PREVALENCE OF MALARIA AND ANAEMIA AMONG HIV INFECTED PREGNANT WOMEN USING CO-TRIMOXAZOLE PROPHYLAXIS IN KINONDONI MUNICIPALITY

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By

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A dissertation submitted in partial fulfillment of the requirements for the Degree of Master of Pharmacy (Hospital and Clinical Pharmacy) of the Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: "Malaria Infection Among HIV Infected Pregnant Women Using Co-trimoxazole Prophylaxis in Kinondoni Municipality" in partial fulfillment of the requirements for the degree of Master of Pharmacy (Hospital and Clinical Pharmacy) of the Muhimbili University of Health and Allied Sciences.

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Dr. Omary Minzi

(Supervisor)

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(Co-Supervisor)

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Date

DECLARATION AND COPYRIGHT

I, **Manyanga, Vicent P.** declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature…….. **Date**……...

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DEDICATION

This work is a dedication to my lovely wife Fortunata and my children Brian and Ivanna. They are the reason for my happiness!

ABSTRACT

Background: HIV-infected pregnant women are particularly more susceptible to the deleterious effects of malaria infection particularly anaemia. The Ministry of Health introduced a daily co-trimoxazole prophylaxis among HIV infected pregnant women to prevent opportunistic infections and malaria in 2011.

Objective: To determine the prevalence and the associated factors of malaria infection and anaemia among HIV infected pregnant women using co-trimoxazole prophylaxis.

Methodology: This was a cross sectional study conducted among HIV-infected pregnant women in eight health facilities in Kinondoni Municipality from February to April 2013.

Results: A total of 420 subjects with the mean±SD age of 28.2 ±5.2 years were recruited and analyzed. The prevalence of malaria infection was 4.5% (19/420). The proportion of subjects with poor adherence to co-trimoxazole was 50.5% (208/320). Factors that were significantly associated with malaria infection were poor adherence to co-trimoxazole prophylaxis [Adjusted Odds Ratio (AOR) = 6.81, 95%CI=1.35-34.43, P=0.02] and severe anaemia (AOR=10.77, 95%CI=1.38-84.05, P=0.022). The prevalence of anaemia was 54 % (227/420). Factors associated with anaemia were WHO clinical stage II (AOR=3.08, 95%CI=1.46-6.49, P=0.003), WHO clinical stage III or IV (AOR=2.65, 95%CI=1.18-5.95, P=0.018), poor adherence to co-trimoxazole prophylaxis (AOR=1.75, 95%CI=1.03-2.98, P=0.039), malaria infection (AOR=10.36, 95%CI=1.33-80.8, P=0.026) and history of episodes of malaria illness during current pregnancy (AOR=1.75, 95%CI=1.00-3.03 and P=0.048).

Conclusion: The study showed a low prevalence of malaria; however, a significant proportion of subjects had anaemia. Efforts for monitoring of adherence to co-trimoxazole prophylaxis and mitigation of advanced HIV/AIDS are needed in order to reduce the burden of malaria and anaemia among HIV infected pregnant women.

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ABBREVIATIONS

3TC - Lamivudine

95%CI - Ninety five percent confidence interval

ABC - Abacavir

AIDS - Acquired Immunodeficiency Syndrome

ANC - Antenatal Clinic

AOR - Adjusted Odds Ratio

ART - Anti-Retroviral Treatment

ARV - Anti-Retroviral

AZT - Zidovudine

CTX - Trimethoprim plus Sulphamethoxazole (Co-trimoxazole)

DNA – Deoxyribonucleic acid

EDTA - Ethylenediaminetetra Acetic Acid

FACS - Fluorescent Activated Cell Sorting

FTC - Emtricitabine

HAART - Highly Active Anti-Retroviral Treatment.

Hb - Haemoglobin

HIV - Human Immunodeficiency Virus

HRP-2 - Histidine Rich Protein-2

IPT - Intermittent Preventive Treatment

IRS - Indoor Residual Spraying

ITN - Insecticide Treated Nets

LPV/r - Lopinavir/ritonavir

MMAS - Morisky Medication Adherence Scale

MoHSW - Ministry of Health and Social Welfare (of Tanzania)

MRDT - Malaria Rapid Diagnostic Test

NACP - National AIDS Control Programme

NNRTI - Non-Nucleoside Reverse Transcriptase Inhibitor

NVP - Nevirapine

PCR - Polymerase Chain Reaction

pLDH - Plasmodium Lactate Dehydrogenase

PMTCT - Prevention of Mother to Child Transmission of HIV infection

SD - Standard Deviation

SP-IPT - Intermittent Preventive Treatment by Sulphadoxine plus Pyrimethamine

SPSS - Statistical Package for Social Sciences or Statistical Product and Service Solutions

TACAIDS - Tanzania Commission for AIDS

TDF - Tenofovir

WHO - World Health Organization

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DEFINITIONS

i. Anaemia: Haemoglobin concentration is <11g/dl.
ii. Malaria infection: For the purpose of this study is regarded when MRDT tested positive.
iii. Mild-to-moderate anaemia: Haemoglobin level is between 7 to 10.9g/dl.
iv. Multigravidae: Women who are pregnant for at least the third time.
v. Primigravidae: Women who are pregnant for the first time.
vi. Secundigravidae. Women who are pregnant for the second time.
vii. Severe anaemia : Haemoglobin level is <7g/dl.

1.0 INTRODUCTION:

1.1 Malaria infection during pregnancy

Malaria is a major contributor of disease burden in Sub-Saharan Africa, with pregnant women and children of less than five years being the most vulnerable population (1). An estimated 3.3 billion people were at risk of malaria in 2010, although of all geographical regions, populations living in sub-Saharan Africa have the highest risk of acquiring malaria. In the year 2010, 81% of all malaria cases and 91% of all deaths due to malaria were estimated to have occurred in the WHO African Region, with children of under five years of age and pregnant women being most severely affected (2). According to the WHO World Malaria Report of 2011; majority of Tanzania's population (>73%) are residing in areas with high malaria transmission (annual incidence ≥1 per 1000 population). Tanzania is the world's fifth largest population at risk of stable malaria whereby an estimated of more than 43 million people are at risk of malaria infection (2). Malaria accounts for over 30% of the national disease burden, making it a top health priority for allocation of resources for its prevention and control (3).

Malaria is caused by five species of parasites of the genus *Plasmodium* that affect humans *(P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi)*, malaria due to *P. falciparum* is the most deadly, and it predominates in Africa (2). *Plsmodium vivax* is less dangerous but more widespread, and the other three species are found much less frequently. The disease is transmitted to humans by the bite of infected female mosquitoes of more than 30 anopheline species. Malaria is an entirely preventable and treatable disease, provided that currently recommended interventions are properly implemented (4). These include vector control through the use of insecticide-treated nets (ITNs), indoor residual spraying (IRS) and in some specific settings larval control can be implemented. Chemoprevention among the most vulnerable population particularly pregnant women is recommended. Another strategy is the confirmation of malaria diagnosis through microscopy or Malaria rapid diagnostic tests (MRDTs) for every suspected case, and lastly is the timely treatment with appropriate

antimalarial medicines according to the parasite species and any documented drug resistance (4).

In areas with stable malaria transmission most of the populations have a relatively high immunity against malaria. Generally *P. falciparum* infection during pregnancy is not associated with acute symptoms and remains undetected and untreated. This usually results to high frequency of parasitaemia, severe anaemia and parasite sequestration in the placental vascular space, also called placenta malaria (1,5). Worldwide it is estimated that malaria during pregnancy causes up to 10,000 maternal deaths each year mainly as a result of severe anaemia (1,6,7).

During pregnancy, there is a transient depression of cell-mediated immunity that allows fetal allograft retention but as well interferes with resistance to various infectious diseases including malaria (8–11). *Plasmodium falciparum* express variant surface antigens (VSA) on infected erythrocytes that bind to chondroitin sulfate A (also referred as VAR2CSA), allowing them to sequester in the placenta resulting to placental malaria. The placental sequestration results in the accumulation of parasitized erythrocytes in the intervillous space, infiltration by inflammatory cells, and release of pro-inflammatory mediators and subsequently negative maternal and foetal outcomes (12). Malaria infection alone (without HIV) during pregnancy has been shown to have a relationship with gravidity whereby the primigravidae are more affected than the others. The decreased in malaria prevalence in multigravidae has been explained by the development of pre-immunity to malaria with increased number of previous pregnancies. The primigravidae remains susceptible to malaria due to partial development of immunity (1,9,13,14).

1.2 HIV Infection during Pregnancy

In Tanzania, HIV infection is caused by the Human Immunodeficiency Virus type 1(HIV-1), and no infection with HIV-2 has been reported yet (15). The common HIV-1 sub-types in Tanzania are A, C, D and their recombinants (15). HIV infection is acquired by sexual intercourse with an infected partner, exposure to infected blood and blood products, or

transmission from an infected mother to the unborn child in the uterus, during delivery, or from breast milk. More than 90% of adults in sub-Saharan Africa acquired HIV infection from unprotected sexual intercourse with infected partners (15). Transmission of HIV through body fluids other than blood and genital secretions such as CSF (cerebrospinal fluid), pleural fluid, amniotic fluids is also possible (15). Depending on clinical presentations HIV/AIDS is classified into four WHO Clinical Stages (15) as follows:

a. WHO Clinical Stage I

Patients are asymptomatic or have persistent generalized lymphadenopathy (lymphadenopathy of at least two sites [not including inguinal] for longer than 6 months). Patients may remain in this stage for several years .

b. WHO Clinical Stage II.

This is a mildly symptomatic stage. It is characterized by unexplained weight loss of less than ten percent of total body weight (assessment of body weight in pregnant women needs to consider the expected weight gain of pregnancy), upper respiratory infections (such as sinusitis, bronchitis, otitis media, and pharyngitis) and range of dermatological conditions including herpes zoster flares, angular cheilitis, recurrent oral ulcerations, papular pruritic eruptions, seborrhoeic dermatitis, and fungal nail infections .

c. WHO Clinical Stage III.

This is a moderately symptomatic stage. The clinical presentations in this stage includes weight loss of greater than ten percent of total body weight (expected weight gain of pregnancy should be considered), prolonged (more than 1 month) unexplained diarrhoea, pulmonary tuberculosis, severe systemic bacterial infections including pneumonia, pyelonephritis, empyema, pyomyositis, meningitis, bone and joint infections, and bacteremia. Mucocutaneous conditions, including recurrent oral candidiasis, oral hairy leukoplakia, and acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis. Also recurrent fever >1month

d. WHO Clinical Stage IV.

