Metabolic Syndrome following Hypertensive Disorders of Pregnancy and among HIV Infected Adults: Understanding Cardiovascular Disease Risk

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2018

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Public Health-Epidemiology

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Abstract

Metabolic syndrome following hypertensive disorders of pregnancy and among HIV infected adults: understanding cardiovascular disease risk

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In this dissertation we discuss the findings of two studies that describe the burden of the Metabolic syndrome (MetS) in Kenya, a low resource setting with increasing incidence and prevalence of cardiovascular disease (CVD) and other non-communicable diseases (NCD.). In

these two complementary studies, we evaluate the risk of MetS in two different study populations, HIV-infected adults and HIV-uninfected postpartum women.

In the first study, we use recently collected cross-sectional data to examine the link between HIV and NCD specifically CVD by estimating the prevalence of MetS in HIV infected adults and evaluating the association between use of antiretroviral therapy (ART) and MetS. We found that the prevalence of MetS did not differ between ART experienced and ART naïve groups (16.9% versus 15.2%). However, ART experienced patients had higher prevalence of elevated fasting blood sugars and lower prevalence of low high-density lipoprotein (HDL)-cholesterol. The prevalence of the other components of the MetS; abnormal waist circumference, elevated blood pressure and hypertriglyceridemia were comparable between the two groups. Older age, female sex and high body mass index were independently associated with diagnosis of MetS.

The second and main study, is a six-month postpartum prospective cohort nested within a longer 3-year cohort of postpartum women. In this study, the exposed group comprised women with two specific hypertensive disorders in pregnancy (HDP), gestational hypertension and preeclampsia, while the unexposed group were normotensive. In this section of the dissertation we evaluated: 1) the prevalence of MetS between women with and without history of HDP, 2) the sociodemographic and clinical correlates of MetS, and 3) the association between the inflammatory markers, specifically high-sensitivity C-reactive protein (hsCRP) and MetS, overall and stratified by exposure to HDP. All the analyses were conducted at 6 months postpartum. Compared to women without HDP, the risk of MetS was 3 times greater among women with HDP. Also, the risk of 3 of the 5 components of MetS (fasting plasma glucose, triglycerides, and blood pressure) was elevated 3 or more times among those with compared to those without HDP. MetS was independently associated with HDP, higher body mass index, and below secondary level of

education. The mean hsCRP level and proportions of high relative CVD risk hsCRP (>3mg/L) levels were higher in women with MetS compared to those without MetS and were significantly different when women with HDP were compared with those without HDP.

From this dissertation, composed of two studies conducted in a low resource setting of sub-Saharan Africa, we come to two conclusions. First, we find evidence of similar risk of MetS comparing ART experienced and ART naïve adults, suggesting that traditional CVD risk factors rather than HIV may have a larger contribution to the CVD risk in this population. Secondly, increased risk of MetS among women with HDP versus those without HDP provides evidence that HDP is associated with increased risk of CVD. Also, women with MetS and those with HDP in particular were more likely to have elevated hsCRP levels supporting the role of chronic inflammation and evaluation of inflammatory biomarkers following HDP to estimate the risk of MetS and therefore CVD risk. The elevation of specific components of MetS namely triglycerides, fasting blood glucose, and blood pressure and increased levels of inflammatory marker, hsCRP are important in understanding future research priority areas. Interventions to reduce CVD risk among women after HDP may need to modify these specific risk factors, use inflammatory markers to triage risk and could include dietary changes, exercise, and use of lipid and blood pressure lowering medications, especially statins because they can also reduce inflammation.

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ACKNOWLEDGEMENTS

First, I would like to highly acknowledge my dissertation committee led by the Chair, Carey Farquhar, MD, MPH, and members, Stephanie Page, MD, PhD, Brandon Guthrie, PhD, Barbra Richardson, PhD, and Stephen Polyak, PhD, Graduate Student Representative, for their support, availability, patience, mentorship and encouragement.

I would also like to recognize the Metabolic Syndrome after Delivery (MetSA) Study Team at Kenyatta National Hospital, Nairobi Kenya (Nurses: Margaret Nyawira, Emma Maitha; Community Health Workers: Grace Achieng, Emily Makokha; Coordination and Management: Teresiah Njambi; and Data Manager: Mark Sigei). I am indebted to the study participants and their families for their time and dedication towards the success of this research.

I thank the University of Washington and especially the Department of Epidemiology for the comprehensive provision of academic resources, skills and knowledge during my graduate training without which the academic rigor and scope would have not been realized.

I would like to acknowledge the funding for the study by the Fogarty International Center and the Office of Research on Women's Health (ORWH) of the National Institutes of Health under Award Number K43 TW010363 and the International AIDS and Research Training Program (IARTP)– under the award number D43 TW000007. Without their fiscal support my Graduate School training and this study would have not been successful.

Lastly, I would like to offer special acknowledgement to my family, my lovely boys and my parents for their support and sacrifices over the years I spent studying especially towards this degree.

DEDICATION

This dissertation is dedicated to all pregnant and postpartum women with and without hypertension in pregnancy who sacrifice themselves knowing the risks of pregnancy to their own lives for the sake of their unborn children, their newborns and their male partners.

To my late grandmother Peres Agik "Nyajanandi", a prayer warrior, my mother Hellen Osoti (great upbringing and sacrifices, and now long-term effects of hypertension during pregnancy). You provided Godly upbringing and environment that nurtured me from the shores of Lake Victoria to that of Pacific Ocean.

To my family, the boys Randy, Rowley, Raanan, and Ryker you all kept me going not forgetting the supporting, loving, caring and committed mom. To my father Bernard Osoti Agik, you laid the academic foundation and sacrificed for me to be who I am, my sister Irene and all my siblings for encouragement and support.

Chapter 1. INTRODUCTION

1.1 HYPERTENSIVE DISORDERS IN PREGNANCY AND CARDIOVASCULAR DISEASE RISK

Approximately 90% of the 16 million premature annual deaths from non-communicable diseases (NCDs) occur in low- and middle-income countries (LMIC), and 37% of these are from cardiovascular diseases (CVD)[1]. CVD is the leading cause of mortality globally, and more than three quarters of these deaths occur in LMIC[2,3]. Despite the progress in reducing CVD mortality, this decline has not been sustained in younger adults below 55 years and especially women[4]. CVD causing 1 in 3 deaths in women and remains the leading cause of death among women globally[5-7]. Compared to high-income countries where the causes are predominantly atherosclerotic, more than half of CVD in sub-Saharan Africa (SSA) is thought to be non-atherosclerotic and a large portion is mainly from inflammatory pathology[7]. However, data from SSA is scarce. Because CVD occurs among younger people in SSA, it disproportionately affects women of childbearing and childrearing age[2,7]. High antenatal clinic attendance, facility delivery and postnatal follow-up in SSA provides an opportunity to identify, follow and manage women at risk of CVD by identifying those with pregnancy related CVD risk factors. Hypertensive diseases in pregnancy (HDP) is associated with more than 2-fold increased risk of future CVD[5,6,8-10], however, it is not known how or why this is the case and there is no data from SSA on this topic.

How HDP leads to CVD is not established, however women with metabolic syndrome (MetS) are at higher risk for HDP and CVD, suggesting a shared pathway[11-13]. MetS refers to a complex cluster of at least three of the following 5 components that increase the risk of CVD and

type 2 diabetes: low high-density lipoprotein (HDL) cholesterol, high triglycerides, elevated arterial blood pressure, abdominal obesity and high fasting blood glucose[14,15]. Whereas each of these components increase the risk of CVD, when present together the risk is multiplied[15-17].

The underlying mechanisms linking MetS and CVD have not been fully understood. However, chronic, low-level inflammation that includes angiogenesis, endothelial injury, and oxidative stress are thought to play a role in this shared pathophysiology[18,19]. Thus, elevated levels of inflammatory markers like high-sensitivity C-reactive protein (hs-CRP) and cytokines such interleukin-6 (IL-6), in patients with HDP and CVD, suggest a shared pathogenesis[20-22]. Also, the three conditions, HDP, MetS and CVD share in their pathology increased inflammatory markers and immunological factors[20,23]. Although these inflammatory processes reverse postpartum, some women may have slow reversal or continued chronic inflammation, which may be seen in elevated inflammatory markers. Such markers may be used to identify women with MetS[18,24].

The prevalence of MetS is increasing worldwide and is closely linked to the presence of obesity, which now averages 20-39% in the non-pregnant adult population in high-income countries[25]. Similar prevalence has been reported in Kenya and Ghana where the prevalence of MetS is also high, especially among non-pregnant women (40%) compared to men (29%)[26-28]. Although high-income countries have recommendations for postpartum care and follow-up of women with HDP that target prevention of MetS and CVD, the LMICs lack studies on CVD risk postpartum and therefore do not have mechanisms or policies to identify and manage high-risk women for CVD prevention.

1.2 HIV AND METABOLIC SYNDROME

In high income countries, CVD has become more prevalent among people living with HIV infection [29,30] and this is likely the result of many factors, including antiretroviral therapy, CVD risk factors, such as tobacco use, as well as chronic inflammation and immune activation associated with HIV infection.[29,30]. The twin burden of CVD and HIV in sub-Saharan Africa has not been studied well, yet both may present major challenges to individuals, families and health systems as HIV infected individuals live longer. The prevalence of MetS provides the first step in estimating the risk of CVD among HIV infected individuals.

Depending on the period of study, study population and study design, the prevalence of MetS in HIV infected individuals may range from 11.2% to 45.4%[31]. Similar to the HIV uninfected, MetS in the HIV-infected population increases CVD risk and CVD-related mortality especially as most HIV infected individuals continue to live longer globally[32]. Initiation and global scale up of antiretroviral therapy (ART) have been associated with an increase in insulin resistance, fat redistribution and dyslipidemia[33]. Although this has reduced significantly with newer regimens of highly active antiretroviral therapy[30,34], it is evident that CVD risk in HIV infected adults also arises from non-ARV related causes. Since Mets is associated with chronic inflammation, any factors that increase inflammation among HIV infected adults may therefore increase their risk of CVD. It is therefore essential to evaluate the contribution of ART versus non-ART factors on the risk of CVD so as to inform CVD prevention especially in low resource settings.

1.3 MOTIVATION AND PUBLIC HEALTH IMPACT

The expected outcome and public health impact of our first study is to evaluate whether adults living with HIV and sub-Saharan Africa at are increased risk of CVD, the leading cause of premature death from NCD and whether this varies with ART. Since MetS is a direct predictor of CVD, its diagnosis in HIV infected individuals can inform treatment and therefore prevention of future CVD, especially in this setting.

The expected outcome and public health impact of our second study will rely on determining whether HDP increases the risk of and early development of CVD, the leading cause of premature death from NCD in women in SSA. CVD prevention is difficult because at diagnosis, the majority of patients have established disease. However, MetS occurs much earlier prior to established CVD and may be used to screen, and administer interventions to prevent, delay onset, or reduce severity of CVD following pregnancies complicated with HDP. Our study findings will inform use of maternal health programs in SSA, where 1 in 10 women have HDP, and CVD disproportionately affects women, to create a window of opportunity for post-delivery strategies for identifying women with MetS for timely CVD prevention interventions.

