The Prevalence of HIV/Malaria Co-Infection during Pregnancy in Adama Hospital and 'Awash Sebat Kilo' Health Center, Ethiopia

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By

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DEDICATION

To My family

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TABLE OF CONTENT

LIST OF TABLES

Page

LIST OF FIGURE

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ART	Antiretroviral Therapy
ADCC	Antibody dependent cell cytotoxicity
AIDS	Acquired Immuno Deficiency Syndrome
APC	Antigen Presenting Cells
CSA	**C**hondroitin Sulfate A
CSF	Cerebro spinal fluid
DC	Dendritic cells
gp	Glycoprotein
gp120	Glycoprotein 120
Hb	hemoglobin
HIV	Human immunodeficiency virus
iRBC	Infected Red Blood Cell
IUGR	Intra Urine Growth Retardation
LBW	Low Birth Weight
MHCII	Major histo-compatibility class two
NK	Natural killer cell
NPV	Negative predictive value
PM	Placental malaria
PPV	Positive Predictive Value
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
RNA	Ribonucleic acid
SD	Standard deviation
TH1	T-helper 1, cells
WHO	World health organization
HPR2	HIstidine rich protein 2

DEFINITIONS

Primigravid women: - Women who got pregnant for the first time

Secundigavid women: - Women who got pregnant for the second times

Multigravid women: - Women who got pregnant for the third and above times

First trimester: - Pregnancy up to the third month

Second trimester: - Pregnancy from the third to six months

Third trimester: - Pregnancy from six to ninth months

Severe anemia: - Hemoglobin level less than 5g/dl for pregnant

Mild anemia: - Hemoglobin level between 5g/dl and 11g/dl for pregnant

Non-anemic: - Hemoglobin level greater than or equal to 11g/dl for pregnant

Severe parasitaemia: - Parasite density greater than 10, 000 parasite/ µl

Abstract

The study was undertaken to determine the prevalence and severity of malaria in HIV positive pregnant and non-pregnant women who receive antiretroviral therapy (ART). The level of malaria prevalence, disease severity (as measured by parasite density and Hb level); and immune status (as measured by $CD4^+$ T cell count) were determined for 500 HIV positive women from Adama hospital and 'Awash Sebat Kilo' health center. 18.4% of the HIV positive women were pregnant and a total of 22.2% were malaria infected. Among the pregnant HIV positive women, 44.6% were malaria infected. Compared to the non-pregnant HIV/malaria co-infected, pregnant HIV/malaria co-infected women, on the average, had a significantly higher ($P<0.001$) parasite densities ($26,595 \pm 15,309$ versus $15,400 \pm 12,278$) and a significantly ($P=0.05$) lower Hb values (7.49 ± 3.34 versus 8.37 ± 3.13). The HIV/malaria co-infected pregnant women also had a lower, but statistically non-significant, mean $CD4^+$ T cell count (195 ± 123 versus 220 ± 140) than the non-pregnant HIV/malaria co-infected women. Compared to pregnant women infected with only HIV, malaria/HIV co-infected pregnant women had significantly lower ($P=0.005$) $CD4^+$ T cell count (195 ± 123 versus 279 ± 151) and significantly lower ($P<0.001$) mean Hb level (7.49 ± 3.34 versus 10.53 ± 2.96). Lower $CD4^+$ T cell count and Hb level and higher parasite density were recorded in primigravid HIV/malaria co-infected pregnant women than in the multigravid ones. Furthermore, a significant improvement in the mean $CD4^+$ T cell count, Hb level and parasite density for HIV/malaria co-infected pregnant women was observed with increasing duration of the use of ART. That is, receiving ART for more than 6 months improved the health condition of HIV/malaria co-infected pregnant women whereby their $CD4^+$ T cell count and Hb levels were increased and malaria parasite densities were decreased ($P=0.001$). The study on the whole has indicated that, in malaria endemic areas, extra care must be taken to protect HIV positive pregnant women from malaria infection.

***Key words**: Malaria, HIV, HIV/malaria co-infection, Pregnancy, Severe malaria, ART*

1. Introduction

1.1. Malaria

1.1.1. Malaria Parasite

The parasites that cause malaria are protozoa that belong to the genus *Plasmodium*, the species of which infect many animal groups including primates, lizards and birds. The four *Plasmodium* species responsible for human malaria are *P. falciparum, P. vivax, P. ovale* and *P. malariae. P. falciparum* is the most virulent parasite and responsible for majority of malaria related death and morbidity. It is found in most malaria endemic regions of the world and is the most common human malaria parasite in Africa. *Plasmodium vivax* has a limited distribution in Africa, but is the most common species outside Africa (Barry, 2005).

Malaria infection is initiated by the transmission of sporozoites to the vertebrate host by the bite of an infected mosquito. The sporozoites enter the blood stream, reaching the liver where they invade and undergo schizogony within hepatocytes. In *P. falciparum* and *P. malariae* infections all schizonts mature and merozoites are released into the blood stream and invade RBC, whereas, in *P. vivax* and *P. ovale* infection some become deeply arrested (hypnozoited). After multiplication, new merozoites will be formed and will re-invade the red blood cells (RBC). During this cycle, sexual forms of the parasite are generated and can consequently be taken up by a mosquito during blood meal. These sexual forms will fuse and undergo sporogonic development in the mosquito mid gut forming new sporozoites that traveled to the salivary glands of the vector Anopheles mosquitoes. The pre erythrocytic stage of infection, which lasts between 5.5 and 14 days depending on the human *Plasmodium* species, is asymptomatic. The blood stage, which is associated with clinical disease, can last up to a year with *P. falciparum* and close to 50 years with *P. malariae* if not treated (Renia and Potter, 2006).

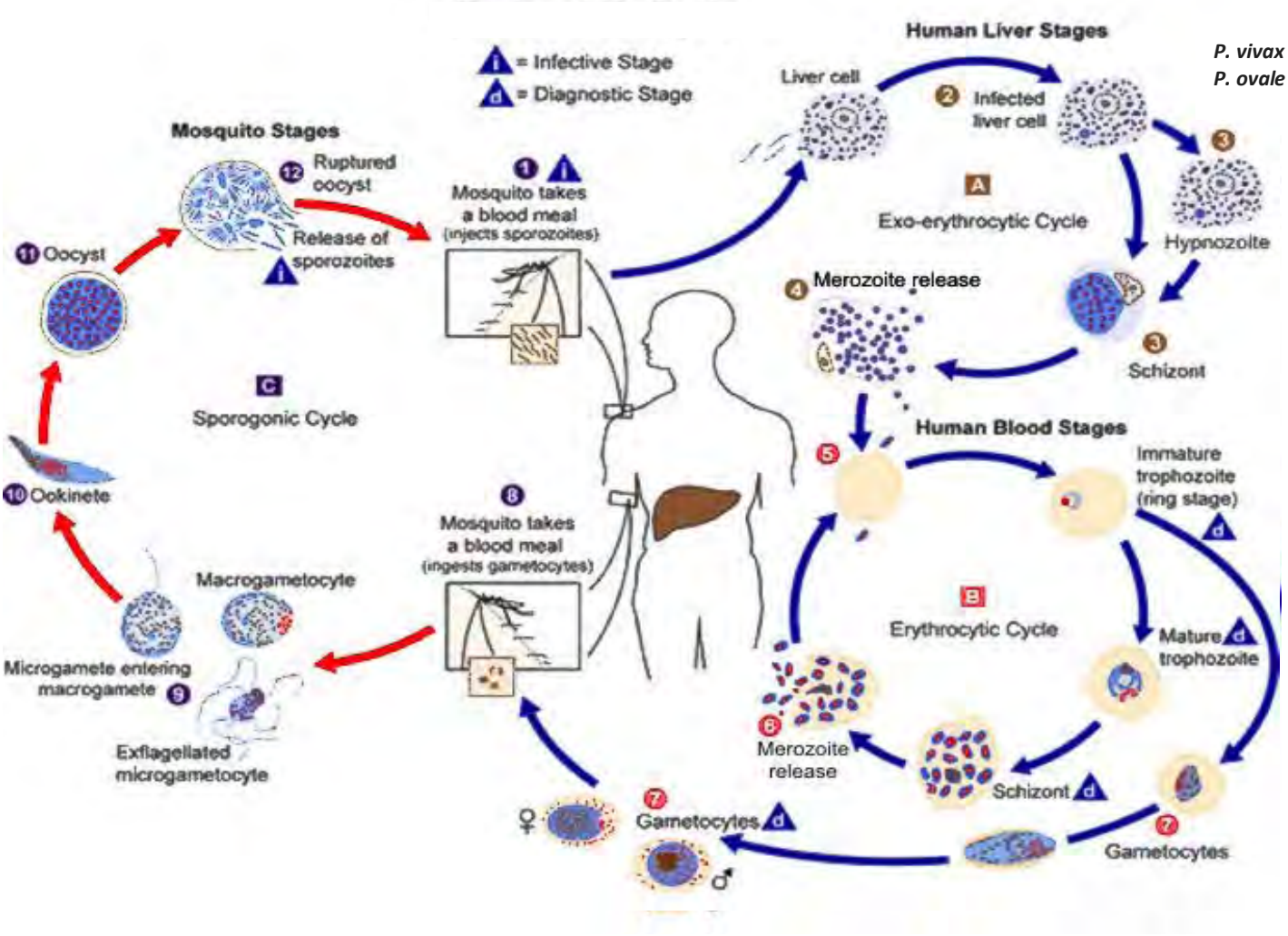

Figure 1 Life cycle of malaria parasite (CDC, 2006)

1.1.2. Malaria Disease

Malaria is an ancient disease, although its exact origins and evolutionary history are unclear. It was described in China 5 thousand years ago. It is thought to have originated in Africa and to have spread subsequently into Asia and Mediterranean. Greek writers recognized the disease and its symptoms, and some reports suggest that malaria was responsible for the decline of city-state populations and depopulation of rural areas. These days, fewer different strains of malaria are found in the Americas than in Africa and Asia (www.cdc.gov/malaria/history).

Patterns of malaria disease vary widely and partly depending on the endemicity of the

parasite/s within the geographical setting. Where individuals have constant and repeated exposure to infection, natural immunity develops slowly. This immunity is first efficient only against clinical malaria disease, but immunity strengthens progressively, leading to reduced blood stage parasite development. However, even long term exposure can never completely counter either re-infection or low (sub-clinical) parasitaemia. This suggests that the pre-erythrocytic stage of the infection does not induce an efficient natural immune response (McGregor, 1987). Moreover, the disease varies with the infecting species of *Plasmodium* and with the individual's prior health. Typically, it causes fever and chills, along with headache, vomiting and diarrhea. It may also cause long-term anemia, liver damage and neurological damage. The most dangerous species, *P. falciparum* can cause cerebral malaria, a frequently fatal condition involving the brain and central nervous system. Those who survive cerebral malaria may experience lasting brain damage (Tagelsir *et al.,* 2008).