This is a severely symptomatic stage pronounced with the AIDS-defining illnesses including HIV wasting syndrome, pneumocystis pneumonia (PCP), recurrent severe or radiological bacterial pneumonia, extrapulmonary tuberculosis, HIV encephalopathy and central nervous system (CNS) toxoplasmosis. Others includes chronic (more than 1 month) or orolabial herpes simplex infection, esophageal candidiasis, kaposi's sarcoma, cytomegaloviral (CMV) infections (CMV retinitis or infection of organs other than the liver, spleen or lymph nodes), extrapulmonary cryptococcosis and disseminated endemic mycoses (e.g. coccidiomycosis, penicilliosis, histoplasmosis). It also includes cryptosporidiosis, isosporiasis, disseminated non-tuberculous mycobacteria infection, tracheal, bronchial or pulmonary candida infection, visceral herpes simplex infection, acquired HIV-associated rectal fistula, cerebral or B cell non-Hodgkin lymphoma, progressive multifocal leukoencephalopathy (PML) and HIV-associated cardiomyopathy or nephropathy

1.3 Prevention of Mother to Child Transmission of HIV Infection

There are two approaches of using ARV for the prevention of vertical transmission of HIV infection from the mother to her baby depending on the pregnant women eligibility (16,17).

a. HIV infected pregnant women eligible for ART

For HIV-infected pregnant women, the initiation of ART for their own health is recommended for all women who have CD4 cell counts of ≤350 cells/μL irrespective of WHO clinical stage or WHO clinical stage 3 or 4 irrespective of the CD4 cell count. The preferred first-line ART regimen in pregnancy comprises of an AZT + 3TC backbone combined with a non-nucleoside reverse transcriptase inhibitor (NNRTI): AZT + 3TC + NVP or AZT + 3TC + EFV. Alternative recommended regimens are TDF + 3TC (or FTC) + NVP and TDF + 3TC (or FTC) + EFV.

b. HIV infected pregnant women not eligible for ART

All HIV-infected pregnant women who do not need ART for their own health require an effective ARV prophylaxis to prevent HIV transmission to their unborn child during pregnancy, labour, delivery, postpartum and during breast-feeding period. ARV prophylaxis should be started from as early as 14 weeks of gestation or as soon as possible afterward. Two options are recommended:

Option A: Antepartum twice-daily AZT starting from as early as 14 weeks of gestation and continued during pregnancy. At the onset of labour, a single dose of Nevirapine (sd-NVP) and initiation of twice daily AZT + 3TC for 7 days postpartum. If maternal AZT was provided for more than 4 weeks during antenatal clinic, omission of the sd-NVP and AZT + 3TC tail can be considered; in this case, maternal AZT is continued during labour and stopped at delivery.

Option B: Triple ARV prophylaxis starting from as early as 14 weeks of gestation and continued until delivery, or, if breastfeeding; continued until 1 week after all infant exposure to breast milk has ended. Recommended regimens include:
AZT + 3TC + LPV/r or AZT + 3TC + ABC or AZT + 3TC + EFV or TDF + 3TC (or FTC) + EFV

1.4 Combined Malaria and HIV During pregnancy

Combined Malaria and HIV infection during pregnancy increases the susceptibility of the pregnant women to the negative effects of malaria suggesting a synergistic interaction between HIV infection and Malaria (18,19). Some studies have shown that HIV-infected pregnant women have significant alterations in immunity to malaria which render them more vulnerable to the adverse effects related to malaria infection (20,21). Results from 11 studies showed that HIV infected pregnant women experienced consistently more peripheral and placental malaria, higher parasite densities, more febrile illnesses, severe anemia, and adverse birth outcomes than HIV uninfected women, particularly in multigravidae (19). It has been shown that HIV

alters the typical gravidity specific pattern of malaria risk by shifting the burden from primarily primigravidae and secundigravidae to all pregnant women (19).

The prevalence of HIV infection among women attending ANC in Tanzania is higher at around 8.2% as compared to general prevalence among women (7%) and men (5%) (17). In other countries within the Sub-Sahara Africa, the reported prevalence of HIV among pregnant women attending Antenatal Clinic has been as high as 40% (22).

The above mentioned severe effects of malaria in pregnant women and the adverse perinatal outcomes associated with it, makes the prevention of malaria infection during pregnancy absolutely imperative. WHO has recommended three strategies in the prevention and control of malaria in pregnancy; these include the use of Intermittent Preventive Treatment (IPT), sleeping under insecticide treated nets (ITNs) and effective case management for both malaria and anaemia (4). To date, Sulphadoxine-Pyremethamnie (SP) is the only treatment with data on effectiveness and safety in the prevention of malaria during pregnancy among HIV un-infected pregnant women. WHO recommends at least 2 doses of SP-IPT after the first trimester (4), however several studies have shown that SP-IPT is ineffective in the prevention of malaria infection and its consequences among HIV infected pregnant women living in areas with high malaria transmission (23,24).

WHO has promoted a daily dose of co-trimoxazole as an alternative to SP-IPT to HIV-infected pregnant women during the whole pregnancy period (25). Concurrent administration of SP and co-trimoxazole is not advised due to its association with increased incidence of severe adverse skin reactions due to overlapping sulpha related toxicity because both drugs contains sulpha compounds (25). The current Tanzania National PMTCT guideline recommends a daily dose of co-trimoxazole to HIV infected pregnant women regardless of their CD4 count or WHO Clinical Stage for prevention of opportunistic infections and malaria during all trimesters (17). Co-trimoxazole is a fixed-dose combination of sulphamethoxazole and trimethoprim with broad spectrum antimicrobial activity that targets a range of aerobic gram-positive and gram-negative organisms, fungi and protozoa (25,26). Unfortunately, there

is limited information about the effectiveness of daily co-trimoxazole for preventing malaria among HIV infected pregnant women.

1.5 Adherence to Co-trimoxazole Prophylaxis

Adherence has been defined as the *'active, voluntary, and collaborative involvement of the patient in a mutually acceptable course of behavior to produce a therapeutic result'* (27). Another definition of adherence proposed by WHO is '*the extent to which a person's behaviour i.e. taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider'* (28). The implicit in the concept of adherence is the choice and mutuality in goal setting, treatment planning, and implementation of the regimen. The patient internalizes the treatment recommendations and then either adheres to these internal guidelines or partially adheres or rejects completely. Furthermore, the definition implies that the patient has a choice and that both patients and providers mutually establish treatment goals and the medical regimen. In practice, studies have shown that the consequences of poor adherence with prescribed medication regimens are in increased morbidity and mortality from a wide variety of illnesses, as well as increased health-care costs (28). Adherence has been found to be a single most important modifiable factor compromising treatment outcome across diseases (28). Therefore ensuring adherence to co-trimoxazole prophylaxis is an important factor in order to achieve good health outcomes among HIV infected pregnant women.

There are different methods for assessing adherence to medications; Osterberg et al (29) categorized the different methods into either direct or indirect. Direct methods include directly observed therapy, measurement of the level of medicine or metabolite in blood, and measurement of the biological marker in the blood. Indirect methods of adherence assessment include patient questionnaires, self-reports, pill counts, rate of prescription refills, assessment of the patient's clinical response, electronic medication monitors, measurement of physiological markers and patient diaries (29). One of the most commonly indirect methods is by assessment of patient's medication taking behaviors by using Morisky Medication Adherence Scale(29–31). The MMAS is a structured self-report 8-item scale that has been

updated from the previously validated 4-item Morisky Medication Adherence Scale with greater sensitivity due to the additional of items addressing the 8 circumstances surrounding adherence behavior. It is considered as the most commonly used self-reporting method to determine adherence(30,31). The theory underlying MMAS-8 is that failure to adhere to a medication regimen could occur due to several factors that can be explored by using structured questions. Each item is measuring a specific medication-taking behavior that could eventually affect the patient's overall adherence.

1.6 Diagnosis of Malaria in Pregnancy

In pregnant women, *P. falciparum* express variant surface antigens (VSA) on infected erythrocytes that bind to chondroitin sulfate A (also referred as VAR2CSA); which is a key receptor for placental sequestration (12). This type of infection is known as placental malaria (12). Therefore, in pregnant women peripheral parasitaemia can be absent or below the detection limit of microscopic method (32,33). Accurate detection of parasite infection in the placenta requires histological examination of the placenta as a 'gold standard' or by microscopic examination of the placental blood (14,32,33). Unfortunately both of these methods can only be performed after delivery when the placenta is available for examination.

Alternatively, malaria during pregnancy whether is due to peripheral or placental infection or both can be detected by using a qualitative method of selected Malaria Rapid Diagnostic Tests (MRDT) (34). MRDTs are made up specific monoclonal antibodies that detect certain parasite's antigens in the blood which are released by red blood cells infected by the malaria parasite. MRDTs for malaria can detect one or more of the following antigens: Histidine Rich Protein 2 (HRP-2), Plasmodium Lactate Dehydrogenase (pLDH) or Aldolase. In a recent systematic review comparing diagnostic accuracy of MRDTs for uncomplicated P. falciparum infection, it was reported that HRP-2 specific MRDTs have better sensitivity than pLDH-based MRDT; however specificity is better for pLDH-based tests (35). *Plasmodium falciparum* specific HRP-2 are most commonly used, because they are less expensive, more stable across a wider temperature range and have a lower detection threshold than pLDH-

based tests (36,37). Moreover, the HRP-2 antigen can be beneficial for the diagnosis of placental malaria because it can be detected in the peripheral circulation even when the parasite is sequestered within the placenta (33,34).

2 LITERATURE REVIEW

A systematic review by Skeketee et al (1) showed that pregnant women in malaria endemic areas are highly susceptible to malaria infection, and they experienced both high frequency and the severity of the disease specifically anaemia. Further studies have reported that malaria and HIV co-infection during pregnancy expands the susceptibility of the pregnant women to the negative effects of malaria suggesting a synergistic interaction between HIV and Malaria (18,19). In a meta-analysis that involved eleven studies conducted by ter Kuile et al (19) showed that HIV-infected women particularly the multigravidae, experiences consistently more peripheral and placental malaria, higher parasite densities, more febrile illnesses, severe anemia, and adverse birth outcomes than HIV-uninfected women (19). Thus, HIV alters the typical gravidity specific pattern of malaria risk by shifting the burden from primarily primigravidae and secundigravidae to all pregnant women (19,38,39).

Since the introduction of co-trimoxazole prophylaxis to all HIV infected pregnant women in Tanzania in 2011 (17), there is limited information with regard to its effectiveness in the prevention of malaria and its consequences particularly anaemia. There are few studies that were done in neighbouring countries among HIV infected pregnant women, and several other studies were done targeting children and adults (including non-pregnant HIV infected women). A malaria prevalence of 2.7% and 5.5% by blood smear and PCR method respectively was reported in a recent study by Kapito-Tembo et al (38) conducted in Malawi. The study was conducted among 1,121 HIV infected pregnant women aimed at determining the effectiveness of co-trimoxazole at reducing malaria parasitaemia and anaemia. Co-trimoxazole prophylaxis was associated with significant reduction in malaria parasitaemia and anaemia (38). Other factors associated with malaria infection were sleeping without ITN, enrollment into the study during rainy season and not using antiretroviral drugs. Newman et al (39) reported a prevalence of placental malaria of 6% and 19% by blood smear and PCR method respectively among HIV-infected pregnant women in an area with high malaria transmission in Uganda. In that study, the use of co-trimoxazole prophylaxis did not increase the risk for placental malaria as compared to the standard SP-IPT.