1.4 REMAINING CHAPTERS

The remaining chapters of this dissertation present the results of the studies. The second chapter presents risk factors and correlates of metabolic syndrome among HIV infected adults and has been published in *AIDS Patient Care and STDs*. The third chapter presents risks of metabolic syndrome after HDP and this manuscript was submitted for publication to the journal, *Hypertension in Pregnancy*. The fourth chapter is on the inflammatory marker hsCRP and risk of

metabolic syndrome and this manuscript will be submitted to the journal, *Hypertension in Pregnancy*. The final chapter summarizes these findings and give directions for further research.

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Chapter 2. METABOLIC SYNDROME AMONG ANTIRETROVIRALTHERAPY NAÏVE VERSUS EXPERIENCED HIV INFECTED ADULTS

2.1 MANUSCRIPT TITLE AND TITLE PAGE

TITLE: METABOLIC SYNDROME AMONG ANTIRETROVIRAL THERAPY NAÏVE VERSUS EXPERIENCED HIV-INFECTED PATIENTS WITHOUT PREEXISTING CARDIO-METABOLIC DISORDERS IN WESTERN KENYA

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Abstract word count: 244 **Total word count:** 3594 **Number of references: 44**

Running title: Metabolic Syndrome in HIV-infected adults in Kenya

Key words: HIV, Cardiovascular diseases, antiretroviral therapy, sub-Saharan Africa, Metabolic syndrome, Kenya

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2.2 ABSTRACT

Metabolic syndromes (MetS), a cluster of cardiovascular disease (CVD) risk factors, is increasingly common in people living with HIV, however, data on prevalence and the role of antiretroviral therapy (ART) as a risk factor for MetS in sub-Saharan Africa are lacking. We conducted a cross-sectional study to assess the prevalence and risk factors for MetS among ART naïve and ART experienced adults living with HIV and without pre-existing cardio-metabolic disorders in Western Kenya. Validated questionnaires and laboratory tests after overnight fasting were used to assess MetS and CVD risk factors. We used logistic regression to identify associations between traditional risk factors, HIV disease characteristics, ART use and MetS. Study participants

included 164 ART experienced and 136 ART naïve patients. The median age was 40 (interquartile range 33,46) years and 64% were women. Median HIV infection and ART use durations were 4.6 (1.7,7.9) and 4.8(2.7,7.8) years, respectively. Prevalence of MetS did not differ between ART experienced and naïve groups (16.9% vs.15.2%), respectively. ART experienced patients had higher rates of elevated fasting blood sugars and lower rates of low HDL-cholesterol. The prevalence of abnormal waist circumference, elevated blood pressure and hypertriglyceridemia were comparable between the two groups. Older age, female sex and high body mass index were independently associated with diagnosis of MetS.

Traditional risk factors rather than ART-related effects were more important predictors of MetS in this cohort. HIV-infected patients without preexisting cardio-metabolic disorders should be regularly monitored for metabolic abnormalities regardless of ART status.

2.3 INTRODUCTION

The global scale up of antiretroviral therapy (ART) has resulted in the reduction of AIDS-related mortality in low-resource settings including Kenya.[1-4] As HIV-infected patients live longer, they are exposed to traditional environmental and behavioral risk factors known to increase risk of metabolic and cardiovascular diseases (CVD) prevalent in their communities.[5] Studies from developed countries have shown that in addition to the traditional risk factors, some ART regimens and HIV infection itself may increase the risk of CVD and diabetes.[6, 7,8] In these studies, patients with HIV were twice as likely to develop stroke or myocardial infarction compared to their HIV-uninfected counterparts.[9, 10] Despite sub-Saharan Africa being a region highly affected by HIV, few studies have assessed aging-related HIV co-morbidities, particularly CVD, metabolic

syndrome (MetS), and their associated risk factors.

Metabolic syndrome is a complex cluster of factors, including hyperglycemia, elevated blood pressure, dyslipidemia and abdominal obesity, that are shown to predict risk of CVD and type 2 diabetes.[11-15] Prevalence of MetS is increasing worldwide and is closely linked to the presence of obesity and dyslipidemia in the adult population in high-income countries.[16] Among HIV-infected individuals, the prevalence of MetS worldwide ranges from 7-52% depending on the defining criteria, study population, study design, and sample size.[17] Recently published meta-analysis data show a similar prevalence of MetS in the general population as in those with HIV.[18] Other studies, however, have reported an increase in the incidence of MetS in HIV-infected patients on ART compared to ART naïve and HIV-uninfected counterparts, indicating that antiretroviral drugs may be associated with increased risk of MetS.[6] Most of these studies were conducted in higher income countries and a greater number of patients were obese, consuming tobacco and on protease inhibitors compared to populations in low and middle-income (LMIC).[19, 20] There has been limited information from SSA particularly on the role ART in the development of MetS. Moreover, most existing SSA studies have focused primarily on HIV-infected adults on ART, making it difficult to determine the independent effect of ART drugs on MetS. The present work examines the prevalence and associated risk factors for the development of MetS in HIV-infected adults without previous clinical diagnosis of CVD who are seeking care at one of the largest HIV clinics in Western Kenya.

2.4 METHODS

This was part of a larger study to assess cardiovascular disease (CVD) risk factors, knowledge, perception and attitudes towards CVD among HIV-infected patients in Western Kenya who had no previous diagnosis of cardiovascular related disease.21 We conducted a cross-sectional survey between July and September 2014 in a sample of HIV-infected adults attending an HIV clinic at the Academic Model Providing Access to Healthcare (AMPATH) program within the Moi Teaching and Referral Hospital (MTRH) in Western Kenya. AMPATH program provides care to over 150,000 HIV-infected people with a broad mix of urban middle class, urban poor and rural populations.22 The AMPATH program is a collaboration between MTRH, Moi University School of Medicine, and a consortium of North American universities that focuses on improving the health of the people of Western Kenya as previously described.23 Moi University is the hub of clinical research in cardiopulmonary diseases in Western Kenya24.

Data Collection

Our data collection methods have been previously described in detail.21 In brief, data were collected by structured questionnaires, physical measurements and venous blood sample analysis. A trained research assistant administered the questionnaire in English, Swahili or a local language.21 Each interview was followed by physical measurements including height, weight, and blood pressure. Participants were asked to return to clinic the following day after fasting for eight hours for blood sample collection. All participants were tested for fasting blood lipids and glucose level. The research participants were expected to complete all the components of the research examination on the second visit. HIV related characteristics and information including the use of

ART, the duration and type of ART regime, previous WHO clinical stage, pre-ART nadir, lowest and highest CD4 T cell count and HIV RNA viral load were obtained from the participant and/or the medical record. Any findings that warranted immediate medical attention were reported to the participant and their physician.

We used the harmonized consensus criteria for metabolic syndrome to define high BP was as systolic BP ≥ 130 mm Hg, diastolic BP ≥ 85 mm Hg, or currently on antihypertensive drug treatment, central obesity as waist circumference of ≥80 cm (women) and ≥94 cm (men), and elevated fasting blood glucose as fasting blood glucose (FBG) ≥ 7mmol/L (126 mg/dl).25 Dyslipidemia was defined as total cholesterol (TC) ≥5.2mmol/L (200 mg/dl) or HDL cholesterol <1.03mmol/L (40 mg/dl), LDL cholesterol ≥ 3.4mmol/L (130 mg/dl), and triglycerides> 4.0mmol/L (350 mg/dl) according to the American Heart Association and American College of Cardiology Foundation.26 Overweight referred to body mass index (BMI) greater than or equal to 25 kg/m2 to 29.9 kg/m2 and obesity was BMI greater than or equal to 30 kg/m2.

HIV-infected patients fulfilling Kenyan national criteria for ART are started on treatment and seen monthly at the AMPATH clinic. Criteria for starting ART at the time of the study included all HIV-infected adults with CD4 T cell count <350 cells/mm3 irrespective of WHO stage and stage III/ IV disease regardless of CD4 T cell count. The first-line ART regimen consisted of either tenofovir/lamivudine or zidovudine/lamivudine + nevirapine or efavirenz. Protease inhibitors (PIs) were only given as second-line ART in accordance with Kenya national guidelines for antiretroviral drug therapy.27

Primary outcome

Our primary outcome, metabolic syndrome (MetS) was defined according to International Diabetes Federation (IDF); central obesity; waist circumference ≥80 cm in women and ≥ 94 cm in men plus two of the following: triglycerides≥150mg/dL (1.7mmol/L), HDL cholesterol <50mg/dL (1.29mmol/L) for women or ≤40mg/dL (1.03mmol/L) for men, fasting blood glucose≥100mg/dL (5.6mmol/L), systolic blood pressure ≥130 mmHg, or diastolic blood pressure ≥85 mmHg.25

Statistical Analysis

Baseline characteristics of study participants were compared between ART experienced and ART naïve participants. Continuous variables were summarized using means (standard deviation [SD]) or medians (interquartile range [IQR]) and compared using two-sample t-tests if normality assumptions were met. The primary outcome of the study was diagnosis of MetS which was compared between the HIV-infected ART experienced group and the HIV-infected ART naïve control group. We estimated the prevalence of MetS and its components by ART status. Categorical variables were summarized using counts and proportions and compared using Pearson's chi-square tests or Fisher's exact tests, as appropriate. Using bivariate and multivariate logistic regression, we also obtained unadjusted and adjusted odds ratios (OR), respectively, for the association between known risk factors and diagnoses of MetS. Data analysis was done using STATA® version 13 (San Antonio, Texas). P values less than 0.05 were considered significant.

Ethical Statement

The Institutional Research and Ethics Committee of Moi University School of Medicine approved the study. All participants provided a written informed consent.

2.5 RESULTS

A total of 300 HIV-infected patients (36% male) were enrolled in the study. Table 1 shows characteristics of HIV-infected adult participants. Fifty-four percent were receiving combination ART and 45.3% were ART naive. Mean (SD) age of ART experienced and ART naïve participants were 43 (9) and 38 (9) years, respectively. Forty percent of the participants were aged between 35,44 years, 42.7% for the ART experienced and 41.9% for the ART naïve group. ART experienced participants were more likely to be older and male, and less likely to have received any formal education (Table 1). In addition, ART experienced participants had significantly higher mean waist circumference and BMI, and were more likely to have dyslipidemia and higher fasting plasma glucose; they had similar rates of alcohol intake and tobacco use as ART naïve adults (Table 1).

Patients on ART had worse history of immune function, with lower nadir CD4 cell count, more WHO stage 4 disease, and longer mean duration of HIV infection compared to the ART naïve adults (6.6 vs. 3.7 years), respectively (see Table 1). Among the ART experienced, mean duration on ART treatment was 5.4 years, with a large number on first line ART (82.3%). Current ART use included 92%, non-nucleoside reverse transcriptase inhibitors (NNRTI) and 6.7% protease inhibitor. Nearly all (99%) were on nucleoside reverse transcriptase inhibitors (NRTI).

Metabolic syndrome prevalence and risk factors

Metabolic syndrome (MetS) frequencies by ART status are presented in Figure 1. MetS prevalence among ART experience and ART naïve were 16.9% and 15.2% respectively. Although Mets prevalence was slightly higher in patients receiving ART, it was not statistically different

(p>0.05). The distribution of individual components of MetS by ART status is shown in Figure 1. Over half of our participants met the criteria for abnormal waist circumference with the pattern not varying by ART status. ART experienced were more likely to have elevated blood glucose, whereas ART naïve were more likely to have low HDL levels (fig. 1) Frequencies of hypertriglyceridemia and elevated blood pressure were similar between the two groups. Significantly more ART experienced (24%) met the criteria for abnormal waist circumference and one additional component of MetS compared to the naïve group (14%), p=0.03.