A malarial attack is characterized by recurrent peaks of fever during the acute phase and can be associated with adverse range of syndromes, including cerebral malaria, severe malarial anemia, metabolic acidosis, shocklike syndrome and placental malaria. It has been proposed that these syndromes result from differential parasite specificity (for example, organ specific sequestration of parasite infected RBCs (iRBC), parasite toxin and the host immune response (including cytokine and chemokine production), and recruitment and sequestration of inflammatory cells to the target organs (Renia and Potter, 2006).

The role of $CD4^+$ T cells against blood stage parasites has been principally defined in rodent models, and it appears that $CD4^+$ T-cells have a dual function in anti-malarial immunity. TH1 cells are required for the control of the acute phase through the production of pro-inflammatory cytokines such as IFN γ and IL-2 while TH2 cells are required for the clearance of the parasite by their interaction with B-cells using IL-4 (Renia and Potter, 2006).

1.1.3. Malaria Epidemiology

Malaria is a life threatening parasitic disease transmitted by a female Anopheles mosquito. It is the most highly prevalent tropical disease, with high morbidity and mortality and high economic and social impact (Adefioye *et al.*, 2007). It continues to be one of the most devastating infectious diseases of our time, next to HIV and TB as a killer disease in tropical and sub-tropical regions (WHO, 2005).

The epidemiology of malaria is governed by characteristics of transmission, which can be described in terms of intensity, stability and seasonal variation. In areas of stable transmission, the pattern of transmission remains roughly unchanged from year to year, whereas areas with unstable malaria are characterized by considerable variation in the intensity of transmission between years (Theander, 1998). All age groups are vulnerable to malaria in areas of unstable malaria transmission and it is generally believed that under such conditions most infections tend to be followed by clinical diseases (Tagelsir *et al*., 2008).

Around 3.2 billion people are at risk of malaria each year, with around 500 million people proceeding to clinical disease and 1-3 million death occurring (Snow e*t al.*, 2005). The burden of this morbidity and mortality is biased towards young children, not yet immune to clinical symptom and pregnant women where parasites are sequestered in the placenta (Barry, 2005 and Rathnam, 2007).

Approximately 90% of all malaria illness and death in the world today occurs in Sub-Saharan Africa, where the most virulent species of malaria parasite, *P. falciparum* thrives (WHO, 2004). In Africa, as in other part of the world, according to Enato and Okhamafe (2005), the malaria situation is not homogenous. In spite of its wide distribution in the continent, it is actually a focal disease. It varies from country to country and in the same country, it may vary from one part to another. Therefore the distribution of malaria in Africa is classified broadly into four epidemiological areas, the hypo-endemic, meso-endemic, holo-endemic and hyper endemic. The epidemiological profile is usually

determined in any given location by several factors including the ecosystem, climate, state of the environment, human behavior and vector and parasite bionomics.

Regarding the epidemiological profile of malaria in Ethiopia, it constitutes a major public health problem and impediment to socio-economic development. It is estimated that about 75% of the total area of the country and 65% of the population is estimated to be at risk of infection (WHO, 2005). An important feature in the epidemiology of malaria in Ethiopia is the variety of transmission season that precludes the development of immunity that favors periodic epidemics attended by high mortality. Seasonal transmission of malaria occurs following the main rainy season from June to August and light rains in March and April. Thus, peak transmission of malaria occurs during the months of September to December. In Ethiopia, *P. falciparum* and *P. vivax* account for about 60% and 40% of infections, respectively, during the peak transmission periods (WHO, 2000a).

1.2 Human Immunodeficiency Virus

1.2.1. HIV Biology

Human immunodeficiency virus (HIV1 and 2), are retroviruses with two strands of genetic material and a few protein molecules with an outer wrapping to hold it together. Each virus particle or virion is shaped as tiny sphere and measures only $1/10{,}000^{th}$ of a millimeter in diameter. The viral core contains the HIV genetic material ribonucleic acid (RNA) and several enzymes, which help the virus start copying itself inside the human cell it has infected. The core and matrix of viral proteins are surrounded by a lipid envelop which is derived from the host cell; with molecules or the HIV envelop proteins sticking out from the surface. The envelop protein is made of two parts, gp120 and gp41 (Fig. 2) (Knipe, *et al.*, 2001).

HIV uses the envelop protein gp120 to bind to cellular receptors ($CD4^+$ T cell and a co-receptor) and fuse with target cells, particularly T-cell and Macrophages. Once inside the host cell, the viral genome and reverse transcriptase are unpacked and the RNA is reverse transcribed in to DNA. Reverse transcriptase is relatively error prone, so viral variants are

continually produced. This variability underlies many of the features of HIV infection. The transcribed DNA is then transported to the nucleus and randomly integrated into the host's genome, and new viral copies are made by hijacking the host's cellular machinery. This process requires the cell to be activated (e.g., through the T-cell receptor or via the cytokine TNFα), so we can immediately see the activation of the immune response stimulates virus production. This leaves the host in an impossible situation in attempting to eliminate the virus or other opportunistic infection it can actually trigger further virus production (Cowley, 2001).

HIV has a very high mutation rate, giving rise to hugely variable viral populations, both within an individual and between individuals. Variation is most prevalent and most important in a major immunologic envelope protein gp120, where there are many hyper-variable regions with in an individual. The virus can change over time and in different location within the body, with in an individual strains can vary by around 10% of their total sequence and by as much as 40% of amino acid sequence in gp120. This has lead to the classification of global HIV-1 strain in to 11 sub-types (A-J and O), also called clades (http//www.phil.cdc.gov//defpefoult.asp)

It was quite early in HIV research, in 1984 that $CD4^+$ T cell was shown to be the major cellular receptor for the virus binding directly to gp120 (Dagleish *et al.,* 1984). $CD4^+$ T cell is found at high level on most T-cells and at lower level on many antigen-presenting cells (APC), such as macrophages. It associates with the T-cell receptor complex during antigen presentation, binding with major histo-compatibility class II (MHCII) proteins on the APCs and acting as co-stimulatory molecules.

However, the cellular distribution of $CD4^+$ T cell went most, but crucially not all the way to explaining the cellular range of susceptibility to the HIV virus. It was found that some viral isolates were tropic for T-cell (especially those that had been passaged in T-cell lines in the laboratory). Others were tropic for macrophages. Isolates from patients in early stages of the disease tend to be M tropic, T-tropic strain appearing in more advanced disease. M tropic strains are also more able to infect neural cells in patient

succumbing to AIDS-related dementia (Cowley, 2001).

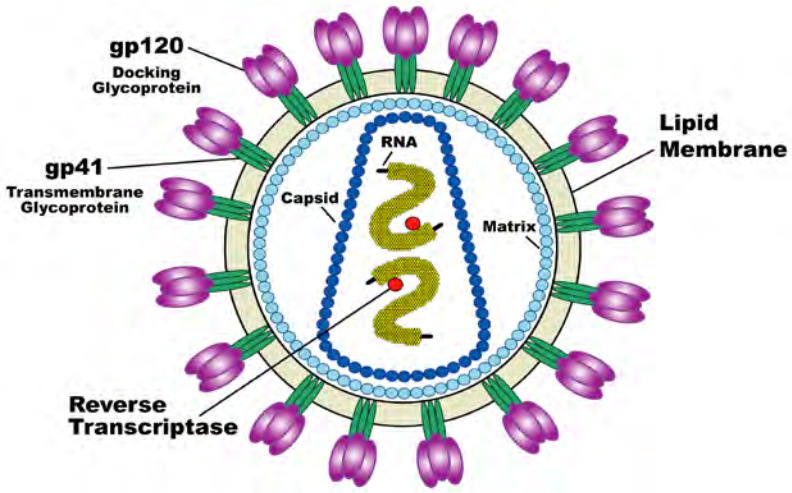

Figure 2. Diagram of HIV
http://en.wikipedia.org/wiki/Image:HIV_Viron.png [accessed on July 08, 2008]

1.2.2. HIV Disease

The typical M-tropic virus usually enters an individual by infecting macrophages, dendritic cells expressing $CD4^+$ T-cells, and chemokine receptors in mucosal tissue. The virus is passed on to systemic activated T-cells and a period of acute infection, develops, where virus replication is high and can be easily detected in the blood and lymph nodes. An initial antiviral immune response then causes viral titers to drop drastically and a persistent state is established, which can continue for years. During this time, there is low level of viral production (when new variants will be continually produced), kept in temporary check by the immune system. However, there is slow, persistent decline in $CD4^+$ T lymphocytes. Eventually, more virulent viral strains (often T-tropic, syncytium inducing) emerge which leads to a second high level of viraemia. This is usually coupled with a decline in $CD4^+$ T-cell count to below 300 cells/µl, and is the point at which opportunistic infections manifest themselves (Feinberg, 1996).

In the absence of ART (antiretroviral therapy), the time between HIV-1 infection and AIDS varies from 5-15 years and appears to be dependent on host genetic factors. During the first two months, after initial infection, the virus multiplies rapidly and causes a decrease in the $CD4^+$ T-cell population. This acute phase of HIV infection is controlled by an active CD8 T-cell response, which peaks at around 2 months post infection.

However, CD8 T-cell number also decrease over time, concomitant with a decrease in $CD4^+$ T cell count and an alteration of the function of dendritic cells and natural killer cells and this initiates a process that leads to progressive destruction of peripheral and recently activated lymphoid $CD4^+$ T-cell populations and leads to AIDS (Fig. 3) (Rinaldo and Piazaza, 2004).

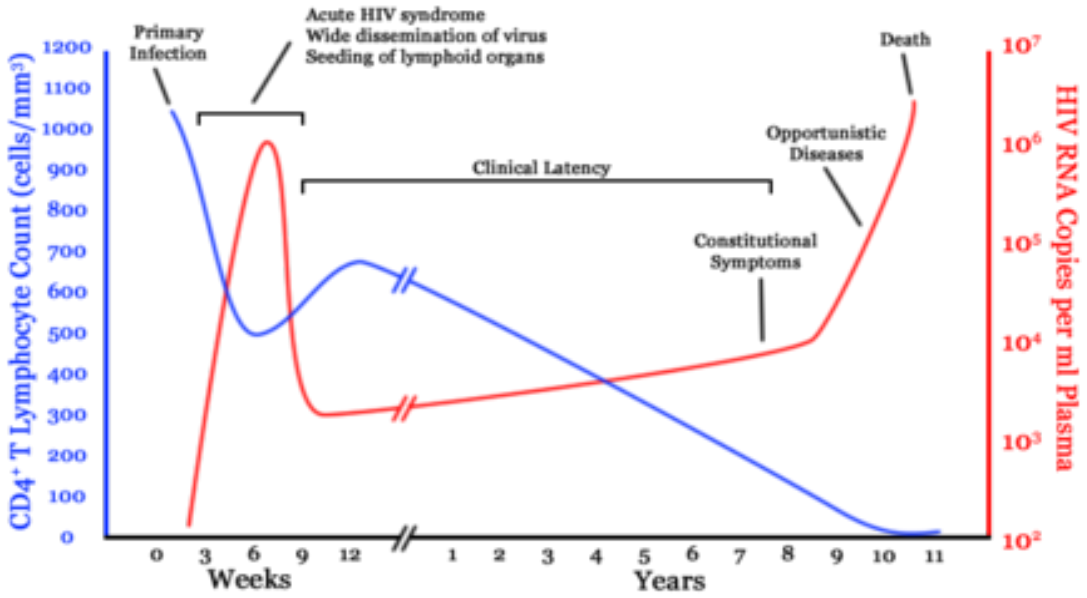

Figure 3. A generalized graph of the relationship between HIV copies (viral load) and $CD4^+$ T cell counts over the average course of untreated HIV infection.