Several studies conducted among HIV infected pregnant women have reported the shifting of malaria infection from primarily primigravidae to all gravidities (19,38–40). In the Malawian study (38), the prevalence of malaria infection among HIV infected pregnant women with regard to the gravidity were 5.7%, 8.8% and 4.7% for primigravidae, secundigravidae and multigravidae respectively. All the gravidities had a statistically similar risk of malaria infection. In the Ugandan study (39), the reported prevalence of placental malaria by smear method were 7.4 %, 13.6% and 4% for primigravidae, secundigravidae and multigravidae respectively. Another study conducted in Kisumu, Kenya by van Eijk et al (41) showed similar pattern of shifting the Malaria burden among all the gravities. The prevalence were 37.3%, 25.9% and 23.1% among primigravidae, secundigravidae and multigravidae respectively.

Looking into other HIV infected populations living in areas with high malaria transmission; several studies have shown that co-trimoxazole prophylaxis was effective in the prevention of malaria. In one study that was carried out in Uganda, a daily co-trimoxazole prophylaxis was found to be effective in conferring protection against malaria in HIV-infected children despite a high prevalence of resistance mediating *P. falciparum* polymorphisms in Uganda (42). Another study conducted in an area with high malaria transmission in Uganda by Mermin et al (40) which involved 1035 HIV infected adults. Compared with the baseline malaria incidence of 50.8 episodes per 100 person-years; co-trimoxazole prophylaxis was associated with 9.0 episodes per 100 person-years.

Previous studies have reported high prevalence of anaemia among HIV infected pregnant women in Dar Es Salaam City. Finkelstein et al (43) reported a prevalence of 83% in 1997, while Mehta et al reported a prevalence of 73% in 2003. In other countries within Sub-Saharan Africa, varied values of prevalence of anaemia among HIV infected pregnant women have been reported (13,19,38,44). In Malawi; Kapito-Tembo et al (38) reported a prevalence of 35.6%; while Nkhoma et al (44) reported a prevalence of 27.4%. In Nigeria, a striking prevalence of 83.8% was reported by Uneke et al (13). Among the factors associated with

anaemia mentioned by Finkelstein et al (43) were malaria infection, low CD4 count and worm infestations.

There is a lack of information about the level of adherence to co-trimoxazole prophylaxis among HIV infected groups including the pregnant women. However several studies have shown that adherence to medication for chronic conditions are usually poor (29). Osterberg et al (29) reported that the average adherence rates among patients receiving treatment for chronic conditions in clinical trials was between 43% and 78% despite of the selection of the patients and attention they received.

3. PROBLEM STATEMENT

Malaria is a major contributor of disease burden in Sub-Saharan Africa, with pregnant women being one of the most vulnerable population (1). HIV-infected pregnant women are particularly more susceptible to the deleterious effects of malaria infection particularly anaemia as compared to the non-infected ones (1,18,19). Moreover, pregnancy increases the risks for opportunistic infections among the HIV infected pregnant women (45); thus creating a need for interventions to prevent both malaria and opportunistic infections among this population.

Following the overlap of HIV and Malaria epidemics during pregnancy in Sub Sahara Africa; WHO has recommended a daily co-trimoxazole prophylaxis to prevent both the opportunistic infections and malaria among all HIV infected pregnant women (25). This is given regardless of the clinical stage, CD4 count or the pregnancy age (25). Additionally, HIV-infected pregnant women receiving daily co-trimoxazole prophylaxis should not be given SP-IPTp so as to avoid adverse drug reactions associated with sulfa drug toxicity. The policy of daily co-trimoxazole prophylaxis without the standard SP-IPT among HIV infected pregnant women was introduced in Tanzania in 2011 (17).

This WHO recommendation was not based on empirical evidence (45). Nonetheless, there were studies that suggested that the standard two doses of SP-IPT could be inadequate in prevention of malaria infection among HIV infected pregnant women found in areas with high malaria transmission (23,24). In view of that, the need to research the effectiveness of co-timoxazole prophylaxis in the prevention of malaria infection and its consequences is imperative.

There is limited information about the effectiveness of co-trimoxazole prophylaxis in preventing malaria infection among HIV infected pregnant women (38–40,42). The current available information is from few studies that were conducted outside Tanzania (38,39). The difference in geographical locations, seasonal variations and hence malaria transmission rates between Tanzania and the countries where studies were done justified the current study to be done.

4. STUDY RATIONALE

HIV-infected pregnant women are particularly more susceptible to the deleterious effects of malaria infection particularly maternal anaemia. As an intervention for preventing opportunistic infections and malaria among HIV infected pregnant women; the Tanzania Ministry of Health and Social Welfare adopted a policy of daily co-trimoxazole prophylaxis in the year 2011. This was in line with the WHO recommendation.

This study was conducted to determine the prevalence of malaria and anaemia, and the associated factors among HIV infected pregnant women. Assessment of the level of adherence to co-trimoxazole prophylaxis among HIV infected pregnant women was also carried out.

The findings of this study can be used by policy makers to reflect whether the current policy has improved the problem of malaria and anaemia among the targeted population as compared to the previous policies.

The findings can be used by health care providers in order to improve the adherence to co-trimoxazole prophylaxis among the HIV infected pregnant women. Additionally, the findings can be used as the baseline for future research among this population.

5. OBJECTIVES

Broad Objectives

To determine the prevalence of malaria and anaemia among HIV infected pregnant women using co-trimoxazole prophylaxis in Kinondoni Municipality.

Specific Objectives

i. To determine the prevalence of Malaria among HIV-infected pregnant women

ii. To determine the factors associated with malaria infection among HIV infected pregnant women using co-trimoxazole prophylaxis.

iii. To determine the prevalence of anaemia among HIV-infected pregnant women who are using co-trimoxazole prophylaxis.

iv. To determine the level of adherence to co-trimoxazole prophylaxis among HIV-infected pregnant women

6.0 METHODOLOGY

6.1 Study Design and Study Area

The study was designed as a facility based cross sectional study. It was conducted in eight government's health facilities in Kinondoni Municipality.

Kinondoni is one of the three Municipalities of Dar Es Salaam City. It is located in northern part of Dar Es Salaam City. It borders the Indian Ocean to the east, Ilala Municipality to the south and Coast Region to both north and west sides. Kinondoni has an estimated population of 1,775,049 (46). It has 33 public health facilities; whereby 2 are Hospitals, 1 is a Health canter and 30 are dispensaries. Malaria is the leading cause of both the outpatient visits and in-patient admission (47).

6.2 Study Population and Sample Size Calculation

The targeted subjects were HIV infected pregnant women using co-trimoxazole prophylaxis who are attending Antenatal Clinic in Health Facilities found in Kinondoni Municipal.

Sample size for this study was calculated using the formula for cross-sectional study based on the study done in Uganda (39). In that study; the prevalence of malaria infection among HIV infected pregnant women using co-trimoxazole prophylaxis (X) was found to be 19%. The formula is:

$$\boldsymbol{n = \frac{z^2\, X(100 - X)}{\varepsilon^2}}$$

n = Minimum sample size

z = Point on standard normal distribution curve corresponding to significance level of 5%. Its value is 1.96

X = Previous prevalence of malaria infection among HIV infected pregnant women using co-trimoxazole prophylaxis (19%)

ε = margin of error on X (set at 4%).

Adding 20% for non-responders, the sample size became 444. A total of 450 subjects were recruited into the study. However, only 420 subjects were included in the final analysis.

6.3 Study Duration:

This study was carried out from February to April 2013.

6.4 Inclusion Criteria

i. HIV infected pregnant women.
ii. Co-trimoxazole prophylaxis use for more than four weeks.
iii. Informed consent from the research subjects.

6.5 Exclusion Criteria:

i. Known haematological condition e.g. sickle cell disease.
ii. Abdormal medical conditions e.g. vaginal bleeding.
iii. Mental disorders.

6.6 Sampling Strategy and Sampling Technique

Kinondoni municipality has a total of 33 government health facilities, including 2 hospitals, 1 health centers and 30 dispensaries.

Cluster sampling was used for selection of health facilities included in the study. The health facilities were divided into three clusters namely hospitals, health center and dispensaries. All the two hospitals and one health center were included in the study. Five dispensaries were obtained using simple random sampling without replacement technique. Each name of the 30 dispensaries was written in a small piece of paper, and then the paper was folded to ‘a ball like’ figure and put in a mug. The mug was thoroughly shaken and five ‘paper balls’ were randomly picked to select the five dispensaries. Table 1 shows the selected health facilities that were involved in the study.

Table 1: Health facilities involved in the study

S/N	Hospital	Health centre	Dispensary
1.	Mwananyamala	Magomeni	Kambangwa
2.	Sinza		Tandale
3			Mburahati
4.			Kimara
5.			Mbezi

Due to limited number of HIV infected pregnant women, all the subjects in the selected clusters who met the inclusion criteria were included in the study.

Antenatal Clinic in all the eight health facilities operates on Monday through Friday from 8:30am to 3:30pm. In each facility, two Research Assistants were deployed after being trained on data collection. One Research Assistant (a nurse working at the PMTCT unit) was responsible in identifying the subjects who met the inclusion criteria. In order to avoid interference with the routine flow of service; subjects who met the inclusion criteria were approached one by one for consenting immediately after they were through with the routine service.

6.7 Data collection methods

HIV infected pregnant women who consented to participate in the study were subjected to a face-to-face interview with the Principal Investigator or the Research Assistant. A structured interview schedule (Appendix 1-2) was used to obtain information on various characteristics of the subjects and supplemented by information from patients files. The subjects were then tested malaria for infection and anaemia. Blood samples were collected from some qualified subjects for CD4 assay.

6.7.1 Research Assistants training:

Two nurses at each clinic were deployed as Research Assistants for this study. They were trained on how to use *HemoCue Hb 201+*® machine (manufactured by HemoCue AB Ängelholm, Sweden) and *SD BIOLINE Malaria Ag P.f/Pan*® MRDT (manufactured by Standard Diagnostics, Inc, Korea) for measuring the Haemoglobin and malaria testing respectively.

6.7.2 Piloting the Interview Schedule:

This was carried out prior to actual data collection to test its effectiveness. Items which were misunderstood were corrected so as to bring out the intended information.

6.7.3 Socio-demographic and background information of the subjects

Data obtained from the direct interview and from files included: age, weight, height, gravidity, marital status, education level, employment status, WHO clinical stage, use of insecticide treated nets (ITN), history of episodes of malaria illness, use of iron supplements, use of deworming drugs, ART Category (Prophylaxis or life-long), duration of zidovudine use and levels of adherence to co-trimoxazole prophylaxis.

6.7.4 Adherence to Co-trimoxazole prophylaxis

Adherence was measured using questions adapted from Morisky Medication Adherence Scale (MMAS) (30,31).

The score for the response to the items are shown in the table 2 below:

Table 2: Adherence Scale to co-trimoxazole prophylaxis:

No.	Questions	YES	NO
i	Do you sometimes forget to take your co-trimoxazole tablets?	0	1
ii	People sometimes miss taking their medicines for reasons other than forgetting. Thinking over the past 2 weeks, were there any days when you did not take your co-trimoxazole tablets?	0	1
iii	Have you ever cut back or stopped taking co-trimoxazole without telling your doctor because you felt worse when you took it?	0	1
iv	When you travel or leave home, do you sometimes forget to bring along co-trimoxazole tablets?	0	1
v	Did you take your co-trimoxazole tablets yesterday?	1	0
vi	Taking medicines every day is a real inconvenient for some people. Do you ever feel hassled about sticking to your treatment plan?	0	1
vii	How often do you have difficulty remembering to take co-trimoxazole tablets? □ Never/Rarely = 1 □ Once in a while = 0.75 □ Sometimes = 0.5 □ Usually = 0.25 □ All the time = 0		
TOTAL SCORE			

The total score of >6 was interpreted as Good adherence, 4 to 5.9 as Average Adherence and <4 as Poor Adherence.