In unadjusted analyses, HIV-infected patients with MetS were more likely to be female, have a higher level of LDL cholesterol, BMI of at least 25 kg/m2 and age above 55 years (see Table 2) compared to those without MetS. After adjustment for demographic characteristics and HIV related characteristics in a multivariate logistic regression analysis, female sex, older age ≥55 years and higher BMI remained independently associated with presence of MetS (see Table 3). We did not observe any association between the use of ART, specific ART drugs, HIV infection duration and MetS.

2.6 DISCUSSION

With introduction to ART, HIV infection has now evolved into a chronic disease with increased morbidity and mortality due to non-AIDs defining conditions, including cardiovascular diseases (CVD).28-30 The consequences of HIV related co-morbidities have not been well documented in SSA but are likely to be of significant magnitude considering the existing poor health systems, lack of qualified health professionals and the sheer number of HIV-infected patients on ART in this region. Since metabolic syndrome (MetS) is a known risk factor for CVD and diabetes,31, 32 in this study we document the burden of MetS among HIV patients with no

previous screening or diagnosis of cardiovascular related diseases. These data identify factors associated with MetS including the role of antiretroviral drugs treatment (ART) and draw attention to the substantial prevalence of unrecognized and untreated risk factors for CVD in this population.

In this study of relatively young HIV-infected adults, MetS was common and similar among HIV-infected adults who were on ART and who were ART naïve. The most common MetS criteria were increased waist circumference, low HDL and high triglyceride levels for both ART naïve and ART experienced. The specific component of MetS most influenced by ART exposure was fasting glucose and HDL cholesterol. In addition to older age, high BMI and female sex were independently associated with MetS and no association was found between ART exposure or specific antiretroviral drugs and MetS. Our results suggest that traditional risk factors may be stronger predictors of MetS than antiretroviral therapy use. Given the projected increase of the aging HIV-infected population, our findings highlight the need to incorporate CVD risk factor screening within the HIV care packages across Kenya to identify those in need of CVD risk reduction interventions irrespective of ART status.

Estimates of MetS prevalence among HIV-infected individuals ART naïve/ART treated in SSA region is limited and the use different diagnostic criteria for MetS has made the comparison difficult. The 16% prevalence of MetS in our study is similar to what has been reported in other studies conducted in African countries with comparable diagnostic criteria, including in Cameroon (16%)33, Burkina Faso (18%)34 and Nigeria (17%)35. However, it is lower than what was reported in other studies.20, 36 A larger study of 850 HIV-infected adults in the US reported MetS prevalence of 26%.20 In another study among 250 HIV-infected adults on non-nucleoside-based ART regimens in Uganda, MetS prevalence was reported at 58%, similar to our study, factors associated with MetS included older age, higher BMI and female sex.36The discrepancy in the

results is likely due to the MetS diagnosis criteria used in the Uganda study which used two rather than three components in defining MetS and the limited number of patients on protease inhibitor (PI) class of drugs in our study (7%) versus US (31%). The Mets prevalence observed among our participants was substantially lower than prior estimates among Kenyan adults from the urban areas whom majority of the participants qualified for MetS due to hypertension and abdominal obesity.37 The difference in patient's characteristics (urban vs. rural) and exclusion of patients with pre-existing CVD related diseases including the known hypertensives and diabetics may therefore have led to underestimation of MetS prevalence in our study.

Antiretroviral therapy has been frequently associated with numerous metabolic alterations. Our finding of similar MetS frequencies between ART experienced and naïve HIV-infected adults was therefore unexpected and contradicts those of Jantarapakde et al who reported a higher prevalence of MetS among ART experienced patients compared to ART naïve in Thailand.14 In that study and like in many similar studies, MetS risk was strongly associated with PI and stavudine use likely due to their effects on triglycerides and blood glucose.38,39 Of note only 15 out of 164 patients were receiving PI or/and stavudine hence we may not have had adequate power to detect any significant effect of these drugs on MetS or its component. Nevirapine drug was associated with increased levels of HDL, an observation that has been well described by others and could partially explain the lower than reported prevalence of MetS among the ART exposed group consistent with a randomized control trial in Spain which reported an increase of HDL levels by 44% in ART naïve patients after 12 months of nevirapine therapy.41, 42 Similarly, ACTG Longitudinal Linked Randomized Trials found no association between NNRTI use, NRTI use and MetS.39 Therefore, increased use of NNRTIs, a known metabolic friendly drug in our setting, may partially explain the lack of difference in MetS rates between the two groups. Our study further

supports the protective nature of ART towards cardiovascular diseases as reported in the SMART study where patients randomized in the interrupted course of ART had increased risk CVD and cardiovascular related mortality.42 Nevertheless, a higher proportion of ART experienced than ART naïve had 1 or 2 components of MetS, suggesting a potential effect of ART on specific rather than all components of MetS. Longitudinal studies are warranted to confirm these results.

Previous studies have demonstrated that longer duration of HIV infection, high viral load and longer duration of ART confers an excess risk of MetS. In our analysis, we did not find any significant association between the duration of HIV-infection and duration of ART use with MetS. A possible explanation for the results is that the duration of HIV was self-reported hence the accuracy of this data could not be confirmed. We also did not find any association between self-reported alcohol intake and tobacco use with MetS, consistent with previous reports from other studies in the region.20, 35,36, 43

The finding of a two-fold risk of MetS in those with high BMI suggests that both overweight/obesity was the main driver for the MetS in our study. This is consistent with previous finding from the region that showed increase risk of MetS among overweight and obese patients both in general and HIV-infected populations.35, 43 A high proportion of women in our study were overweight/obese compared to men, which may partially explain increased prevalence of MetS among females. Kenya, like many other African countries, is undergoing epidemiological transition, and epidemic rates of obesity and other metabolic abnormalities have been noted during recent years44. A recent population wide survey in Kenya reported overweight/obesity prevalence of 28%, which disproportionally affected women.5 This prevalence was lower than that found in our study (41%) raising a concern about the possible influence of HIV infection and other unique

HIV related characteristics in addition to lifestyle behaviors (i.e. poor diet and sedentary lifestyle) on obesity and consequently MetS.

Our study had several strengths. It is the first study of prevalence of MetS among HIV-infected adults stratified by ART use in Kenya. Since it was conducted prior to universal treatment, it provides a rare opportunity to compare the effect of commonly used antiretroviral drugs on risk of MetS, a risk factor for CVD and diabetes. Also, it examined adults without known diagnosis of CVD related diseases, thus showing the true risk of MetS prior to onset of CVD. We had complete data for all parameters required to make diagnosis of MetS. Thus, our study results can be generalizable to similar settings. Limitation of our study included the cross-sectional design, which prohibited a definitive determination of the temporal association between some independent variables and MetS. This current study examined the role of current self-reported behaviors (i.e. smoking, alcohol consumption etc.), which may not entirely reflect past behaviors that may have influence the development of MetS. We were not able to assess the association of current CD4 cell count, viral load with MetS in our cohort because that data was not available. The exclusion of patients with pre-existing CVD related diseases including the known hypertensives, a component for MetS may have results in underestimation of the true prevalence of MetS in this population. Likewise, inclusion of HIV negative adults would have made it possible to compare biochemical changes due to HIV infection in the absence of treatment.

2.7 CONCLUSION

Metabolic syndrome (MetS) was prevalent among ART naïve and ART experienced HIV-infected adults without preexisting cardio-metabolic disorders and traditional risk factors most strongly influenced the MetS diagnosis. Our findings emphasize the importance of addressing

traditional risk factors for metabolic abnormalities as the HIV-infected populations age. Future longitudinal studies comparing HIV-uninfected and infected adults are needed to confirm these results and to assess the predictive role of MetS towards CVD in HIV infected populations in SSA.

2.8 ACKNOWLEDGMENTS

This project was supported by NIH Research Training Grant # R25TW009337 funded by the Fogarty International Center. We acknowledge Belinda Korir and the Cardiovascular and Pulmonary Disease Center of Excellence in Kenya (HHSN268200900031C), President's Emergency Plan for AIDS Relief (PEPFAR)/USAID (AID-623-A-12-0001). AO is supported by the Fogarty International Center and The Office of Research on Women's Health (ORWH) of the National Institutes of Health under Award Number K43 TW010363. TT is supported by grant R21TW010459-02S1 from the Fogarty International Center of the National Institutes of Health (NIH).

Author Disclosure Statement: All authors declare no financial conflicts of interest.

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Table 1. Characteristics of HIV-infected participants by ART status

	ART experienced (N=164)	ART naïve (N=136)	P value
Demographics			
Female	94 (57.3)	98(72.1)	0.008
Age, years	43.1 (9.4)	37.9 (8.9)	<0.001
Age groups, years			<0.001
18-34	29 (17.7)	48 (35.3)	
35-44	70 (42.7)	57 (41.9)	
45-54	37 (22.6)	23 (16.9)	
>55	28 (17.1)	8 (5.9)	
Education level			
High school or more	55 (33.5)	15 (11)	0.03
Cardiovascular risk factors			
Current tobacco use	5 (3)	5 (3.7)	0.95
Current alcohol drinking	37 (22.6)	24 (17.7)	0.29
Diabetes	2 (1.5)	5 (3.1)	0.35
Overweight/obese	71 (43.3)	50 (36.8)	0.25
Physical activity, work related	122 (74.4)	89 (65.4)	0.09
HDL, mmol/L	1.5 ± 0.6	1.1 ± 0.5	<0.001
LDL, mmol/L	2.8 ± 0.9	2.5 ± 0.1	0.001
Triglycerides, mmol/L	1.6 ± 1.1	1.5 ± 0.9	<0.21
Total cholesterol, mmol/L	4.8 ± 1.1	4.1 ± 1.0	<0.001
BMI, kg/m^2	25.1 ± 5.9	23.8 ± 5.2	0.05
Waist circumference, cm	87.8 ± 12.7	84.6 ± 9.4	0.01
SBP, mm Hg	116.3 ± 5.5	115.7 ± 5.4	0.40
DBP, mm Hg	65.5 ± 5.6	65.9 ± 6.1	0.57
FBS, mmol/L	5.0 ± 1.0	5.1 ± 1.0	0.34
HIV related factors			
Nadir CD4[§], cells/mm^3	191.4 ±127.8	417.9 ± 206.2	<0.001
Peak viral load[ξ], copies/ml	120,646.1 (538-112.9)	189,710 (199-678.9)	0.5861
WHO Clinical stage 4	15 (9.2)	4 (2.9)	<0.001
HIV duration, years	6.6 ± 4.7	3.7 ± 4.4	<0.001
ART duration, years	5.4 ± 3.3		
Type of ART			
PI-based			
Lopinovir/Ritonavir	11 (6.7)		
NRTI-based			
Stavudine	4 (2.4)		
Zidovudine	57 (34.8)		

Lamuvidine	152 (92.7)
Tenofovir	109 (66.5)
NNRTI-based	
Nevirapine	99 (60.4)
Efavirenz	51 (31.1)
Others	9 (5.5)

Values are n (%), median (interquartile range) or mean ± SD.
ART, Antiretroviral therapy; SBP, Systolic BP; DBP, Diastolic BP; HDL, High-density lipoprotein; LDL, low-density lipoprotein; NNRTI, Non-nucleoside reverse transcriptase inhibitors; NRTI, Nucleoside reverse transcriptase inhibitors; BMI, Body mass index; FBS, Fasting blood sugar.
[§] N = 188
[ξ] N = 38