$CD4^+$ T Lymphocyte count (cells/mm³)
HIV RNA copies per ml of plasma
http://en.wikipedia.org/wiki/AIDS [accessed on May 30, 2009]

Invasion and multiplication of the virus can be inhibited by cytokines including IFNα, TNF-α, and IL-13, and by those chemokines which interact with the chemokine receptors, CCR5, CXCR4 or CCR2. IFN-γ, IL-4 and TGF-β can either enhance or inhibit HIV, depending on prevailing conditions. During asymptomatic HIV infection, infected circulating cells harbor unexpressed HIV in vivo, while the virus is continually produced within the lymph nodes and in the central nervous system (Schrager and D'Souza, 1998).

1.2.3. HIV Epidemiology

The AIDS pandemic can also be seen as several epidemics of separate subtypes; the major factors in its spread are sexual transmission and vertical transmission from mother to child during pregnancy, at birth and through breast milk. The estimated number of persons living with HIV worldwide in 2007 was 33.2 million [30.6–36.1 million], a reduction of 16% compared with the estimate published in 2006 (39.5 million [34.7–47.1 million]) (UNAIDS/WHO, 2006).

HIV incidence is the key parameter that prevention efforts aim to reduce, since newly infected persons contribute to the total number of persons living with HIV; they will progress to disease and death over time; and are a potential source of further transmission. Global HIV incidence likely peaked in the late 1990s at over 3 million new infections per year, and was estimated to be 2.5 million [1.8–4.1 million] new infections in 2007 of which over two thirds (68%) occurred in Sub-Saharan Africa. This reduction in HIV incidence likely reflects natural trends in the epidemic as well as the result of prevention programs resulting in behavioral change in different contexts (UNAIDS/WHO, 2007).

AIDS remains a leading cause of mortality worldwide and the primary cause of death in Sub-Saharan Africa, illustrating the tremendous, long-term challenge that lies ahead for provision of treatment services, with the hugely disproportionate impact on Sub-Saharan African countries. More than two out of three (68%) adults and nearly 90% of children infected with HIV live in this region, and more than three in four (76%) AIDS deaths in 2007 occurred, illustrating the unmet need for antiretroviral treatment in Africa. The region's epidemics, however, vary significantly in scale, with national adult (15–49 years) HIV prevalence ranging from less than 2% in some countries of to above 15% in most of southern Africa. Southern Africa alone accounted for almost one third (32%) of all new HIV infections and AIDS deaths globally in 2007 (http://www.who.int/media).

A total of 1.7 million [1.4 million–2.4 million] people in Sub-Saharan Africa became infected with HIV in the past year, declining from 2.2 million [1.7 million–2.7 million] new infections in 2001.There are currently an estimated 22.5 million [20.9 million–24.3 million] people living with HIV in the region in 2007-compared with 20.9 million [19.7 million–23.6 million] in 2001. In Sub-Saharan Africa, adult (15–49 years) HIV prevalence declined from 5.8% [5.5%–6.6%] in 2001 to 5.0% [4.6%–5.5%] in 2007 (UNAIDS/WHO, 2007). AIDS continues to be the single largest cause of mortality in Sub-Saharan Africa since of the global total of 2.1 million [1.9 million–2.4 million] adult and child deaths due to AIDS in 2007, 1.6 million [1.5 million–2.0 million] occurred there. There are an estimated 11.4 million [10.5 million–14.6 million] orphans due to AIDS in this region and unlike other regions the majority of people living with HIV in Sub-Saharan Africa (61%) are women (UNAIDS/WHO, 2007).

Ethiopia is one of the hardest hit Sub-Saharan African countries by the HIV pandemic. In 2005, it was estimated that a total of 1,320,000 people were living with HIV/AIDS. Out of this, 634,000 were living in rural areas and 686,000 in urban areas. It was estimated that 128, 900 new HIV infections (353 a day) including 30,300 HIV positive births, and 134,450 (368 a day) AIDS deaths (including 20,900 children <15 years) occurred in 2005, in Ethiopia (FMOH-Ethiopia, 2006).

According to the most recent estimates, about 1 million people were living with HIV in Ethiopia in 2008. In the same year, approximately 290,000 people needed ART (FMOH-Ethiopia, 2007). A fee-based ART program was officially started in 2003. Moreover, a number of initiatives have been undertaken to expand the availability of ART in Ethiopia, including those by the Global Fund, the Ethiopian North American Health Professionals Association, the Clinton Foundation, and the Ethiopian Red Cross Society. A free ART program was launched in early 2005. Under the guidance of the strategic plan for the multi-sectoral response, 2004–2008 and the road map for accelerated access to ART, 2004–2006 and 2007–2008/10 the ART roll-out plan has been implemented (FMOH-Ethiopia, 2008).

1.3. The Effect of HIV on Malaria

Two important variables are necessary to consider when analyzing potential interactions between HIV and malaria. First, in contrast to HIV infection, repeated malaria infections can induce a degree of acquired immunity. However, this immunity is not complete, and does not totally prevent the incidence of parasitaemia, but instead prevent the development of severe clinical disease. Thus, the impact of HIV infection on malaria immunity differ in non-immune and semi-immune individuals, where malaria infection, has a different pathogenic pattern, secondly the timing of the co-infection and the stage at which observation of the interaction between the infections are made, may also influence the conclusion of a particular study. There are different hypothesis on the impact of these different variables on the interaction between HIV and malaria (Renia and Potter, 2006).

If infection with the two pathogens occurs simultaneously, there is a rapid induction of a strong anti-HIV cellular immune response, which curbs virus development (Feinberg, 1996). It is possible that, early in the co-infection there would be an effect on parasite development and/or against immune mediated malaria pathology. This type of effect might be non-specific through the activation of cytokine dependent mechanisms. At later time points (between few months to a year), if the malaria infection is not treated, it is probable that the effects of HIV on $CD4^+$ T-cell members might impede the development of a natural immunity against the parasite. When the malaria parasite infects an individual who has been already infected with HIV, the interactions between the infections will probably depend on the duration of the HIV pre-infection. In individuals who have been recently infected with HIV, it is possible that the effect on malaria immunity will be similar to that described in the first scenario, with the malaria infection being controlled by non-specific mechanisms. It is also possible that the increased induction of polyclonal B-cells could lead to greater production of inhibitory anti-malarial antibodies (Feinberg, 1996).

In individuals who have been infected with HIV for longer periods before malaria infection, two separate situations are discernible. In the first situation, where individuals are infected with HIV, activation of immune system towards TH2 type responses is

observed (Douek *et al.*, 2003). These could therefore have a negative influence on the development of anti-malarial immunity. In the second situation, where individuals have profound immuno suppression due to a decline in peripheral $CD4^+$ T-cells, and the destruction of recently activated lymphoid $CD4^+$ T-cells, malaria infection is characterized by relatively increased parasitaemia and number of clinical episodes. This has been observed in several studies in African adults leading to an increase in parasite density and clinical episode was associated with lower $CD4^+$ T-cell counts (Whitworth *et al.*, 2002).

It has been shown that HIV infection could hypothetically modify malaria infection through the induction of regulatory T-cells (Sakaguchi, 2003). Furthermore, HIV infection in asymptomatic individuals or patients, undergoing antiviral treatment has been shown to induce $CD4^+$, CD25 T-cells to secrete suppressive production and an increase in circulating cytokines such as IL-10 or TGFβ-CD25 T-cells (Walter *et al.*, 2005). Thus, through the induction of T regulatory cell activity, a pre-existing HIV infection could lead to an increase in malaria parasite density.

Where HIV infection occurs in individuals who are already infected by malaria parasites, two different kinds of interactions, depending on the timing of the disease are likely. In individuals recently infected with malaria, the situation might be similar to that of HIV/malaria co-infection occurring at the same times, as described above. Secondly, in individuals with chronic malaria infection, HIV infection might inhibit parasite development in non-specific manner. This non-specific inhibition might synergize with an ongoing anti-malarial immunity and thus parasitaemia would be inhibited (Walter *et al.*, 2005).

An additional effect of HIV infection on patients pre-infected with malaria is the potential reduction of the response to anti-malarial treatments. According to Targett (1992), that efficient clearance of drug sensitive parasites in treated patients requires the participation of different arms of the immune system. Moreover, age acquired anti-malarial immunity can be abrogated by chloroquine resistant parasites in some

individuals (Djimde *et al.,* 2003). Thus it might be expected that the multiple effects of HIV on immune system would interfere with immune mediated clearance of blood parasites and would increase the risk of anti-malarial treatment failure by diminishing the role that the immune system plays in the clearance of parasites. In addition, HIV-infected patients may be at increased risk of malarial disease due to a newly acquired infection. A greater number of episodes of parasitaemia at presentation, which is associated with a less favorable response to treatment, is observed in HIV-infected populations, could also adversely influence the response to anti-malarial treatment (Kamya *et al.*, 2006).

However, as clearance of malaria parasite is mostly mediated by B-cell dependent mechanisms, this effect would be mostly secondary and initial studies with non-immune African children and semi-immune adults did not reveal significant difference in the level of treatment failure (Colebunders *et al.*, 1990).

$CD4^+$ T-cells provide help in the production of anti-malarial antibodies and they may help to control parasitaemia through the production of cytokines (Brown *et al.,* 1998). Thus, HIV, by depleting $CD4^+$ T-cells, would have impact on the immune response towards malaria. Thus, HIV causes an increased incidence of severe malaria (Chandramohan and Greenwood, 1997, Ambroise, 2001).

1.4. The Effect of Malaria on HIV

As malaria infection induces the systemic production of pro-inflammatory cytokines and chemokines, it is likely that these mediators would have an impact on HIV infection. In fact, HIV infected adults acutely infected with *P. falciparum* were shown to have higher plasma viral loads (Hoffman *et al.*, 1999). The greater viral load in those patients co-infected with malaria correlated with a marked increase in the pro-inflammatory cytokine response to the parasite, and a generalized systemic immune activation shown to originate from macrophages (Pisell *et al.,* 2002). However, conflicting results have been obtained from different studies. An increase in HIV replication in blood mononuclear cells exposed to either malaria antigens or malaria pigment has been observed, and this was shown to be associated with increased production of INFα (Xiao *et al.,* 1998). It was

also reported that, short stimulations with *P. falciparum* antigens down regulated CCR5 but not CXCR4. However, longer stimulation up regulated CCR5 through the induction of IFN γ production of which blocked HIV replication (Moriuchi *et al.,* 2002).

