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FACULTY OF HEALTH SCIENCES

SCHOOL OF PUBLIC HEALTH

RESEARCH REPORT

INCIDENCE OF MALARIA IN HIV-INFECTED AND UNINFECTED RWANDAN WOMEN FROM 2005 TO 2011

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Research report submitted to the School of Public Health, University of Witwatersrand Johannesburg in partial fulfilment of the requirements for the degree of Masters of Science in Epidemiology and Biostatistics

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Declaration

I, ***Jacqueline Umunyana***, declare that this is my own work. It is being submitted for the degree of Master of Science in Epidemiology and Biostatistics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature:

Full names: Jacqueline Umunyana

Date: 7th November 2013

Abstract

Background

Malaria in HIV-infected (HIV+) persons is associated with reduced immunity due to a decrease in CD4+ cells count and an increase in viral load, and immunity becomes more compromised in HIV-infected ART-naïve patients. However, the relationship between treatment of HIV infection with antiretroviral therapy (ART) and malaria among HIV co-infected individuals has not been widely reported in Africa, in particular amongst Rwandan women. In this study, the investigator examined malaria incidence and its associated potential risk factors in a cohort of HIV-uninfected, HIV-infected on ART and HIV-infected naïve Rwandan women.

Method

The data used in this research consists of 936 women enrolled in the Rwandan Women's Inter-association Study and Assessment (RWISA) study. Follow-up visits were carried out every 6 months for a period of 5 years. Incidence of malaria was considered as self-reported if it occurred during the 6 months prior to the study visit**.** Incidence rates (IRs) and Hazard ratios (HRs) with 95% CI were determined in HIV-uninfected, HIV-infected ART-naïve and HIV-infected on ART groups. Predictors of malaria incidence in these groups were estimated by Hazard ratios (HR, 95% CI) using Cox regression adjusted for potential confounders.

Results

Of the 936 women enrolled in the study (226 HIV-uninfected and 710 HIV-infected), almost 90% of the women reported malaria during the follow-up period. At the baseline visit, the median age of the participants was lower among HIV-infected women at 34 years ([IQR] 30-39), compared to that of HIV-uninfected women at 43 years ([IQR] 34-49), $P<0.01$.

In both groups of HIV-infected and HIV-uninfected women, a large number were widowed i.e. 49% vs. 42%, P<0.01

The HIV-infected women had lower educational status (67% vs. 57%, P<0.01) and lower employment opportunities (68% vs 72%, P=0.002) than HIV-uninfected women. Of the HIV-uninfected women, 174 (77%) and of HIV-infected women 596 (84%) reported that they did not have enough food to eat.

Malaria incidence was higher in HIV-infected ART-naïve women [adjusted HR= **1.2, 95% CI (1.01-1.36), P=0.03**], when compared to HIV-infected women on ART. However, when malaria incidence was compared according to HIV status, HIV-infected women showed a significantly lower incidence when compared to their HIV-uninfected counterparts [adjusted HR= **0.8, 95% CI (0.69- 0.97), P=0.02]**. The independent predictors of malaria incidence in the cohort were unemployment, lower level of education, age and season.

Conclusion

HIV-infected antiretroviral-naïve women in malaria-endemic areas are at higher risk of malaria than HIV-infected women on antiretroviral therapy. In countries where both diseases overlap, the indirect effect of HIV treatment with combination antiretroviral therapy could reduce malaria burden. These findings suggest that additional malaria prevention efforts should be aimed at the untreated HIV-infected population.

Keywords

HIV; Antiretroviral Therapy; Malaria Incidence

Dedication

I dedicate this dissertation to my mother, Fortunate Kamanyana who has been a teacher, nurse and counsellor, ensuring that I should be where I am now. I also dedicate this work to my late father, Gerald Sempabwa, without whose support and inspiration, I would never have had the courage to follow my dreams.

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List of Abbreviations and Acronyms

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
CI	Confidence Interval
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
IQR	Inter-quartile range
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
RASD	Regional Alliance for Sustainable Development
RWISA	Rwanda Women's Association Study and Assessment
TRAC Plus	Centre for Treatment and Research on AIDS, Malaria, Tuberculosis and other Epidemics
WE-ACTx	Women's Equity in Access To Care and Treatment
WHO	World Health Organisation

Definition of terms

Malaria: a mosquito-borne infectious disease of humans and other animals caused by protists of the genus plasmodium.

Malaria incidence: The number of new cases of malaria per time at risk.

Stable Malaria: Malaria is said to be stable if it is transmitted throughout the year by long-lived, anthropophilic vector anopheline mosquitoes.

Unstable Malaria: Amount of malaria transmission changes from year to year.

Epidemic Malaria: periodic or occasional sharp increase of malaria in a given indigenous community.

Endemic Malaria: Constant incidence of Malaria over a period of many successive years in a given area.

Chapter 1 : Introduction

1.1. Research Report Outline

This research report is organized into five chapters. The first chapter covers the background of the study, the literature review, the aims and objectives and the justification of the study.

The second chapter describes the study methodology, provides a brief description of the study setting, study design, study population, data collection and statistical analysis. The third chapter presents the study results while the fourth chapter provides a discussion of the study results. The final chapter presents the conclusion of the results as well as remarks and policy recommendations based on the study findings.

1.1.2. Background

Infection with malaria and with the Human Immunodeficiency Virus (HIV) that causes Acquired Immune Deficiency Syndrome (AIDS) are two of the most common infections in many parts of the world, especially in Sub-Sahara Africa (1). It is estimated that over 34 million people are living with the HIV-infection globally, with estimates of 216 million cases and 655 000 deaths of malaria in 2010 (2), of which 91% were reported from within the African region (3). The most severely affected areas with HIV include Zambia, Zimbabwe, Mozambique, Malawi and the Central African Republic (3), where in addition to a high prevalence of HIV (> 10%), 90% of the population is also at risk of malaria (4).

The 2005 Demographic and Health Survey (DHS) done in Rwanda, found an HIV prevalence of 3% among adults nationwide, with marked differences in prevalence by region, age and gender (5),

In the capital city of Rwanda, Kigali, prevalence was 6.7% among all adults, and 8% among adult women (5).

According to a 2002 census, Rwanda is among the 'malaria endemic' countries (6), which puts the entire population of Rwanda at risk from malaria, including an estimated 1.8 million children under five as well as 450,000 pregnant women a year. The Malaria Unit in Rwanda has classified 19 of the country's 30 districts as malaria 'endemic' and the remaining 11 as 'epidemic prone'. In both areas, transmission occurs year round, with two seasonal peaks (April-May and November-December) following distinct rainy seasons (7).

Malaria is more common in HIV-infected adults, mainly pregnant women and children, compared to among HIV-uninfected adults (1, 8, 9). Even though studies have demonstrated HIV-infection to be a potential risk factor for malaria, some studies showed that febrille illnesses attributed to malaria was lower among HIV-infected patients (10).