Table 2 Characteristics of HIV infected participants by Mets status

	With MetS (N=48)	**Without MetS (N=252)**	**P value**
Gender			
Female	40 (83.3)	152 (60.3)	0.002
Age, years Mean (SD)	43.2 ± 9.83	40.2± 9.42	0.05
Age groups, years			
18-34	8 (16.7)	69 (27.4)	0.12
34-55	30 (62.5)	157 (62.3)	0.98
>55	10 (20.8)	26 (10.3)	0.04
Smokers	0 (0.0)	12 (4.8)	0.12
Current alcohol drinking	8 (16.7)	53 (21.0)	0.49
Dyslipidemia	43 (89.6)	133 (52.8)	<0.001
BMI ≥25 kg/m^2	32 (66.7)	93(36.9)	<0.001
Nadir CD4, cells/mm^3	251.9 (196.0)	261.7 (185.9)	0.79
Peak viral load, copies/ml	195472.3 (185216.3)	126071.9 (86873.08)	0.82
Length of HIV infection, years	5.42 (4.9)	5.3 (4.8)	0.85
ART duration, years	4.7 (3.0)	5.5 (3.3)	0.2
ART use	25 (52.1)	139 (55.2)	0.70
Type of ART			
Lopinovir/Ritonavir	2 (8.0)	9 (6.5)	0.78
Zidovudine	11 (44.0)	46 (33.1)	0.29
Lamuvidine	24 (96.0)	128 (92.1)	0.49
Tenofovir	13 (52.0)	96 (69.1)	0.10
Nevirapine	13 (52.0)	86 (61.9)	0.35
Efavirenz	11 (44.0)	40 (28.8)	0.13
Others	2 (8.0)	7 (5.0)	0.55

Values are n (%) or mean ± SD. ART, Antiretroviral therapy; MetS, Metabolic Syndrome. Other abbreviations as in Table 1.

§ N = 188

ξ N = 38

Table 3. Factors associated with Metabolic Syndrome among HIV-infected Patients

	Univariate analysis			Multivariate analysis		
	Odds ratio	**(95% CI)**	**P value**	**Adjusted Odds ratio**	**95% CI)**	**P value**
Male sex	0.30	0.14-0 .68	0.004	0.22	0.09-0.56	0.001
Age groups[a], yrs						
≥55	2.54	1.08-5.95	0.03	3.35	1.20-9.33	0.02
ART use	0.88	0.48-1.64	0.70	-		
HIV duration	1.01	0.95-1.07	0.84	-		
BMI ≥25 kg/m [b]	3.42	1.78-6.5	<0.001	2.83	1.38-5.82	0.005
LDL	1.02	1.01-1.05	0.01	-		
HIV viral load >100,000 copies/ml	2.88	0.66-12.49	0.16	-		
HIV stage 4[c]	0.96	0.70-1.31	0.79	-		
CD4 nadir[d] < 200 cells/mm^3	1.26	0.64-2.46	0.51	-		

CI, confidence interval. Other abbreviations as in Table 1.

[a] vs. Age groups 18-54 years.

[b] Reference BMI <25 kg/m^2

[c] Reference HIV stages 1-3

[d] Reference Nadir CD4 ≥ 200 cells/mm^3

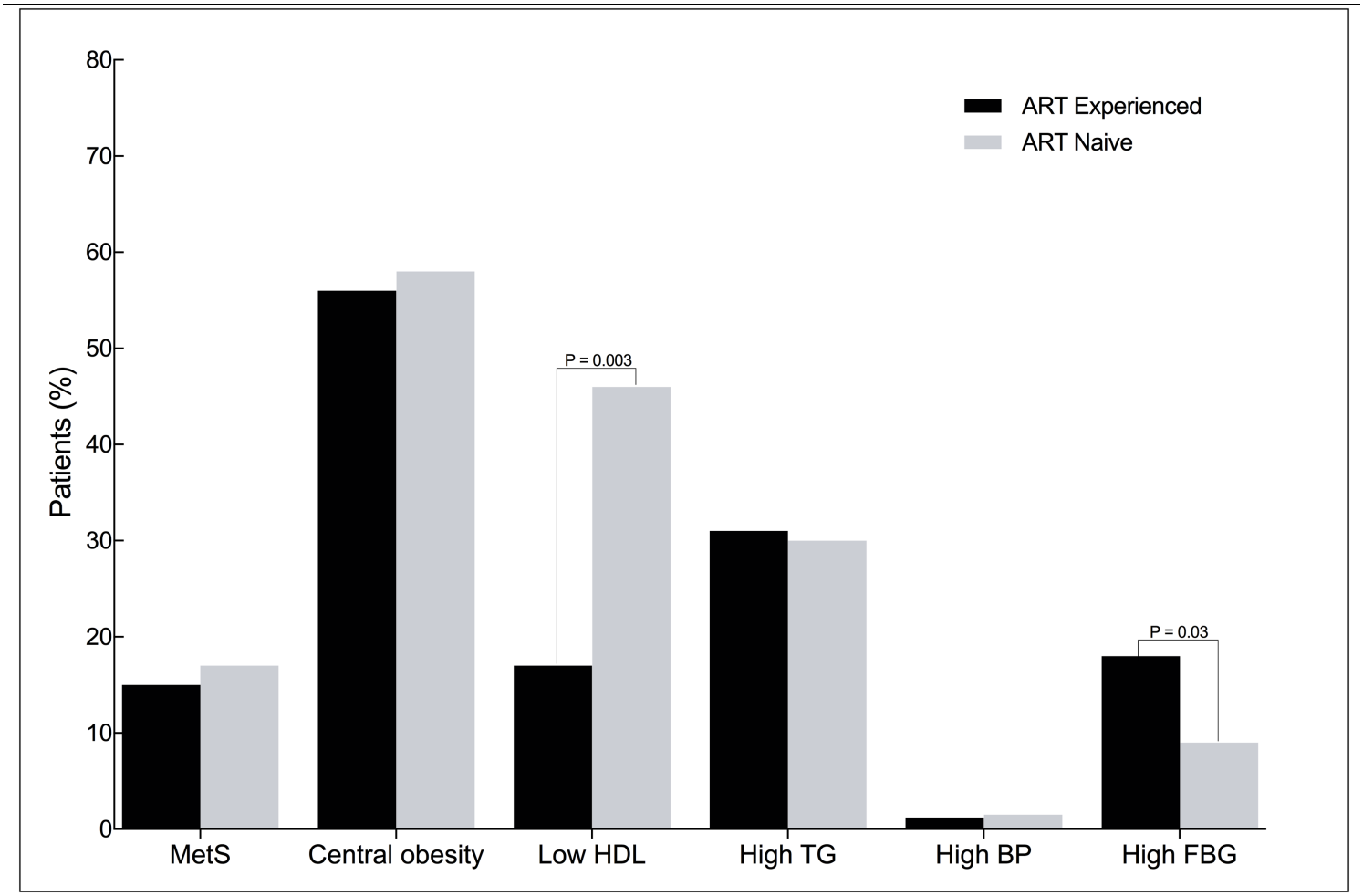

Figure 1. Prevalence of Metabolic Syndrome by ART status.

All ($n = 300$) participants were included. Central obesity was defined as waist circumference of ≥80 cm (women) and ≥90 cm (men). High TG was defined as triglycerides ≥150mg/dL (1.7mmol/L), low HDL was HDL cholesterol levels of <50mg/dL (1.29mmol/L) for women and ≤40mg/dL (1.03mmol/L) for men, high FBG was fasting blood glucose of ≥100mg/dL (5.6mmol/L), High BP was a systolic blood pressure of ≥130 mmHg, or diastolic blood pressure of ≥85 mmHg

Chapter 3. METABOLIC SYNDROME AFTER HYPERTENSIVE DISORDERS IN PREGNANCY

3.1 MANUSCRIPT TITLE AND TITLE PAGE

TITLE: RISK OF POSTPARTUM METABOLIC SYNDROME SIX MONTHS AFTER GESTATIONAL HYPERTENSION AND PREECLAMPSIA, A PROSPECTIVE COHORT STUDY

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This project was supported by the Fogarty International Center and the Office of Research on Women's Health (ORWH) of the National Institutes of Health under Award Number K43 TW010363. A. O. was supported by NIH Research Grant D43 TW009580, funded by the Fogarty International Center, National Institute on Drug Abuse, and National Institute of Mental Health. The NIH was not involved in the study design, collection, analysis and interpretation of data, writing of the report or in the decision to submit the article for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract word count 401 **Text word count** 2993 **References** 40

Condensation

Gestational hypertension and preeclampsia are associated with three-fold or greater risk of metabolic syndrome at 6 months postpartum

Short Title

Preeclampsia, gestational hypertension and postpartum metabolic syndrome

AJOG at a Glance:

A. Why was this study conducted?

To determine the risk of metabolic syndrome, a marker of future cardiovascular disease risk, after gestational hypertension and preeclampsia. The study was conducted in Kenya, a region in sub-Saharan Africa with high fertility rates and significant cardiovascular disease burden and cardiovascular disease mortality.

B. What are the key findings?

Gestational hypertension and preeclampsia are associated with significantly increased risk of postpartum metabolic syndrome.

C. What does this study add to what is already known?

As the first prospective cohort study in sub-Saharan Africa in this area of research, we report that there is a high risk of metabolic syndrome and therefore future cardiovascular disease among women who experience gestational hypertension and preeclampsia.

The study also informs the need for structured postpartum care, as well as mechanistic and intervention studies that focus on lowering cardiovascular disease risk after gestational hypertension and preeclampsia.

3.2 ABSTRACT

Background

The cardiovascular disease (CVD) risk after gestational hypertension or preeclampsia has not been well studied in low and middle-income countries especially in sub-Saharan Africa, a region heavily burdened by CVD and CVD-related deaths. Understanding the risk of postpartum metabolic syndrome, an indicator of CVD risk, can inform care after GH and PE in this setting and globally.

Objective

We sought to evaluate the risk of metabolic syndrome and its components at 6 months postpartum, comparing women whose most recent pregnancies were complicated with new onset of hypertension after 20 weeks of pregnancy, specifically gestational hypertension or preeclampsia with those who had normotensive pregnancies.

Study design

This was a prospective cohort study in which women exposed to gestational hypertension or preeclampsia were actively recruited and enrolled; unexposed normotensive women were also enrolled during the first 12 weeks after delivery in Nairobi, Kenya. Participants were interviewed, and physical examination including anthropometric measurements were obtained at enrollment and 6 months postpartum. Fasting lipid profile and fasting plasma glucose were measured at 6 months postpartum. The main outcome was risk of metabolic syndrome and its components. A generalized linear regression model with Poisson distribution was used to estimate crude and adjusted relative risk of 6-month postpartum metabolic syndrome and its components comparing women with and without gestational hypertension or preeclampsia during their most recent pregnancy.