In addition, the effects of co-infection with malaria and HIV on dendritic cells (DC) have an important role in the induction and maintenance of cellular anti-HIV immunity. Strong poly specific CD8 T-cells sustained $CD4^+$ T-cell responses have been associated with protection against HIV (Renia and Potter, 2006). As with any infection, the initiation of an immune response depends on the presentation of viral antigens by DC to T-cells. DC can present the virus either endogenously when infected or can cross-present it after the capture of viral antigens from infected or apoptotic cells (Feinberg, 1996). Several studies suggested that, DC from malaria infected animals were shown to have impaired cross presentation activity. It is therefore possible that, this inhibitory effect might influence the establishment and/or maintenance of anti HIV immunity.

There is evidence that T-cell function is impaired during acute episodes of malaria. Proliferative responses to a variety of antigens are depressed during acute episodes of malaria when assessed by tests carried out on peripheral blood mononuclear cells, but it is possible that this is due in part to sequestration rather than depletion of competent cells. Thus, one might expect malaria infection to have an adverse effect on HIV infection both by stimulating T-cell turnover and by impairing T-cell cytotoxic function. Finally, malaria infection may damage the placenta in such a way as to facilitate transmission of HIV (Chandramohan and Greenwood, 1997).

1.5. HIV Infection during Pregnancy

The HIV/AIDS epidemic is one of the major factors affecting women's health. Almost half of the 37.8 million people living with HIV globally are women and more than 2 million pregnancies occur in HIV positive women each year (UNAIDS, 2004). The majority of these are in resource constrained settings where the risk of maternal morbidity and mortality is also unacceptably high, and where most of the 529,000 deaths from complications of pregnancy, child birth and abortion occur annually (WHO, 2004).

HIV infection rates in pregnant women vary considerably, ranging from below 1% to over 40% across countries. Africa still has the highest rates, although the HIV prevalence in some Asian countries has risen significantly (Mclntyre, 2005).

The true contribution of HIV/AIDS to maternal mortality is difficult to measure, as the HIV status of pregnant women is not always known, so existing data underestimates, but there is increasing evidence that HIV/AIDS related maternal deaths have increased considerably (McIntyre, 2005). In some areas of high prevalence, HIV/AIDS may influence maternal mortality in several ways. Women living with HIV/AIDS may be more susceptible to direct or obstetric causes of maternal mortality, such as postpartum hemorrhage, puerperal sepsis and complications of caesarean section. AIDS-related deaths may be incidental to the pregnancy or may be true indirect causes of maternal mortality where the infection itself or opportunistic infections such as tuberculosis progress faster in pregnancy. There is growing evidence for the impact of the AIDS epidemic on maternal mortality rates and for the effect of AIDS-related complications on maternal deaths (McIntyre, 2003).

Maternal HIV may also result in transmission of HIV to her child as well as other adverse pregnancy outcomes such as still birth, low birth weight (LBW), prematurity and intra uterine growth retardation (IUGR), which are known risk factors for perinatal and neonatal mortality and morbidity, regardless of the transmission of HIV itself to the fetus/child. Large observational studies in Africa have suggested an association between maternal HIV infection and adverse pregnancy outcome (Miotti *et al.,* 1990).

1.6. Malaria Infection during Pregnancy

Although the problem of malaria during pregnancy was first chronicled in the medical literature approximately 70 years ago, it has been neglected area of research. Early studies defined the epidemiology of malaria in pregnancy in areas with moderate or high level of transmission and studied methods of treatment and prevention using chemo prophylaxis (Greenwood *et al.,* 2007).

Although the vast majority of women with malaria infections during pregnancy remain asymptomatic, it is estimated that each year over 25 million women become pregnant in malarious areas of Africa, with most living in areas of stable malaria transmission (Guyatt and Snow, 2004). In malaria endemic areas *P. falciparum* parasitaemia during pregnancy is associated with anemia in pregnant women, and low birth weight, particularly in primigravidae, which is an important risk factors for early infant mortality and morbidity (Ayisis *et al.,* 2003).

The impact of malaria on pregnant mothers varies depending on the level of transmission of malaria on that particular area and the level of acquired immunity by the mother (Yartey, 2006). According to Desai *et al*. (2007), few infections in healthy adults living in areas of high malaria transmission result in fever, and the same is true for semi-immune pregnant women. Although it is commonly assumed that most parasitaemic pregnant women are therefore symptomless, a study in Ghana suggested that pregnant women were more likely to complain of symptoms compatible with malaria if they were parasitaemic than if they were not, despite the absence of a recent history of fever. The major adverse effect of malaria in pregnancy on the mother, according to Luxemburger *et al. (*2001), is anemia.

Although the pathogenesis of placental malaria is not completely understood, it appears that the initiating factors is the unique capability of some malaria parasite clones to sequester in the placenta (Walter *et al.,*1982), through cytoadherence to specific receptors, such as chondroitin sulfate A and hyaluronic acid present on the placental endothelial cells and syncytiotrophoblasts. This leads to recruitment of inflammatory cells (particularly, monocytes) to the placenta and local chemokines, and a cytokine milieu production of pro-inflammatory cytokines which is determinatal during pregnancy (Wegman *et al.,* 1993). Peripheral and placental malaria, induce higher parasite density, more febrile illness, and more severe anemia (Steketee *et al.,* 1996c)

1.7. HIV and Malaria Infection during Pregnancy

Because of high prevalence of HIV and malaria in Sub-Saharan Africa, co-infections are

common. This has important implications, since both HIV and malaria are among the leading causes of morbidity in pregnancy in Africa, and even modest effects of one infection on the other could lead to a substantial negative impact on the health of pregnant women and their new borne (Whitworth *et al.,* 2002). Studies among pregnant women in Sub-Saharan Africa have shown that HIV infected women particularly multigravidae are more likely to be infected with *P. falciparum* and to have higher parasite densities than HIV uninfected women (Ayisis *et al.,* 2003). HIV infection thus seems to alter the well established parity specific pattern of malaria susceptibility in areas of stable malaria transmission, where in the absence of HIV, primigravidae, and to a lesser extent secundigravidae are more affected than are other parities (Aysis *et al.,* 2003). It is also supported by Steketee *et al.* (1996b) who found that the effect of HIV was principally observed in multigravidae, but there was no significant increased risk of malaria prevalence in primigravidae, although parasite densities are significantly higher.

With the availability of data from more studies it is clear that HIV infected primigravidae are also affected (Terkuile *et al.,* 2004). Nevertheless, the HIV associated risk of malaria is consistently greater in multigravidae suggesting that HIV affects the immune memory mechanism responsible for the parity dependent acquisition of anti-malarial immunity in pregnancy (Mount *et al.,* 2004). Thus, HIV alters, the typical gravidity specific patterns of malaria risk by shifting the burden from primarily primigravidae and secundigravidae to all pregnant women, placing HIV infected multigravidae in western Kenya at similar risk of malaria as HIV uninfected women in their first and second pregnancies after adjusting for maternal age (Van Eijk *et al.,* 2003).

The clearest evidence for interactions between HIV and malaria has been obtained from studies of placental malaria (Ned *et al.,* 2005). HIV infection diminished the development of parity specific immunity, which is typically observed in multigravidae and this, was also observed in women without active AIDS. Impairment of humoral response to malaria antigens was induced by HIV infection during pregnancy confirming that parity-specific immunity is mediated by antibodies to specific variant antigens expressed by infected RBC which sequester in the placenta (Mount *et al.,* 2004).

Malaria infection in the placenta was also shown to increase RNA viral load. In particular, intervillous and peripheral blood mononuclear cells increased RNA viral load and induce the secretion of IFNγ and α, two cytokines that are determinal during pregnancy (Moore, *et al*., 2004). Increase expression of the chemokine receptor CCR5, the main co-receptor for mother to child HIV transmission (Salvator and Scarlatti, 2001), has been observed on maternal placental macrophages during malarial infection. This suggests that there may be an increase in the rate of mother to child transmission, especially in conjunction with increased viral load.

Both HIV and malaria are known causes of maternal anemia. Studies in western Kenya and Malawi on the prevalence of anemia for HIV infected and HIV/malaria co-infected women show that women with single infection with HIV/malaria were more at risk than un-infected women. However, dually infected women were at considerably greater risk of having anemia (Hb<11g/dl) or moderate to severe anemia (Hb<8g/dl) than those with single infection (Aysis *et al.*, 2003, and Van Eijk *et al.*, 2001). There was evidence between synergistic interactions between the effect of malaria and HIV, suggesting that the degree of malaria associated anemia is worse in HIV infected women, possibly reflecting, the higher parasite densities and longer duration of malaria (Terkuile *et al*., 2004).

The hypothesis of the present study is that dual infection of HIV/malaria during pregnancy is common in a malaria endemic locality and hence, malaria will be more severe in HIV/malaria co-infected pregnant women under Ethiopian condition.

2. Objective of the Study

2.1. General Objective

To determine the prevalence of HIV/malaria co-infection in pregnant women and to assess the associated disease outcomes.

2.2. Specific Objectives

- To determine the prevalence of malaria infection among HIV positive pregnant women
- To assess the difference in the Hb level and $CD4^+$ T cell count between HIV/malaria co-infected pregnant women and women infected only with HIV or malaria.
- To assess the level of disease severity indicators (parasite density, Hb level) caused by malaria infection in HIV positive pregnant and non-pregnant women

3. Materials and Methods

3.1. Description of the Study Area

Adama, also known as **Nazareth**, is a city in central Ethiopia and the previous capital of the Oromia Region. It is located in the Misraq Shewa Zone of Oromia, at 8.55° N 39.27° E at an elevation of 1622 meters, approximately 100 km southeast of Addis Ababa. The city sits between the base of an escarpment to the west, and the top of the Great Rift Valley to the east. Based on figures from the Central Statistical Agency in 2009, this city has an estimated total population of 422,490 of whom 210,168 were males and 212,322 were females. The place has latitude of $39_0\ 17^{'}$ and longitude of $8_0.33^{'}$.