The fact that HIV-infected adults are more vulnerable for malaria is not unexpected, because CD4+ T cells, together with B cells and antigen-presenting cells (which are all decreased in the case of HIV infection), are essential for the immune response to malaria (11, 12). However, HIV treatment with antiretroviral therapy (ART) that is increasingly available in most African malaria endemic countries is linked to HIV viral suppression, reconstitution of immune response, and decreased mortality in HIV-infected people (3, 13). Hence, the chance for co-infection with malaria decrease.

Once a HIV-infected adult is infected with malaria, the disease becomes associated with an increase in HIV viral load and a fall in CD4+ cell count, hence implicates in worsening the clinical course of people infected with HIV (14, 15).

Knowledge of the association of HIV-infection and ART in HIV-infected Rwandan women with a simultaneous incidence of malaria will contribute to our understanding of co-infection and co-morbidity, and inform policy and public health strategies.

1.2. Literature Review

1.2.1. The dual burden of HIV and malaria in Africa

Countries in Sub-Saharan African are home to the 12% of the world's population most heavily affected by HIV-infection. About 68% of all people living with HIV-infection live in Sub-Saharan Africa, with 70% of new infections occurring in this region during 2010 (16).

A recent systematic review of 41 Sub-Saharan African countries estimated HIV-infection to have caused an average increase of 1.3% in the prevalence of malaria and 4.9% in malaria-related mortality (17). As a result, an additional 3 million of clinical malaria cases and 65 000 malaria related deaths in total, may be attributed to HIV infection in Africa every year (4).

Co-infection of HIV and malaria is a common occurrence in many areas of Sub-Saharan African and other countries, where more than 90% of the population is exposed to malaria, and the prevalence of HIV is above 10% (4, 18).

In 2009, the Centre for Disease Control (CDC) put forward guidelines on opportunistic infections in adolescents and adults and included malaria on the list of HIV/AIDS-related opportunistic infections (19).

Despite the fact that malaria is not the main cause of death among HIV-infected patients (20), it has been identified to be the third highest cause of HIV-related morbidity in Africa (1). HIV-infection is expected to increase the morbidity and mortality attributed to malaria.

This is due to the fact that immunosuppression is believed to become impaired as result of the immune response to the malaria parasites, increasing the occurrences of clinical malaria in HIV-infected individuals (14, 21).

1.2.2. Immunologic response to malaria by HIV-infected adults with and without Antiretroviral Therapy (ART)

Studies have shown that HIV-infection results in a higher incidence (22, 23) and more severe manifestations (11) of malaria, particularly amongst patients who are significantly immunosuppressed. According to the available findings, T-cell immunity in HIV-infection is impaired, which is of importance for antimalarial responses (18). In a cohort of adults in rural Malawi, a high parasite density with fever was associated with HIV-1 seropositivity and low CD4+ cell count. Furthermore, HIV-1 RNA concentrations and CD4+ cell counts were moderately but inconsistently associated with parasitemia.

HIV and malaria interaction was further established in a Ugandan population-based cohort study, where parasitemia and clinical malaria were observed to be more common among HIV-infected patients when compared to the HIV-uninfected individuals (15). Clinical malaria was significantly higher in HIV-infected individuals when compared to those who were HIV-uninfected, and the odds of clinical malaria increased with decreasing CD4+ cell counts and an advancing clinical stage. Parasitemia was significantly more common in the group with HIV-infected individuals at 11.8%, as compared to 6.3% in HIV-uninfected adults (15).

Subsequently, the effect of HIV-infection on malaria was observed by examining malaria markers including susceptibility, prevalence of malaria infection, malaria severity and disease response to treatment (21, 24, 25). More recent studies have shown that HIV-infected patients are predisposed to more frequent episodes of symptomatic severe/complicated malaria, including death (11, 14, 26).

Malaria infection seems to be rare in HIV-infected individuals who continuously use *Cotrimoxazole* and antiretroviral therapy (4, 13, 27, 28).

Although the findings on the effect of antiretroviral therapy on malaria are limited, HIV protease inhibitors were shown to have antiplasmodial effects on erythrocytic stage parasites (29). The antiretroviral protease inhibitors *Ritonavir* and *Saquinavir* affect CD36-mediated cyto-adherence, which is thought to play a role in cerebral malaria and other end-organ damage in the case of severe malaria (25)**.**

In a study done in Uganda, malaria incidence was significantly lower during ART and *cotrimoxazole* regimen than it was during *cotrimoxazole* regimen alone (IRR 0·36 [95% CI 0·18–0·74], p=0·0056) (27). The ART regimen did not include protease inhibitors.

Similarly, initiation of continuous ART with immune system recovery resulted in the reduction of malaria incidence rates (30).

A common methodological limitation to these studies was the measurement of CD4+ cell count during malaria episodes (15, 21), which may not accurately represent a given individual's immune system because malaria infection itself may cause changes to the CD4+ cell count. Thus, malaria incidence can be considered to be inversely associated to CD4+ T cell count and ART seem to reduce malaria infection rate.

1.3. Aims and objectives of the study

The principal aim of the current study was to assess the effect of HIV-infection on malaria incidence amongst a female cohort enrolled in the RWISA (Rwanda Women's Association

Study and Assessment) study. Furthermore, the aim was to assess the effect of ART on malaria incidence in HIV-infected women.

Our research aimed to answer the following questions:

- Is HIV-infection associated with higher incidence of malaria?
- Does ART utilization lower the incidence of malaria among HIV-infected Rwandan women?

1.3.1. Specific objectives

1. To describe baseline socio-demographic and clinical factors of women participating in the RWISA study;
2. To determine predictors of malaria incidence in HIV-uninfected Rwandan women;
3. To determine predictors of malaria incidence in HIV-infected women on ART against HIV-infected ART-naïve patients;
4. To compare the first, second and overall incidence of malaria in:
   a. HIV-infected against HIV-uninfected women
   b. HIV-infected women on ART against HIV-infected ART-naïve patients.

1.4. Justification of the study

HIV and malaria co-infection are common in Sub-Saharan Africa, including Rwanda, and this substantial dual burden with both malaria and HIV co-infection is the cause of increased morbidity and mortality among the individuals who contract these diseases (31, 32).

Rwanda lies within the regions where HIV-infection and malaria overlap, with 40% of Rwanda's districts classified as 'malaria epidemic prone' and the rest as 'endemic'. Although previous studies have focused on the prevalence of malaria and HIV-infection and ART utilization (33), so far, there is a paucity of information documenting the incidence of malaria in HIV patients and the effect of ART on malaria incidence in Rwanda.

Furthermore, the risks of severe malaria and malaria-related death appears to be increased significantly in HIV-infected patients of all ages that live in regions where malaria transmission is unstable (34). Therefore, it is argued here that further investigation of the effect of HIV on malaria infection and the severity of malaria is warranted. So, it is important to study and assess the factors responsible for the occurrence of malaria infection in HIV-infected patients.

Chapter 2 : Methodology

2.1. Study Design and Setting

This is a retrospective cohort study that was conducted in Kigali, Rwanda. The data was collected and analyzed from participants in the RWISA study, an observational prospective cohort study that assessed the effectiveness and toxicity of HAART (35). RWISA enrolled participants on 15 May, 2005 with follow-up visits every six months that took place through to 25 February, 2011.