Results

Among 194 postpartum women enrolled and followed until 6 months postpartum, 63 (32%) had experienced gestational hypertension or preeclampsia. Prevalence of metabolic syndrome at 6 months postpartum was higher among women who had gestational hypertension or preeclampsia (22 of 63, 34.9%) compared to those who were normotensive (15 of 131, 11.5%) during their most recent pregnancy ($p<0.001$). Gestational hypertension or preeclampsia was associated with a three-fold increased risk of metabolic syndrome at 6 months postpartum (Relative risk [RR] 3.01; 95% Confidence interval [CI] 1.58, 5.71; $p=0.001$) and increased risk of three of the five components of metabolic syndrome: hypertension (RR 3.35 95% CI [2.04, 5.51], $p<0.001$), hypertriglyceridemia (RR 3.25 95% CI [1.16-9.10], $p=0.01$), and fasting hyperglycemia (RR 6.20 95% CI [1.07-35.76], $P=0.03$), compared to having normal blood pressures during pregnancy. Both waist circumference and high-density lipoprotein cholesterol were elevated at 6 months postpartum among those with gestational hypertension or preeclampsia but were not statistically significantly different for those who had versus those who did not have these conditions.

Conclusion

At 6 months postpartum, gestational hypertension and preeclampsia were associated with three-fold or higher risk of postpartum metabolic syndrome and its components, hypertension, fasting hypertriglyceridemia, and fasting hyperglycemia. Thus, gestational hypertension and preeclampsia could help identify women at risk of future cardiovascular disease in this resource-limited sub-Saharan African setting.

Key words: prospective cohort, metabolic syndrome, gestational hypertension, dyslipidemia, preeclampsia, hyperglycemia, postpartum, cardiovascular disease, prevention, mortality, pregnancy, Kenya, sub-Saharan Africa

3.3 INTRODUCTION

Metabolic syndrome (MetS) is defined by the presence or treatment of 5 cardiovascular disease (CVD) risk factors: atherogenic dyslipidemia (elevated triglycerides [TG], reduced high-density lipoprotein cholesterol [HDL-C] levels), elevated blood pressure (BP), central obesity and elevated fasting plasma glucose (FPG)[1]. MetS is associated with an estimated 2-fold increase in risk of developing ischemic heart disease and cerebrovascular disease, and has been associated with increased CVD-related mortality, as well as all-cause mortality[2]. While each of the five components of MetS independently increases CVD risk, when present together the risk is multiplied. There is substantial data supporting this association, however the underlying mechanisms linking the development of MetS and CVD have not been conclusively determined, MetS and CVD both have genetic and environmental origins[3,4], but progression of MetS to CVD may also result from insulin resistance, neurohormonal activation and chronic inflammation processes. These can be delayed by lifestyle changes, risk factor modification and pharmaceutical therapy of components of MetS and could become targets for CVD prevention in this populatio[55].

From studies in high income countries, pregnancies complicated by gestational hypertension (GH) or preeclampsia (PE) are associated with increased risk of MetS postpartum[6-11]. PE is new onset (after 20 weeks of pregnancy or within 12 weeks postpartum) hypertension (systolic blood pressures of 140 or higher and/or diastolic blood pressures of 90 or greater) with symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications while GH is new onset hypertension without end organ damage[12]. GH and PE have been associated with a 2-fold or greater risk of future CVD, premature CVD related deaths and type 2 diabetes compared with normotensive pregnancies[13-16]. Although CVD-mortality has declined in both men and women in the United States, there has been stagnation in younger adults,

especially women under the age of 55 years[17], partly as a result of female-specific CVD risk factors such as GH and PE[18]. This increased risk for MetS and CVD after GH or PE suggests a shared pathway[9,19,20]. Both GH and PE have been recognized as cardiac risk factors[21] that identify women for close postpartum follow-up in high-income countries[10,22,23]. The association between GH and PE and CVD risk has not been well studied in low and middle-income countries (LMIC) especially in sub-Saharan Africa, a region heavily burden by CVD and CVD-related deaths[24]. Understanding the risk of postpartum MetS in LMIC can inform care after GH and PE in this setting and globally.

In this prospective cohort study, we evaluated the risk of MetS postpartum, comparing women whose pregnancies were complicated with GH or PE with those who had normotensive pregnancies in Nairobi, Kenya. Women were enrolled in the immediate postpartum period and followed until 6 months postpartum. We compared the prevalence of MetS and that of its components between women who had versus those who did not have GH or PE hypothesizing that women with recent GH or PE would have a higher risk of MetS at 6 months postpartum when compared to women without these pregnancy complications.

3.4 MATERIALS AND METHODS

Study Design and Setting

This was a prospective cohort study to compare the risk of MetS among women with versus those without GH or PE at 6 months postpartum at Kenyatta National Hospital (KNH), the largest national teaching and referral hospital in Nairobi, Kenya. KNH provides low and high-risk obstetric care and conducts more than 10,000 deliveries annually, 5% of which are complicated by GH or PE.

The study was approved by the Kenyatta National Hospital/ University of Nairobi Ethical Research Committee (#P325/04/2016) on July 04, 2016 and University of Washington

Institutional Review Board (#STUDY00001151) on February 15, 2017. All participants provided written informed consent.

Recruitment

Postpartum women with or without GH or PE, who were stable and ready for discharge were screened for eligibility at the postnatal wards. Women were eligible if they were: HIV uninfected, not intending to become pregnant for at least 6 months, aged 28 years or older and did not have conditions suggestive of prepregnancy MetS such as pregestational diabetes, chronic hypertension, malignancy, renal, hepatic or biliary disease. We excluded women who used statins, insulin, oral hypoglycemic agents, or antihypertensives before onset of their most recent pregnancy. Only women who had medical records, were planning to live within 50 km from the hospital, were willing to be followed for at least 6 months and to undergo physical examination and blood sample collection were recruited.

Potential participants were categorized as exposed if they had GH or PE and unexposed if they were normotensive using in-patient records and BP measurements at screening prior to discharge. We used the International Society for the Study of Hypertension in Pregnancy (ISSHP) classification guidelines to define PE as new onset elevation of blood pressure ([BP] ≥ 140/90 mm Hg at least 2 hours apart at rest, after 20 weeks of pregnancy and before 12 weeks postpartum) with symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications and GH as new onset elevation of BP without evidence of end organ damage[12]. Efforts were made to recruit participants in a ratio of 1:2 for exposed to unexposed groups respectively.

We enrolled women who returned to the study clinic not more than 12 weeks postpartum, and who provided written informed consent. Each participant underwent a structured interview to collect sociodemographic, family, past medical, obstetric, gynecological and contraceptive history followed by measurements of BP (mm Hg), weight (kilograms), height (centimeters), hip circumference (centimeters), and waist circumference ([WC]) centimeters). BP was measured using a calibrated automatic recording device, OMRON, at least 10 minutes after the participant arrived at the clinic. As per the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, three readings were taken at least 10 minutes apart using a cuff that encircled at least 80% of the arm while seated at rest and then averaged[25]. We measured weight using a calibrated electronic scale with subjects in light clothing and without shoes, height using a dropdown ruler, WC using a non-stretchable tape at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest and widest hip circumference. Measurements were repeated twice and averaged if within 1 cm but repeated if exceeded 1 cm (discrepant). We then computed the waist-hip-ratio and the body mass index (BMI).

All study procedures were conducted by trained research study nurses. A trained community health worker and research study nurse contacted enrolled study participants monthly to update contacts and physical addresses and reminded the participants to return to the clinic at 6 months postpartum.

Six-month postpartum follow-up and study procedures

Study subjects underwent structured interviews, measurements of BP, weight, height and WC and blood draws. Blood samples were collected from the antecubital vein 9-12 hours after

fasting into evacuated blood collection tubes with ethylenediaminetetraacetic acid anticoagulant (BD Vacutainer) for lipids or a serum separator gel for glucose. Samples were inverted gently 5–10 times, stored on ice, centrifuged in a refrigerated centrifuge at 1,500 rpm for 30 minutes to isolate the plasma fraction at 4°C and then placed into an ice bath at 2-4°C within 2 hours of collection. Plasma and serum aliquots were kept frozen at -80°C at the University of Nairobi laboratory, then batched and shipped to Seattle, USA for testing by the University of Washington' Department of Laboratory Medicine's Research Testing Service. On the day of analysis, the specimens were thawed and mixed thoroughly. Serum lipids and glucose were quantified using the Beckman Coulter AU5812 automated chemistry and immunochemistry analyzer using enzymatic assays.

Outcome

The primary outcome, MetS was diagnosed if a participant had 3 or more of the 5 components of the 2009 consensus criteria: 1) abdominal obesity (WC≥ 88 cm), 2) elevated fasting TG (≥150 mg/dL) or its treatment, 3) low HDL-cholesterol (<50 mg/dL) or its treatment, 4) elevated BP (systolic BP ≥130 or diastolic BP ≥85 mm Hg or treatment for hypertension), and 5) elevated FPG ≥100 mg/dL or its treatment[26].

Statistical analysis

To compare the baseline sociodemographic, physical and reproductive characteristics and the 6-month postpartum risk of MetS between women with and without GH or PE, we used Student t-tests and nonparametric Wilcoxon rank sum tests for continuous variables and Pearson's chi-square tests or Fisher's exact tests for categorical variables. We obtained crude and adjusted

relative risk estimates using a generalized linear model with a Poisson distribution link and adjusted for potential confounding variables that were determined a priori to be related to both the exposure and the outcome. These potential confounding variables included maternal age, level of education, body mass index, hormonal contraception, breastfeeding, marital and employment status. P-values <0.05 were considered statistically significant. All analyses were conducted using STATA® version *13*.

3.5 RESULTS

Baseline characteristics of study participants

From November 2016 to August 2018, we followed 213 women from enrollment until 6 months postpartum. Overall, 194 (91%) completed all the study procedures (Figure 1). Of these, 63 (32%) had been exposed to hypertension after 20 weeks of pregnancy as either GH or PE while 131 (68%) had not. Most baseline sociodemographic, reproductive characteristics and physical measurements at enrollment were similar between the two groups (Table 1). However, we observed some differences; specifically, women with prior GH or PE were more likely to have delivered an infant with a lower mean birthweight (2516.1g versus 3323.6 g, p<0.001) and at a lower mean gestational age (36.1 versus 38.8 weeks, p<0.001), and were more likely to: have a stillbirth or neonatal death (28.6% versus 6.1%, p<0.001), be of higher parity (mean 4 versus 3, p=0.049), higher body mass index (BMI) ≥ 30 kg/m^2 (60.7% versus 43.8%, p=0.03) and exclusively breastfed their infants (75.6 % versus 54.5%, p=0.01) compared to women who were normotensive during their most recent pregnancy. The mean systolic blood pressure (BP) and diastolic BP were also significantly higher among exposed compared to unexposed women (134.0 versus 116.8 and 90.1 versus 78.7 mm Hg respectively, p<0.001) (Table 1).

Physical and biochemical characteristics of participants at 6 months postpartum

At 6 months postpartum, exposed women had significantly higher mean systolic (132.6 versus 119.1 mm Hg) and diastolic (89.2 versus 79.1 mm Hg) BP compared to unexposed women ($p<0.001$) (Table 2). Nearly one quarter (n=15 out of 63[23.8%] of exposed women versus less than one tenth of unexposed women (n=12 out of 131 [9.1%], p=0.006) had BP≥ 140/90 mm Hg, $p<0.001$. Exposure to GH or PE was associated with a trend towards higher mean serum triglycerides (TG) levels (95.7 versus 80.2 mg/dl, p=0.05) and higher mean serum remnant cholesterol levels (19.1 versus 16.1 mg/dl, p=0.05) compared to no exposure. Exposed women had higher but not statistically significantly different mean levels of serum total cholesterol, high density lipoprotein (HDL)-cholesterol, fasting plasma glucose (FPG), weight, waist circumference (WC), body mass index (BMI) and proportion with BMI ≥30 Kg/m^2 when compared to normotensive women (Table 2).

