomic biomarkers associated with malaria, to determine predictors of low birth weight, to assess the awareness on the intermittent preventive treatment and long lasting insecticide treated mosquito nets, to assess the level of knowledge of healthcare workers on the intermittent preventive treatment and long lasting mosquito nets, and finally to assess the practices of intermittent preventive treatment and LLIN among pregnant women.

The following were the major socio-demographic findings after computing Chi-square and Fisher’ Exact tests.

The first objective revealed that the majority of the respondents were in the 18-25 years’ age-group, primigravida, married, with secondary level of education, middle income level, resident of rural areas and were in the second trimester. There was a strong statistical association observed with marital status (p-value <0.001), gestation age in

weeks (p-value < 0.001) and a weak relationship to the area of residence (p-value <0.028). The research found that malaria test was strongly associated with anemia (p-value <0.001).

The second objective revealed that the prevalence of low birth weight in this study was 4.6%. Low birth weight was not influenced by malaria or any other associated conditions during pregnancy (p-value >0.923). Malaria was not a risk factor for low birth weight with a relative risk (0.999, CI 0.926-1.077).

The third objective revealed a pioneering finding based on the biomarkers discovery showing that in multidimensional scaling, the proteins diversity, abundance, interaction and separation were perfectly observed. In addition, there was a strong statistical association between proteins and multivariate ANOVA whereby gestation, parity and test result were strongly associated (p-value<0.001), whereas mode of delivery and education were associated with proteins (p-value<0.05) with 0.019 and 0.031 respectively. A strong normalized protein expression signal was observed for 16 proteins in the cohort of malaria positive, and 6 proteins in the cohort of malaria negative women.

The fourth objective revealed that there was good knowledge in the proportion of malaria positive respondents as compare to the proportion of malaria negative pregnant women (52.9% versus 25.7%). In fact, 59 (42.1%) pregnant women reported that the attitude of healthcare workers was very good. Pregnant women knew that intermittent preventive treatment sulfadoxine-pyrimethamine prevents malaria in 75 (76%), protect malaria in pregnant women and their babies 24 (13%) while only 3% said that it is used for deworming. There was a statistical association between the number of intermittent preventive treatment doses and malaria test (p-value < 0.001). The coverage of 2 doses and plus of intermittent preventive intermittent treatment was higher among the malaria positive pregnant women 48 (68.6%) against 29 (41.4%) respondents.

The fifth objective revealed that the majority of healthcare worker's knowledge on when to start intermittent preventive treatment were good (n=28, 71.8%), side effects (n=28, 71.8%), benefits of sleeping under the long lasting mosquito nets (n=37, 94.5%). **The sixth objective** found that the practices of intermittent preventive treatment as expressed through the use of 2 doses of IPT--SP were good in the cohort of positive malaria as compare to the cohort of negative malaria (n=35, 50%; n=11, 15.7%) respectively. There was no significance difference in both groups in regard to the use of intermittent preventive treatment 3 doses (n=18, 25.7% in negative; n=13, 18.6% in positive) respectively. Indeed, these findings have been similarly corroborated in previous studies while contradicted in others, reason as to why the discussion was carried out scrupulously to determine the boundaries in the contribution of each author's idea before giving own view.

The current research study found that there was a strong association between socio-demographic factors such as marital status (p-value <0.001), gestation in weeks (p-value < .0001) while area of residence (p-value <0.028) had a weak association. The majority of the respondents were aged between 18 to 25 years' age-group, primigravida, married, with secondary level of education, middle income level, resident of rural areas in the second trimester. Malaria test was strongly associated with anemia (p-value <0.001). The low birth weight prevalence was estimated at 4.6 %. Strikingly, low birth weight was not associated with malaria or other associated conditions during pregnancy (p-value =0.923). In fact, malaria was not found to be a risk factor for low birth weight but a protective factor (RR: 0.999, CI 0.926-1.077). The number of live births were 129 (92.2%) out of whom 4 (2.29) were admitted, stillbirth 1 case (0.7%). It was recorded 10 (7.1%) miscarriages, caesarian section 15 (11.5%). Females 78 (60%) against 52 (40%) male infants. There was pioneering finding about biomarkers revealed in

multidimensional scaling that proteins diversity, abundance, interaction and separation were perfectly observed. In addition, there was a strong statistical association between proteins in multivariate ANOVA with gestation, parity and test result (p-value<0.001), whereas mode of delivery and education were associated with proteins at p-value <0.019 and <0.031 respectively.

A strong normalized protein expression signal was observed for 16 proteins in the cohort of malaria positive, and 6 proteins in the cohort of malaria negative women. Concerning awareness on intermittent preventive treatment sulfadoxine-pyrimethamine and long lasting insecticide treated nets, Chi-square test found that good knowledge (intermittent preventive treatment sulfadoxine-pyrimethamine and long lasting insecticide treated nets) was observed in the proportion of malaria positive respondents as compare to malaria negative (52.9% versus 25.7%). In fact, 59 (42.1%) pregnant women reported that the attitude of healthcare workers was very good (Likert scale). Pregnant women knew that intermittent preventive treatment sulfadoxine-pyrimethamine prevents malaria in 75 (76%), protect malaria in pregnant women and their babies 24 (13%) while 3% only said that it is used for deworming. There was a statistical association between the number of intermittent preventive treatment sulfadoxine-pyrimethamine doses and malaria test (p-value < 0.001). The coverage of 2 doses and above was higher among the malaria positive pregnant women 48 (68.6%) against 29 (41.4%). With regard to the qualitative data, the awareness of intermittent preventive treatment captured during focus group discussion indicated that pregnant women 7 (58%) were aware that intermittent preventive treatment sulfadoxine-pyrimethamine protects against malaria "*This medicine helps to reduce the effects of malaria and prevents mother to contract malaria so that the baby can be protected". Mother three focus group discussion one.*

"It prevents the mother from malaria and other illnesses so that the baby remains safe". Mother four focus group discussion one.

"It prevents the mother from miscarriages and at the same time prevent the foetus to get malaria". Mother five focus group discussion one (Text box 1).

However, 5 (42%) respondents were unaware that intermittent preventive treatment sulfadoxine-pyrimethamine prevents malaria during pregnancy were to say; *"I have never heard about it before although it helps to prevent malaria and keep the baby healthy and treat at the same time other diseases". Mother one focus group discussion one.*

"I have never been told about something like that but it prevents diseases". Mother two focus group discussion one (Text box 2).

Respondents 24 (34.3%) in malaria negative and 5 (7.1%) did not take intermittent preventive treatment sulfadoxine-pyrimethamine. The reason given by some pregnant women was that "*it is not a problem since I use mosquito net and burn some leaves to chase away mosquitos in my house*" during focus group discussion (Text box 6). Pregnant women transpired that they have never heard about the importance of this medicine called sulfadoxine- pyrimethamine, this was enough a reason for concern to have an important number of participants not knowing the benefit of a key intervention during antenatal.

Chi-square test showed that the majority of healthcare worker's knowledge on IPT/SP administration were good (n=28, 71.8%), side effects (n=28, 71.8%), benefits of sleeping under the long lasting mosquito nets (n=37, 94.5%). Finally, practices were computed using percentage of respondents and focus group discussion sessions. The compliance or good practices were considered when intermittent preventive treatment sulfadoxine-pyrimethamine 2 doses and plus *"I am sure it is almost two time I have been given this*

medicine when going to the antenatal clinic. I took it with water after the nurse explained to us and I have never refused to swallow it". Mother two focus group discussion one.