6.7.5 Malaria Testing, Haemoglobin Measurement and CD-4 Count Assay.

After the direct face-to-face interview, the subjects were tested for Malaria infection using *SD BIOLINE Malaria Ag P.f/Pan® MRDT* manufactured by Standard Diagnostics, Inc, Korea. Haemoglobin was measured by *HemoCue Hb 201+®* machine manufactured by HemoCue AB Ängelholm, Sweden. The two tests were done at the research site and results were obtained immediately. Samples for CD4 Count assay were collected from some subjects and sent to Mwananyamala Hospital. Details for the procedures are explained below:

a. Haemoglobin measurement

The tip of the middle finger was cleaned by alcohol swab; then a blood sample was obtained by finger pricking procedure. The *Haemocue 201+®* cuvette was filled with blood, excess blood was cleaned from the cuvette and air bubbles were removed. The cuvette was placed in the device tray and the holder was pushed gently into the photometer. The results were recorded from the digital display.

b. Diagnosis of Malaria

The blood sample was collected by finger pricking method after the tip of the middle finger was cleaned by alcohol swab. The test device was placed on a clean flat surface. About 5 µl of whole blood were added into the 'Sample Well' of respective test device using a micropipette supplied with the test device. Four drops of assay diluent were added into the 'sample diluent well'. All the test results were recorded within 30 minutes.

c. CD-4 Count

CD-4 Count was done at Mwananyamala Hospital's laboratory using the *BD FACS Count®* Machine. Samples were collected from the subjects who had never done previously, or who had done in the past ≥6 months. For those who had done in the period of less than 6 months, their latest results were recorded in our study. This is the policy in Kinondoni Municipal that only samples from patients who met the above mentioned criteria are to be accepted. That policy is based on WHO recommendation that maternal monitoring of immunological status through the measurement of CD-4 cell counts should be done every 6 months (16). About 4ml

of whole blood (4ml) was collected in EDTA collection tubes by standard venipuncture procedure. The collected samples were kept at room temperature (18-25° C) and then transported in special container to Mwananyamala Hospital for analysis. All samples were processed within 30 hours from the time of collection. Blood was then put in a test tube and mixed properly to avoid clotting. Using a pipette, 50µl of blood was drawn from a test tube and mixed with *BD FACS count*® reagent in a test tube and closed with a cap then the mixture was put in a dark place, at room temperature for 60 to 120 minutes. After about 1 to 2 hours 50µl of fixative solution was drawn and mixed with an incubated blood then vortexed for 5 seconds. Fixative solution is used to stop reaction and protect the cells from damage. The mixed solution was put into the machine for analysis. Printed results were produced automatically.

6.8 Management of patients

Pregnant women who were diagnosed with malaria or anaemia during the study were referred back to the clinician where they were managed as per the Standard Treatment Guideline. In order to make the process of 'internal referral' seamless, information was communicated to the clinicians before the study began.

Likewise, subjects who were found with CD4 count ≤350 cells/µL were referred to clinician for initiation of Anti-retroviral treatment as per the National guideline for the management of HIV/AIDS (15)

6.9 Data Analysis

Data was entered, cleaned and analyzed using computer software called Statistical Package for Social Sciences (SPSS) version 20. Various characteristics of the research subjects were summarized using frequency distribution tables and the data were described by percentage. The variables were classified as categorical data.

Dependent variables were malaria infection and anaemia. While the independent variables were socio-demographic factors, WHO clinical stage, CD-4 Count, levels of adherence to co-trimoxazole prophylaxis, gravidity, pregnancy age (trimester), sleeping under insecticide

treated bed nets (ITN), history of episodes of malaria illness, use of iron supplements, use of de-worming agent and duration of AZT use (categorized as <3months, 3 to <6months and ≥6months).

Classification of anaemia was based on the recommendation by WHO (48); normal (Hb ≥11g/dl), mild anaemia (Hb=10-10.9g/dl), moderate anaemia (Hb=7-9.9g/dl) and severe anaemia (Hb<7g/dl). CD4 count was categorized according to the four bands of HIV related immunodeficiency proposed by WHO (49). These includes: no significant immunodeficiency (≥500 cells/µL), mild immunodeficiency (350-499 cells/µL), advanced immunodeficiency (200-349 cells/µL) and severe immunodeficiency (<200µL).

Pearson Chi-square Test and Fischer's Exact Test were used in the univariate analysis between the dependent and independent variables where applicable. Independent variables which showed a statistical significant difference with the outcome variable by univariate analysis were subjected to multivariate logistic regression to determine the predictors of the outcome. P-value of < 0.05 was considered significant to provide evidence of significant difference or association.

7. ETHICAL CONSIDERATION

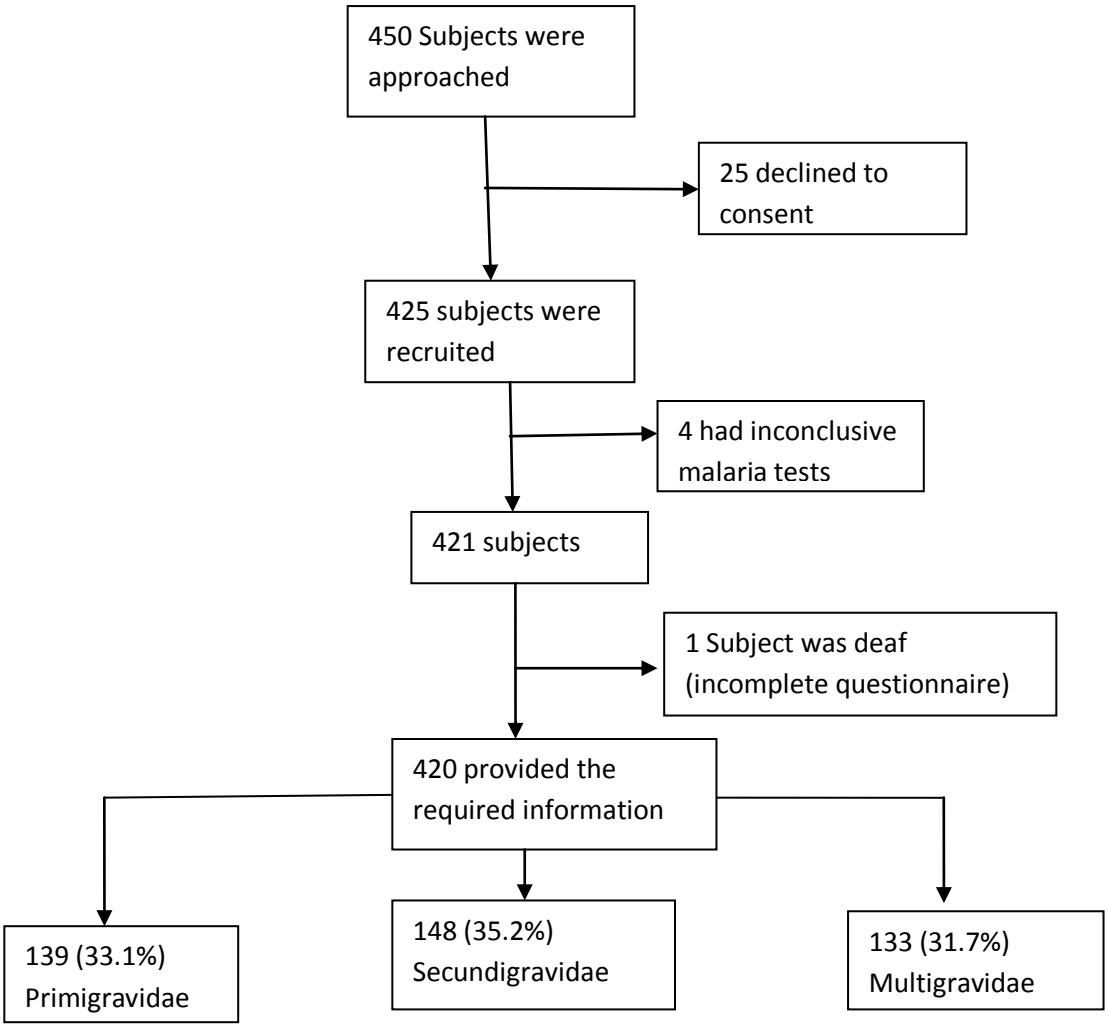
Ethical approval for the study was given by the Ethical Committee of the Muhimbili University of Health and Allied sciences (MUHAS). Permission from Kinondoni Municipal Council to conduct the study in the health facilities was granted. Information about the study was delivered to the patients and a written consent was obtained before the study was conducted. The confidentiality was ensured to all individuals who participated in the study.

8.0 RESULTS

8.1 Socio-demographic and background characteristics of the study subjects

A total of 450 HIV infected pregnant women in various trimesters were approached for recruitment into the study. Data from 420 subjects was entered in the final analysis (Figure 1).