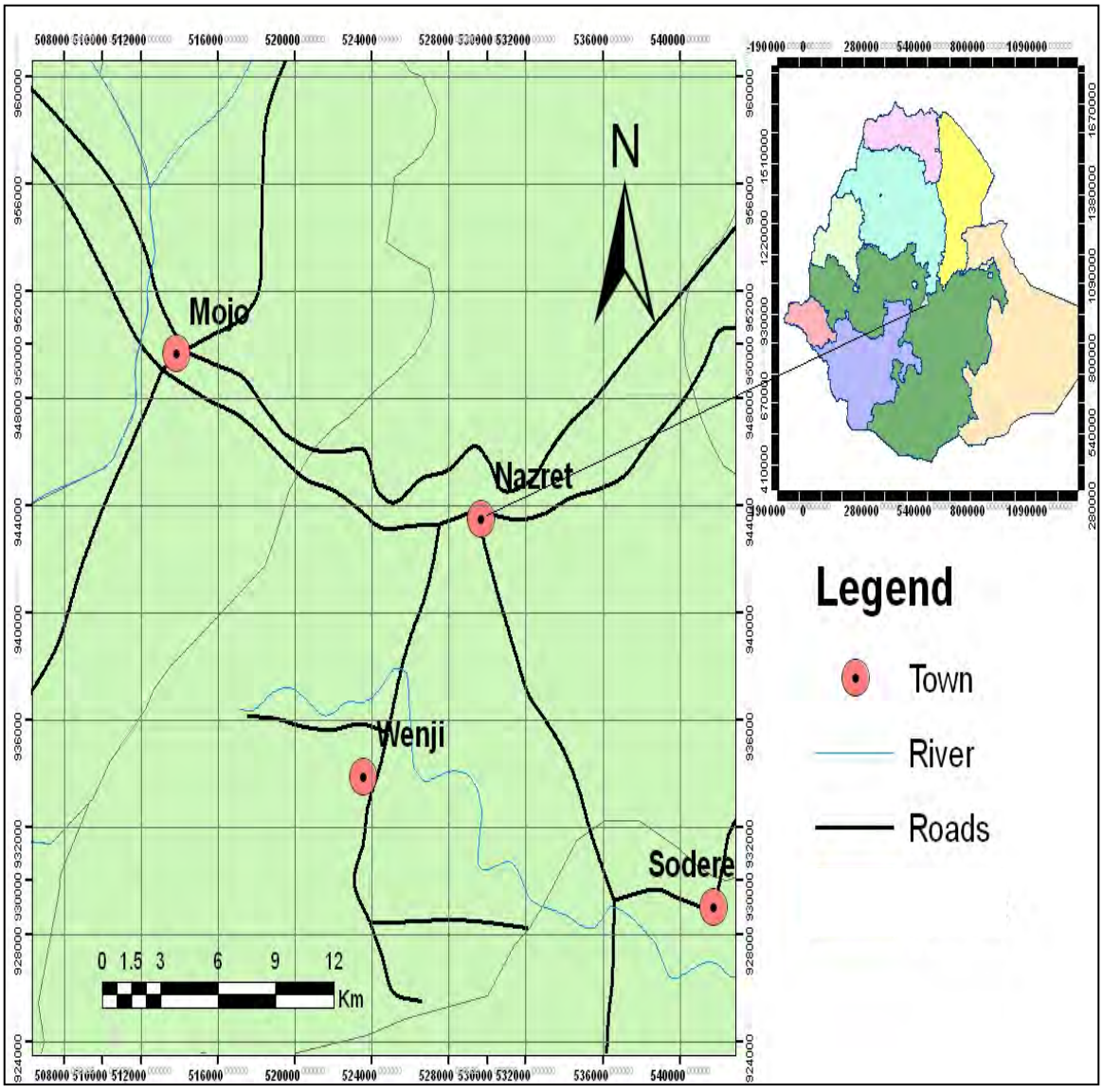

Figure 4 Map showing the location of Nazreth (Adama)
(Source: Ethiopian Mapping Service Agency, June 27, 2008)

According to information from the natural metrology agency, the mean monthly rainfall, maximum and minimum temperature of Adama/ Nazreth is 70.71mm, 27.24^{o}c and 14.40 respectively.

Awash Sebat kilo, is a market town in central Ethiopia. Located in Administrative Zone 3 of the Afar Region, above a gorge on the Awash River, after which the town is named. The town lies on the Addis Ababa - Djibouti Railway. Awash lies outside the Awash National Park, which is known for its wildlife, for the Mount Fentale caldera and for the Filwoha Hot Springs. Based on figures from the Central Statistical Agency in 2009, Awash Sebat Kilo has an estimated total population of 11,053, of whom 5,748 were males and 5,305 were females. It is the largest settlement in Awash Fentale woreda. It has an elevation of 960m above sea level. The place has latitude of 8^0.59 and a longitude of 40.^{0}9.

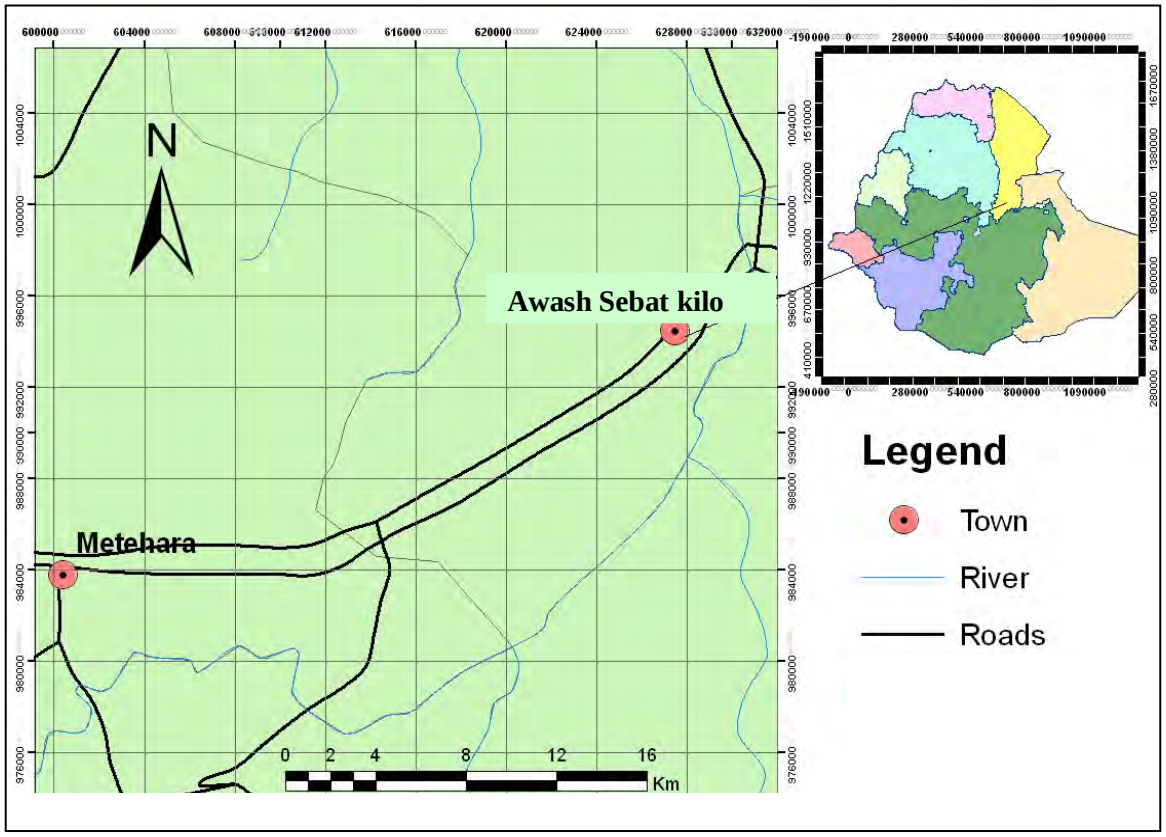

Figure 5 Map showing the location of 'Awash Sebat Kilo'

(Source: Ethiopian Mapping service agency, June 27, 2008)

According to information from the natural metrology agency, the mean monthly rainfall, maximum and minimum temperature of Awash Sebat Kilo health center are 37.25mm, 33.6^{o}c and 17.3^{o}c respectively.

3.2. Source Population and the Study Participants

The source populations were people visiting both Adama hospital and 'Awash Sebat Kilo' health center. The target population was HIV positive women who receive ART services in Adama hospital and 'Awash Sebat kilo' health center during the study period. It included all HIV positive women of reproductive age (15-45 years).

3.3. Sample Size Determination

Sample size was calculated by using the following formula: -

$$n = \frac{Z^2 x P(1-P)}{d^2}$$
$$n = \frac{1.96^2 x 0.5(1-0.5)}{d^2}$$
$$n = \frac{1.96^2 x 0.5 x 0.5}{0.05^2}$$
$$n = 384$$

P=prevalence (malaria and HIV co-infection in pregnant women) = 0.5, (50%)

Z= Confidence interval =1.96

d= Marginal error=5% = 0.05

However, as all women may not be co-operative for the study a sample size of 384 may not be adequate; to account for this 10% additional samples were included.

$$n_1 = 10\% \, n + n$$
$$n_1 = 10\% \, (384) + 384$$
$$n_1 = 38.4 + 384$$
$$n_1 = 422.4$$

Moreover, as all study participants were HIV positive women and the likelihood of being pregnant in that condition may be significantly low because of the various awareness creation services being provided to the population, 18% additional sample were included to optimize the chances of including adequate study participants to arrive at a reasonable conclusion from the study.

$$N = 18\%n_1 + n_1$$
$$N = 18\%(422.4) + 422.4$$
$$N = 76.032 + 422.4$$
$$N = 498.432$$
$$N \approx 500$$

3.4. Measurements

Bio-Data Collection

A pre-tested structured questionnaire was administered requesting demographic information on age, history of the pregnancy and gravidity and socio-demographic variables were collected. All patients fulfilling the inclusion criteria were interviewed.

Blood Sample for Malaria Diagnosis

After reading verbal consent and getting the signature from each study participants, blood sample was collected. Thick blood smears were directly stained using Giemsa method for 20 minutes, while thin blood smears were initially fixed with methyl alcohol for a few seconds and then stained for 20-30 minutes. After that, clean water was poured on the surface of stained films for 10-15 seconds and then the films were dried. The slides were examined under an ordinary light microscope. At least 100 oil immersion fields were checked before reporting a negative result. Malaria parasite counting on Giemsa-stained thick blood film done against 200 WBCs. Malaria parasite density calculation was based on the

assumption of 8,000 WBC per micro-liter blood (Prudhomme, *et al.*, 2005).

$$parasite/\mu l = \frac{WBC/\mu l \;\; X \quad\quad parasite\ count\ against\ 200\ WBC}{200}$$

Malaria slides read in the study sites were checked in the biomedical science laboratory of Biology Department for confirmation of diagnosis.

Rapid Diagnostic Test (RDT)

Malaria parasite detection also performed by using RDT. The RDT available for this study was *Paracheck-Pf* (Orchid Biomedical Systems, Goa, India, manufactured date June 2006 with an expiry date of April 2008), detects the histidine rich protein 2 (HRP2), a protein uniquely synthesized by *P. falciparum* and present in the bloodstream of an infected individual (Howard, *et al.*, 1986). Studies have shown that HRP2 remains in the bloodstream for an extended time following successful eradication of the parasite, contributing to false positive results and limited specificity. A study by Humar *et al.* (1997) on *Paracheck-Pf* (early HRP2 version utilizing IgG antibodies) showed detectable levels of HRP2 in 27% of patients 28 days following successful treatment. Blood sample was taken by pricking the fingertip of the patient using a needle in the test kit. Then the blood was dropped to the test device on its special place. This was followe by addition of seven drops of buffer which helps the blood run through the device. Then the result was read with formation of two dark red lines means the sample is positive for *P. falciparum* malaria or if only one red line, the control was seen, the sample is either malaria negative or positive for other malaria parasites.

Hemoglobin and CD4 cont

The hemoglobin measure is performed in the Spectro-photometric channel of the CELL DYN 18. The whole blood sample from each patient was aspirated to the analyzer by the aspiration peristaltic pump. The pump aspirates the sample through the shear valve. The reading from the CELL DYN 18 printed out. Whole blood sample from the patients also used for the $CD^{+}4$ count by using BD FACS Count. Staining of the blood sample was the first procedure. Then sample from the BD FACS Count control was pressed and the reagent lot code and bead counts were entered. After that confirm button from the machine was pressed and patient accession number entered to it. The reagent pair was vortex up right for 5 seconds and the $CD^{+}4$ tube was uncap and the reagent pair was placed in the sample holder. Then the run button on the machine was pressed and the reagent pair was removed and the $CD^{+}4$ tube was recap. Finally the reading was printed out.