"The nurse told me that it is very paramount to take each month until I will be close to deliver for me to stop malaria disturbance I am going regularly to clinic and I received one dose so far". Mother three focus group discussion one.

"I am confident that I took three times those tables somehow they were not friendly but the nurse explained it clearly that it was to curb malaria infection". Mother four focus group discussion one.

The same applied for pregnant women who reported sleeping under long lasting mosquito nets every day, they had to say *"I Sleep under the net and it is good and when I get sick immediately I visit the doctor" Mother one focus group discussion one.*

"It is advisable to sleep under the net and I do it frequently, I visit the doctor earlier, clear stagnant water around my house". Mother two focus group discussion one.

"It is good to sleep under the net, clear bushes, visit the hospital whenever she suffers from malaria". Mother three focus group discussion one (Text box 8). It was established that with regard to the practices all participants complied and used long lasting insecticide treated nets. When many members of the community are using this method, mosquito will not be able to feed from human blood and in turn they cannot sustain for long.

5.2 Conclusions

Detection of pregnancy associated malaria among pregnant women as a strategy to improve mother child health outcomes in Bungoma County, Kenya was a nested prospective cohort study design with both mixed methods. A total of 140 (100%) were tested for malaria of which 70 (50%) were negative. The main objective was to detect and determine the influence of malaria on low birth weight, to assess the knowledge,

attitude and practices of pregnant women, as well as the knowledge of healthcare workers on the intermittent preventive treatment and long lasting insecticide treated nets. Overall, the majority were aged 18-25 years, were primigravida, were married, had secondary level of education, had middle income status, were self-employed, were residents of rural areas and in their second trimester. The study found that there was significant association between marital status (p-value <0.001), gestational age in weeks (p-value <0.001) and area of residence (p-value < 0.028).

First objective: with regard to the socio-demographic characteristics such as marital status, residence and gestational age which were considered as important variables significantly associated with malaria among pregnant women, while the remaining of variables were not significantly associated. However, in the absence of a required sample size to compute for regression analysis which could have helped to determine the strength of the association for the above enumerated variables, these findings yield strong results which can inform the audience on the meaningful parameters such as marital status. In previous studies, marital status has been largely associated with a lot of benefits for the health status of pregnant women such as economic and moral support from the husband, attention of the male partner for the regular antenatal care attendance, etc.

Second objective: with regard to the association between malaria and the low birth weight, the study found that malaria test was significantly associated with anemia (p-value <0.001) but there was not significant difference in both cohorts with regard to low birth weight (RR=0.999). Whether malaria was associated with HIV, hypertension or gestational diabetes there was no negative influence with regard to the low birth. Therefore, this study revealed a low birth weight prevalence estimated at 4.6% as compare to 11% in the study by Eisele *et al*., (2012) and 24% by WHO, (2020). The two factors such as the utilization of intermittent preventive treatment and good practices of

long lasting insecticide treated nets found in this research study could have contributed to the improvement of low birth weight and minimized the negative effect of malaria in this study. Therefore, knowing that there was no prior statistical analysis on the pooled effect on the two factors can inform on further research targeting the pooled effect.

Third objective: despite the limitations of commonly used malaria test methods at the study area in Webuye hospital, the main tests used to detect malaria before the enrollment were essentially rapid test and microscopy. Proteins diversity, interaction and separation were observed in multidimensional scaling using Simpson index and richness or abundance of proteins observed with Chao index. Normalized protein expression of 16 potential biomarkers among the positive malaria cohort and 6 potential proteins in the negative malaria cohort. Indeed, these findings constitute an undoubted progress for malaria proteomic profiling, which still needs more effort to make the potential discovered biomarkers into a useful cost-effective diagnostic test appropriated for the low constrained settings. This test will contribute besides intermittent preventive treatment and mosquito treated nets method a strong preventive and detective strategy towards the improvement of maternal and child health outcomes in the prone malaria endemic areas.

Four objective: When comparing knowledge of expectant women affected by malaria on the study area to the knowledge of malaria negative pregnant women, it was noted a significant difference in knowledge of both groups. Side effects p-value <0.001, knowledge of doses p-value <0.012, expectant who reported having been informed prior the DOT (Strategy consisting in taking sulfadoxine-pyrimethamine in the presence of the healthcare worker) with p-value <0.003. Knowledge on side effects and doses were higher among malaria positive group as compare to malaria negative group with 52.9% versus 25.7% and 20.0% versus 5.7% respectively. Among the reasons of not using

sulfadoxine-pyrimethamine, respondents reported that it causes abortion 30.9%, vomiting 10.9%, premature labour 91%, fatigue7.3%. The common source of information on the DOT strategy "intermittent preventive treatment-sulfadoxine-pyrimethamine" cited by the respondents were 85.7% nurses, 6.4% doctors. Therefore, good knowledge can contribute to achieve better malaria preventive strategies and improve IPT/SP trends as well as complementing the net method via the household use of LLIN.

Fifth objective: When referring to level of knowledge of healthcare workers on IPT/SP and LLNI, the current results describe a trend where by 20 (51.3%) healthcare workers had disclosed having participated in the training process with regard to the two enumerated methods. Furthermore, they agreed that the training received was beneficial to their work. 18 (46.2%) reported possessing new guidelines for antenatal clinics on intermittent preventive treatment. Different components focused on the knowledge of IPT/SP on the side of professionals were assessed, and the majority (n=28; 71.8%) reported that IPT/SP should be started with assurance that it is employed in the indicated pregnancy term which is the second. The reason is that there is no proved induction of malformation during the use of this medicine. Therefore, healthcare good knowledge of malaria preventive strategies can be transformed into successful accomplished interventions, which would be targeting at the same time the performances of IPT/SP, as well as the barrier method based on the use of LLIN toward disease prevention to protect their unborn infants and guarantee vital health status during this crucial period.

Sixth objective: When inferring about practices of the two protective methods "LLIN and IPT/SP" it was wise to inform the difference yielded between the two proportions of pregnant women who possessed and used their barrier method based on the LLIN the night before the survey. Respondents who had malaria positive were 70(100%) and all

of them used their long-lasting treated nets as compare to their counterparts without malaria 68 (97.1%). Statistics show that there was no significant association on the side of LLIN and malaria test computed (p-value >0.05), whereas malaria test computed against IPT/SP yielded a statistical significance at p-value less than 0.001. In fact, the results of this research study demonstrated that there were distinctive features between practicing LLIN as compare to knowledge of intermittent preventive treatment.

5.3 Recommendations

With regard to the current findings, the following are policy research recommendations to:

i. The Government of Kenya to provide technical and continuous medical support to the healthcare institutions and healthcare providers through professional development, formative training, supply of amenities to improve preventive interventions among pregnant women in endemic malaria areas (IPT/SP, long lasting insecticide treated nets).

ii. To the partners and donors to avail research funds for continuous support to spearhead malaria cost effective test innovation.

iii. To the communities and families: to encourage and support pregnant women to attend early antenatal clinic specifically in the endemic malaria areas for early interventions, education, information and communication for the adoption of good behavioral practices and sustainable individual activities prompt to fight malaria infection.

iv. To the researchers, scientists and academics to conduct further research in pregnant women targeting a high level sampling in various areas including non-pregnant population to establish the relevance of biomarkers identified and to enhance better understanding on the existing knowledge in this field. Therefore,

this study recommends further research to be conducted on a cost-effective test from the discovered novel biomarkers, which can be useful for low constrained resource settings as an alternative option to complement the existing methods facing gene deletion challenges.