Figure 1 : Flow chart of the research subjects

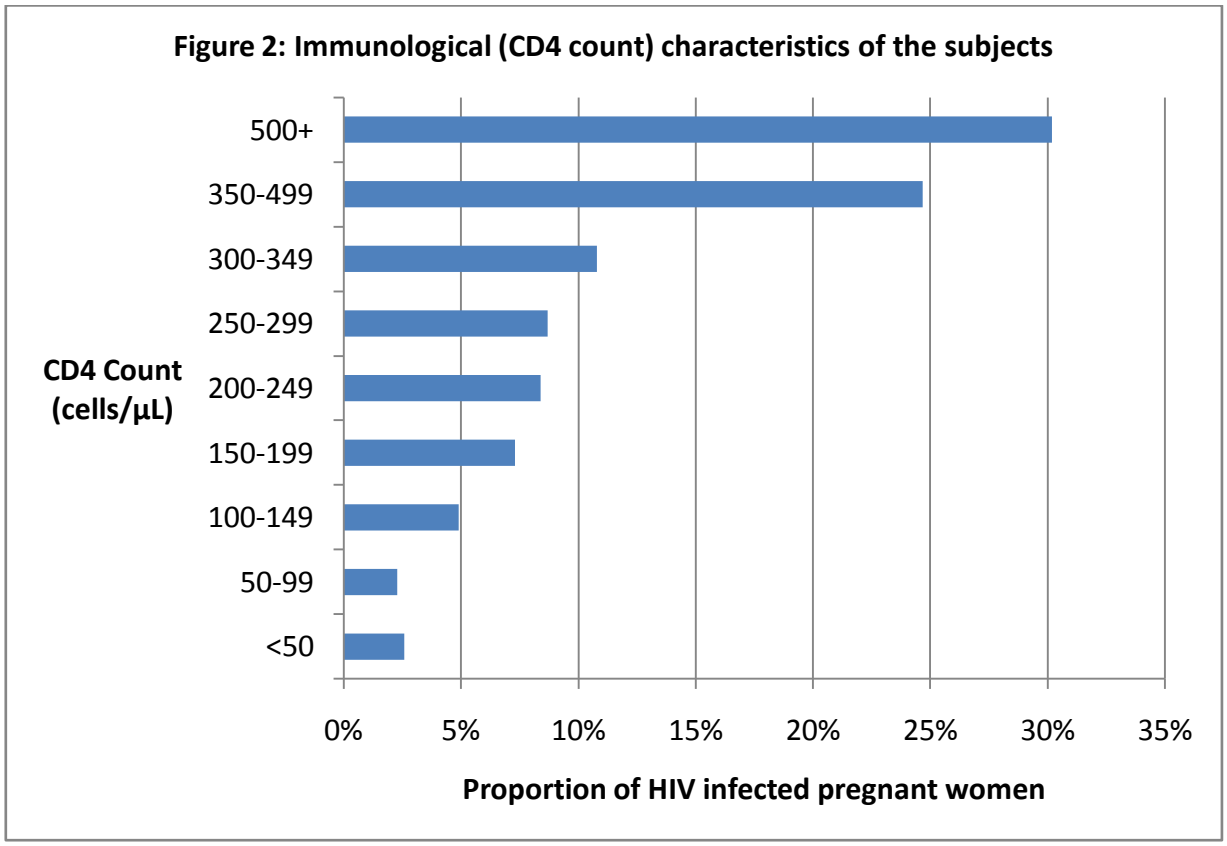


The socio-demographic characteristics of the study population are summarized in table 3 below. The mean±SD age of the subjects was 28±5.2 years.

Table 3: Socio-demographic characteristics of HIV infected pregnant women who were recruited into the study (N=420)

Characteristics	Number of respondents(n)	%
Age group (years)		
<20	16	3.8
20-34	341	81.2
≥35	63	15.0
Marital Status		
Single	35	8.3
Cohabiting	69	16.4
Married	316	75.2
Level of Education		
No formal education	33	7.9
Primary	276	65.7
Secondary	87	20.7
Post-secondary	24	5.7
Employment		
Employed	123	29.3
Business/self-employed	188	44.8
Not employed	109	26

CD4 count results of 344 subjects out of 420 were obtained. The result showed that, 17.2% (59/344) had CD4 counts <200cells/µL and 30.2% (104,/344) had CD4 count ≥500cells/µL. Figure 2 shows the distribution of CD4 count among the subjects.



8.2 Prevalence of Malaria and associated factors

The study revealed a prevalence of malaria infection of 4.5% (19/420). All the malaria positive subjects were infected with *P. falciparum.*

No any socio-demographic factor was found to have a statistically significant association with malaria infection. Tables 4 describe the distribution of malaria infection by the socio-demographic characteristics.

Table 4: Distribution of malaria infection by the socio-demographic characteristics (N=420):

Characteristics	n	Prevalence of malaria n(%)	P-Value
Age group (years)			
<20	16	0(0)	
20-34	341	16(4.7)	
≥35	63	3(4.8)	0.674[a]
Marital status			
Single	35	2(5.7)	
Cohabiting	69	3(4.3)	
Married	316	14(4.4)	0.939[a]
Level of education			
No formal education	33	3(9.1)	
Primary	276	10(3.6)	
Secondary	87	5(5.7)	
Post-secondary	24	1(4.2)	0.490[a]
Employment			
Employed	123	3(2.4)	
Business/self-employed	188	10(5.3)	
Not employed	109	6(5.5)	0.416[a]

[a] Calculated by Pearson chi-square

The study showed a pattern towards the increase in the prevalence of malaria infection as the levels of adherence to co-trimoxazole prophylaxis decreased from good to poor. Table 5 describes the distribution of malaria according to the levels of adherence to co-trimoxazole prophylaxis. The subjects with poor adherence were 6.8 times more likely to have malaria infection as compared to those with good adherence (AOR=6.81, 95% CI =1.35-34.43 and P= 0.02) (Table 7).

Table 5: Distribution of malaria infection by the levels of adherence to co-trimoxazole prophylaxis (N=420)

Adherence Levels	n	Malaria Prevalence n(%)	P-Value
Good	208	2(1)	
Average	80	4(5)	
Poor	132	13(9.8)	0.001[a]

[a] Calculated by Pearson chi-square

Table 6 and 7 describe the univariate and multivariate analysis between various factors (obstetrical, clinical, ART and ITN use) and the prevalence of malaria infection among the HIV infected pregnant women. The prevalence of malaria was higher among the subjects who had severe anaemia as compared to those who had mild-to-moderate or no anaemia. Subjects who had severe anaemia were eleven times more likely to have malaria infection as compared to those without anaemia (AOR=10.77, 95% CI = 1.38-84.05 and P = 0.023).

Another finding of our study was the prevalence of malaria infection in different gravidities had no statistically significant difference (P = 0.563), that is to say the prevalence of malaria was statistically similar in all gravidities. This has clinical significance because it shows the shifting of malaria burden from primarily primigravidae to all gravidities

Table 6: Malaria prevalence by selected factors (clinical, obstetrical, ART and ITN use) among the subjects (N=420)

Characteristics	n	Malaria prevalence n (%)	P-Value
WHO clinical stage			
Stage I	338	12(3.6)	
Stage II	43	2(4.7)	
Stage III-IV	39	5(12.8)	0.031[a]
CD4 count (cells/µL)*			
≥500	104	4(3.8)	
350-499	85	3(3.5)	
200-349	96	8(8.3)	
<200	59	4(6.8)	0.417[a]
Pregnancy trimester			
1st trimester	20	1(5)	
2nd trimester	206	9(4.4)	
3rd trimester	194	9(4.6)	0.986[a]
Gravidity			
Primigravidae	139	6(4.3)	
Secundigravidae	148	5(3.4)	
Multigravidae	133	8(6)	0.563[a]
ITN use			
Yes	380	15(3.9)	
No	40	4(10)	0.096[b]
ART Use Category			
Prophylaxis	288	11(3.8)	
Life Long	132	8(6.1)	0.305
History of malaria			
Yes	107	10(9.3)	
No	313	9(2.9)	0.005[a]
Anaemia			
Severe	21	2(9.5)	
Mild-to-moderate	207	16(7.7)	
Normal	192	1(0.5)	0.001[a]

[a] Calculated by Pearson Chi Square, [b] Calculated by Fischer's Exact Test, *Results of CD4 count were available for only 344 subjects out of 420 (81.9%)

Table 7: Predictors of malaria infection among HIV infected pregnant women:

Characteristic	AOR	95%CI	P-Value
WHO clinical stage			
Stage I (reference)	1		
Stage II	0.851	0.171-4.226	0.844
Stage III-IV	2.305	0.699-7.597	0.170
Adherence to co-trimoxazole			
Good (reference)	1		
Average	3.578	0.611-20.955	0.157
Poor	6.806	1.346-34.429	0.02
History of malaria			
Yes	1.298	0.451-3.736	0.629
No (reference)	1		
Anaemia			
Severe	10.767	1.379-84.048	0.022
Mild-to-moderate	11.059	0.859-142.396	0.065
Normal (reference)	1		

8.3 Prevalence of Anaemia and associated factors

Out of 420 HIV infected pregnant women, the prevalence of any anaemia (Hb <11g/dl) was found to be 54 % (227/420); while that of mild-to-moderate anaemia and severe anaemia were 49.3% (207/420) and 5% (21/420) respectively. The overall mean±SD haemoglobin concentration was 10.3±1.5 g/dl.

No any socio-demographic factor was found to have a statistically significant association with anaemia. Tables 8 below describe the distribution of anaemia (Hb<11g/dl) by the socio-demographic characteristics.

Table 8: Distribution of anaemia prevalence by the socio-demographic characteristics (N=420):

Characteristics	n	Prevalence of anaemia n(%)	P-Value
Age group (years)			
<20	16	9(56.2)	
20-34	341	180(52.8)	
≥35	63	38(60.3)	0.536[a]
Marital status			
Single	35	21(60)	
Cohabiting	69	40(58)	
Married	316	166(52.5)	0.544[a]
Level of education			
Primary	276	144(52.2)	
Secondary	87	52(59.8)	
Post-secondary	24	10(41.7)	
No formal education	33	21(63.6)	0.237[a]
Employment			
Employed	123	69(56.1)	
Business/self-employed	188	106(56.4)	
Not employed	109	52(47.7)	0.303[a]

[a] Calculated by Pearson chi-square

The prevalence of anaemia among subjects with malaria infection was 94.7% (18/19) as compared to 52.1% (209/401) among malaria negative subjects. Table 9 below describes the prevalence of anaemia according to the status of malaria infection. Malaria infected subjects were 10.4 times more likely to have anaemia as compared to those who had negative malaria test (AOR=10.36, 95% CI=1.33-80.8, P = 0.026) (Table 11). The median haemoglobin concentration among subjects with malaria infection was 9.1g/dl and the range was between 6.8g/dl to 11.6g/dl (figure 3). The subjects who had at least one episode of malaria illness during the current pregnancy were 1.8 times more likely to have anaemia as compared with those without a history (AOR=1.75 95%CI=1.01-3.03, P=0.048).

Table 9: Prevalence of anaemia according to the status of malaria infection

Characteristic	n	Prevalence	P-Value
Malaria infection			
Yes	19	18(94.7)	
No	401	209(52.1)	0.0001[a]
History of episodes of malaria illness			
Yes	107	76(71)	
No	313	151(48.2)	0.0001[a]

[a] Calculated by Pearson chi-square

Fig 3: Haemoglobin counts distribution according to the status of malaria infection:

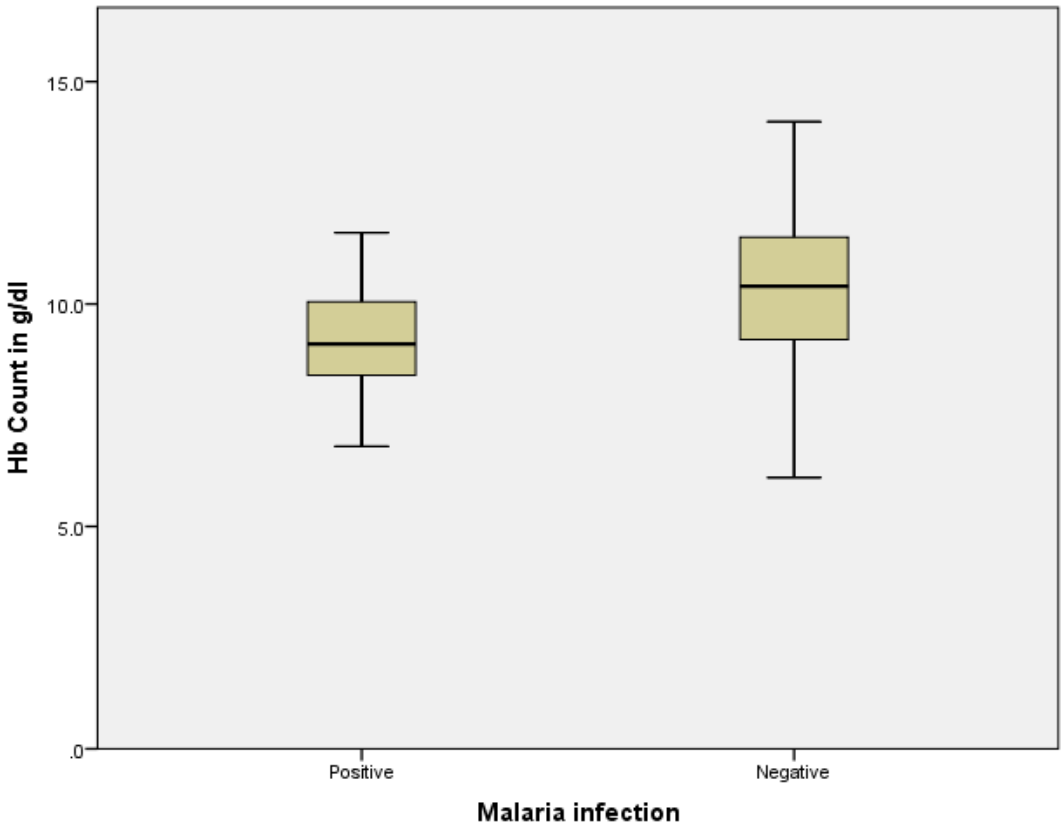


Table 10 below show the prevalence of anaemia according to various factors (clinical, obstetrical, ITN use, ART, Iron supplements, de-worming drug use and adherence to co-trimoxazole prophylaxis) among HIV infected pregnant women. The prevalence of anaemia increased as the HIV/AIDS advanced from lower to higher WHO clinical stages (i.e. from stage I to IV). Pregnant women who were on WHO clinical stage III or IV were 2.7 times more likely to have anaemia as compared to those on WHO clinical stage I (AOR= 2.65, 95%CI=1.18-5.95 and P=0.018) (Table 11).

The prevalence of anaemia increased as the levels of adherence to co-trimoxazole prophylaxis decreased from good to poor. Subjects who had poor adherence were 1.8 times more likely to have anaemia as compared to those with good adherence (AOR=1.75, 95%CI=1.03-2.98 and P=0.041).

A pattern was seen towards the increase in the prevalence of anaemia as the CD4 Count decreased among the subjects (P = 0.006); however the CD4 Count was obtained from only 81.9% (344/420) of the total subjects.

Table 10: Prevalence of anaemia according to various factors among HIV infected pregnant women (N=420):

Characteristics	n	Prevalence of anaemia n(%)	P-Value
WHO Clinical Stage			
Stage I	338	165(48.8)	
Stage II	43	32(74.4)	
Stage III-IV	37	30(76.9)	0.0001[a]
CD4 Count (cells/µL)*			
≥500	104	48(46.2)	
350-499	85	45(52.9)	
200-349	96	62(64.6)	
<200	59	42(71.2)	0.005[a]
Pregnancy trimester			
1st trimester	20	9(45)	
2nd trimester	206	107(51.9)	
3rd trimester	194	111(57.2)	0.404[a]
Gravidity			
Primigravidae	139	72(51.8)	
Secundigravidae	148	83(56.1)	
Multigravidae	133	72(54.1)	0.767[a]
ITN use			
Yes	380	201(52.9)	
No	40	26(65)	0.144[a]
ART category			
ARV Prophylaxis	288	146(50.7)	
Life-long ART	132	81(61.4)	0.142[a]
Duration of AZT use (months)			
<3	162	93(57.4)	
3-5.9	168	92(54.8)	
≥6	90	42(46.7)	0.253[a]
Iron supplements			
Yes	338	185(54.7)	
No	82	42(51.2)	0.567[a]
Use of de-worming drug			
Yes	327	171(52.3)	
No	93	56(60.2)	0.176[a]
Adherence to co-trimoxazole			
Good	208	92(44.2)	
Average	80	47(58.8)	
Poor	132	88(66.7)	0.0001[a]

[a] Calculated by Pearson Chi Square, [b] Calculated by Fischer's Exact Test, *Results of CD4 Count were available for only 344 subjects out of 420 (81.9%)

Table 11: Predictors of anaemia among HIV infected pregnant women:

Characteristic	AOR	95%CI	P-Value
WHO Clinical Stage			
Stage I (reference)	1		
Stage II	3.076	1.458-6.491	0.003
Stage III-IV	2.653	1.184-5.945	0.018
Adherence to CTX			
Good (reference)	1		
Average	1.478	0.848-2.578	0.168
Poor	1.752	1.03-2.979	0.039
History of episodes of malaria illness			
Yes	1.746	1.005-3.033	0.048
No (reference)	1		
Malaria infection			
Yes	10.363	1.329-80.798	0.026
No (reference)	1		

8.3 Adherence to co-trimoxazole prophylaxis

The levels of adherence to co-trimoxazole prophylaxis among the 420 HIV infected pregnant women were 49.5% (208/420) and 50.5% (212/420) for good and average-to-poor respectively. Table 12 below shows the levels of adherence to co-trimoxazole prophylaxis according to the socio-demographic characteristics. No any socio-demographic factor was found to have a statistically significant association with the levels of adherence.

Table 12: Levels of adherence to co-trimoxazole prophylaxis by socio-demographic characteristics (N=420)

Characteristics	n	Average-to-poor adherence n(%)	P-Value
Age group (years)			
<20	16	6(37.5)	
20-34	341	168(49.3)	
≥35	63	38(60.3)	0.156[a]
Marital status			
Single	35	18(51.4)	
Cohabiting	69	40(58)	
Married	316	154(48.7)	0.378[a]
Level of education			
No formal education	33	17(51.5)	
Primary	276	135(48.9)	
Secondary	87	47(54)	
Post-secondary	24	13(54.2)	0.837[a]
Employment			
Employed	123	67(54.5)	
Business/self-employed	188	91(48.4)	
Not employed	109	54(49.5)	0.564[a]

[a] Calculated by Pearson Chi Square

Table 13 describes levels of adherence to co-trimoxazole prophylaxis according to different characteristics of the subjects. Only WHO clinical stages had statistically significant association with the level of adherence to co-trimoxazole prophylaxis. Subjects who were on WHO clinical stages III or IV were 2.3 times more likely to have average or poor adherence as compared to those in the WHO clinical stage I (OR=2.31, 95%C.I=1.12-4.77 and P= 0.023).

Table 13: Levels of adherence to co-trimoxazole prophylaxis according to various characteristics of the subjects (N=420):

Characteristics	n	Average-to-poor adherence n (%)	P-Value
WHO Clinical Stage			
Stage I	338	164(48.5)	
Stage II	43	21(48.8)	
Stage III-IV	39	27(69.2)	0.049[a]
CD4 Count (cells/µL)*			
<200	59	35(59.3)	
200-499	181	87(48.1)	
≥500	104	61(58.7)	0.132[a]
Pregnancy trimester			
1st trimester	20	14(70)	
2nd trimester	206	105(51)	
3rd trimester	194	93(47.9)	0.168[a]
Gravidity			
Primigravidae	139	69(49.6)	
Secundigravide	148	75(50.7)	
Multigravidae	133	68(50.2)	0.969[a]
ITN use			
Yes	380	193(50.8)	
No	40	19(47.5)	0.692[a]
Co-current Use of ≥4 drugs			
Yes	132	66(50)	
No	288	146(50.7)	0.895[a]

[a] Calculated by Pearson Chi Square, [b] Calculated by Fischer's Exact Test, *Results of CD4 Count were available for only 344 subjects out of 420 (81.9%)

9 DISCUSSION

The study shows that the prevalence of malaria infection is 4.5% (19/420) among HIV infected pregnant women who were using co-trimoxazole prophylaxis. Similar results were obtained in a previous study conducted in Malawi by Kapito-Tembo et al (38). In that study, the prevalence of malaria infection among HIV infected pregnant women who were using co-trimoxazole prophylaxis was 2.7% and 5.5% by blood smear and real time PCR method respectively. Malawian study differs from the present study in the methods of malaria diagnosis. Real-time PCR targets parasite DNA and has a higher sensitivity than the MRDT which targets antigens released to the blood by the malaria parasites (33,34,37). Microscopy method in practice varies a great deal in different settings, owing to variable techniques of blood film preparation, staining, film reading standards and most important is the level of expertise of the examining microscopist (33). Therefore, like the findings from the two studies tell; real-time-PCR is expected to yield higher prevalence, followed by MRDT and microscopy.

Another study that was done among HIV infected pregnant women who were using co-trimoxazole prophylaxis in high malaria transmission area in Uganda by Newman et al (39) showed the prevalence of placental malaria of 6% and 19% by using blood smear and PCR method respectively. The difference between the Ugandan prevalence and the present study could be explained by several reasons. Firstly, in the Ugandan study, the blood sample was collected from the placenta while in our study it was from a finger prick. In malaria endemic areas, placental blood is expected to harbour more parasites than the peripheral blood due to the tendency of *P.falciparum* to sequester in the placenta (14,33,34). As a result, the use of placental blood would yield higher prevalence than the one done on the peripheral blood. The second reason could be the difference in the methods of malaria diagnosis, like we have already seen earlier in the Malawian study (38), the PCR method is expected to yield a higher prevalence as compared to MRDT. However the large difference seen between the two studies could be contributed by other factors including the difference in the levels of adherence to co-

trimoxazole prophylaxis, use of ITN and the difference in transmission rates between the two areas (39).

The prevalence of malaria infection increased as the levels of adherence to co-trimoxazole decreased from good to poor. The subjects with poor adherence were almost seven times more likely to have malaria infection as compared to those with good adherence. We could not find any previous study that investigated the association between the malaria infection and levels of adherence to co-trimoxazole prophylaxis among similar subjects. An explanation for the pattern showed in our study could be the fact that the good adherents had higher exposure to co-trimoxazole and therefore were more protected as compared with the poor adherents. Had it not been the effect of poor adherence to co-trimoxazole prophylaxis among some subjects, the prevalence of malaria could have probably been minimal.

The present study showed a higher prevalence of malaria among subjects with anaemia (Hb <11g/dl) as compared with those without anaemia. Subjects who had anaemia were ten times more likely to have malaria infection as compared to those without anaemia. Due to association between anaemia with advancement of HIV/AIDS (13,43,50,51), it is likely the subjects with anaemia had poor immunity and therefore were more susceptible to malaria infection as compared with non-anaemic one.

In consistent with previous studies (19,38–40), malaria infection was distributed to all the gravidities. HIV infection altered the typical gravidity specific pattern of malaria risk by shifting the burden from primarily primigravidae and secundigravidae to all pregnant women. In the Malawian study (38), the prevalence of malaria infection among HIV infected pregnant women with regard to the gravidity were 5.7%, 8.8% and 4.7% for primigravidae, secundigravidae and multigravidae respectively ($P>0.05$). Therefore all the gravidities had similar risk of malaria infection. In the Ugandan study (39), the reported prevalence of placental malaria by smear method were 7.4 %, 13.6% and 4% for primigravidae, secundigravidae and multigravidae respectively ($P >0.1$).