Risk of postpartum metabolic syndrome and its components at 6 months postpartum

The prevalence of MetS was significantly higher in women who were exposed to GH or PE (n=22 of 63, 34.9%) compared to those who were not ([n=15 of 131, 11.5%], $p<0.001$) (Figure 2). Compared to the unexposed, exposed women were significantly more likely to have elevated BP (systolic BP≥ 130 or diastolic BP ≥ 85 mm Hg or treatment for hypertension [n=28, 44.4% versus n=20, 15.6%, $p<0.001$]), TG ≥150 mg/dl (n=10, 15.9% versus n=7, 5.5%, p=0.02) and FPG≥ 100 mg/dl (n=5, 7.9% versus n=2, 1.6%, p=0.03). WC ≥ 88 cm and HDL-cholesterol < 50 mg/dl the most common MetS criteria. However, they were not statistically significantly different between women with and without GH or PE.

In unadjusted analysis, the risk of MetS was three times greater in women exposed to GH or PE compared to those who were not exposed (Relative Risk (RR) 3.05, 95% Confidence interval (CI) [1.70,5.47], P<0.001 (Table 3). These associations persisted when adjusted for maternal age, level of education, hormonal contraception, breastfeeding, BMI, marital and employment status. In adjusted analysis, the risk remained three times greater for MetS (RR 3.01, 95% CI [1.58,5.71], p=0.001, BP ≥130/85 mm Hg or treatment for hypertension (RR 3.35 95% CI [2.04,5.51], p<0.001, hypertriglyceridemia (RR 3.25 95% CI [1.16,9.10] p=0.01, and was 6 times higher for fasting hyperglycemia (RR 6.20 95% CI [1.07,35.76], p=0.03) when comparing women with versus without exposure to GH or PE. There was no statistically significant difference in the prevalence of low HDL-cholesterol and high WC.

3.6 COMMENT

In this prospective study, we found that at 6 months postpartum, relatively young women exposed to new onset hypertension (after the first 20 weeks of pregnancy) as either gestational hypertension (GH) or preeclampsia (PE) had more than three times greater risk of metabolic syndrome (MetS) compared to women who had normal blood pressures during pregnancy in this low- and middle-income countries (LMIC) setting. We also observed three-fold or greater risk of 3 components of MetS at 6 months postpartum after GH or PE compared with normotensive pregnancies.

The prevalence of MetS of 35% among women exposed to GH or PE in this setting is slightly higher than those reported in previous prospective cohorts in high-income settings that used similar criteria. However, this is mainly due to the differences in study populations and duration of postpartum follow-up. Van Rijin et al reported prevalence of MetS of 15.2% after early onset PE versus 4.3% following normotensive pregnancies. MetS was diagnosed at 6 months

postpartum[6], as in our study; however important differences between this study and ours is that we included early and late onset preeclampsia, as well as gestational hypertension regardless of parity. Similary, Smith et al observed a MetS prevalence of 18%-18.6% in exposed compared to 5.7-7% in unexposed women[22,27]at one year postpartum in a study population that excluded gestational diabetes or GH. Notably, the relative risk of MetS was similar between the two studies. Utrecht and Hypitat Risk Assessment Study cohorts found a 14-16% prevalence of MetS among exposed women[23,28] but did not have a control group. There are no published studies on the prevalence of MetS among postpartum women in SSA for comparison. This high prevalence of MetS in our study is consistent with the 40% or greater prevalence of MetS among non-pregnant adult female population regionally, in Kenya and Ghana[29-31]. These and our findings suggest that insulin resistance, dyslipidemia, and inflammation, the risk factors for MetS, are highly prevalent after GH or PE. Since MetS is a known risk factor for CVD[2,32], our study demonstrates that without intervention women exposed to GH or PE in this setting are at extremely high risk of CVD.

The components of MetS that were elevated after GH or PE were hypertriglyceridemia, high systolic and diastolic BP and high FPG. In a recent metanalysis of 24 case control and 5 cohort studies, hypertriglyceridemia was associated with preeclampsia, suggesting a role of TG in the development of PE, MetS and CVD[33-35]. Since hypertriglyceridemia may also reflect hyperglycemia, studies using glycated hemoglobin may evaluate this association and identify women with long standing insulin resistance postpartum. Apart from low HDL-cholesterol, elevated serum TG may play a significant role in CVD and a dose dependent increase in risk of CVD and all-cause mortality from hypertriglyceridemia has been described[33-35]. The dyslipidemia in postpartum MetS in other settings have been characterized by low HDL-cholesterol, high triglycerides and high total cholesterol. We did not observe a similar trend probably due to the

high prevalence of low HDL-cholesterol and high total cholesterol in our population and that our study population was only 6 months postpartum. Postpartum hypertension and fasting hyperglycemia have been reported in multiple studies estimating the risk of MetS from 3 months to decades postpartum[19,36,37]. Our results therefore suggest that hypertriglyceridemia, hypertension and insulin resistance are the most affected components of MetS after GH or PE in this setting and should be considered in monitoring and potential interventions to reduce risk of CVD in these women. We will follow this cohort until 3 years postpartum for these outcomes.

Whereas the evidence that pregnancies complicated with GH and PE increase the risk of CVD continues to grow, this risk has not previously been studied in sub-Saharan Africa (SSA), where three quarters of global annual CVD-related deaths occur[24,38]. Younger adults, especially women below 55 years in the United States, continue to experience high CVD morbidity and mortality rates[17] partly due to female-specific CVD risk factors, including GH or PE[2,18,39]. Our findings further support the need to reduce CVD risk after GH or PE[6-8,11,18,19,22,37,40].

Our study had several strengths. This is the first study to assess the risk of MetS among a cohort of postpartum women following exposure to GH or PE in Kenya and the rest of SSA to the best of our knowledge. Secondly, since GH has also been associated with increased CVD risk, our inclusion of GH and not PE alone makes our findings more representative of the risk of MetS after new onset hypertension in pregnancy.

The major limitation of our study was that by enrolling women postpartum and not before 20 weeks of pregnancy, we may have not completely excluded preexisting MetS or misclassified some cases of preexisting hypertension as GH or PE. Also, due to lack of universal screening for gestational diabetes, we were not able to completely identify women with both gestational diabetes and PE or GH. However, if the measurement error was adjusted for it would make the true risk estimates even higher further supporting our hypothesis of increased risk of MetS after GH and

PE. Due to their rarity, we did not include other CVD risk behaviors such as smoking, alcohol consumption, physical inactivity and dietary patterns in our adjusted models.

In conclusion, MetS and its components (elevated blood pressure, fasting hypertriglyceridemia and fasting hyperglycemia) were highly prevalent at 6 months postpartum following GH or PE. Since the presence of MetS suggests an increased risk of future CVD, additional longitudinal incidence, mechanistic and intervention studies targeting CVD prevention, such as blood pressure and lipid lowering medications for women with GH or PE may help lower the CVD burden in LMIC and globally.

Acknowledgment(s)

We gratefully acknowledge the contribution of the study staff, study participants and the National Institute of Health through the Emerging Global Leader Award (K43) for making this study possible.

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Table 4: **Baseline characteristics of study participants by gestational hypertension and preeclampsia**

Characteristic	GH or PE N=63 n (%) or mean ± SD	Normotensive N=131 n (%) or mean ± SD	P value
Age, years	33.4 ± 3.6	32.8 ± 4.1	0.33
≥ 35	18 (28.6)	45(34.3)	0.42
Married	61(96.8)	124(94.7)	0.50
Secondary or higher education	41(65.1)	95(72.5)	0.33
Employed	45(71.4)	103(78.6)	0.27
Preconception hormonal contraception	33(52.4)	7 (58.8)	0.40
Parity	4 ± 2	3 ± 1	0.049
Gestation, weeks	36.1 ± 3.7	38.8 ± 2.0	<0.001*
Maternal weight, Kilograms	74.5 ± 14.1	72.2 ± 13.1	0.25
Height, centimeters	141.4 ± 42.1	141.8 ± 41.1	0.94
Waist circumference, centimeters	97.0 ±17.0	94.5 ± 9.4	0.29
Hip circumference, centimeters	109.3 ± 16.2	107.2 ± 9.5	0.35
Waist-hip ratio >0.85, centimeters	44 (68.6)	98 (76.2)	0.41
Infant birthweight, grams	2516.1 ± 995.3	3323.6 ± 617.7	<0.001*
Male infant sex	33 (52.4)	69 (52.7)	0.97
Stillbirths or neonatal deaths	18 (28.6)	8 (6.1)	<0.001*
Exclusive breastfeeding	34 (75.6)	67 (54.5)	0.01*
SBP, mm Hg	134.0 ± 21.7	116.8 ± 13.9	<0.001*
DBP, mm Hg	90.1 ± 15.8	78.7 ± 10.7	<0.001*
Body mass index (kg/m^2)	31.9 ± 5.7	30.7 ± 5.9	0.19
≥30 (obese)	37 (60.7)	56 (43.8)	0.03*

Values are in n (%) or mean ± SD. SD, standard deviation;

Abbreviations: GH, Gestational hypertension, hypertension occurring after twenty weeks of pregnancy or within 12 weeks postpartum without symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; PE, Preeclampsia, hypertension accompanied by symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; *p<0.05

Table 5. **Physical and biochemical characteristics by prior gestational hypertension and preeclampsia at six months postpartum**

Characteristic	GH or PE N=63	Normotensive N=131	P value
BP≥140/90, mmHg	15 (23.8)	12 (9.2)	0.006*
SBP, mmHg	132.6 ± 18.4	119.1 13.7	<0.001*
DBP, mmHg	89.2 ± 13.1	79.1 10.3	<0.001*
Total cholesterol, mg/dL	177.8 ± 36.6	174.6 ± 31.9	0.56
Triglycerides, mg/dL	95.7 ± 50.6	80.2 ± 53.5	0.05
¶HDL-cholesterol, mg/dL	50.6 ± 11.1	49.9 ± 10.1	0.65
#LDL-cholesterol, mg/dL	108.0 ± 30.4	108.7 ± 26.7	0.88
Remnant-cholesterol, mg/dL	19.1 ± 10.1	16.1 ± 10.7	0.05
Fasting plasma glucose, mg/dL	81.2 ± 18.8	77.1 ± 8.1	0.10
Weight, kilograms	76.8 ± 15.3	74.7 ± 13.4	0.34
Waist circumference, cm	95.6 ± 12.4	95.2 ± 11.4	0.79
Body mass index (kg/m^2)	32.8 ± 6.2	31.9 ± 6.0	0.31
≥30 (obese)	41 (65.1)	76 (58.9)	0.30

Values are in n (%) or mean ± standard deviation;

Abbreviation: GH, Gestational hypertension, hypertension occurring after twenty weeks of pregnancy or within 12 weeks postpartum without symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; PE, Preeclampsia, hypertension and symptoms or signs of end organ damage such as proteinuria, renal, liver,

cardiac or neurological complications; BP, Blood pressure; mmHg, millimeters of mercury; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; mg/dL, milligrams per deciliter; HDL, high density lipoprotein, LDL-low density lipoprotein; kg, kilograms; cm, centimeters; kg/m^2, kilograms per square meters; *p<0.05