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APPENDICES

Appendix I: Consent Form

INFORMED CONSENT QUESTIONNAIRE FORM FOR RESPONDENTS

Dear Madam,

My name is Joseph Mukala Nkongolo a PhD student of Mount Kenya University. I am currently carrying out a research study on detection of malaria in pregnant women. This research has benefits of raising awareness in pregnant women to seek early antenatal clinic for detecting malaria and any other infection during pregnancy. On the other hand, to remind the expectant women on the advantages of taking IPT/SP and sleeping under the insecticide treated nets to prevent adverse effects of malaria in pregnancy. Feel free to provide answers concerning the beneath aspects:

1. Your bio-data and individual informations.

2. Sample of blood (4 mls) will be withdrawn and you may experience mild itching on the pricking point, which will not last. Information you will provide in this study will remain anonymous, and will be kept secret and not disclosed to any person rather than the researcher. In the case you consent to be recorded and taken picture, this will limit only for the purpose of the study and will be safely kept from intruder and upon study completion they will be discarded. Further, if you would like to withdraw from the ongoing study, then feel free to do so.

Thank for your participation
Name ……..
Phone no…….
Signature……
Date…….
Fomu ya Shaha

Fomu ya kibali itatafsiriwa kwa Kiswahili kwa wale ambao hawawezi kuzungumza kwa kiingereza.Ushiriki wako ni kamilifu kwa hiari na wewe una uhuru wakati wowote wa kuacha uhusiano . Ungependa kunipa majina yako, alafu kukubali vipimo .

Taarifa zozote utakazozitoa zitahifadhiwa kwa siri. Mimi nimechagua, baada ya kuelezwa kwa madhumuni ya utafiti huu kukubali kushiriki.

Jina…….
Nambari ya simu ……
Sahihi…….
Tarehe……
Informed Consent Form for Phlebotomy and Malaria Test

Kindly sign in the consent form as to testify that you agree to be part of this process before starting the research study titled “Detection of pregnancy associated malaria among pregnant women as a strategy to improve mother child health outcomes in Bungoma County, Kenya”. A registered laboratory technologist will draw your blood by pricking a vein in your right or left arm for the purpose to test only malaria. The excess of blood will be transferred to the Uppsala University to test for potential proteomic biomarker. The disinfectant will be used prior removal of blood to prevent infection and after the syringe will be discarded safely. The risks such as temporary pain and bruise are minimal and disappear fast. In the case you have a preoccupation and would like to suspend your voluntary participation in the ongoing research process or the removal of blood, feel not under any pressure or coercion for exiting from the study.

Thank for your participation
Name ……..
phone no…….
Signature…….
Date……..…….

Appendix II: Questionnaire

Section A. To establish socio-demographic factors associated with pregnancy-associated malaria among pregnant women.

1. Address of the respondent.

Phone No……../ Contact of Next of Kin……...

County [ ]

Sub-County [ ]

Village [ ]

Rural…… [ ] Urban [ ]

No	Questions	Answers {Tick }	
1	Date of last menses		
2	Age of respondent	18-25 [ ] 26-33 [ ] 34-41 [ ] 42-49 [ ]	
3	Parity	Primigravida [ ] Second gravida [ ] Multigravida [ ] Grand multigravida [ ]	
4	Marital status	Married [ ] Single [ ] Divorced [ ] Widow [ ]	
5	Gestational age	1-12 weeks [ ] 13-25 weeks [ ] 26-38weeks [ ]	Trimester 1 Trimester 2 Trimester 3
6	School attainment of respondent	None [ ] Primary [ ] Secondary [[College/university [ ]	0 1 2 3
7	Occupation	Housewife [ ]	1 2

		Employed [ ] Self-employed [ ]	3
8	Income level	Low (10-20 k) [ ] Middle (21-35 k) [ ] High (> 35 k)	1 2 3
9	Distance to the health facility	…….hours	…….minutes

SECTION B

1. Predictors of low Birth weight

No.	**Questions**	**Answers**	
1	SP-IPT use	Dose 1 Dose 2 Dose 3	
2	Smoking	N_O=1 Yes=2	
3	Alcohol	No=1 Yes=2	
4	Blood pressure	Values : ……mmHg	
5	Weight	Values:……Kg	
6	Height	Values :……..Cm	
7	Blood sugar	Values in mmol/l	
8	HIV status	NR=non reactive R =reactive	

2. Outcomes Collection Tool

No.	**Code patient**	**Phone (2 lines)**	**Phone for next of kin**	**Complete address**	**Normal Delivery**	**Low birth weight**	**Weight**	**Sex Male (1) Female (2)**	**Weeks at delivery**	**APGAR score**

Section C. To ascertain key potential proteomic biomarkers associated with malaria among pregnant women.

Respondent Code.	Malaria positive	Malaria negative	AGE	MODE OF DELIVERY	NEWBORN OUTCOME	CHILD SEX	MARITAL STATUS	RESIDENCE	Newborn weight	Education level	Parity	Gestation
	Study ID	**Study ID**										
PANEL												
Olink Target												
ASSAY												
UNIPROT												
OLINK ID												

Informations of participants were to be captured in sheets with proteomic results or Uniprot in malaria/non-malaria participants after analysis.

Section E. To assess the awareness among pregnant women on the intermittent preventive treatment and the insecticide treated nets.

Focus Group Discussion on Intermittent Preventive Treatment of Malaria among Pregnant Women.

Technique

The focus group discussion session timing was 60 minutes. It comprised one group of six participants. Participation criteria were based on free and voluntarily acceptance. There were pregnant women with current or past malaria infection history, or not at all. The history of using IPT/SP and LLIN or not at all. The FGD was preceded by signing informed written consent after clear explanation on voice recording, pictures and videotape. A research team was composed with two moderators, two note takers and a person for recording.

A. Topic/Theme: Intermittent Preventive Treatment

Q.1. What are the reasons for use of the IPT/SP in pregnancy?

Q.2. How many doses should intermittent preventive treatment/sulfadoxine-pyrimethamine given, at which interval and what are the side effects during pregnancy?

Q.3.What do you know about the direct observed strategy of IPT/SP?

Q.4. Which category of pregnant women do not qualify to be given IPT/SP?