3.5. Quality Assurance

Quality assurance of drugs and supplies in the laboratory maintained using simple visual methods: a First-In-First-Out (FIFO) system used to avoid expiration and ensure fresh supplies at all levels. Quality assurance for the laboratory procedures are undertaken based on the general guidelines, for $CD4^{+}$ T cell count, there is BD FACS Count Controls on the $CD4^{+}$ T cell count machine. Quality control measure for hemoglobin is based on CELL DYN 18 plus control measures.

3.6. Ethical Clearance

The following was considered:

- Ethical clearance was approved and written by ethical clearance committee of the department of Biology of Addis Ababa University.

- A letter informing the medical directors of Adama hospital and 'Awash Sebat kilo' health center about the objective of the study was written from the university prior to actual data collection.
- Prior to sample collection, the objective of the study was explained to each of the study participants and their consent signature was obtained.
- All malaria positives were treated for free as per Ministry of Health (MOH) protocol.

3.7. Data Analysis

Data was entered using SPSS version software for possible association between and among variables. Chi-square (X^2) analysis and Mann-Whitney Test were used for comparison of proportions. Independent sample T-test (Student T-test) and one way analysis of variance with 95% confidence interval were used for mean comparison. Prevalence rate was calculated as the sum of the numbers of positive cases per examined subjects. Intensity of infection was determined by counting parasite density per slide. To determine the number of parasite per micro liter of blood, the number of parasite obtained pre 200 WBC, was multiplied with 8000 and the result divided by 200. All statistical values are with 95% confidence interval i. e., with $P<0.005$ significant level.

4. Results

4.1. Description of the Study Participants

A total of 500 HIV positive women age 15 to 45 years and receiving ART were included in the study (Table 1). This included 400(80%) from Adama hospital, and 100(20%) from 'Awash Sebat Kilo' health center (Table 2). Among the study participants 92 (18.4%) were pregnant and the rest (81.6%) were non-pregnant.

Table 1: Age and Pregnancy among HIV positive women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

Age	Pregnant n (%)	Non-pregnant n (%)	Total n (%)
15-19	0(0)	17(100)	17(3.4)
20-24	14(19.4)	58(80.6)	72(14.4)
25-29	35(23.3)	115(76.7)	150(30)
30-34	33(23.6)	107(76.4)	140(28)
35-39	9(12.5)	63(87.5)	72(14.4)
40-45	1(2)	48(98)	49(9.8)
Total(N)	92(18.4)	408(81.6)	500

Table 2: Socio-demographic characteristics of HIV positive women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

Socio demographic variables		**Pregnant n (%)**	**Non-pregnant n (%)**	**Total n (%)**
Occupation	Farmers	7(26.9)	19(73.1)	26(5.2)
	Students	0(0)	12(100)	12(2.4)
	House wives	26(18.7)	113(81.3)	139(27.8)
	Daily workers	10(16.7)	50(83.3)	60(12)
	Teachers	4(26.7)	11(73.3)	15(3)
	Business	23(29.9)	54(70.1)	77(15.4)
	Unemployed	16(11.7)	121(88.3)	137(27.4)
	Other	6(17.6)	28(82.4)	34(6.8)
Educational status	Illiterate	22(15.8)	117(84.2)	139(27.8)
	Elementary school	38(18.9)	163(81.1)	201(40.2)
	Secondary school	25(18.1)	113(81.9)	138(27.6)
	Higher education	7(31.8)	15(68.2)	22(4.4)
Marital status	Married	68(28.6)	170(71.4)	238(47.6)
	Divorced	1(1.3)	75(98.7)	76(15.2)
	Widowed	23(15.2)	128(84.8)	151(30.2)
	Never married	0(0)	35(100)	35(7)
Place	Adama	73(18.3)	327(81.7)	400(80)
	'Awash Sebat kilo'	19(19)	81(81)	100(20)

Most of the women that participated in the study were house wives (27.8%), and unemployed women (27.4%) and the rest were engaged in different occupations. Regarding the educational status, women that have completed elementary school make up the largest portion (40.2%); with illiterate women (27.8%) and those who had completed secondary school (27.6%) were of equal proportion. There were only few (4.4%) women that had chance for higher education among the study participants. Furthermore, the married (47.8%) and the widowed (30.2%) together make up the largest proportion of the study participants, while the divorced (15.2%) and women who never married (7%), were small proportions. The proportion of HIV positive pregnant women in Adama hospital (18.3%) and 'Awash Sebat Kilo' health center (19%) was almost the same (Table 1).

4.2. Malaria Case Detection

4.2.1. Microscopic Diagnosis

Among the study participants, 111 (22.2%) were positive for either of the two *Plasmodium spp*. (*P. falciparum and P. vivax)* infections with *P. falciparum* identified in 57 (51.4%) of the positive cases and *P. vivax* making up for the rest (54 (48.6%)) of the positive cases (table 5), and with no mixed infection was detected. Among the malaria positive cases, 41 (36.9%) of the women were pregnant (Table 4). Regarding the place where the women came from and the distribution of malaria, 35 (35%) of HIV positive women attending medication in 'Awash Sebat Kilo' health center were positive for malaria, and 76 (19%) HIV positive women attended medications in Adama hospital were positive for malaria. Result from chi-square analysis revealed that there is a significant difference between place of medication and malaria positive cases *(X^2*=4.741 and P=0.029) (Table 4).

4.2.2. Rapid Diagnostic Test (RDT)

Examination of blood by using RDT shows that, out of a total of 57 microscopically confirmed *P. falciparum* positive cases, the RDT test showed positive result for only 25 (43.9%) of them. Moreover, from negative *P. falciparum* malaria cases, which was

confirmed by the microscope examination, the RDT give positive result for 17(4.4%) of them (Table 3). RDT was 43.9% [CI: 31.0-57.6] sensitive and 95.6% [93.0-97.4] specific in the diagnosis of *P. falciparum* infection, with a positive predictive value and negative predictive values of 59.5% [43.3-74.0] and 92.1%, [88.9-94.4] respectively.

Table 3: *Plasmodium falciparum* parasite detection: Microscopy versus RDT among HIV positive women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

		Microscopy		**Total**
		Positive n (%)	Negative n (%)	
RDT				
	Positive	25(43.9)	17(4.4)	42
	Negative	32(56.1)	372(95.6)	404
	Total	57	389	446

Table 4: Comparison of malaria prevalence between study sites in pregnant and non-pregnant HIV positive women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

Variables	Total examined(n)	Malaria positive n (%)	Statistics
Study site			
Adama	400	76(19)	X^2= 4.741
'Awash Sebat kilo'	100	35(35)	P= 0.029
Pregnancy			
Pregnant	92	41(44.6)	X^2 =32.652
Non-pregnant	408	70(17.2)	P= 0.000

4.2.3. Malaria prevalence among HIV positive women under ART in different trimester and gravidity

From the total 111 malaria/HIV co-infected women 41 were pregnant and the rest 70 were non-pregnant women. From 41 HIV/malaria co-infected pregnant women 25 (61%), was due to *P. flaciparum* and the rest 16 (39%) was due to *P. vivax*. The distribution of *P. falciparum* and *P. vivax* malaria among women at different trimester indicates that there distribution is more or less the same, but the distribution varies among different gravid women with highest prevalence of *P. falciparum* malaria (83.3%) recorded in multigravid women with 16.7% of *P. vivax* infection in the same group (Table 5)

Table 5: Malaria prevalence among HIV positive pregnant women under ART in different trimesters and gravidity in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

Pregnancy		Malaria n (%)	*Pf* n (%)	*Pv* n (%)
Pregnancy				
	Pregnant	41	25(61)	16(39)
	Non- pregnant	70	32(45.7)	38(54.3)
	Total	111	57 (51.4)	54 (48.6)
Trimester				
	1st	15	9(60)	6(40)
	2nd	16	10(62.5)	6(37.5)
	3rd	10	6(60)	4(40)
	Total	41	25 (61)	16 (39)
Gravidity				
	1st	16	8(50)	8(50)
	2nd	13	7(53.8)	6(46.2)
	3rd	12	10(83.3)	2(16.7)
	Total	41	25 (61)	16 (39)

4.3.1. Comparing mean hemoglobin level, $CD4^+$ T cell count and parasite density between HIV/malaria co-infected pregnant and non-pregnant women

The mean $CD4^+$ T cell count for HIV/malaria co-infected pregnant women was 195±123 cells/µl and that of the non-pregnant women was 220±140 cells/µl, this result shows that, the means of these groups were not statistically different from one another (P=0.335). The mean Hb level of HIV/malaria co-infected non-pregnant women was 8.73±3.13 g/dl and that of the pregnant one was 7.49±3.34 g/dl, this result shows that the means of the two groups were significantly vary from each other (P=0.05). The mean parasite density of dually infected pregnant women, with HIV and *P. falciparum* malaria, was 32,362±17,153 parasites/µl and that of the non-pregnant women was 18,863±15,155 parasites/µl, this result shows that the two means differ significantly from one another (P=0.003), and the mean parasite density of pregnant women infected with HIV and *P. vivax* parasite was 17,585±3484 and that of the non-pregnant women was 12,484±8344, statistical analysis for this result revealed that the two means does not differ significantly form one another (P=0.230)(Table 6).

Table 6: Comparison of hemoglobin (Hb) level, $CD4^+$ T cell count and parasite density between malaria/HIV co-infected pregnant and non-pregnant women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

Pregnancy		No. examined	Mean $CD4^+$ Tcells/µl± SD	Mean Hbg/dl± SD	Mean parasite density ±SD	
					Pf	*Pv*
	Pregnant	41	195±123	7.49±3.34 *	32,362±17,153 **	17,585±3484
	Non-pregnant	70	220±140	8.73±3.13	18,863±15,155	12,484±8344
Total		111				

* P=0.05
** P=0.003

4.3.2. Malaria/HIV Co-Infected Pregnant Women

4.3.2.1. Comparing the mean hemoglobin and $CD4^+$ T cell count between malaria positive and negative HIV positive pregnant women

The mean Hb level of HIV/malaria co-infected pregnant women and pregnant women infected only with HIV was 7.485±3.34 g/dl and 10.53±2.96 g/dl, respectively. Statistical analysis revealed that the means of these study groups were significantly differ from one another (P<0.001). The mean Hb level for HIV positive pregnant women infected with *P. falciparum* was 6.69±3.4 g/dl and that of infected with *P. vivax* was 8.24±3.17) g/dl. Results from statistical analysis revealed that there is no significant variation between the two groups (P=0.134). In addition, comparison of means between HIV positive pregnant women with no malaria infection and HIV positive women infected with *P. falciparum* was performed and the statistical analysis revealed that there is significant difference between the two means (P=0.000), similar analysis was performed between HIV positive pregnant women infected with *P. vivax* and HIV positive pregnant women without malaria infection, the statistical analysis revealed that there is significant variation between these two means (P=0.012). Moreover, the mean $CD4^+$ T cell count for HIV/malaria co-infected pregnant women was 195±124 cells/µl, and that of infected with only HIV was 280±151 cells/µl. Statistical analysis revealed that means $CD4^+$ T cell count of pregnant women co-infected with HIV and malaria and those infected with only HIV were significantly vary from one another (P=0.005). The mean $CD4^+$ T cell count in HIV positive pregnant women infected with *P. falciparum* was 172±114 cells/µl and that of women infected with *P. vivax*, was 216±132 cells/µl. Results from statistical analysis revealed that there is no significant variation between this two groups (P=0.226). Furthermore, mean comparison of $CD4^+$ T cell count between HIV positive pregnant women with no malaria infection and HIV positive women infected with *P. falciparum* was performed and the statistical analysis revealed that there is significant difference between the two means (P=0.001), similar analysis was done for those women infected with *P. vivax* and women without malaria infection and the statistical analysis revealed that there is no significant variation between the two means (P=0.157) (Table 7).