Another study in Kenya (41) showed similar pattern of shifting the Malaria burden among all the gravities. The prevalence of malaria infection in Kisumu, Kenya was 37.3%, 25.9% and 23.1% among primigravidae, secundigravidae and multigravidae respectively (P>0.05). The explanation for this outcome is that HIV affects the immune memory mechanism which is responsible for the parity-dependent acquisition of antimalarial immunity in pregnancy and therefore predisposes the secundigravidae and multigravidae to similar risk of malaria infection as the primigravidae (19). Alternatively, ter Kuile et al (19) gave an explanation that multigravidae probably have longer sexual experience and they could be more immunosuppressed because there is a possibility that they had been infected with HIV longer than younger primigravidae women.

Surprisingly, our study could not find association between the prevalence of malaria infection and low CD4 count or advanced WHO clinical stages; which is known to increase the vulnerability to malaria infection (19). This could be explained by the impact of malaria control interventions particularly the use of co-trimoxazole prophylaxis and sleeping under ITN among HIV infected pregnant women. Kapito-Tembo et al (38) reported a similar findings in Malawi, whereby HIV infected pregnant women at all levels of CD4 counts (i.e. <200, 200-499 and ≥500 cells/μL) had similar risk to malaria infection. In the same Malawian study (38), subjects who were in WHO clinical stage I or II were 4 times more likely to have malaria infection as compared to the one in clinical stage III or IV.

The present study showed that the prevalence of anaemia among HIV infected pregnant women who are using co-trimoxazole prophylaxis was 54 %. Previous studies that were conducted in Dar Es Salaam city reported higher prevalence compared to the present study. Finkelstein et al (43) reported a prevalence of 83% in 1997, while Mehta et al (52) reported a prevalence of 73% in 2003. The decrease in the anaemia prevalence over time could be explained by the improvement of antenatal care in Tanzania among HIV infected pregnant women e.g. changes in malaria prevention strategies from chloroquine and SP-IPT to daily co-trimoxazole prophylaxis, introduction of free ITNs, availability of ARV, use of deworming agents and ferrous sulphate supplementation. Other studies that were conducted in different

countries within Sub-Saharan Africa have reported varied values of prevalence of anaemia (13,19,38,44). In Malawi; Kapito-Tembo et al (38) reported a prevalence of 35.6%; while Nkhoma et al (44) reported a prevalence of 27.4%. In Nigeria, a prevalence of 83.8% was reported by Uneke et al (13). The reasons for this variation are not clear, but it may be connected to the complexity and multifactorial etiology of anemia in pregnancy in sub-Saharan Africa including HIV/AIDS, malaria, protein and micronutrients deficiency and endemic diseases e.g. hookworm and schistosomiasis (13,43).

Good adherence to co-trimoxazole was associated with reduced prevalence of anaemia. A pattern was seen towards the increase in the prevalence of maternal anaemia as the levels of adherence to co-trimoxazole prophylaxis decreased from good to poor. Poor Adherents were almost 1.8 times more likely to have anaemia as compared with good adherents. This could be due to the fact that, good adherent subjects had more exposure to the co-trimoxazole drug and therefore they were more protected from malaria infection and its sequelae particularly anaemia as compared to the poor adherent one. Walker et al (53) have previously reported the benefits of co-trimoxazole in reducing morbidities among HIV infected population; this could similarly explain the reduction in anaemia among good adherents.

CD-4 Count and WHO clinical staging are parameters which are used to monitor progression of HIV/AIDS (15,25). In consistent with a previous study by Finkelstein et al (43), advanced HIV/AIDS clinical stages (clinical stage III or IV) or CD4 Count of < 200 had a strong association with anaemia. The subjects who were on WHO clinical stage III or IV were 3 times more likely to have anaemia as compared with those in the WHO clinical stage I. Similarly, the prevalence of anaemia increased as the CD4 Count decreases among the subjects. This pattern could be explained by the fact that subjects with advanced clinical stages or low CD4 count had poor immunity against malaria and other infections; therefore they were more vulnerable to anaemia. Furthermore, advanced HIV/AIDS is associated with local diseases along the gastrointestinal tract which eventually results to poor absorption of nutrients necessary for the formation of haemoglobin. Likewise the bioavailability of co-trimoxazole which is necessary for malaria prevention could as well be affected.

Low CD-4 count and advanced HIV/AIDS stages reflect the chronicity (prolonged existence) of the disease; therefore anaemia could also be due to chronic diseases (50,51). Anemia of Chronic Disease (ACD) develops in subjects with a prolonged illness. Infections with pathogens normally activate macrophages triggering a strong cytokine production among which are tumor necrosis factor (TNF), γ-interferon (IFN-γ) and nitric oxide (NO). The immune response mounted against such infections is required for parasite clearance but its persistence can cause collateral damage to the host with occurrence of anemia as the major pathology (50). The inflammation triggers the release of chemicals e.g. hepcidin, that signal the iron regulation mechanism to adopt a defense mode. This type of anemia is usually characterized by an imbalance between erythro-phagocytosis and erythropoiesis (50,51).

Similar to previous studies (13,19,44), dually infected (HIV plus malaria infection) pregnant women were at considerably greater risk of anaemia as compared with those with HIV infection alone. HIV infected pregnant women who had positive malaria test were ten times more likely to have anaemia as compared with those who had negative malaria test. Both HIV (particularly with advanced immunosuppression) and malaria infection are individually known causes of anaemia (19); thus, dually infected subjects are expected to be affected more by anaemia as the result of the synergistic interaction between malaria and HIV/AIDS. Anaemia associated with malaria is caused by hemolysis of the red blood cells and hypersplenism, a condition characterized by the exaggeration of inhibitory or destructive function of the spleen (48).

The present study also showed the prevalence of anaemia was higher among the subjects who had at least one episode of malaria illness during the current pregnancy as compared with those who had no history of malaria. Subjects who had at least one episode of malaria illness during the current pregnancy were nearly two times more likely to have anaemia as compared with those without a history. In consistent with this study, Nkhoma et al (44) reported a significant association between number of previous malaria episodes and maternal anaemia; having two or more episodes was associated with increased risk to anaemia. The reason that

Both HIV (particularly with advanced immunosuppression) and malaria infection are individually known causes of anaemia (19) could explain this outcome.

In disagreement with previous knowledge (16,54,55); our study could not find significant association between anaemia and zidovudine use. This could be due to the positive role of the antiretroviral drugs and co-trimoxazole in controlling the HIV infection and other morbidities and subsequently outweighed the anaemia inducing effect of zidovudine. Furthermore, the multi-etiologies of anaemia in HIV infected pregnant women in Sub-Saharan Africa (43) e.g. HIV infection itself, malaria infection, nutrients deficiency and worm infestations could be the reason for the high prevalence in both groups and consequently lack of significant difference. Sinha et al (56) reported similar findings in a study conducted in India. In that study (56), pregnant women who used zidovudine were surprisingly 70% less likely to be anaemic compared with women not receiving zidovudine.

Similarly to a study by Finkelstein et al (43), anaemia prevalence was high in both the subjects who used iron supplements and those who did not; likewise to the use of de-worming agents. Lack of significant difference between the groups could be explained by the complexity and multifactorial etiology of anemia apart from the iron deficiency or worm infestation among HIV infected pregnant women in Sub-Saharan Africa (43,50,51).

Adherence levels to co-trimoxazole prophylaxis among the 420 HIV infected pregnant women were 49.5% and 50.5% for good and average-to-poor respectively. We could not find any previous study that assessed the levels of adherence to co-trimoxazole among HIV infected pregnant women. In the present study; there was a pattern towards the decrease in the levels of adherence as the HIV/AIDS advances from lower to higher WHO clinical stages (i.e. from clinical stage I to IV). This could be explained by the fact that as the disease progressed to higher clinical stages patients tends to experience more discomfort and more gastrointestinal side effects because of the local opportunistic infections in the gut as a result patients would end up skipping doses. Furthermore, as the disease progress to severe condition, patients tends to loose trust to the prescribed medication, and may decide not to take them or to look for alternatives (28).

10. CONCLUSION AND RECOMMENDATIONS

10.1 CONCLUSION

There is a low prevalence of malaria infection among HIV infected pregnant women using co-trimoxazole prophylaxis in Kinondoni Municipality. More than half of the subjects had average-to-poor adherence level to co-trimoxazole prophylaxis.

Poor adherence to co-trimoxazole prophylaxis and anaemia were significantly associated with malaria infection; additionally all gravidities had similar risk to malaria infection.

A significant proportion of HIV-infected pregnant women had anaemia. Malaria, advanced HIV/AIDS and poor adherence to co-trimoxazole prophylaxis were significantly associated with anaemia among HIV infected pregnant women.

10.2 RECOMMENDATIONS

Measures should be taken to maintain the low prevalence of malaria among HIV infected pregnant women shown by the current study. One of these measures should focus on improving and monitoring the adherence to co-trimoxazole prophylaxis. Lucky enough, adherence to co-trimoxazole is also significantly associated with anaemia; thus improving it would be killing two birds by one stone.

Involvement of both the policy makers and direct health care providers is needed in order to address the issue of adherence to co-trimoxazole prophylaxis. Policy makers should prepare standardized tools that would be used in assessing and monitoring adherence. Direct health care providers should be involved in daily activities that would improve the adherence to co-trimoxazole prophylaxis e.g. counseling and provision of health education on importance of good adherence to medications and the consequences of poor adherence.

Steps should be taken to mitigate advanced HIV/AIDS among pregnant women; which is significantly associated with anaemia. These include early diagnosis and timely initiation of anti-retroviral treatment.

Furthermore, other measures should be taken aboard to address anaemia among HIV infected pregnant women taking into consideration its complexity and multiple etiologies. These should include de-worming programs, schistosomiasis control and nutritional supplementation.