B. Topic/ Theme: LLIN "Long lasting Insecticide Treated Nets"

Q. 1 What are the reason for use of insecticide treated nets in pregnant women?

Q. 2 Why do pregnant women refuse to sleep under LLIN?

No	Questions	Answers	
1	Do you know the benefits of IPT/SP	No=1 Yes=2	If yes, give the benefits of IPT/SP
2	Do you know the side effects of IPT/SP	No=1 Yes=2	If yes, give the side effects of IPT/SP
3	Do you know different doses of IPT/SP "intermittent preventive treatment/sulfadoxine-pyrimethamine"	No=1 Yes=2	If yes, give the number of doses of IPT/SP
4	Is the IPT/SP a safe drug to be given during the first trimester pregnancy?	No=1 Yes=2	If yes give reason…… If no give reason……
5	Have you ever refused to take intermittent preventive treatment/sulfadoxine-pyrimethamine because of any reason?	No=1 Yes=2	If yes, give the reason……
6	Have you been informed about intermittent preventive treatment/sulfadoxine-pyrimethamine before taking it?	No=1 Yes=2	
7	What is the attitude of healthcare workers towards healthcare provision?	Negative=1 Positive=2	
8	From which source did you hear about IPT/SP and LLIN	1. Nurses 2. Doctors 3. Media 4. Friends 5. Schools 6. Church 7. Other source:……	
9	Have you ever been given a long lasting treated mosquito nets during antenatal clinic for free for this pregnancy or before	No=1 Yes=2	
10	Do you trust informations given by healthcare workers	No=1 Yes=2	
11	Do you own a long lasting net	No=1 Yes=2	
12	Did you use the net last night	No=1 Yes=2	

13	Give reason why you do not use the net	Not told No=1, Yes=2 No breathing well No=1, Yes=2 Toxicity No=1, Yes=2 Name another reason:	

SECTION D. To assess the level knowledge of healthcare workers on intermittent preventive treatment and insecticide treated nets.

N	Question	No=1	Yes=2	Answers (Tick)
Intermittent preventive treatment				
1	Sex	Female=1	Male=2	
2	Age 1.18-32 2.33-57 3. above 58			
3	Highest level of Education	1.Primary 2.Secondary 3.Tertiary/University		
4.	Professional training	1.Nurse 2. Clinical officer 3.Doctor 4.Midwife		
5	Have you ever worked in antenatal clinic?	1.no 2.yes		
6	If yes, for how long did you work there?	1.Less than2yrs 2.Between 2 to 5 yrs 3.More than five yrs 4.Don't Know		

7	Are you currently working in mother child health	1.no yes		
8	Have you received any training on malaria?	1.no 2.yes		Write here: the year…… Name training……
9	If yes, as the training beneficial for your work	1.no 2.yes 3.not sure		
10	Do you have a new guideline for antenatal clinic on IPT/SP and LLIN	1.no 2.yes		
11	Please state when the IPT-SP should be started during pregnancy	State here…… …..		…… …….. …….
12	Do you known how many doses of IPT/SP should be received during pregnancy	1.no 2.yes 3.dont know		…… ……Doses
13	Do you know side effects of IPT/SP on the fetus	Please briefly explain		Cite here: …… ……

		---------- ---------- --------		…… …
14	Do you know the advantage of taking intermittent preventive treatment/sulfadoxine-pyrimethamine during pregnancy	1.no 2.yes 3.dont know		write here: …… …….…… …….
15	Do you know that women under septrin in PMTCT should not take intermittent preventive treatment/sulfadoxine-pyrimethamine	1.no 2.yes 3.dont know		
16	In which other cases IPT/SP is contradicted during pregnancy	1.no 2.yes 3.dont know		…… … …… ….. …… ….
17	Do you know that SP-IPT should be associated with folic iron at 0.4 mg daily	1.no 2.yes 3.dont know		

18	Do you know the reason why folic iron dose of 5 mg should not be given at the same time with sulfadoxine-pyrimethamine	1.no 2.yes 3.dont know		Write here : ……..
19	Do you know that IPT/SP needs to be taken in the presence of a nurse/medical worker	1.no 2.yes 3.dont know		
20	Direct observed strategy " DOT" faces which challenges in your routine care service delivery	1.no 2.yes 3.dont know		Cite here: …… … …… … …… ……..
21	Do you know the importance of giving IPT-SPduring pregnancy	1.no 2.yes 3.dont know		Cite here: …… …… …… ……
22	Do you know that when a pregnant women is confirmed with malaria,			

	IPT/SP should be avoided?			
23	Do you know the composition of IPT/SP			Cite here: …… ……..…… ……..
	LLIN "Long Lasting Insecticide treated nets"			
24	Do you know the importance of sleeping in the insecticide treated nets during pregnancy	1.no 2.yes 3.dont know		Write here: …… …… …… …… ……
25	Do you distribute Long-lasting insecticide nets to pregnant women	1.no 2.yes 3.dont know		Write here if no: The reason is…….. …… ……..…… ……..
26	Do you run (short) out of stock in the long lasting insecticide treated nets	1.no 2.yes 3.dont know		Write here if yes, How many days. …… ……..

27	Do you know that long lasting insecticide treated nets efficacy can last for at least three years?	1.no 2.yes 3.dont know		Write here…….

SECTION F: To assess the practices of pregnant women on intermittent preventive treatment and insecticide treated nets.

Focus Group Discussion

A. Topic/Theme: Practice of preventing malaria during pregnancy

Q1. What do you do to prevent malaria during pregnancy?

Q2. What traditional medicine do you use to prevent malaria during pregnancy?

Q3. What are your concerns about use of insecticide treated net in the house?

Appendix III: Ethical Clearance Letter from Mount Kenya University - 1

Mount Kenya University

REF: **MKU/ERC/2027**
TO: **DR. JESSE GITAKA**

Date: 10 December 2021

REG: **FACULTY**

Dear Sir/Madam,

RE: INTERDISCIPLINARY RESEARCH FOR AN INTEGRATED COMMUNITY DIRECTED STRATEGY TO ELIMINATE MALARIA IN TROPICAL AFRICA

This is to inform you that **Mount Kenya University** has reviewed and approved your above research proposal. Your application approval number is **1100.** The approval period is **10/12/2021 - 09/12/2022.**

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used
ii. All changes including amendments, deviations and violations are submitted for review and approval by **Mount Kenya University**
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **Mount Kenya University** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affect the safety or welfare of study participants and others or affect the integrity of the research must be reported to **Mount Kenya University** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal
vii. Submission of an executive summary report within 90 days upon completion of the study to **Mount Kenya University**

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

The Chairman
Mount Kenya University
Ethics Review Committee
P. O. Box 342 - 0100, Thika

Dr. Peter G. Kirira
Chairman, Mount Kenya University IERC

Appendix IV: Ethical Clearance Letter from Mount Kenya University - 2

Mount Kenya University

REF: **MKU/ERC/2100** Date: 24 February 2022

TO: **JOSEPH MUKALA NKONGOLO**

REG: **PHDPH/2019/48569**

Dear Sir/Madam,

RE: DETECTION OF PREGNANCY ASSOCIATED MALARIA AMONG PREGNANT WOMEN AS A STRATEGY TO IMPROVE MOTHER CHILD HEALTH OUTCOMES IN BUNGOMA COUNTY, KENYA.

This is to inform you that **Mount Kenya University** has reviewed and approved your above research proposal. Your application approval number is **1173.** The approval period is **22/02/2022 - 21/02/2023.**

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used
ii. All changes including amendments, deviations and violations are submitted for review and approval by **Mount Kenya University**
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **Mount Kenya University** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affect the safety or welfare of study participants and others or affect the integrity of the research must be reported to **Mount Kenya University** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal
vii. Submission of an executive summary report within 90 days upon completion of the study to **Mount Kenya University**

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

The Chairman
Mount Kenya University
Ethics Review Committee
P. O. Box 342 - 0100, Thika

Dr. Peter G. Kirira
Chairman, Mount Kenya University IERC

Appendix V: Introductory Letter from Mount Kenya University

Mount Kenya University

DIRECTORATE OF GRADUATE STUDIES

PHDPH/2019/48569

27th May, 2022

The Director, Research Coordination Division
National Commission for Science, Technology & Innovation
Utalii House, 8th & 9th Floor
P.O Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: JOSEPH MUKALA NKONGOLO - REGISTRATION NUMBER. PHDPH/2019/48569

The purpose of this letter is to introduce the above named student who is pursuing Doctor of Philosophy in Public Health in the Department of Epidemiology and Biostatistics in the School of Public Health.