Table 7: Comparison of $CD4^+$ T cell count and hemoglobin (Hb) level between malaria positive and malaria negative HIV positive pregnant women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

	Malaria –ve n=51	Malaria +ve n=41	Pf n=25	Pv n=16
Mean Hb ± SD (g/dl)	10.53±2.96	7.49±3.34*	6.69±3.4	8.24±3.17
Mean $CD4^+$ ± SD (cells/μl)	279±151	195±123**	172±114***	216±132

* P=0.000 *** P=0.001

**P=0.005

4.3.2.2. Determination of $CD4^+$ T cell count at different trimesters and gravidity of malaria infected HIV positive pregnant women

Among the HIV/malaria dually infected pregnant women there were 26 (63.4%) women with $CD4^+$ T cell count less or equals to 200 cells/μl and 15 (36.6%) with $CD4^+$ T cell count greater than 200 cells/μl. Also, there were 15 (36.6 %) and 26 (39 %) first trimester and trimester greater than one HIV/malaria co-infected pregnant women respectively. Mann-Whitney Test was done to determine the frequency of $CD4^+$ T cells less or equals to 200 and greater than 200 between first trimester pregnant women and women with greater than one trimester. The statistical analysis revealed that there was no relationship between $CD4^+$ T cells count and number of trimester in HIV/malaria co-infected pregnant women. But similar investigations were made for different gravid HIV/malaria co-infected pregnant women. Here also there are 16 (39%) and 25 (61%), first gravid women and women with gravidity greater than one. Similar investigation was made to determine the frequency of $CD4^+$ T cells count less or equals to 200 and greater than 200 between primigravid women and women with gravidity greater than one. The Mann-Whitney Test revealed that there is a significant relationship between number of gravidity and $CD4^+$ T cells count (U = 112.5, P=0.000), with lower $CD4^+$ T cell count recorded in primigravid women (Table 8).

Table 8: Determination of $CD4^+$ T cell count at different gravidity of HIV/malaria co-infected pregnant women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

		Gravidity		Total
		1^{st} n (%)	$> 1^{st}$ n (%)	
$CD4^+$ T cell	≤200	17 (61.2)	11 (38.5)	28
	>200	1 (5.9)	16 (94.1)	17
	Total	18	27	45*

U = 112.5, P=0.000

* Adjusted total

4.3.2.3. Determination of severity of anemia at different trimesters and different gravidity in malaria positive pregnant women

In malaria/HIV co-infected pregnant women, there were 20 (48.8%), 14 (34.1%), and 7 (17.1%) severe, mild and non-anemic women. From 15 HIV/malaria co-infected pregnant women with first trimester of pregnancy, there were 7 (47%), 5 (33%) and 3 (20%) women with severe, moderate and non-anemic conditions respectively. From 26 HIV/malaria co-infected women with trimester greater than one, there were 13 (50%), 9 (34.6%) and 4 (15.4%) severe, mild and non-anemic conditions respectively. The Mann-Whitney Test for the determination of frequencies of severe, mild and non-anemic conditions between first trimester and trimester greater than one revealed that there is no relationship between trimester and severity of anemia. Similar analysis was done on severity of anemia among different gravid women. From 16 primigravid women there were 13 (81.25%), and 3 (18.75%) women with severe and mild anemia respectively, there was no women with non-anemic situation in this group of women. From 25 women with gravidity greater than one, there were 7 (28%), 11 (44%) and 7 (28%) severe, mild and non-anemic conditions respectively. The Mann-Whitney Test for the distribution of malaria severity for different gravid women revealed that, there is no relationship between severity of anemia and gravidity.

4.4. Determination of parasite density at different trimesters and different gravidity

In HIV/malaria co-infected pregnant women, there were 4 (9.8%) and 37 (90.2%) pregnant women with parasite density less than 10,000 parasites/μl and greater or equals to 10,000 parasites/μl respectively. Mann-Whitney Test was done to determine the frequency of parasite density with in different trimesters in dually infected pregnant women, with malaria and HIV. Statistical analysis revealed that there is no significant difference in parasite density between first trimester pregnant women and pregnant women with greater than one trimester (U = 184, P=0.779) (Table 9). Similar analysis was made between HIV/malaria co-infected first gravid women and women with gravidity greater than one. Nonetheless, there was no statistically significant difference between these two groups.

Table 9: Determination of malaria parasite density at different trimesters in HIV/malaria co-infected pregnant women under ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007-Jan. 2008.

		Trimester		
		1^{st} n (%)	$>1^{st}$ n (%)	Total
Parasite density	<10,000	2 (50)	2 (50)	4
	≥10,000	13 (35.1)	24 (64.9)	37
	Total	15	26	41

U = 184, P=0.779

4.5. Anti Retroviral Therapy (ART)

In HIV/malaria co-infected pregnant women, those who attend ART services for less than 6 months got a mean $CD4^+$ T cell count 85 cells/µl mean Hb level 4.95 g/dl and mean parasite density 35,702 parasites/ µl. Those women who attend the services for 6-12 months got mean $CD4^+$ T cell count 203 cells/µl mean Hb level 7.99 g/dl and mean parasite density 23,846 parasites/ µl. Furthermore, women who took ART for more than one year got mean $CD4^+$ T cell count 387 cells/µl mean Hb level 11.44 g/dl and mean parasite density 13,975 parasites/µl. All the three variables $CD4^+$ T cell count, Hb level and parasite density vary significantly with the duration of ART ($P<0.001$) (Table 10).

Table 10: Determination of mean $CD4^+$ T cell count, hemoglobin level and malaria parasite density among HIV/malaria co-infected pregnant women based on the duration of ART in Adama hospital and 'Awash Sebat Kilo' health center, Sept. 2007- Jan. 2008.

	ART Duration				
Mean	1-6months (n=17)	7-12months (n=15)	>one year (n=9)	F	P
$CD4^+$(Tcells/µl) ± SD	85 ± 40.79	203 ± 34.2	387 ± 63.1	136.56	0.000
Hb(g/dl) ± SD	4.95 ± 2.15	7.99 ± 2.85	11.44 ± 0.74	25.16	0.000
Parasite density ± SD	35,702 ± 16,970	23,846 ± 10,717	13,975 ± 5026	8.75	0.001

5. Discussion

This study showed that HIV positive women with higher education and those engaged in business activity, had a relatively higher pregnancy rate, which probably is due to the fact that these women have a relatively better source of income and hence may have developed better confidence to bear and raise children in spite of the relative risk of congenital transmission of HIV to their children.

The finding that the majority of HIV positive women in the study were either married or are widowed is a confirmation to the long held view (Drain *et al.,* 2006) that HIV transmission in Sub-Saharan African countries is heterosexual. This is a matter of serious concern indication that a one-to-one sexual relationship is not being maintained among the study population.

In this study, because of the poor diagnostic efficiency of RDT malaria diagnosis was based on light microscopy. The poor performance of RDT in Ethiopia, (poor sensitivity and predictive value) has also been reported by Endeshaw *et al.* (2008) which is in contrast to the report of Moonasar *et al.* (2007), where they showed RDT to have a comparable level of accuracy to microscopy in South Africa. The low sensitivity and false predictive value of RDT may have been due to insufficient antigenaemia (WHO, 2000b) and due to lack of optimization of the RDT for the conditions in Ethiopia.

Due to possible differences in malaria and HIV/AIDS control measures, the variation in the peoples' behavior towards seeking treatment and prevention in the two study sites, the proportion of malaria/HIV co-infected women was much higher in 'Awash Sebat Kilo' than in Adama. Adama is a city with better environmental hygiene and has a better organized anti-AIDS services compared to 'Awash Sebat Kilo'. Furthermore, 'Awash Sebat Kilo' is known for its higher malaria transmission intensity, according to the information from the local health center, which has been reporting malaria cases through most of the months of the year unlike Adama, where malaria peaks occur only after the big (June-August) and small (March-May) rainy seasons. (Abeku *et al.,* 2003).

The finding in this study that malaria co-infection was higher in HIV positive pregnant women than in the non-pregnant HIV positive women is supported by earlier reports of Uko *et al.* (1998), Adefioye *et al.* (2007) and HImeidan (2004) who reported a higher malaria prevalence in pregnant women than the non-pregnant. Moreover, this study found that there were more *P. falciparum* infections than *P. vivax* in HIV infected pregnant women, which is inline with earlier reports of Singh *et al.* (2001), where, among 151 malaria infected pregnant women, *P. falciparum* was the predominant species (88%). The increased susceptibility of pregnant women to malaria is not well understood, but is presumed to be related to modification of systemic and placental immunological parameters (Moore *et al.,* 2000). Additionally, increased attractiveness towards the malaria vector due to pregnancy might also be the reason for more number of malaria positive pregnant women then the non pregnant ones (HImeidan 2004). Furthermore, more number of *P. falciparum* cases than *P. vivax* during pregnancy is presumed to be due to the higher prevalence of *P. falciparum* (60%), than *P. vivax* (40%) in Ethiopia (WHO, 2000a) and (Mirjam *et al.,* 2006). Moreover, cytoadherance properties of *P. falciparum* to chondroiton Sulfate A (CSA) a ligand abundant in the syncytiotrophoblast may account for it (Gitau and Eldred, 2005).

The present study showed that among HIV/malaria co-infected women, absolute $CD4^+$ T-cell counts were lower in the pregnant than in the non-pregnant and this is in agreement with a report from India by Dayama *et al.* (2003). Similarly, an earlier study among African women had demonstrated reduced absolute values of $CD4^+$, $CD8^+$, and total T lymphocytes in pregnancy (Bisalinkumi *et al.,* 1992). Thus, since pregnancy is a stage of generalized immune suppression, as it has been shown by Dayama *et al.* (2003) this may contribute to a more rapid progression of HIV infection than expected in the absence of pregnancy.

The lower mean Hb value in HIV/malaria co-infected pregnant women compared to the non-pregnant one is supported by earlier reports of Lu *et al.* (1991) and Brabin (1992). The lower Hb concentration during pregnancy is due to the problem in the production of

RBC and their principal component, Hb, affected by many congenital and acquired conditions during pregnancy (Yip, 2000).