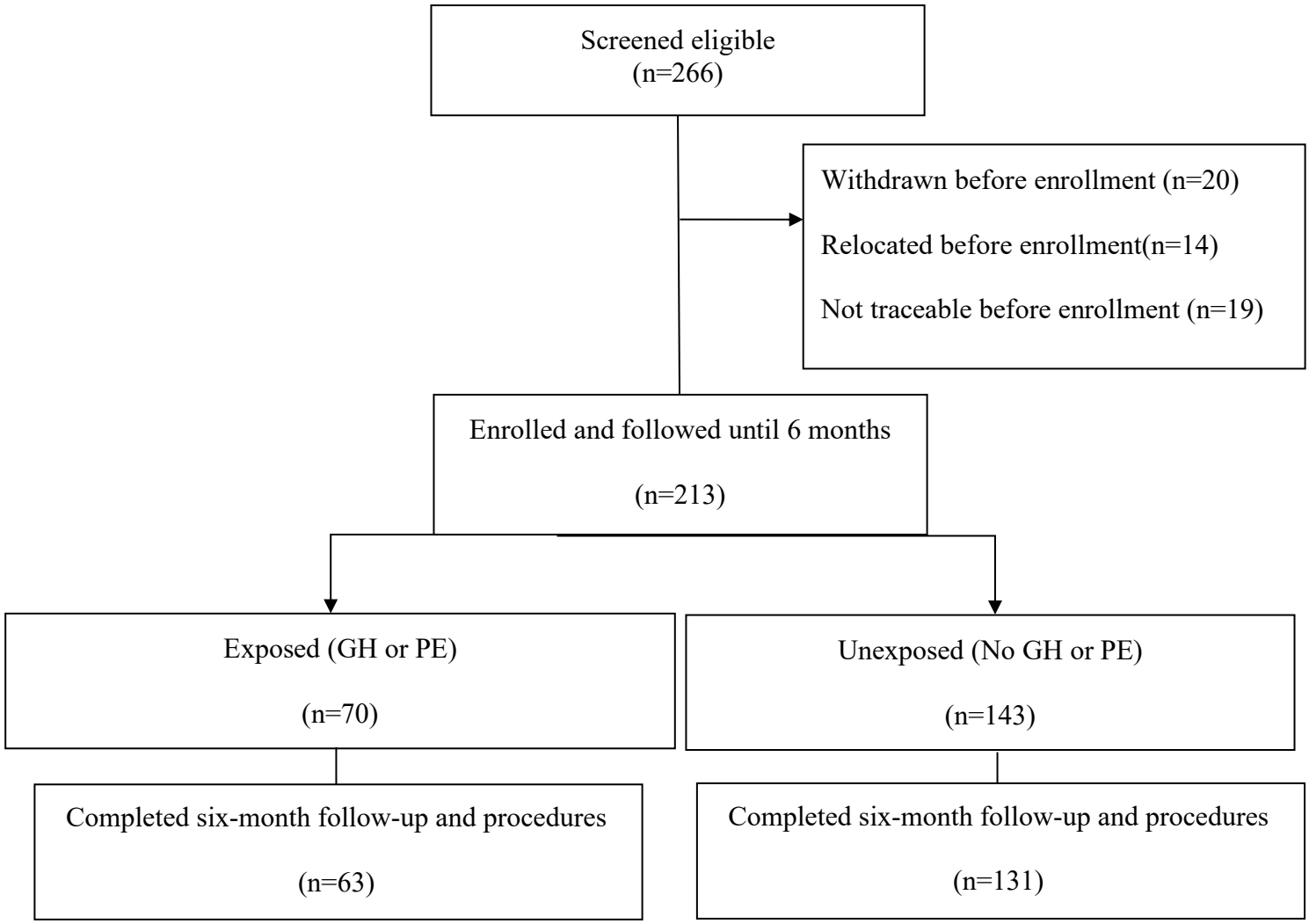
Table 6. **Risk of metabolic syndrome and its components at 6 months postpartum**

	GH or PE N=63	Normotensive N=131	P value	RR	95% CI	aRR *	95% CI	P value
MetS (3 or more components)	22 (34.9)	15 (11.5)	<0.001‡	3.05	1.70-5.47	3.01	1.58-5.71	0.001‡
BP ≥130/85 mmHg†	28 (44.4)	20 (15.6)	<0.001‡	3.14	1.97-5.01	3.35	2.04-5.51	<0.001‡
Triglycerides ≥150 mg/dl	10 (15.9)	7 (5.3)	0.02‡	2.97	1.19-7.44	3.25	1.16-9.10	0.03‡
HDL-cholesterol ≤40 md/dl	32 (50.8)	66 (50.4)	0.96	1.01	0.75-1.36	0.97	0.72-1.31	0.85
Fasting plasma glucose ≥100 mg/dl†	5 (7.9)	2 (1.5)	0.03‡	5.20	1.04-26.06	6.20	1.07-35.76	0.04‡
Waist circumference ≥88 cm	47 (74.6)	96 (73.3)	0.85	1.02	0.85-1.22	0.91	0.77-1.06	0.22

Values are in n (%) or mean ± standard deviation;

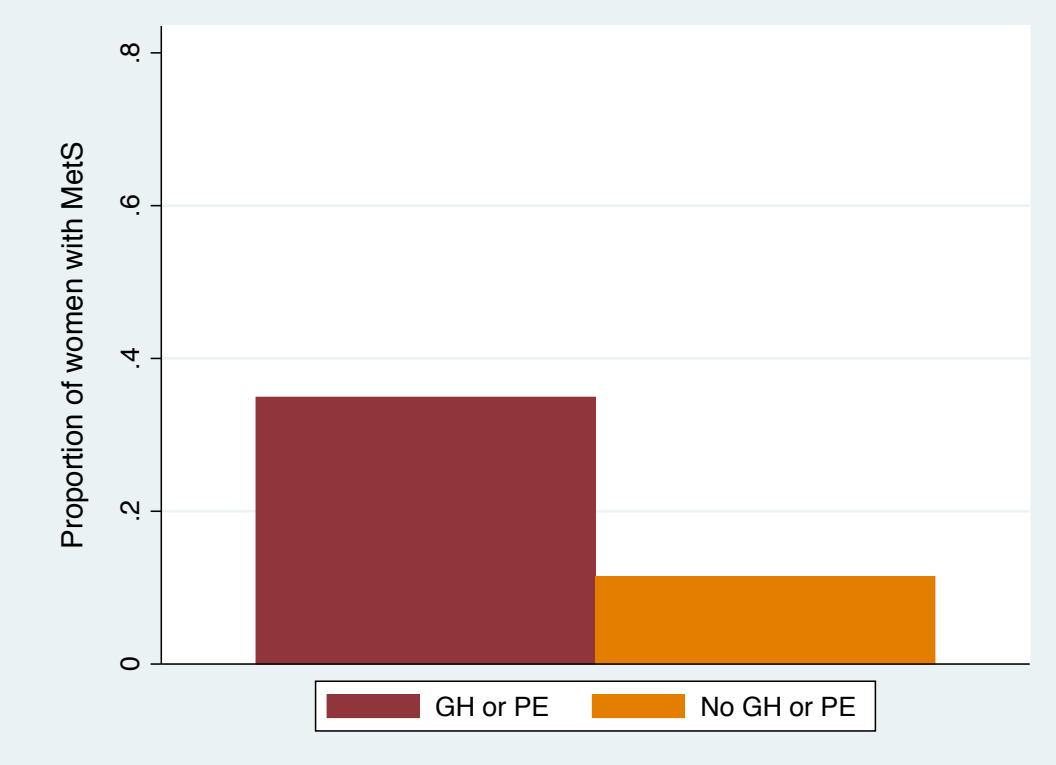
Abbreviations: GH, Gestational hypertension, hypertension occurring after twenty weeks of pregnancy or within 12 weeks postpartum without symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; PE, Preeclampsia, hypertension and symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; RR, relative risk; aRR; adjusted relative risk; MetS, metabolic syndrome, BP, blood pressure; mmHg, millimeters of mercury; mg/dL, milligrams per deciliter; HDL, high density lipoprotein; cm, centimeters;

*Adjusted for age, education, body mass index, marital status, employment status, hormonal contraception and breastfeeding, ‡ P<0.05, ‡p<0.05; †Or treatment



Abbreviations: GH, Gestational hypertension, new onset hypertension after twenty weeks of pregnancy or within 12 weeks postpartum without symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; PE, Preeclampsia, new onset hypertension after twenty weeks of pregnancy or within 12 weeks postpartum with symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications.

Figure 2. **Enrollment and follow-up of postpartum women with and without gestational hypertension and preeclampsia**



Abbreviations: MetS, Metabolic syndrome; GH, Gestational hypertension, new onset hypertension after twenty weeks of pregnancy or within 12 weeks postpartum without symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; PE, Preeclampsia, new onset hypertension after twenty weeks of pregnancy or within 12 weeks postpartum with symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications

Figure 3. **Prevalence of metabolic syndrome by gestational hypertension and preeclampsia at 6 months postpartum**

Chapter 4. INFLAMMATORY MARKERS AND POSTPARTUM METABOLIC SYNDROME AFTER HYPERTENSIVE DISORDERS IN PREGNANCY

4.1 TITLE AND TITLE PAGE

METABOLIC SYNDROME AND HIGH-SENSITIVITY C-REACTIVE PROTEIN AFTER GESTATIONAL HYPERTENSION AND PREECLAMPSIA, A PROSPECTIVE COHORT STUDY

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This project was supported by the Fogarty International Center and the Office of Research on Women's Health (ORWH) of the National Institutes of Health under Award Number K43 TW010363. A. O. was supported by NIH Research Grant D43 TW009580, funded by the Fogarty International Center, National Institute on Drug Abuse, and National Institute of Mental Health. The NIH was not involved in the study design, collection, analysis and interpretation of data, writing of the report or in the decision to submit the article for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. S.T.P. is supported by the Robert B. McMillen Professorship in Lipid Research.

Abstract word count 401 **Text word count 2993** **References 40**

4.2 ABSTRACT

Background

The high risk of metabolic syndrome, cardiovascular disease and premature cardiovascular disease mortality after preeclampsia and gestational hypertension suggests a shared pathophysiologic pathway marked by chronic inflammation. High-sensitivity C-reactive protein, a biomarker of chronic inflammation and an independent predictor for cardiovascular disease, may identify women with postpartum metabolic syndrome who are at increased risk of cardiovascular disease after preeclampsia and gestational hypertension.

Objective

We determined whether hsCRP levels were different in women who had versus those who did not have metabolic syndrome six months postpartum and then evaluated whether this association varied by history of gestational hypertension and preeclampsia during their most recent pregnancy.

Study design

In this prospective cohort study women with and without gestational hypertension or preeclampsia were enrolled during the first 12 weeks after delivery and followed until 6 months postpartum in Nairobi, Kenya. We obtained physical and anthropometric measurements, and measured fasting lipid profile, plasma glucose and High-sensitivity C-reactive protein levels. We categorized as high relative cardiovascular disease risk high-sensitivity C-reactive protein levels greater than 3 mg/L compared to a combined category of low and intermediate risk (<3 mg/L). Generalized linear regression model with Poisson distribution was used to estimate crude and

adjusted relative risk of elevated high-sensitivity C-reactive protein levels comparing women with and those without metabolic syndrome. In a subgroup analysis, we stratified the association between high-sensitivity C-reactive protein levels and metabolic syndrome by recent diagnosis of preeclampsia and gestational hypertension.