The title of his research is *"Detection of Pregnancy Associated Malaria among Pregnant Women as a Strategy to Improve Mother Child Health Outcomes in Bungoma County, Kenya."*

He has been cleared by the University's Ethics Review Committee (Certificate attached) and now has to proceed to the field to collect data for his research between May and November, 2022.

Any assistance accorded to his will be highly appreciated.

Thank you.

Mount Kenya University
P.O. Box 342-01000, THIKA
Office of the Director
Graduate Studies

Dr. Samuel M. Kareithi, PhD
Director, Graduate Studies
Enc.

Appendix VI: NACOSTI Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **685048**

Date of Issue: **25/March/2022**

RESEARCH LICENSE

This is to Certify that Mr.. Joseph MUKALA NKONGOLO of Mount Kenya University, has been licensed to conduct research in Bungoma on the topic: DETECTION OF PREGNANCY ASSOCIATED MALARIA AMONG PREGNANT WOMEN AS A STRATEGY TO IMPROVE MOTHER CHILD HEALTH OUTCOMES IN BUNGOMA COUNTY, KENYA for the period ending : 25/March/2023.

License No: **NACOSTI/P/22/16233**

685048

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix VII: Authorization from County Director of Education

REPUBLIC OF KENYA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
State Department of Basic Education and Early childhood Bungoma County

When Replying please quote
e-mail: bungomacdc@gmail.com

Ref No: BCE/DI/19/VOL.III/207

County Director of Education
P.O. Box 1620-50200
BUNGOMA

Date: 14TH APRIL 2022

TO WHOM IT MAY CONCERN

RE: AUTHORITY TO CARRY OUT RESEARCH.

NACOSTI/P/22/16233.

The bearer of this letter ***Mr. Joseph Mukala Nkongolo of Mount Kenya University*** has been authorized to carry out research on ***"Detection of pregnancy associated malaria among pregnant women as a strategy to improve mother child health outcomes in Bungoma County, Kenya"*** for the period ending 25th March 2023.

Kindly accord him the necessary assistance.

PHILIP K. CHIRCHIR
COUNTY DIRECTOR OF EDUCATION
BUNGOMA COUNTY

COUNTY DIRECTOR OF EDUCATION
BUNGOMA
P.O. Box 1620,
BUNGOMA - 50200

SGS

Appendix VIII: Authorization from County Commissioner

REPUBLIC OF KENYA

THE PRESIDENCY
MINISTRY OF INTERIOR AND COORDINATION OF NATIONAL GOVERNMENT

Telephone: 055-30326.
Fax: 055-30326.
E-mail: ccbungoma@yahoo.com
When replying please quote

Office of the County Commissioner
P.O. Box 550-50200
BUNGOMA.

REF:ADM.15/13/VOL.III/173

14th April, 2022

Deputy County Commissioner

WEBUYE WEST SUB-COUNTY

RE: RESEARCH AUTHORIZATION - JOSEPH MUKALA NKONGOLO

The above named has requested for authority to conduct research on the topic ***"Detection of pregnancy Associated Malaria among pregnant Women as a strategy to improve mother child health outcomes ."*** for a period ending 25th March, 2023.

Authority is hereby granted for the specific period as per research license letter dated 25th March, 2022 License No. NACOSTI/P/22/16233 signed by Director General, National Commission for Science, Technology and innovation.

Any assistance accorded to him in this pursuit would be highly appreciated by this office.

Christine W. Chacha
For: County Commissioner
BUNGOMA COUNTY.

COUNTY COMMISSIONER
BUNGOMA

Appendix IX: Authorization from County Health Director

REPUBLIC OF KENYA

COUNTY GOVERNMENT OF BUNGOMA
MINISTRY OF HEALTH
OFFICE OF THE COUNTY DIRECTOR HEALTH

Telephone: 0725393939
E-mail: heallth@bungoma.go.ke
When replaying please quote

COUNTY DIRECTOR OF HEALTH
BUNGOMA COUNTY
P. O. BOX 18-50200
BUNGOMA

OUR REF: CG/BGM/CDH/RESRC/VOL.1

DATE: 19th April, 2022.

Joseph Mukala
Mount Kenya University
Thika.

RE: PERMISSION TO CARRY OUT RESEARCH IN BUNGOMA COUNTY

Following your application for authority to carry out research on **"Detection of Pregnancy Associated Malaria Among Pregnant Women as a Strategy to Improve Mother Child Health Outcomes in Webuye County Hospital, Bungoma County",** I am pleased to inform you that you have been authorized to undertake the research for the period ending 23rd March, 2023.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the County Director of Health. The soft copy of the same should be submitted through the online Research Information system.

Thank you.

COUNTY DIRECTOR OF HEALTH
BUNGOMA COUNTY
P.O. Box 18-50200
BUNGOMA

Robert Mose
For, County Director Of Health
BUNGOMA COUNTY

CC. Medical Superindendant Webuye County Hospital

Appendix X: Authorization from Webuye Sub county Hospital

REPUBLIC OF KENYA
COUNTY GOVERNMENT OF BUNGOMA
MINISTRY OF HEALTH
OFFICE OF THE MEDICAL SUPERINTENDENT
WEBUYE COUNTY HOSPITAL

Telephone: 0732333682/0759932327
Email: webuyedistricthospital@yahoo.com

Medical Superintendent
Webuye County Hospital
P.O. Box 25 – 50205
WEBUYE

RE: WBY/DH/GA/95/VOL. I/102

Date: 27TH MAY, 2022

Dr. Joseph Mukala Nkongolo
Mount Kenya University
Thika Main Campus
P.O. Box 342- 00100
NAIROBI

Dear Sir,

RE: APPROVAL TO CARRY OUT RESEARCH AT WEBUYE COUNTY HOSPITAL

Following your letter dated 26th May, 2022 requesting to carry out research at Webuye County Hospital refers.'

I am pleased to inform you that you can carry out your research; ***'Detection if Pregnancy Associated Malaria Among Pregnant Women as a Strategy to Improve Mother Child Health Outcomes in Webuye County Hospital."***

Yours faithfully,

MEDICAL SUPERINTENDENT P.O BOX 25-50205 WEBUYE
27 MAY 2022
WEBUYE COUNTY HOSPITAL

DR. SIMON KISAKA
FOR: MEDICAL SUPERINTENDENT
WEBUYE COUNTY HOSPITAL

Appendix XI: Material Transfer Agreement

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by Mount Kenya University, Thika, Kenya and Uppsala University, Department of Immunology, Genetics and Pathology, in all transfer of research material (samples, derivatives, and specimens) related to the protocol; "PEA-based proteome profiling for prediction of Preeclampsia".

Provider: Dr. Jesse Gitaka, Mount Kenya University

Recipient: Prof. Masood Kamali-Moghaddam, Uppsala University

1. Provider agrees to transfer to recipient's designated (Provider) research materials/specimens related to the investigation of human proteomics, including dried blood spots, and serum, ("Research Material"). These materials will be accompanied by meta data, including time of collection.