A significantly higher parasite density in HIV/malaria co-infected pregnant women than in the non-pregnant co-infected, recorded in this study is in line with reports from elsewhere. The studies by Shulman *et al.* (1996), Steketee *et al.* (1996a), Lander *et al.* (2002) and Van Eijk *et al.* (2003) had shown an increased risk of higher malaria parasitaemia in HIV infected pregnant women than in the non-pregnant. The increased susceptibility of HIV/malaria co-infected pregnant women to higher density of malaria parasitaemia is presumed to be related to the generalized immune suppression that occurs during the course of pregnancy (Dayama *et al.*, 2003).

The significantly increased malaria parasitaemaia in HIV infected pregnant women due to *P. falciparum* not *P. vivax* compared to the non-pregnant ones, shown in this study is also supported by earlier reports of Singh *et al.* (1996), Mcgready *et al.* (2004). The significant increase in malaria parasitaemia during *P. falciparum* infection might be due to placental changes (increased number of inflammatory cells, fibrin deposition and cytotrophoblastic prominence) were significantly greater in women infected with *P. falciparum* compared with women infected with *P. vivax* (Jelliffe, 1968).

Similar to that detected in this study, other findings from Western Kenya and Malawi had shown HIV/malaria co-infected women to be at a considerably greater risk of having mild anemia (Hb<11g/dl) or severe anemia (Hb<5g/dl) than those with single infection with malaria or HIV (Van Ejik *et al.,* 2001 and Aysis *et al.,* 2003). The destruction of parasitized RBC, reduced RBC production in the bone marrow and immune complex mediated phagositosis of uninfected RBCs are some of the mechanisms known to cause anemia during malaria infection (Trape, 1985). Furthermore, bone marrow infection, gastrointestinal bleeding and soluble factors in the serum, which affect hematopoiesis are some of the mechanisms by which HIV infection affects Hb (Greenwood, 1997). As both HIV and malaria have impact in reducing the level of hemoglobin in the blood, infection with both will drastically decrease the level of hemoglobin than single infection with only HIV.

A significantly lower $CD4^+$ T cell count in HIV infected pregnant women with *P. falciparum* infection and a non-significant relationship of $CD4^+$ T cell count between HIV positive women infected with *P. vivax* infection and HIV positive controls with out malaria recorded in this study was similar to the report by Kassa *et al.* (2006). This could be due to the relative difference in the nature of activation of the immune system by the two malaria parasites (Chotivanich *et al.,* 2000).

The lack of association between trimester in pregnancy and $CD4^+$ T cell count in this study is similar to the report from Kenya by Temmerman *et al.* (1995). However, it was contradicted by another study that showed $CD4^+$ T cell decrease during the third trimester that levels off after delivery in both HIV infected and uninfected pregnant women, whereas it increases in uninfected mothers (Lander *et al,* 2002). Further studies are required to determine the association of $CD4^+$ T cell count and number of trimester during pregnancy.

A significantly different $CD4^+$ T cell count between HIV/malaria co-infected primigravid women and those with gravidity greater than one, with lower $CD4^+$ T cell count recorded in primigravid women is not supported by earlier reports of Dembo *et al.* (2008) where $CD4^+$ T cell count does not differ significantly with gravidity. The result found in this study might be due to all women were on ART, so HIV/malaria co-infected multigravid women show better improvement towards the treatment than the primigravid ones, moreover, further studies are required to determine the importance of these observations.

The absence of difference in the severity of anemia among HIV positive women on ART, of different gravidities, is in agreement with the findings of Ticconi *et al.* (2003), which showed that, the risk of severe anemia is not different between primigravids and multigravids. The lack of difference in severity of anemia between primigravid and multigravid women observed in this study might be due to the fact that all the HIV/malaria co-infected pregnant women were receiving ART, which will improve their immunity against malaria parasites leading to reduced RBC destruction with a

concomitant reduction in anemia. However, there are contradicting reports by Aysis *et al*. (2003) who had shown the prevalence of severe anemia in HIV/malaria co-infected multigravid women to be higher (32.9%) than that of primigravids (23.5%). And similarly, Van Eijk *et al*. (2003) found the prevalence of anemia in HIV/malaria co-infected primigravid pregnant women to be lower (23.8%) than that in the multigravid women (34.0%) with repeated pregnancy, nutritional demand of the women increased that intern increase anemia in multigravid women. The contradicting result found in the studies indicates that subject requires further investigation.

The findings by Schulman *et al*. (1990), Steketee *et al.* (1996b), Lander *et al.* (2002), and Van Ejik *et al.* (2003) that showed no significant relationship between gravidity and parasitaemia in HIV/malaria co-infected pregnant women is also supported by the findings of the present study. The absence of association between gravidity and parasite density suggests that HIV alters the typical gravidity-specific pattern of malaria risk by shifting the burden from primarily primigravidae and secundigravidae to pregnant women of all gravidities (Terkuile *et al.,* 2004). This relatively recent study had provided clarity for any early reports of Verhoeff *et al.* (1999) who reported dually infected primigravid women with HIV and malaria to be more parasitaemic than multigravid women.

The luck of association between trimester and mean malaria parasite density in HIV/malaria co-infected pregnant women is also supported by (Coulibaly et al., 2007) and (Achidi *et al.,* 2007). The systemic cytokine profile was found to be biased towards Th2 responses; a prerequisite for a successful pregnancy, which is associated with increased malaria parasitaemia. This pattern was unaffected by gestational age (Achidi *et al.,* 2007).

The use of ART by HIV infected patients resulted in a significant increase in $CD4^+$ T-lymphocyte counts, which is similar to the finding by Martin *et al.* (2001). Moreover, WHO (2003) report showed that after getting ART treatment for 12 months, there is an overall increase in the mean $CD4^+$ T cell count by 221 cells per mm^3. Similarly, a study

by Ormaasen *et al.* (2003) showed an increase in $CD4^+$ T cell count after six month ART. The increasing count of $CD4^+$ T cells with duration of ART has been shown to be associated with decrease in the viral load, which would then enhance the immune competence of the patient so as to curb malaria parasitaemia, (Tatfeng *et al.*, 2007). This is due to the fact that $CD4^+$ T cells play a central role in the immune system against many pathogens including malaria parasites.

Therefore, it is proper to provide ART to HIV/malaria co-infected pregnant women even if their $CD4^+$ T cell counts are above 200 cells/µl. This would be necessary because pregnancy complicated with malaria would lead to a fast deterioration of the immune status of such patients, possibly resulting in an accelerated progression to AIDS.

6. Conclusions

- The study has identified the increased risk of malaria severity in HIV positive pregnant women; a finding that has a considerable public health implication in areas where both diseases are prevalent.
- The study revealed high malaria parasite density, reduced Hb level and $CD4^+$ T cell count in HIV positive pregnant women, indicating that pregnancy has an adverse effect leading to severe malaria in HIV positive women.

7. Recommendations

Based on the present findings we recommend that:

- Extra attention must be given to protect HIV positive women from malaria infection i. e.,
  - ❖ Pregnant women with HIV living in malaria endemic areas should be screened for malaria infection during their Antenatal Care (ANC) follow up visits and explain the role of managing malaria in PMTCT (Pregnant mother to child transmission of HIV)
  - ❖ Full coverage of HIV infected pregnant women by providing insecticide treated bed nets (ITNs)
- More studies in different parts of the country with varied eco-epidemiological malaria transmission patterns must be conducted to confirm the present findings.
- Investigation on anemia in HIV/malaria co-infection and gravidity in pregnant women should be considered as further study subjects.

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Appendix

Appendix I Questionnaire

Prevalence of HIV/malaria co-infection during pregnancy

Interviewer name_____, today's date: ____/____/____ (EC)

Interview number_____

Woman's name_____

Demographic Information

1. Age (years):.....[ ]

(If the mother does not know her age, then estimate her age using the categories below)

Less than 15 years.....[ ] 30-34 years..... [ ]

15-19 years.....[ ] 35-39 years.....[ ]

20-24 years.....[ ] 40-45 years.....[ ]

25-29 years.....[ ]

2. Woreda: _____

Kebele: _____

3. What language do you usually speak with family members at home?....[ ]

Amharic

Tigrigna

Afan Oromo

Other (Specify) _____

4. What is your occupation?.....[ ]

Education:

5. Can you read?..... [ ]

Yes... [ ] No... [ ]

6. Have you ever attended school?..... [ ]

Yes... [ ] No... [ ]

If yes:

7. How many years of school have you completed?..... [ ]

Marital status

8. Are you married?..... [ ]

Yes, married or living with a man

Was married or living with a man, but, divorced

Widowed

Never married or lived with man

Reproductive and Clinical history:

9. Are you pregnant now?..... [ ]

If no, end the question and go to the examination part. If yes, go to question no

10. How many times have you been pregnant? (Including this one)..... [ ]

How many months?..... [ ]

History of Fever or Malaria

11. Have you had a fever or malaria during this pregnancy?..... [ ]

Yes [ ] No [ ] Unknown [ ]

If yes:

12. How many episodes of fever or malaria did you have? .....[ ]

13. Did you sleep under a bed net during this pregnancy?..... [ ]

Yes [ ] No [ ]

13a. If yes how frequently?..... [ ]

All the time

Most of the time

Sometimes

Rarely

14. Did you receive blood transfusion in this pregnancy?..... [ ]

Yes [ ] No [ ]

15. Have you had malaria during the past week?..... [ ]

Clinical Examination and Laboratory Findings

16. Hemoglobin (Hb).....____ ____ ___ (g/dl)

17. Weight.....____ ____ ___ (k.g.)

18. Height..... _____ _____ (cm.)

19. Smear result..... [ ]

Positive [ ] Negative [ ] Undetermined [ ]

20. Rapid test result..... [ ]

If negative, skip to end of questionnaire

21. Malaria Species..... [ ]

Plasmodium falciparum

Plasmodium vivax

Plasmodium malariae

Undetermined

22. Parasite density..... [ ]

PLEASE CHECK OVER THE QUESTIONNARIRE NOW TO MAKE SURE THAT ALL QUESTIONS HAVE BEEN ANSWERED, AND THEN CHECK THIS BOX..... [ ]

Thank the women for their time!

Appendix II Consent Statement

PREVALENCE OF HIV/MALARIA CO-INFECTION DURING PREGNANCY

Purpose of the Assessment

The knowledge we learn about women's ideas and actions about malaria/HIV co-infection and what they do when they are pregnant will help in planning programs to decrease malaria/HIV co-infection in pregnant women.

Procedures

Participating in interview (or focus group) is up to you. If you agree to participate, we will ask you some questions about yourself and your community. We will also ask where you have received health care during pregnancy. Moreover, we will take small blood sample for some laboratory procedures. You do not need to answer any questions that we ask that you do not want to and can refuse to give your blood. Besides, even after you decide to participate and start to answer the question, and you decide that you do not want to finish it, you can leave at any time.

Benefits

You will receive treatment if you are positive for malaria.

Risk and Discomforts

We will not be telling anyone about your individual answers to the questions. We will not be recording your name in any permanent record.

I agree to participate in the study.

_____ _____

Signature of study participants Signature of witness