2.2. Study population

RWISA enrolled a cohort of 936 women, 710 of whom were HIV-infected, and 226 were HIV-uninfected. The women were recruited from the Women's Equity in Access to Care and Treatment (WE-ACTx) clinical site in Kigali and community-based organizations, along with other HIV clinical care sites in Kigali. To be eligible, participants had to be 25 years of age or older, willing to undergo voluntary counselling and testing for HIV infection, able to complete an interview in the local Kinyarwanda language, and were required to have lived in Rwanda in 1994. All HIV-infected women enrolled in RWISA were naïve to ART at baseline, but those with clinical indications for treatment were initiated on ART after the baseline visit. HIV-infected women with prior history of receiving ART were excluded from the study, except for those women who had had a single dose *Nevirapine* to prevent mother to child transmission of HIV.

2.3. Data collection

The data was collected from all participants who met the study criteria, using standard forms at baseline every six months. The data was also collected from women who had just been initiated on ART after three months. At baseline as well as at each subsequent visit, participants underwent a physical examination and provided blood samples for CD4+ lymphocyte and a complete blood count. These measurements were undertaken at each visit for the women taking ART.

Socio-demographic characteristics such as: age; marital status; level of education; employment status; ART use history for HIV-infected women; having enough food; and residence, were collected. In addition, participants were asked whether they had contracted malaria within six months prior to the study visit. See more information on each of the variables collected can in Appendix A.

2.4. Exposure and Outcome

The effect of HIV-infection on malaria incidence among the HIV-infected women, and the effect of ART on malaria incidence were investigated and compared. Only malaria events reported during follow-up visits were considered for incidence estimation. 3 different incidence rates of reported malaria were calculated.

A malaria incident event was only considered if it occurred during/within the previous 6 months of the study visit (1). The incidence rate of first reported malaria episode was considered without considering the reported malaria at baseline visit (2).

The incidence rate of second malaria episode was established (an episode that occurred after the period of the last 6 months of the study visit, without considering the first episode) and (3) the overall incidence rate of reported malaria throughout the study period, by incorporating the first, second and all subsequent episodes of malaria.

The first 2 episodes and overall incidence were calculated according to HIV-status and ART-utilization. The third rate of malaria incidence was a combination of the first two and all-subsequent episodes.

There was no laboratory based diagnosis for malaria infection, hence malaria incidence was defined as the number of new reported malaria episodes among the study population per time at risk.

2.5. Statistical analysis

Both descriptive and analytical analyses were performed. Descriptive analysis was used to describe the study population using frequencies and proportions for categorical variables. A summary of the median and interquartile range was used for variables that are not normally distributed. Bar graphs were used for graphical display of categorical variables.

The incidence rates were calcualted using non-parametric methods by using the analysis of the survival data estimating incidence rates and to compare incidence rates across the groups (36).

Specifically, incidence rates were calculated by obtaining the number of newly reported malaria cases per person years followed by HIV status and by ART use or not from the time the participant joined the study until to the end of the study.

Kaplan-Meier curves were used to compare the probability of being malaria–free according to women's HIV status and ART-use.

In this analysis, we used log-rank, to test the level of equality across the strata from which the overall estimate of the patients' probability of malaria-free survival was drawn.

Participants that were lost to follow-up before the end of the study period and those who were still not experiencing the event at the end of study period were considered as censoring events. It was decided that the choice of origin of time would be the calendar time after the baseline visit.

Cox proportional hazard models were used to determine predictors of malaria in HIV-uninfected and HIV-infected women who were on ART and those who were ART naïve.

Univariable Cox proportional hazard models were used to model the association between independent factors of interest with the hazard of malaria.

In the multivariable Cox proportional hazards models, predictors were included in the model if the test had a p-value of 0.25 or less in the univariate analysis, except for age that was included in all multivariable models regardless of its statistical significance.

The final multivariable model was determined using a backward regression method and only those covariables were retained that had a two-sided p-value of equal to or less than 0.05. Interaction terms were tested in all the models by comparing models with and without interaction terms using likelihood ratio tests.

Unadjusted hazard ratios (HRs) and adjusted HRs were obtained with their 95% confidence intervals (CI) in all the models. The proportional hazard assumptions were assessed using Sconefeld residual test.

2.6. Ethical consideration

The RWISA study was reviewed and approved by the Rwandan National Ethics Committee and the Institutional Review Board at Montefiore Medical Centre in the Bronx, New York, USA. Informed consent included a video describing and demonstrating the study, followed by a group question and answer period, and a subsequent individual written informed consent process. Participants received transportation support, a meal while waiting, and a modest monetary incentive.

The data collected for this study did not include any personal identifiers, and a unique identifier (participant ID) identified all study participants. Permission and approval for the current study was granted by the University of the Witwatersrand Human Research Ethics Committee (Appendix B1).

Chapter 3 : Results

3.1. Introduction

This chapter presents results from the prospective observation of the RWISA cohort study, and on the association of HIV infection and HIV treatment on the incidence of malaria infection. The analysis consists of two parts. The first part describes the cohort and compares the first, second and overall malaria incidence in HIV-infected versus uninfected women with HIV-infected patients who are on ART versus patients who are ART naïve. Graphical presentations of time to event are given using Kaplan-Meier plots. The second part determines the predictors of malaria incidence according to women's HIV status and ART utilization among HIV-infected women using the Cox Proportional Hazard Models.

3.2 Baseline Characteristics of RWISA Participants

A total of 936 adult women participants were enrolled in the study by 15 May, 2005. Out of these enrolled women, 76% (710) were infected with HIV, whereas 24% (226) were not infected with the disease. Table 3.1 presents the demographic characteristics of these subjects at study entry by HIV status.

The median age of the participants was lower among HIV-infected women, at 34 years ([IQR] 30-39), when compared to that of HIV-uninfected women, at 43 years ([IQR] 34-49).

Overall 407 (43%) of the women were widowed, 111 (49%) in the HIV-uninfected group and 296 (42%) in the HIV-infected group.

A total of 527(74%) HIV-infected and 111 (49%) uninfected women said they were not living in their own houses. HIV-uninfected, 153 (68%) and 512 (72%) HIV-infected women were not employed. A high proportion in both groups indicated they never have enough food to eat: 174 (77%) of HIV-uninfected and 596 (84%) of HIV-infected women. Amongst HIV-uninfected and infected women, 123 (57%) and 473 (67%) respectively had only a primary level of education. There was a high proportion of participants who already reported having had malaria before enrolment into the study. About 185 (82%) among the HIV-uninfected women, while 563 (79%) among the HIV-infected women.