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APPENDICES

Appendix 1: Interview Schedule in English

Part I: Sociodemographic and Background Information

1. Identification number: _____
2. Age (years): _____
3. Body weight (Kg) _____
4. Height (m) _____
5. BMI(kg/m2)_____
6. Marital Status:
   - Single
   - Cohabiting
   - Married
   - Divorced
   - Widowed
7. Education Level:
   - Primary
   - Secondary
   - Post-secondary
   - No formal education
8. Type of employment:
   - Employed
   - Business/self-employed
   - Not employed
9. WHO Clinical Stage
   - Clinical stage I
   - Clinical stage II
   - Clinical stage III

- Clinical stage IV

10. ART Category
    - HAART
    - PMTCT Prophylaxis
11. Is Zidovudine among ARV drugs the patient is using?
    - Yes
    - No

[If the response in question number 11 is "Yes" then go to question number 12; but if the response is "No" then go to question number 13]

12. What is the duration of Zidovudine (AZT) use (months)? _____
13. What is your gravidity?
    - Primigravidae
    - Secundigravidae
    - Multigravidae
14. What is the age (months) of this pregnancy _____
15. Some pregnant women do not prefer to sleep under Mosquito treated nets (ITNs), Do you sleep under the ITN?
    - Yes
    - No
16. Have you ever suffered from Malaria illness and use anti-malaria drug(s) during this pregnancy?
    - Yes
    - No
17. Some pregnant women do not prefer to use ferrous tablets. Have you used ferrous tablets during this pregnancy as directed at the Antenatal Clinic?
    - Yes
    - No

18. Have you used any drug(s) for combating worms during the past three months?

- Yes
- No

Part II: Use of Co-trimoxazole Prophylaxis

19. Individuals have identified several issues regarding their medication-taking behavior and we are interested in your experiences. There is no right or wrong answer. Please answer each question based on your personal experience with the use of co-trimoxazole tablets

No.	Questions	YES	NO
a	Do you sometimes forget to take your co-trimoxazole tablets?		
b	People sometimes miss taking their medicines for reasons other than forgetting. Thinking over the past 2 weeks, were there any days when you did not take your co-trimoxazole tablets?		
c	Have you ever cut back or stopped taking co-trimoxazole without telling your doctor because you felt worse when you took it?		
d	When you travel or leave home, do you sometimes forget to bring along the co-trimoxazole tablets?		
e	Did you take co-trimoxazole tablets yesterday?		
f	Taking medicines every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?		
g	How often do you have difficulty remembering to take the co-trimoxazole tablets? - Never/Rarely - Once in awhile - Sometimes - Usually - All the time		
TOTAL SCORE			

Part III: Investigational Results

20. HB count (g/dL) is _____

21. Result of Malaria Rapid Diagnostic Test is

    - Positive
    - Negative

22. CD4 Cell count (Cells/µL): _____

Appendix 2: Interview Schedule in Kiswahili

Sehemu I: Maelezo ya Mama Mjamzito

1. Namba ya utambuzi: _____
2. Umri (years): _____
3. Uzito wa Mwili (Kg) _____
4. Kimo (m) _____
5. BMI(Kg/m^2)_____
6. Hali ya ndoa:
    - Hajaolewa
    - Unaishi na mpenzi
    - Umeolewa
    - Umetaliki
    - Mjane
7. Kiwango cha elimu:
    - Msingi
    - Sekondari
    - Zaidi ya sekondari
    - Hujasoma
8. Ni kazi gani unaifanya?
    - Umeajiriwa
    - Biashara/umejiajiri

- Hauna kazi

9. WHO Clinical Stage
   - Clinical stage I
   - Clinical stage II
   - Clinical stage III
   - Clinical stage IV
10. Je, upo kwenye kundi lipi la matumuzi ya dawa za kupunguza makali ya VVU?
    - Matibabu yasiyo na kikomo kwa mama (HAART)
    - Matibabu ya muda mfupi ya kumkinga mtoto na VVU
11. Je, unatumia dawa ya kupunguza makali ya VVU aina ya Zidovudine?
    - Ndiyo
    - Hapana

    [Kama amejibu swali namba 11 "ndiyo" nenda swali namba 12; na kama amejibu "hapana" rukia swali namba 13]
12. Je, ni kwa muda gani (miezi) ametumia dawa ya zidovudine (AZT) _____
13. Je, ujauzito huu ni wa ngapi?
    - Wa kwanza
    - Wa pili
    - Wa tatu au zaidi
14. Je, ujauzito huu una miezi mingapi? _____
15. Wamama wajawazito wengine huwa hawapendelei kulala china ya chandarua kilichotibiwa na dawa (ITN), je wewe huwa unatumia chandarua kilichotibiwa?
    - Ndiyo
    - Hapana
16. Je ulishawahi kuugua malaria na kutumia dawa ya malaria katika kipindi cha ujauzito huu?
    - Ndiyo
    - Hapana
17. Wamama wajawazito wengine huwa hawapendi kutumia dawa ya vidonge za kuongeza dawa. Je wewe katika ujauzito huu umeshatumia dawa za vidonge za kuongeza damu kama ulivyopewa kliniki?

- Ndiyo
- Hapana

18. Je umetumia dawa za kuondoa minyoo katika kipindi cha miezi mitatu iliyopita?
    - Ndiyo
    - Hapana

Sehemu II: Matumizi ya Dawa ya Vidoge ya co-trimoxazole (septrin):

19. Wagonjwa wengi huwa wanapata changamoto mbalimbali katika kuzingatia matumizi ya dawa ya co-trimoxazole. Sisi tunataka kujua kutoka kwako kwa kujibu maswali yafuatayo hapo chini. Kumbuka kuwa huu sio Mtihani

No.	SWALI	NDIYO	HAPANA
a	Je, kuna wakati huwa unasahau kumeza dawa ya co-trimoxazole?		
b	Ukiacha sababu ya usahaulifu, kuna watu bado huacha kutumia dawa. Ukifikiria katika kipindi cha wiki mbili zilizopita; je, kuna siku zilipita bila kumeza dawa ya co-trimoxazole?		
c	Je, kuna wakati baada ya kujisikia vibaya kufuatia kutumia dawa uliamua kupunguza dozi au kuacha kutumia bila ya kumtaarifu Daktari wako?		
d	Ukiwa na safari inayokupasa kulala ugenini; je, kuna wakati unasahau kubeba dawa zako za co-trimoxazole?		
e	Je, jana ulimeza dawa zako za co-trimoxazole?		
f	Kwa baadhi ya watu, kumeza dawa kila siku inawapa usumbufu. Je, wewe huwa unahisi usumbufu kumeza dawa ya co-trimoxazole kila siku?		
g	Je, ni mara ngapi umekuwa ukisahau kumeza dawa ya co-trimoxazole? - Sijawahi/ni nadra sana kusahau - Kuna wakati huwa nasahau - Imekuwa mazoea kusahau - Siku zote huwa nasahau		
JUMLA YA ALAMA			

Sehemu III: Matokeo ya vipimo vya uchunguzi

20. HB count (g/dL) ni _____

21. Majibu ya kipimo cha MRDT ni

- Positive = 1
- Negative = 2

22. CD4 Cell count (Cells/µL) ni _____

Appendix 3: Consent Form in English

Title Of The Study: Malaria infection among HIV-infected pregnant women using co-trimoxazole prophylaxis in Kinondoni Municipal.

My name is **Vicent P. Manyanga**, a second year Clinical Pharmacy Student from School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS). I am conducting a study with the above title as part of my study program of Masters of Pharmacy in Hospital and Clinical Pharmacy.

Aims of the study: This study aims to determine the prevalence of malaria infection and anaemia among HIV infected pregnant women. In addition, the study will determine the levels of adherence to co-trimoxazole prophylaxis.

Participation in this study: You can participate in this study if you are Pregnant and HIV infected and attending Antenatal Clinic. The study mainly involves responding to a questionnaire which has general questions about socio-demographic characteristics and simple investigations for anaemia and malaria infection. You will be told by the investigator about other conditions that will limit you from participating in the study. The results are going to be used in the study and for your normal routine care.

If you choose not to participate in this study, you will continue to receive the normal care at the antenatal clinic and you will not be compromised in any way.

Risks: We do not anticipate any risks involved in participating in the study.

Benefits: By participating in this study; you will know whether you are infected with malaria and your haemoglobin level; therefore you will benefit from appropriate treatment and advice according to the findings.

Confidentiality: All information collected during this study will be kept strictly confidential and will not be revealed to anybody outside the research team.

Cost: You will not be required to make any payments to participate in this study and no payment will be made to you.

For further information, questions or queries, you can contact the following:

1. Vicent P. Manyanga (Investigator),
   School of Pharmacy,MUHAS
   P. O. Box 65001,
   Dar es Salaam.
   Cell no: +255 754 47894
   Email: vcenetz@yahoo.com
2. Dr. Omary Minzi (Research Supervisor)
   Senior Lecturer
   School of Pharmacy, MUHAS.
3. Director of Research and Publications,
   MUHAS.
   P.O.Box 65001
   Dar Es Salaam.

I, _____, have read/been told of the contents of this Form and have understood its meaning. I agree with my knowledge to participate in this study.

Signature of the pregnant Mother _____

Signature of Researcher _____

Date _____

Appendix 4: Consent Form in Kiswahili

Fomu ya Ridhaa ya Kushiriki Katika Utafiti

Mada ya Utafiti: Maambukizi ya ugonjwa wa malaria kwa wamama wajawazito wenye maambukizi ya Virusi Vya Ukimwi wanaotumia dawa ya co-trimoxazole katika manispaa ya Kinondoni.

Jina langu ni **Vicent P. Manyanga**, mwanafunzi wa Shahada ya Uzamili ya Ufamasia wa Magonjwa kutoka Shule ya Ufamasia ya Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili (MUHAS). Ninafanya utafiti kuangalia Maambukizi ya ugonjwa wa malaria kwa wamama wajawazito na upungufu wa damu kwa wamama wajawazito wenye maambukizi ya Virusi Vya Ukimwi katika Manispaa ya Kinondoni.

Malengo ya utafiti: Utafiti huu unalenga kujua kiasi cha maambukizi ya malaria na upungufu wa damu kwa wamama wajawazito ambao wameathirika na virusi vya ukimwi. Pia utafiti huu utaangalia ni kwa kiasi gani wamama wajawazito wanazingatia matumizi ya dawa ya co-trimoxazole.

Ushiriki katika utafiti: Unaweza kushiriki katika utafiti huu kama wewe ni mama mjamzito na umeathirika na Virusi Vya Ukimwi, pia uwe unafanya mahudhuria ya kliniki. Utatakiwa kujibu maswali kutoka kwenye fomu maalumu. Vilevile utafanyiwa vipimo kuangalia maambukizo ya malaria na kujua kiwango cha damu ulichonacho. Majibu yatatumika kwenye tafiti huu na pia kukuhudumia wewe. Kama hautapenda kushiriki, utaendelea kupata huduma kama kawaida bila tofauti yeyote.

Hatari: Hatutarajii kwamba kutakuwa na hatari kwa kushiriki katika utafiti huu.

Faida za utafiti: Kupitia utafiti huu utaweza kujua kama una maambukizi ya malaria, pia utaweza kujua kiasi cha damu ulichonacho. Hivyo basi utafaidika kwa kujua afya yako na kupata matibabu au ushauri kulingana na majibu ya vipimo.

Usiri: Taarifa zote zitakazokusanywa katika utafiti huu zitakuwa siri, hivyo ushiriki wako hautajulikana na mtu. Taarifa hizi zitajulikana kwenye timu ya watafiti tu.

Malipo: Kwa kushiriki kwenye utafiti huu, hautalipwa wala hautalipa malipo yeyote. Ukiwa na swali au tatizo lolote, unaweza kuwasiliana na wafuatao:

1. Vicent P. Manyanga (Mtafiti Mkuu),
   School of Pharmacy, MUHAS
   P. O. Box 65001,
   Dar es Salaam.
   Cell no: +255 754 478794
   Email: vcenetz@yahoo.com
2. Dr. Omary Minzi (Msimamizi wa Utafiti)
   Senior Lecturer
   School of Pharmacy, MUHAS.
3. Director of Research and Publications,
   MUHAS.
   P.O.Box 65001
   Dar Es Salaam.

Mimi, _____, nimesoma/nimesomewa maelezo yote yaliyomo kwenye fomu hii na nimeelewa. Nakubali kushiriki katika utafiti huu.

Sahihi ya mama mjamzito _____

Sahihi ya Mtafiti _____

Tarehe _____