   Research Material will only be used for research purposes as described in the protocol by Recipient in an appropriate designated laboratory with suitable biosafety conditions. Research Material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

2. Research Material and its derivatives will be used by Recipient in connection with the research project ("Research Project"), "PEA-based proteome profiling for prediction of Preeclampsia".

3. In all presentations or written publications concerning the Research project, Recipient will acknowledge the Provider's contribution of this Research Material unless requested otherwise.

4. This research material represents a significant contribution on the part of Provider and is considered proprietary to Provider. Recipient therefore agrees to retain control over this Research Material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of Provider.

5. Provider does not take any responsibility for loss, damage, wastage or spoilage of the Research Material during, or after, shipment to the address provided by the recipient under conditions agreed to in the protocol on shipment of the samples. This Research Material is provided as a service to the research community. Research Material is being supplied to Recipient with no warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose.

7. The under-signed Provider and Recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.

9. The Provider maintains ownership of the Research Material and its derivatives unless stated otherwise.

Signatures

For Recipient:

Recipient's Investigator
Prof. Masood Kamali-Moghaddam

Duly Authorized
Signature

Tobias Sjöblom
Digitally signed by Tobias Sjöblom
Date: 2021.09.03 14:13:23 +02'00'

Date September 3, 2021
Mailing Address for Material:

Date September 3, 2021
Mailing Address for Notices:

Uppsala University
Dept. of IGP, BMC
P.O. Box 815
SE-751 08 Uppsala, Sweden
masood-kamali@igp.uu.se

Masood Kamali-Moghaddam
Digitally signed by Masood Kamali-Moghaddam
Date: 2021.09.03 15:39:38 +02'00'

For Provider

Provider's Investigator
Dr. Jesse Gitaka

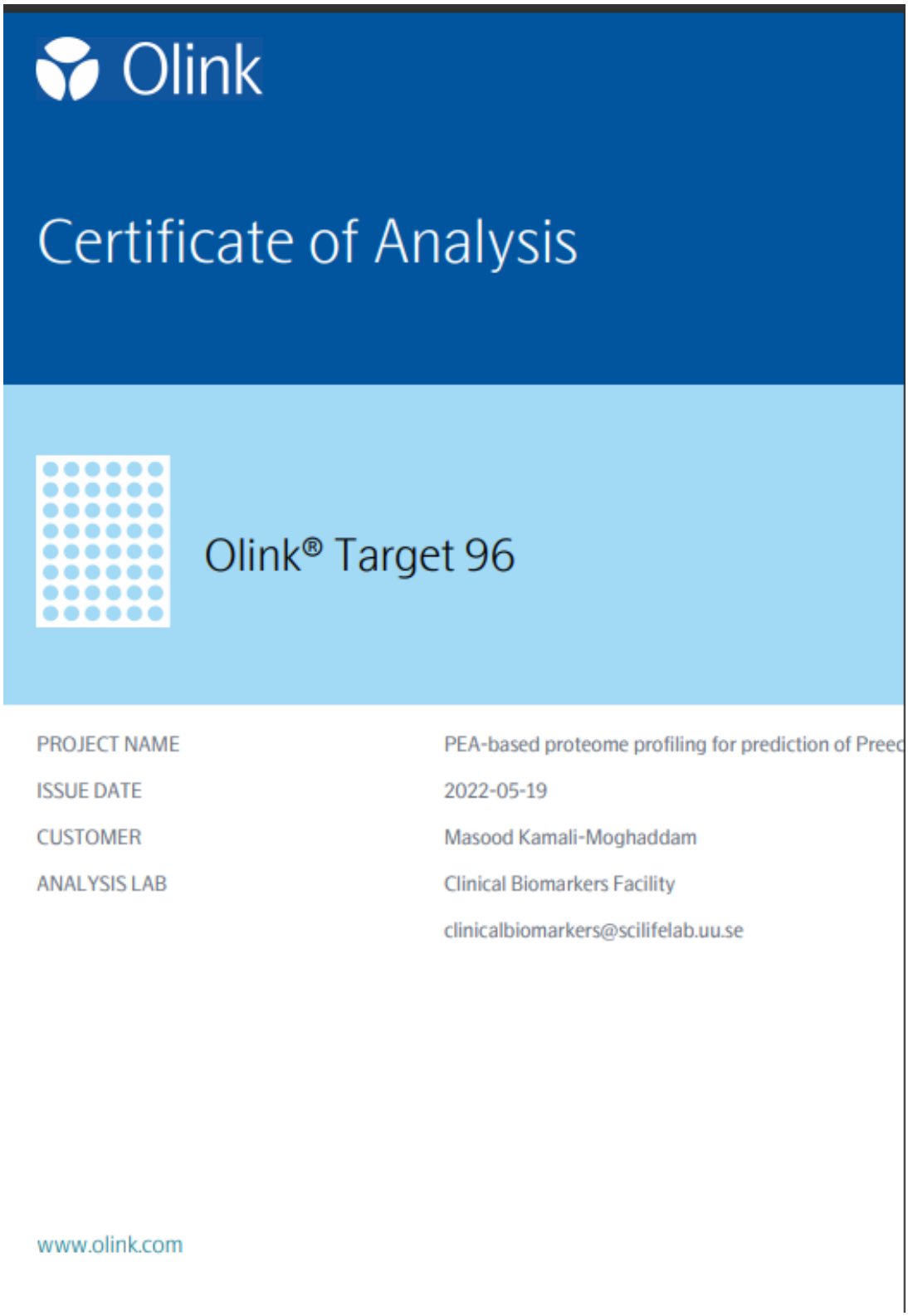
Duly Authorized
Signature

Date September , 2021
Mailing Address:

Date September , 2021 14
Mailing Address for Notices:

Mount Kenya University
P.O. Box 342-01000, Thika, Kenya
Phone (254) 722 425 613
Phone (254) 672 820 000

Appendix XII: Certificate of Analysis I



Olink

Certificate of Analysis

Olink® Target 96

PROJECT NAME	PEA-based proteome profiling for prediction of Preec
ISSUE DATE	2022-05-19
CUSTOMER	Masood Kamali-Moghaddam
ANALYSIS LAB	Clinical Biomarkers Facility
	clinicalbiomarkers@scilifelab.uu.se

www.olink.com

1. Project information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Target 96 Cardiovascular II	24	2	IPC Normalized
Target 96 Inflammation	23	2	IPC Normalized

1.1 Sample type

DBS

1.2 Project specific comments

APU Project No 2021012

2. Quality control

Four internal controls are added to each sample to monitor the quality of assay performance, as well as the quality of individual samples. The quality control (QC) is performed in two steps:

1. Each sample plate is evaluated on the standard deviation of the internal controls. This should be below 0.2 NPX. Only data from sample plate that pass this quality control will be reported.
2. The quality of each sample is assessed by evaluating the deviation from the median value of the controls for each individual sample. Samples that deviate less than 0.3 NPX from the median pass the quality control.