Table 3.1: Baseline characteristics of 936 adult female participants recruited in the study according to HIV status and CD4+ T cell count category

	HIV status (N=936)			**CD4+ T cell count category: HIV-infected patients (n=685)**			
Participant Characteristics	HIV- (n=226)	HIV+ (n=710)	P-value	HIV+CD4+ ≥350 (n=197)	HIV+CD4+ 200-350 (n=269)	HIV+CD4+ ≤200 (n=219)	P-value
Age, (IQR)	43(34-49)	34(30-39)	<0.001	34(29-38)	34(31-39)	34(30-39)	0.20
Marital status: n (%)							
Married	47(21)	88(12)		26(30)	41(47)	20(23)	
Single	39 (17)	192(27)	<0.001	63(34)	66(35)	58(31)	0.12
Widowed	111(49)	296(42)		72(25)	118(41)	95(33)	
Divorced	15(7)	107(15)		26(25)	39(38)	38(37)	
Missing	14(6)	27(4)		10(43)	5(22)	8(35)	
Domicile type: n (%)							
Own house	115(51)	183(26)	<0.001	59(33)	72(40)	47(26)	0.13
Otherwise	111(49)	527(74)		138(27)	197(39)	172(34)	
Employment: n(%)							
Yes	51(23)	171(24)	0.002	138(28)	200(41)	152(31)	0.37
No	153(68)	512(72)		51(30)	63(37)	55(33)	
Missing	22(10)	27 (4)		8(31)	69(23)	12(46)	
Education: n (%)							
None	67(32)	156(23)		47(31)	58(39)	45(30)	
Primary	123(57)	473(67)		132(28)	187(41)	138(30)	0.12
Secondary	25(11)	73(10)	<0.001	16(23)	23(33)	31(44)	
Missing	11(5)	19(2)		2(25)	1(12)	5(62)	
Enough food to eat: n (%)							
Always	29(13)	97(14)		25(26)	35(37)	35(37)	0.60
Never	174(77)	596(84)	<0.001	166(29)	230(40)	178(31)	
Missing	23(10)	17(2)		6(37)	4(25)	6(37)	
Ever had malaria: n (%)							
Yes	185(82)	563(79)		153(28)	14(39)	179(33)	
No	29(13)	136(19)	<0.001	41(32)	51(40)	37(29)	
Missing	12(5)	11(1)		3(30)	4(40)	3(30)	0.89

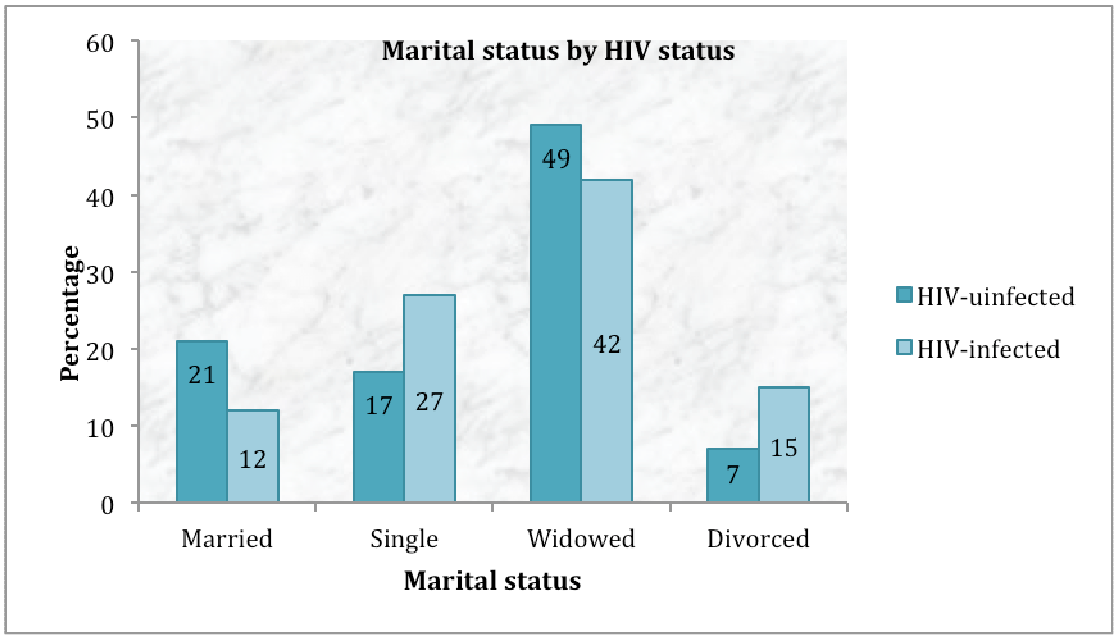

Figure 3.1: Distribution of 936 study participants at baseline according to HIV status and marital status, 2005

From the figure above, it is noted that widowed women were the majority in the cohort.

Table 3.2: Incidence rates (IRs) and hazard ratios (HR's) with 95% confidence intervals (CIs) of first episode and second episode of malaria and overall incidence rate of malaria, by HIV status and ART from 2005 to 2011

Malaria incidence	IR (95% CI) [a]	Unadjusted			Adjusted [b]		
HIV-status		**HR**	**P-value**	**95% CI**	**HR**	**P-value**	**95% CI**
First episode: (N=452)							
Negative (n=115)	149.1 (124.2-178.9)	Ref.			Ref.		
Positive (n=337)	142.5 (128.2-158.6)	0.9	0.71	0.77-1.18	0.9	0.69	0.76-1.19
Second episode: (N=202)							
Negative (n=60)	103.0 (79.9- 132.6)	Ref.			Ref.		
Positive (n=142)	110.8 (94.0-130.7)	1.1	0.44	0.82-1.52	1.0	0.81	0.75-1.43
ART-utilization							
First episode: (N=449)							
ART-use (n= 195)	130.2 (113.2-149.9)	Ref.			Ref.		
ART naïve (n= 254)	156.8(138.7-177.3)	1.0	0.75	0.85-1.24	1.0	0.73	0.85-1.25
Second episode: (N=200)							
ART-use (n= 110)	104.4(86.6-125.8)	Ref			Ref		
ART naïve (n= 90)	113.2 (92.1-139.2)	1.0	0.99	0.75-1.32	1.1	0.55	0.96-1.00
Overall incidence							
HIV-status:							
Negative (n=217)	22.4 (91.6-25.5)	Ref.			Ref.		
Positive (n=552)	18.8(17.3-20.4)	0.8	0.06	0.73-1.00	0.8	0.02	0.69-0.97
ART-utilization:							
ART-use (n=368)	17.0 (15.4-18.8)	Ref.			Ref.		
ART-naïve (n=396)	23.6 (21.4-26.0)	1.2	0.05	1.00-1.33	1.2	0.03	1.01-1.36

[a] Per 1000 person-years CI: Confidence Interval

[b] Cox proportional-hazards models adjusted for age

3.3. Comparison of Malaria Incidence Rates by HIV status

A total of 857 (92%) adult women had follow-up visits of 6 months after the baseline visit. Of these 769 (90%) reported having contracted malaria during the follow-up period.

The number of first malaria episodes was 115 (53%) among 217 HIV-uninfected women, and 337 (61%) among 552 HIV-infected persons.

Among HIV-uninfected women having experienced a first episode of malaria and subsequent follow-up visits, 60 experienced a second episode 180 days after the first episode. This corresponds to an incidence rate of 103.0 per 1000 person-years (95% CI, 79.9–132.6/1000person-years). Among HIV-infected women with a first malaria episode, 142 experienced a second episode, giving an incidence rate of 110.8/1000 person-years (95% CI, 94.0–130.7/1000 person-years). There was, however, no significant association between HIV and malaria first and second episodes (Table 3.2). Nevertheless, a significant association was observed when all episodes occurring during the study period were analysed (overall malaria incidence). After adjustment, it was noted that HIV-infected patients were 20% less likely to report malaria compared to HIV-uninfected women [adjusted HR=0.8, 95 % CI (0.69- 0.97)]. This corresponds to an IR, 22.4 (95% CI, 91.6-25.5/1000 person-years) in HIV-uninfected and IR, 18.8 (95%CI, 17.3-20.4/1000 person -years) in HIV-infected individuals.

3.4 Comparison of Malaria Incidence Rates by Antiretroviral Therapy

Further analysis of HIV-infected women according to ART status, i.e. on ART or ART naïve (Table 3.2) shows a total of 764 (89%) women who have reported malaria infection during the study follow-up period. The number of first malaria episode was 195 (64%) among 305 HIV-infected women who were already on ART, and 254 (45%) among 568 HIV-infected women who were ART naïve.

It was observed that the incidence rate of first reported malaria episode was higher in HIV-infected ART naïve women compared to HIV-infected women on ART.

After an interval of 6 months, the incidence rate of second malaria episode in ART-naïve women was still higher corresponding to an incidence rate of 113.2 (95% CI, 92.1–139.2/1000person -years), as compared to 104.4 (95% CI, 86.6-125.8/1000person -years) HIV-infected women, who were on ART. There was a significant association between ART-naive individuals with the hazard of developing malaria. ART-naïve patients were 1.2 times more likely to develop malaria infection when compared to HIV-infected individuals on ART, [adjusted HR=1.2, 95 % CI (1.01- 1.36)]. This corresponds to an overall incidence rate of 17.0 (95% CI, 15.4–18.8/1000 person-years) in HIV-infected women on ART, and 23.6 (95% CI, 21.4–26.0/1000 person-years) among ART-naïve patients.

Hence, it was observed that malaria incidence was highest in HIV-infected ART naïve participants, followed by HIV-uninfected participants and lowest among HIV-infected participants who were on treatment.

3.5. Cumulative Incidence Rate of Malaria by HIV status

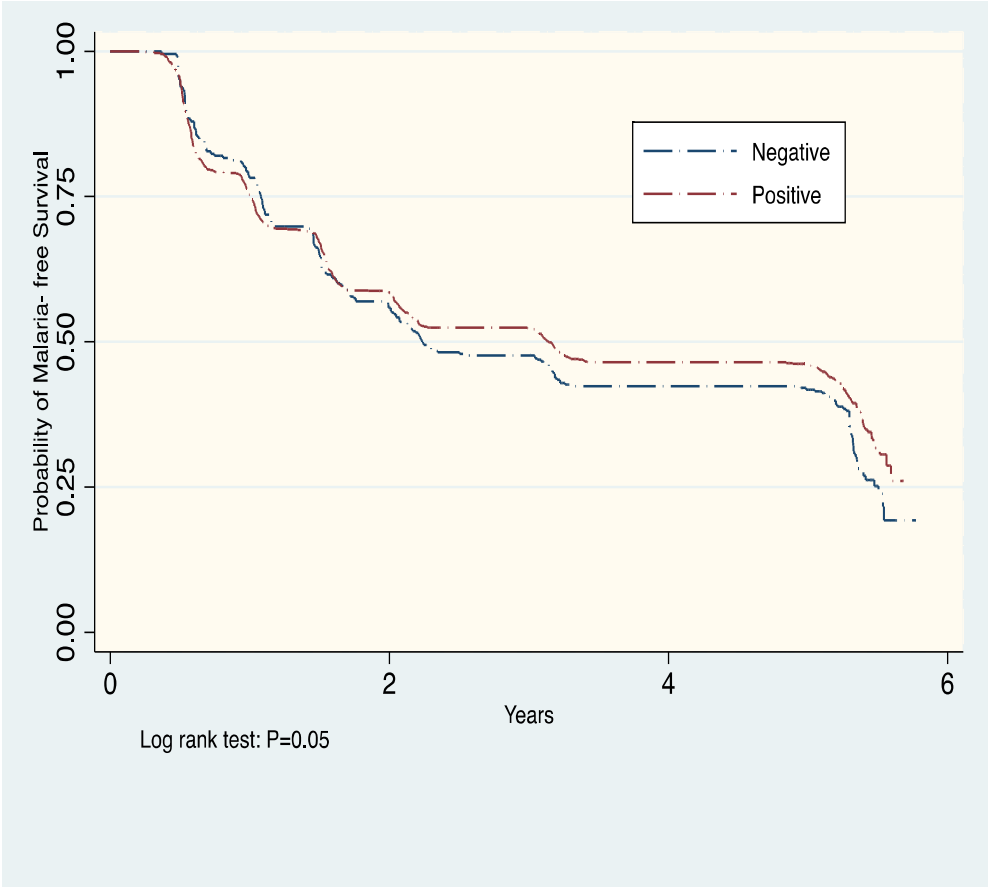

Figure 3.2: Kaplan-Meier curves by HIV status

From the Kaplan Meier curves by HIV status (Figure 3.3), it is noted that until 1 year of follow-up, HIV-infected individuals held a relatively high probability of experiencing malaria, compared to HIV-uninfected individuals. As the time of follow-up went by, HIV-uninfected women experienced higher chances of malaria compared to HIV-infected individuals.