Data from all samples is included in the data output file. Samples that did not pass the QC are indicated in columns named "QC Warning". Data points from samples that do not pass QC should be treated with caution. [See 4]

2.1 Summary of Quality Control

Olink panel	No. of samples that passed QC / Tot no. of samples	Passed samples (%)
Target 96 Cardiovascular II	N/A	N/A
Target 96 Inflammation	N/A	N/A

2.2 Intra- and Inter-Assay Coefficient of Variance (%CV)

Intra and inter CVs are based on control samples (pooled plasma samples) included on each plate. Calculations are made using linear NPX-values. The number of assays with CVs within defined intervals are presented.

2.2.1 Average %CV

Olink panel	Intra-Assay %CV Reference intra CV <15%	Inter-Assay %CV Reference inter CV <25%
Target 96 Cardiovascular II	4	8
Target 96 Inflammation	5	8

2.2.2 Intra-Assay %CV Distribution

	No. of proteins with %CV within defined intervals				
Olink panel	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A
Target 96 Cardiovascular II	64	24	3	1	0
Target 96 Inflammation	40	23	3	0	26

2.2.3 Inter-Assay %CV Distribution

	No. of proteins with %CV within defined intervals				
Olink panel	<10%	≥10 - <20%	≥20 - <30%	≥30%	N/A
Target 96 Cardiovascular II	70	20	1	1	0
Target 96 Inflammation	54	10	1	1	26

3. Protein detection results

3.1 Number of proteins detected in >75% of the samples

Olink panel	No. of detected proteins / Tot no. of proteins	Detected proteins (%)	Expected detectability in EDTA plasma* (%)
Target 96 Cardiovascular II	83 / 92	90	>90
Target 96 Inflammation	58 / 92	63	>75

*The expected detectability is based on EDTA plasma from healthy donors. These values are intended as guidelines only and protein levels may vary depending on different pathological conditions, sample matrices, or sample preparation methods.

3.2 Data output

Data is presented as normalized protein expression (NPX) values, Olink Proteomics' arbitrary unit on log2 scale. [See 4]

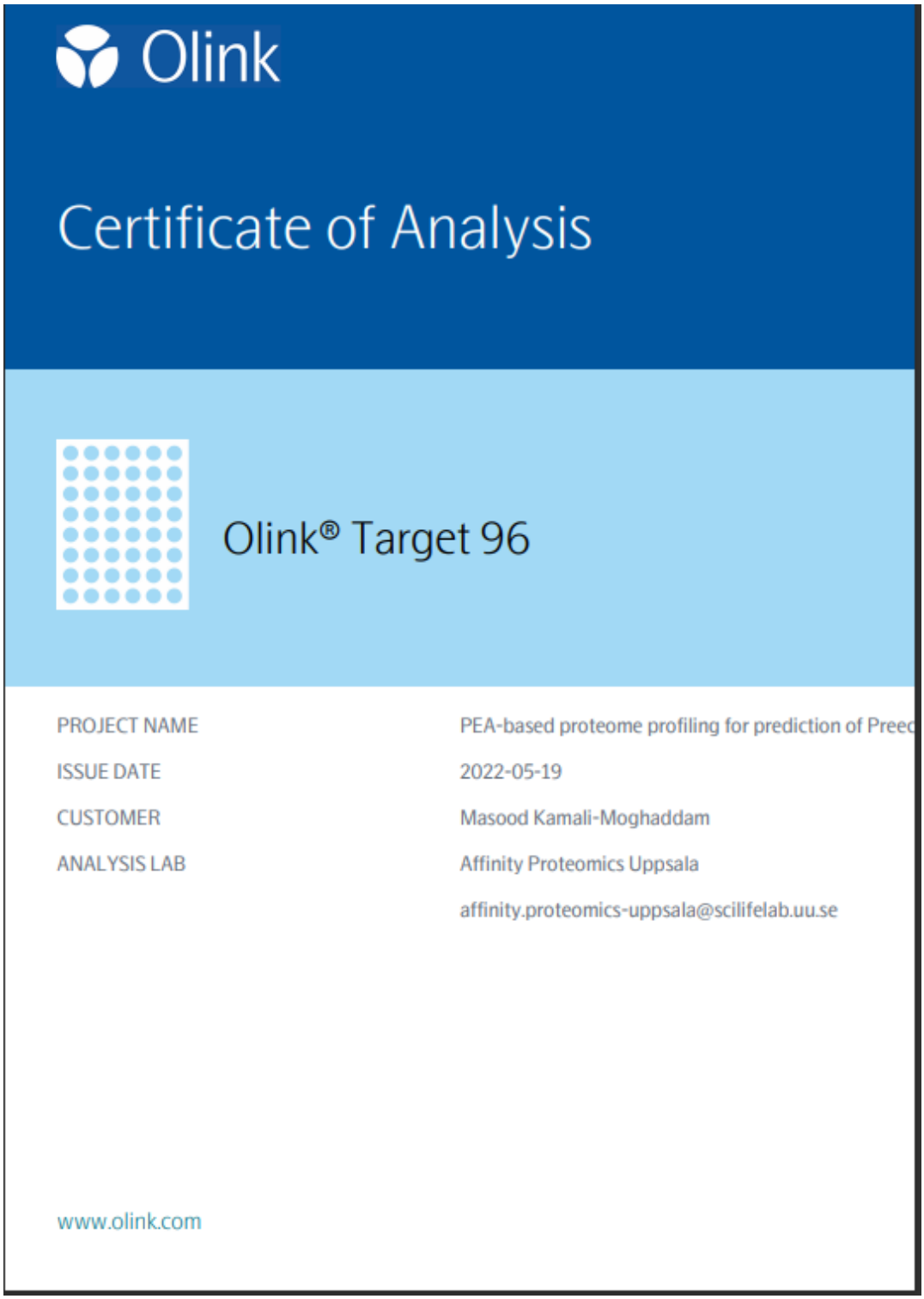
The NPX values are presented in a separate data file. Data points for samples that did not pass QC are written in red text. Data values for measurements below limit of detection (LOD) are reported for all samples. Cells containing data values below LOD are indicated with a pink background. [See 4]

4. Further information

Collection of direct links to pages containing important information relating to Olink data generation and processing, as well as additional support content:

https://www.olink.com/key-links/

Appendix XIII: Certificate of Analysis II



Olink

Certificate of Analysis

Olink® Target 96

PROJECT NAME	PEA-based proteome profiling for prediction of Preec
ISSUE DATE	2022-05-19
CUSTOMER	Masood Kamali-Moghaddam
ANALYSIS LAB	Affinity Proteomics Uppsala
	affinity.proteomics-uppsala@scilifelab.uu.se

www.olink.com

1. Project information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Target 96 Cardiovascular II	150	2	IPC Normalized
Target 96 Inflammation	150	2	IPC Normalized

1.1 Sample type

Serum

1.2 Project specific comments

APU Project No 2021012
Hemolysis in following samples: 4-1,14-1,3-3,23-2, 21-3, 22-3, 6-1, 20-2, 54-1,40-3,37-2, 38-3.
Project has been IPC-normalized due to suspected poor inter-plate randomization between plate 1 and 2.

2. Quality control

Four internal controls are added to each sample to monitor the quality of assay performance, as well as the quality of individual samples. The quality control (QC) is performed in two steps:

1. Each sample plate is evaluated on the standard deviation of the internal controls. This should be below 0.2 NPX. Only data from sample plate that pass this quality control will be reported.
2. The quality of each sample is assessed by evaluating the deviation from the median value of the controls for each individual sample. Samples that deviate less than 0.3 NPX from the median pass the quality control.