3.6. Cumulative Incidence Rate of Malaria according by ART utilization

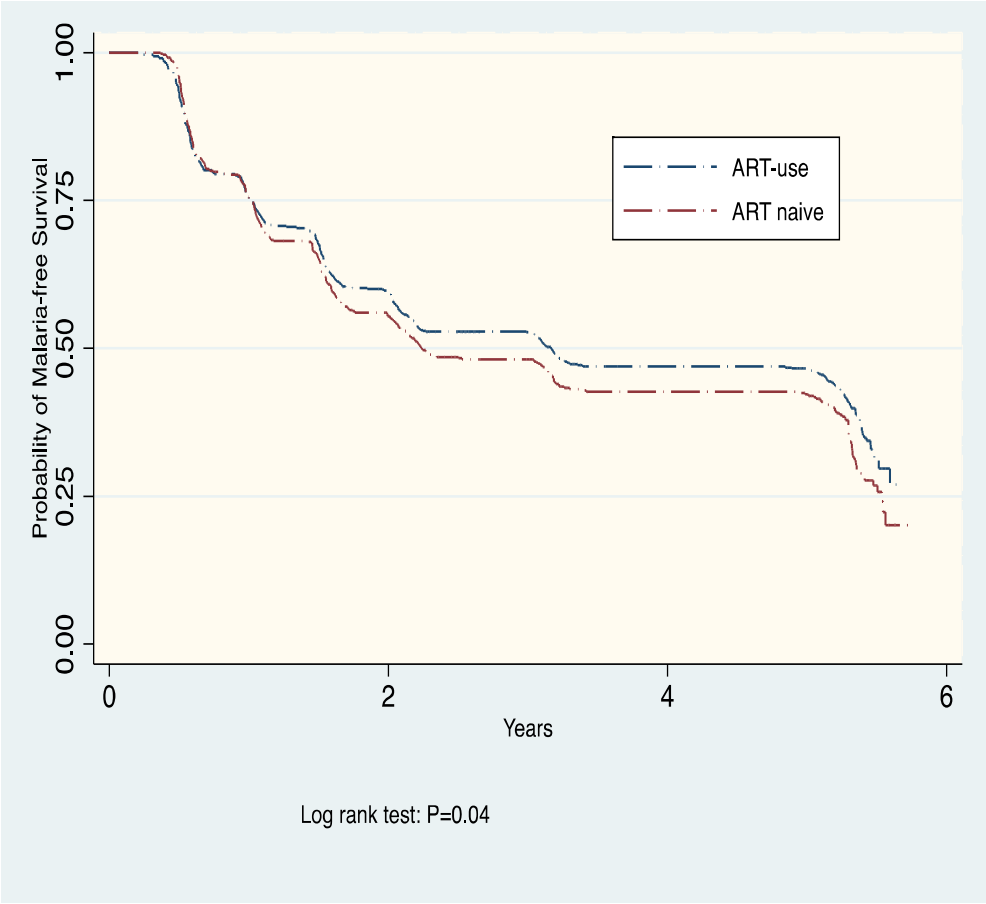

Figure 3.3: Kaplan-Meier curves by Antiretroviral Therapy

Both groups (on ART and ART naïve) shared almost the same malaria infection experience during the early years of follow-up (i.e. the few years after the initiation of ART) (Figure 3.4). From 6 months to 1 year follow-up, HIV-infected women who were not on treatment experienced a higher chance of contracting malaria compared to those who were on ART.

Note that the step function at the beginning is due to the fact that there is no cumulative number of events or number at risk. More specifically, it took 6 months to have a total number of reported malaria events after the baseline visit and the ART initiation.

3.7. Predictors of Malaria Incidence in HIV-uninfected Women

The total follow-up period from 15 May, 2005 to 25 February, 2011 yielded a total of 769 reported cases of malaria, with the rate 119.27 malaria cases per 100 person years in a total of 857 participants followed up upon after the baseline visit. Table 3.3 below gives the Hazard Ratios of reported malaria amongst HIV-uninfected women participants.

In the univariable analysis, season and domicile were marginally associated with the hazard of malaria in HIV-uninfected women. Marital status, level of education, having enough food and employment status was not associated with the malaria hazard in HIV-uninfected women.

The multivariable analysis showed a significant association between age and HIV-uninfected women with the hazard of developing malaria. Every 10-year increase in age was associated with a 10% lower hazard of developing malaria in HIV-uninfected women [adjusted HR=0.9, 95 % CI (0.97- 0.99)].

Patients who were living in their own houses were 30% less likely to report malaria compared to individuals who were living somewhere else [unadjusted HR=0.7, 95 % CI (0.60- 1.02)]. After further adjusting for age, employment and season, however, the effect size was attenuated, and lost its marginal statistical significance [adjusted HR= 0.9, 95 % CI (0.64-1.19]. Similarly, there was no association between employment status and HIV-uninfected individuals with the hazard of developing malaria [adjusted HR=0.7 95 % CI (0.45-1.05)]. During dry seasons, HIV-uninfected individuals were 1.4 times more likely to develop malaria when compared to the rainy seasons [adjusted HR= 1.4, 95 % CI (1.07- 1.93)].

Table 3.3: Univariable and multivariable associations of malaria incidence in HIV-uninfected women from 2005 to 2011 (n=226)

	Univariable			Multivariable		
Characteristic	**HR**	**p-value**	**95 % CI**	**HR**	**p-value**	**95% CI**
Age, per 10 year increase	0.9	0.03	0.97-0.99	0.9	0.01	0.97- 0.99
Marital status:						
Married	Ref.					
Single	0.8	0.45	0.54-1.31			
Widowed	0.7	0.25	0.54-1.07			
Divorced	1.0	0.98	0.56-1.79			
Missing	0.9	0.92	0.56-1.67			
Employment:						
Yes	Ref.			Ref.		
No	0.6	0.11	0.47-1.08	0.7	0.09	0.45-1.05
Education:						
None	Ref.					
Primary	1.0	0.65	0.78-1.46			
Secondary	1.2	0.38	0.77-1.94			
Missing	1.3	0.38	0.72-2.33			
Enough food to eat:						
Always	Ref.					
Not always	1.4	0.73	0.19-10.23			
Never	2.8	0.30	0.39- 20.37			
Domicile:						
Not own house	Ref.			Ref.		
Own house	0.7	0.08	0.60- 1.02	0.9	0.40	0.64-1.19
Season:						
Rainy	Ref.			Ref.		
Dry	1.3	0.06	0.97-1.88	1.4	0.05	1.07-1.93

Ref. indicates Reference group

3.8. Predictors of Malaria Incidence in HIV-infected Women on ART

Since understanding ART use is one of the main aims of this study, the focus was on all HIV-infected women who were initiated on ART after the baseline visit.

Table 3.4 shows the predictors of malaria incidence in HIV-infected patients who were initiated on ART after the baseline. There was a significant association between employment status and education level and HIV-infected patients on ART with the hazard of developing malaria. For those individuals who were on ART and not working, the hazards of developing malaria were 1.5 times greater compared to those individuals who were employed [adjusted HR=1.7,95 % CI (1.17-1.93)].

During the follow-up period, it was observed that the hazard of developing malaria in HIV-infected women on ART was 40% less in individuals who had primary level of education compared to women who had no basic education. This was statistically significant [adjusted HR=0.6, 95 % CI (0.47- 0.75)]. Similarly, women who had secondary level of education ran a 30% lower risk of developing malaria during the follow-up compared to women who had no basic education [adjusted HR=0.6, 95 % CI (0.44- 0.97)]

Table 3.4. Univariable and multivariable associations of malaria incidence in HIV-infected women on ART from 2005 to 2011 (n=586)

	Univariable			Multivariable		
Characteristic	**HR**	**P-value**	**95 % CI**	**HR**	**p-value**	**95% CI**
Age, per 10 year increase	0.9	0.24	0.97-1.00	0.9	0.18	0.97-1.00
CD4+ cell count baseline (cells/µl)	1.0	0.02	1.00-1.02	1.0	0.03	1.00-1.14
Marital status:						
Married	Ref.					
Single	1.2	0.36				
Widowed	1.1	0.52				
Divorced	1.0	0.84				
Missing	0.9	0.85				
Employment:						
Yes	Ref.			Ref.		
No	1.4	0.003	1.13- 1.86	1.5	0.001	1.17-1.93
Education:						
None	Ref.			Ref.		
Primary	0.6	<0.001	0.49- 0.77	0.6	<0.001	0.47- 0.75
Secondary	0.7	0.04	0.45-0.98	0.7	0.03	0.44-0.97
Missing	0.7	0.52	0.38-1.62	0.8	0.43	0.36-1.55
Enough food to eat:						
Always	Ref.					
Not always	1.0	0.91	0.34-3.37			
Never	2.1	0.26	0.67-6.61			
Domicile:						
Not own house	Ref.			Ref.		
Own house	0.8	0.16	0.65-1.07	0.9	0.56	0.71-1.20
Season:						
Rainy	Ref.					
Dry	1.1	0.34	0.88-1.41			

Ref. indicates Reference group

3.9. Predictors of Malaria Incidence in HIV-infected ART-naive Women

From the adjusted hazard ratios (shown in Table 3.5), it was observed that HIV-infected naïve women who were not working were 1.5 more likely to develop malaria compared to women who were working. This was statistically significant, [adjusted HR=1.5, 95 % CI (1.05- 2.08)].

Primary level of education was associated with a 40% lower hazard of developing malaria in patients who were ART naïve, when compared to patients who had no basic education [adjusted HR=0.6, 95 % CI (0.47- 0.89)]. If the level of education is altered from basic to secondary level of education, while holding the effect of other variables constant, the hazard of developing malaria decreased to 50% and this was statistically significant [adjusted HR=0.5, 95 % CI (0.24-0.98)].

HIV-infected naïve patients were 40% less likely to report malaria compared to the malaria hazard estimates during the periods that follow the distinctly dry seasons from May to June and from November to December, after holding the effects of other predictors in the model constant. This effect was statistically significant [adjusted HR=0.6,95 % CI (0.43-0.88)].

Table 3.5: Univariable and multivariable associations of malaria incidence in HIV-infected ART –naïve women from 2005 to 2011 (n=377)

	Univariable			Multivariable		
Characteristic	HR	P-value	95 % CI	HR	P-value	95% CI
Age, per 10 year increase	1.0	0.27	0.99-1.03	1.0	0.84	0.98-1.02
CD4+ cell count baseline (cells/µl)	1.0	0.78	0.99-1.00			
Marital status:						
Married	Ref.					
Single	0.9	0.71	0.53-1.52			
Widowed	1.2	0.42	0.75-1.95			
Divorced	0.9	0.76	0.50-1.65			
Missing	0.4	0.15	0.12-1.38			
Employment:						
Yes	Ref.			Ref.		
No	1.4	0.06	0.98-1.94	1.5	0.02	1.05-2.08
Education:						
None	Ref.			Ref.		
Primary	0.6	0.01	0.47-0.90	0.6	0.008	0.47-0.89
Secondary	0.5	0.10	0.29-1.11	0.5	0.04	0.24-0.98
Missing	0.3	0.29	0.04-2.56	0.2	0.23	0.03-2.18
Enough food to eat:						
Always	Ref.			Ref.		
Not always	1.1	0.86	0.16-8.62	1.1	0.90	0.15-8.26
Never	3.0	0.27	0.41-21.74	3.0	0.27	0.41-21.98
Domicile:						
Not own house	Ref.					
Own house	1.0	0.73	0.77- 1.44			
Season						
Rainy	Ref.			Ref.		
Dry	0.6	0.008	0.44-0.88	0.6	0.008	0.43-0.88

Ref. indicates Reference group

Chapter 4 : Discussion

Few studies have evaluated the effect of HIV-infection and ART on malaria infection, and to the best of the investigator's knowledge only one study has examined prevalence of malaria and helminth infection in HIV-infected pregnant women on ART in Rwanda (33). In this study, the prevalence of, and the protective risk factors for helminth and malaria infections were determined in pregnant HIV-infected Rwandan women receiving ART. The current study specifically investigated the incidence rates of malaria according to HIV serostatus and according to ART use among HIV-infected women. This study further investigated the predictors of malaria incidence in HIV-infected and HIV-uninfected women (not specifically pregnant women) and the predictors of malaria incidence among HIV-infected women on ART with those who were ART naïve.

4.1. Comparison of Malaria Incidence Rates by HIV Status

It was found that HIV-uninfected Rwandan women participating in the RWISA study had a relative high risk of malaria when compared to HIV-infected women. This study shows that overall, incidence rates of reported malaria in HIV-uninfected women was higher than that of HIV-infected individuals, during the study period.

Our findings are consistent with earlier studies conducted in the former Zaire (DRC) (37), Malawi (38) and Uganda (39), which have already provided evidence that malaria is lower in HIV-infected than in HIV-uninfected individuals. In most of these studies however, malaria infection was limited to children.

Recent studies in Maputo, Mozambique (10), have observed malaria incidence to be lower in HIV-infected adults than in HIV-uninfected adults.

The low rate of reported malaria among HIV-infected patients might have been confounded by pre-use of *cotrimoxazole* prophylaxis, which was found to have a protective effect on malaria infection in children (40) and adults (41) and antimalarial drugs (42) and a probable awareness about the nature of transmission of malaria parasites since HIV-infected patients are sensitized in relation to their health. In this case, control and preventive measures must have been put in place.

The reporting of malaria incidence rate was high during the combination of all reported cases during the study period. This supports the notion that the primary reason for the increased incidence may be the lack of making use of malaria prevention measures compared to HIV-infected women who were probably more conscious about their health. However, it is not known whether or not similar results would be found in other malaria endemic regions with different malaria control measures.

4.2. Comparison of malaria incidence rates by Antiretroviral Therapy among HIV-infected Women

First-line ART regimens used in Rwanda included two Nucleoside Reverse-Transcriptase Inhibitors (NRTI) and a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI), either in individual or fixed-dose combination tablets (43).

The current study demonstrated that, after the initiation of ART, the overall incidence rates of reported malaria was significantly higher in HIV-infected ART-naïve women compared to HIV-infected women who were on ART.

The decreased reported incidence of malaria infection among patients on ART may likely be due to the reconstitution of their immune system, associated with the administration of the drugs (44). This result is consistent with a laboratory-based reports from the neighbouring country of Uganda where antiretroviral treatment was associated with a 75% decline in the incidence of malaria in HIV patients on ART (30). Another study in Uganda found a low prevalence of malaria among asymptomatic ART treated HIV-infected patients (45). Similarly, a more recent study from Nigeria (46) revealed a low prevalence of asymptomatic malaria among HIV patients on HAART.

Rwanda has made significant progress in scaling up its malaria control interventions. The statistics from the Ministry of Health shows a decline in malaria cases since 2005 (President's Malaria Initiative, Rwanda, 2012). In the past five years, there has been more than a 50% decline in confirmed malaria cases, admissions and deaths, in district hospitals in Rwanda since 2005. This was due to a marked increase in the use of Insecticide-Treated mosquito Nets (ITNs) as well as in the use of *Artemisinin*-based Combination Therapy (ACT). The decline has occurred across all ages (7)**.**

Even though there were high measures of malaria control in Rwanda – built on performance-based decentralized health system – the high incidence of malarial infection among patients not on ART could be due to their exposure to mosquito bites in their various places of residence, which had not seen the institution of malaria control measures.