Data from all samples is included in the data output file. Samples that did not pass the QC are indicated in columns named "QC Warning". Data points from samples that do not pass QC should be treated with caution. [See 4]

2.1 Summary of Quality Control

Olink panel	No. of samples that passed QC / Tot no. of samples	Passed samples (%)
Target 96 Cardiovascular II	145 / 150	97
Target 96 Inflammation	146 / 150	97

2.2 Intra- and Inter-Assay Coefficient of Variance (%CV)

Intra and inter CVs are based on control samples (pooled plasma samples) included on each plate. Calculations are made using linear NPX-values. The number of assays with CVs within defined intervals are presented.

2.2.1 Average %CV

Olink panel	Intra-Assay %CV Reference intra CV <15%	Inter-Assay %CV Reference inter CV <25%
Target 96 Cardiovascular II	4	8

Olink panel	Intra-Assay %CV Reference intra CV <15%	Inter-Assay %CV Reference inter CV <25%
Target 96 Inflammation	5	8

2.2.2 Intra-Assay %CV Distribution

	No. of proteins with %CV within defined intervals				
Olink panel	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A
Target 96 Cardiovascular II	64	24	3	1	0
Target 96 Inflammation	40	23	3	0	26

2.2.3 Inter-Assay %CV Distribution

	No. of proteins with %CV within defined intervals				
Olink panel	<10%	≥10 - <20%	≥20 - <30%	≥30%	N/A
Target 96 Cardiovascular II	70	20	1	1	0
Target 96 Inflammation	54	10	1	1	26

3. Protein detection results

3.1 Number of proteins detected in >75% of the samples

Olink panel	No. of detected proteins / Tot no. of proteins	Detected proteins (%)	Expected detectability in EDTA plasma* (%)
Target 96 Cardiovascular II	89 / 92	97	>90
Target 96 Inflammation	67 / 92	73	>75

*The expected detectability is based on EDTA plasma from healthy donors. These values are intended as guidelines only and protein levels may vary depending on different pathological conditions, sample matrices, or sample preparation methods.

3.2 Data output

Data is presented as normalized protein expression (NPX) values, Olink Proteomics' arbitrary unit on log2 scale. [See 4]

The NPX values are presented in a separate data file. Data points for samples that did not pass QC are written in red text. Data values for measurements below limit of detection (LOD) are reported for all samples. Cells containing data values below LOD are indicated with a pink background. [See 4]

4. Further information

Collection of direct links to pages containing important information relating to Olink data generation and processing, as well as additional support content:

https://www.olink.com/key-links/

5. Samples that did not pass QC

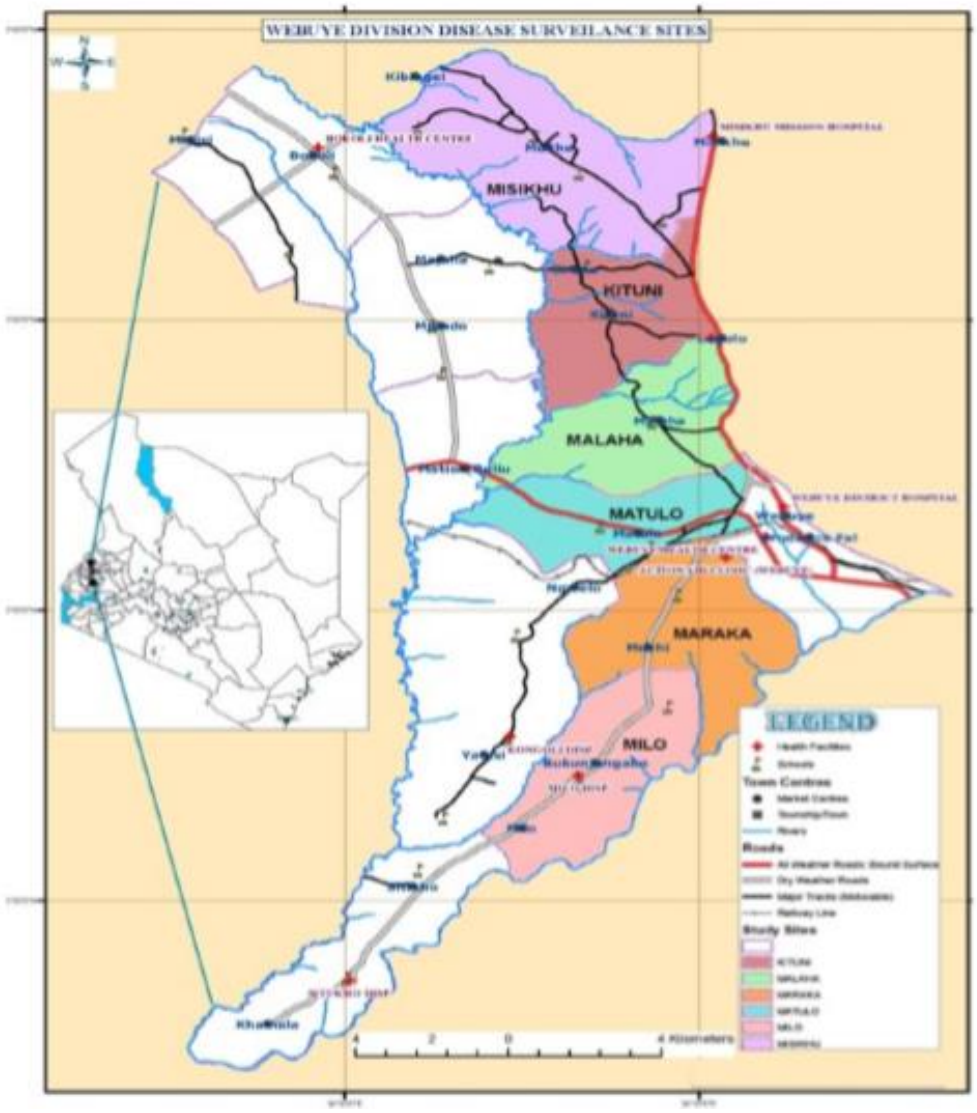
Sample ID	Target 96 Cardiovascular II	Target 96 Inflammation
20-1	x	x
20-2	x	x
12-1	x	
20-3	x	x
3-2	x	
39-1		x

5.1 Observed deviations

Data for these samples/assays are set to "No data" in the results output file.

Datapoint	Plate	Reason
53-2 / IL-24	2021012-INF-2	Datapoint failed

Appendix XIV: Bungoma County Map



Appendix XV: Similarity Report

DETECTION OF PREGNANCY ASSOCIATED MALARIA AMONG PREGNANT WOMEN AS A STRATEGY TO IMPROVE MOTHER CHILD HEALTH OUTCOMES IN BUNGOMA COUNTY, KENYA

ORIGINALITY REPORT

20%	17%	13%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	ugspace.ug.edu.gh Internet Source	1%
2	Submitted to Mount Kenya University Student Paper	1%
3	scholarworks.waldenu.edu Internet Source	1%
4	pure.uva.nl Internet Source	1%
5	erepository.mku.ac.ke Internet Source	<1%
6	Submitted to Chester College of Higher Education Student Paper	<1%
7	ir.jkuat.ac.ke Internet Source	<1%
8	Submitted to University of Central Lancashire Student Paper	<1%