Some of the study participants were from a poor socio-economic background and came from a suburb of the city of Kigali where malaria infection is likely to be high and where there may be poor environmental sanitation with presence of stagnant water and bushes around houses.

4.3.Predictors of Malaria Infection in the Cohort

Lack of employment was found to be a major predictor of malaria infection within the study sample except for HIV-uninfected women group. This may be explained by the fact that the majority of HIV-infected individuals had low levels of education, which is taken as a proxy measure of employment and skills. Similar studies revealed the same findings of notable differences between the lowest and highest income groups, with the annual risk of malaria being greater for the poorest amongst them (47, 48).

Similarly, individuals with no basic education had a higher risk of malaria when compared to individuals with primary or secondary level of education. This finding is consistent with previous studies, which found that higher levels of education were associated with improved knowledge and practices in relation to appropriate prevention and treatment strategies of malaria infection (48, 49). However, the level of education was not a significant risk factor for malaria among HIV-uninfected women.

In 2009, Rwanda introduced 9 Years of Basic Education (9YBE), which provides all Rwandan children with nine years of free and, in theory, compulsory education between the ages of 7 and 15. The subsequent progress in providing access to primary education has been impressive (Rwanda education statistics, 2012). This might have had an impact on the effect of lack of education that it may be having with regards to the risk of developing malarial infection.

It was also found that the hazard of malaria incidence increased with age amongst HIV-uninfected women. The finding agrees with a study in Nigeria (50), where a similar pattern was observed in HIV-uninfected individuals.

There is an indication that increasing age may be a factor for HIV-infected individuals, but the sample size of our analysis may not hold for the analysis of sub-groups.

A positive association between reported malaria and CD4+ cell count among HIV-infected individuals was noticed. There was, however, no statistical difference observed between the two groups. Other studies reported previously have found an association between low CD4+ cell count and malaria infection (46),(40).

Even though malaria transmission occurs all year-round in Rwanda, the findings show that HIV-uninfected, and HIV-infected individuals who were ART-naïve, were at a great risk of malaria infection during periods of higher precipitation, namely between April-May and November-December. This may be explained by individuals who have low immunity (HIV-infected naïve) or who may have lost the naturally acquired immunity to malaria infection which coincides with the 'malaria season' (44).

4.4. Strengths of the Study

This is one of the few adequately powered studies in Rwanda that investigates the incidence of malaria in HIV-infected and uninfected Rwandan women. The study has enabled the investigator to specifically address malaria incidence among HIV infected individuals on ART and ART-naïve in comparison with uninfected group with regard to predictors of malaria incidence.

The investigator believes that these findings are potentially applicable to other African settings where co-existence of malaria and HIV infection is prevalent.

Furthermore, the repetitive measures over a 5-year period at every 6-months interval provides further information about the onset, incidence and prediction of malaria and HIV co-infection, and about within-individual change.

4.5. Limitations of the study

The major limitation of the study was that the study used secondary data rather than conducting a laboratory-based malaria parasite testing. In addition, the investigator was neither able to link malaria history with a participant's medical files, nor use fever as a proxy measure of malaria infection.

Since some participants did not report to the study site, especially during the sixth visit, this study has not possibly captured all the relevant visits and episodes of malaria, resulting in the under-reporting of malaria incidence.

Further information missing for the current analysis such as insecticide-treated net use, and other malaria prevention efforts, might have influenced the rate of malaria observed in our study. Each of these factors may have influenced malaria incidence in certain individuals and may have led to a residual confounding of variables in our measures on effect.

Other limitations reflected the nature of the cohort. It should be taken into consideration that the cohort included only women, so the results do not fully represent the general Rwandan population.

Chapter 5: Conclusion and Recommendations

5.1.Conclusion

HIV treatment was associated with lower incidence of malaria compared to HIV naïve patients. A high incidence rate of reported malaria in HIV-infected women not on treatment compared to those who were on ART during the follow-up period was noted. It was also observed that HIV-uninfected women were more likely to report malaria compared to HIV-infected counterparts.

Generally, low levels of education and illiteracy and unemployment were significantly associated with the risk of malaria in HIV-infected women on treatment and in women who were not on treatment.

Another finding was that an increase in age was significantly associated with the risk of developing malaria in HIV-uninfected women.

5.2. Recommendations and Policy implications

The evidence for higher reported incidence rates of malaria is strong, and is consistent in HIV-infected adult women not on ART. This justifies the need for intensified malaria prevention and control strategies in areas where co-infection with malaria and HIV is common.

In an effort to minimize the risk of malaria infection among HIV-infected women not on ART, there is a need for policy makers to better understand the factors associated with the development of malaria infection.

For the prevention of malaria, empiric deworming in areas where helminth infection is common (51) and the use of insecticide-treated bed nets may be combined with counselling on HIV disease for ART-naïve persons who have malaria.

As a policy, malaria prophylaxis should be considered for HIV-infected persons in order to prevent malarial infection and to reduce malarial illness.

At a minimum, the seasonal variation of malaria in Rwanda should be taken into consideration when planning media campaigns, educational programs and bed-net distributions. These programmes should be designed to make people aware of malaria and of how to prevent it prior to the increase risk of malaria from April to May and from November to December.

Further studies on the duration of effect of ART on malaria after ART initiation in non-pregnant women are recommended.

Severe malaria with HIV co-infection has been shown to be common in settings where the two disease overlap (40). Further studies directly assessing this topic are required in order to validate this association. Furthermore, studies based on clinically diagnosed malaria are highly recommended.

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Appendices

Appendix A: A summary of individual-level variables

Variable	Definition	Time of data collection	Variable type	Variable coding
Age	Continuous variable modelled as per 10 year increase (= age/10)	Baseline	Independent	N/A
Marital status	Ordered variable	Baseline	Independent	Married=1 Single =2 Widowed=3 Divorced=4
Employment status	Categorical variable	Baseline	Independent	0=No 1=Yes
CD4 count at baseline	Continuous variable	Baseline	Independent	NA
Monthly income	Continuous variable	Baseline	Independent	N/A
Education level	Ordered variable	Baseline	Independent	1=None 2=Primary 3=Secondary
Enough food	Ordered variable	Baseline	Independent	1= always 2=not always 3=Never
Domicile type	Categorical	Baseline	Independent	0 = otherwise 1 = own house
Season	Categorical	Baseline	Independent	1 = rainy 2 = non-rainy
HIV status	Categorical	Baseline	Independent	0 = negative 1 = positive
ART use	Categorical	Follow-up visit	Independent	1 = Yes 2 = No
Malaria since last visit	Categorical	Every 6 months	Dependent	1=Yes, 2=No

Appendix B University of the Witwatersrand Human Research Ethics Clearance Certificate for the study

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Jacqueline Umunyana

CLEARANCE CERTIFICATE **M121037**

PROJECT Comparison of Malaria Incidence in HIV-Infected and Uninfected Rwanda Women from 2005-2008

INVESTIGATORS Dr Jacqueline Umunyana.

DEPARTMENT School of Public Health

DATE CONSIDERED 26/10/2012

DECISION OF THE COMMITTEE* Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 26/10/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...