Results

Of the 171 eligible women, 34 (19.9%) were diagnosed with metabolic syndrome at 6 months postpartum (Figure 1). More than half, 20 (58.8%) of participants who had metabolic syndrome compared to one quarter, 35(25.5%) of those who did not have metabolic syndrome ($p<0.001$) were diagnosed with gestational hypertension or preeclampsia during their most recent pregnancy. Median (interquartile range) high-sensitivity C-reactive protein levels were higher in women with compared to those without metabolic syndrome, 4.7 (1.8-5.9) versus 2.2 (1.1-4.4) mg/L respectively. High-sensitivity C-reactive protein were more likely to be high (>3 mg/L) in women with compared to those without metabolic syndrome, (Relative Risk 1.70 95% Confidence interval 1.05,2.73, p=0.03). In the subgroup analysis, the high-sensitivity C-reactive protein remained statistically significantly higher in the hypertensive (Relative Risk 2.16 95% Confidence interval 1.01,4.62, p=0.04) but not in the normotensive group (Relative Risk 1.46 95% Confidence Interval 0.93,2.28).

Conclusion

Women with metabolic syndrome at six months postpartum in urban Kenya are more likely to have elevated high-sensitivity C-reactive protein levels, especially when they have gestational hypertension or preeclampsia. Longitudinal studies to assess whether women with hypertension during pregnancy and persistently elevated high sensitivity-C-Reactive Protein post-partum have increased incidence of cardiovascular morbidity are warranted.

Key words: prospective cohort, metabolic syndrome, gestational hypertension, preeclampsia, dyslipidemia, hyperglycemia, postpartum, cardiovascular disease, pregnancy, Kenya, sub-Saharan Africa, high-sensitivity C-reactive protein, inflammation

4.3 INTRODUCTION

Metabolic syndrome (MetS) is a cluster of five atherosclerotic cardiovascular disease (CVD) risk factors; central obesity, atherogenic dyslipidemia (hypertriglyceridemia, low high-density lipoprotein [HDL] cholesterol), elevated blood pressure, and hyperglycemia. MetS is associated with a two-fold or greater risk of CVD. CVD is the leading cause of mortality globally, and three quarters of deaths attributable to CVD occur in low and middle income countries, including sub-Saharan Africa[2]. Although MetS and CVD may have genetic origins, environmental causes such as excess caloric intake and physical inactivity play a critical role in their etiology and chronic inflammation due to a number of causes is a hallmark of both conditions[3-5]. There is growing evidence that two hypertensive conditions in pregnancy, preeclampsia (new onset hypertension with symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications), and gestational hypertension (new onset hypertension without end organ damage)[6] both increase the risk of developing the MetS[7-12]. Also, gestational hypertension (GH) and preeclampsia (PE) are associated with two-fold or greater risk of future CVD, type 2 diabetes and premature CVD-mortality[13-16]. These associations suggest a shared pathogenesis, that includes inflammation and endothelial dysfunction for these conditions, and suggests a potential role for inflammatory biomarkers in the identification, evaluation and monitoring of treatment for MetS and CVD risk factors, especially after GH and PE [10,17,18].

High-sensitivity C-reactive protein (hsCRP) is a sensitive biomarker of chronic inflammation and an independent predictor for CVD and type 2 diabetes[19-22]. Notably, hsCRP is unlikely a cause of CVD but a downstream product of a chronic inflammatory process initiated by activation of the interleukin 1ß, interleukin 6 and C-reactive protein pathways[23]. The contribution of chronically elevated hsCRP on risk of CVD is comparable to that of total cholesterol, HDL cholesterol, and elevated blood pressure[23]. For this reason, CVD risk scores, like the Reynold Risk Score, that incorporate hsCRP may outperform the traditional Framingham score in prediction of CVD risk[5,23,24]. High levels of hsCRP have also been reported in patients with MetS[20]. Elevated hsCRP and increased risk of MetS have independently been reported months to decades after pregnancies complicated with GH or PE in high income settings[25]. Similar associations have not been studied in sub-Saharan Africa, a region affected by high burden of GH, PE and CVD and where hsCRP might be useful in identification and management of women with increased CVD risk after GH or PE.

We evaluated whether hsCRP values were elevated among women with versus those without MetS at 6 months postpartum and tested if the association varied with history of GH or PE in the most recent pregnancy. We hypothesized that due to underlying chronic inflammation, women with MetS would have higher hsCRP levels compared to those without MetS, and that hsCRP levels would be higher in the subgroup of women with a history of GH and/or PE compared to those without these conditions, as a result of endothelial dysfunction and inflammation during pregnancy complicated with these conditions.

4.4 MATERIALS AND METHODS

Study Design and Setting

This was a prospective cohort study designed to evaluate the risk of MetS and concentrations of inflammatory markers after pregnancies complicated by GH or PE at Kenyatta National Hospital (KNH), the largest national teaching and referral hospital in Nairobi, Kenya. The study population comprised women who had been followed from immediate postpartum to 6 months after delivery. The protocol was approved by the Kenyatta National Hospital/University of Nairobi Ethical Review Committee and University of Washington Institutional Review Board. All participants signed written informed consent prior to any study procedures.

Recruitment

We screened postpartum women just prior to their discharge from the postnatal wards who lived within 50 km of the hospital. Eligible women were HIV uninfected, not planning pregnancy for at least 6 months, aged 28 years or older and without conditions predisposing to or suggestive of prepregnancy MetS such as pregestational diabetes, chronic hypertension, malignancy, renal, hepatic or biliary disease. We excluded women with suspected pregestational MetS, specifically, those who used statins, insulin, oral hypoglycemic agents or antihypertensives before the current pregnancy. Eligible women with and without GH or PE were invited for enrollment within 12 weeks, and only those who returned and provided written informed consent were enrolled.

At enrollment participants underwent structured interviews, physical and anthropometric measurements of BP, weight, height, and waist circumference. All study procedures were conducted by trained research study nurses. A study nurse and community health worker called enrolled participants each month to update contacts and confirm the sixth month visit.

Six-month follow-up and study procedures

At the 6 month follow-up, each participant underwent a structured interview, physical and anthropometric measurements of BP, weight, height, hip and waist circumference and fasting blood draws. Blood samples were collected into evacuated blood collection tubes with ethylenediaminetetraacetic acid anticoagulant (BD Vacutainer), centrifuged in a refrigerated centrifuge, placed into an ice bath at 2-4°C and then kept frozen at -80°C at the University of Nairobi laboratory, in Kenya. Samples were later batched and shipped for testing by the Department of Laboratory Medicine Research Testing Service at the University of Washington in Seattle, United States. The specimens were thawed, mixed thoroughly then analyzed using the Beckman Coulter AU5812 automated chemistry and immunochemistry analyzer for lipids, glucose and hsCRP.

Definition of metabolic syndrome

MetS was diagnosed by presence of 3 or more of the following 5 components of the harmonized 2009 consensus criteria or their treatment: 1) central obesity (waist circumference≥ 88 cm), 2) fasting hypertriglyceridemia (≥150 mg/dL), 3) low fasting HDL-cholesterol (<50 mg/dL), 4) elevated blood pressure (BP) (systolic BP ≥130 or diastolic BP ≥85 mm Hg), and 5) fasting hyperglycemia (≥100 mg/dL)[26].

For the subgroup analysis, we defined PE as new onset hypertension with symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications and GH as hypertension without end organ damage as per the International Society for the Study of Hypertension in Pregnancy classification guidelines[6]. Hypertension was defined as BP ≥ 140/90 mmHg at least 2 hours apart at rest, after 20 weeks of pregnancy and before 12 weeks postpartum.

High-sensitivity levels categories

The primary outcome, high-sensitivity CRP (hsCRP), was categorized as low, intermediate and high relative CVD risk if below 1 mg/L, between 1-mg/L and above 3 mg/L respectively[27]. This was based on the association between hsCRP on vascular risk which has been previously described as linear with levels of hsCRP < 1, 1 to 3, and > 3 mg/L showing evidence of lower, average, and higher relative vascular risk. Our analysis was restricted to women with hsCRP levels ≤ 10 mg/L, first because hsCRP is more sensitive at levels ≤ 10 mg/L compared to traditional CRP for predicting future CVD risk[28], and secondly because hsCRP levels above 10 mg/L indicate an acute inflammatory or infectious process which are frequent in this setting and not with chronic inflammation consistent with poor metabolic health.

Statistical analysis

We used the student t test, medians and interquartile ranges or nonparametric Wilcoxon rank sum test for continuous variables and Pearson's chi-square tests or Fisher's exact tests for categorical variables to compare the characteristics of women with and without MetS at 6 months postpartum and to evaluate the association between MetS and high hsCRP levels. Using generalized linear regression models, we evaluated the risk of having a high CVD risk hsCRP (>3 mg/L) versus a combined intermediate and low CVD risk (≤3 mg/L) level, when comparing women with versus those without MetS. We repeated the above analysis stratified by a diagnosis of GH or PE versus normal blood pressures during the most recent pregnancy.

All multivariable models were adjusted for maternal age and BMI. P value was significant at <0.05. Analyses were conducted using STATA version *13*. College Station, TX: StataCorp LP.

4.5 RESULTS

Between November 2016 to August 2018 we enrolled 213 participants, of whom 194 completed interviews, physical examination and analysis for fasting plasma glucose, lipid profile and hsCRP at 6 months postpartum (Figure 1). Of these, 23 (11.9%) had hsCRP levels above 10mg/L leaving 171 participants (88.1%) for this analysis (Figure 1). Nearly one fifth of women had MetS (34, 19.9%) and 137 (80.1%) did not. Slightly over half of women with MetS (20, 58.8%) also had GH or PE while only one quarter (35, 25.5%) of those without MetS (p<0.001) were also diagnosed with these conditions during their most recent pregnancy.

As expected, there were significant differences in some anthropometric and biochemical measurements between women who had and those who did not have MetS. Women with MetS were more likely to have higher mean systolic blood pressure (BP) (134.8 versus 122.0 mmHg, p<0.001), diastolic BP (90.7 versus 81.5 mmHg, p<0.001), weight (83.6 versus 72.4 Kilograms, p<0.001), waist circumference (102.2 versus 92.8 centimeters, p<0.001), hip circumference (114.4 versus 108.8 centimeters) and body mass index (BMI) (34.5 versus 31.1 kg/m^2 p=0.003), compared to those who did not have MetS. Women with MetS were also more likely to be obese ([BMI≥30 kg/m^2]82.4% versus 50.4%, p<0.001) relative to those without MetS postpartum. MetS was significantly associated with dyslipidemia and hyperglycemia; specifically, women with Mets had higher mean levels of triglycerides (116.8 versus 76.7, p<0.001, remnant-cholesterol (23.4 versus 15.3, p<0.001)and lower mean levels of high-density lipoprotein (HDL)-cholesterol (44.6 versus 51.8, p<0.001) , compared to women without MetS. In addition, the mean fasting glucose

was higher in women diagnosed with MetS (89.2 mg/L) compared to those who did not a diagnosis of Mets (76.5 mg/L), at 6 months postpartum, p<0.001. Although in neither case, the average fasting glucose reached criteria for diabetes (Table 1).

Metabolic syndrome and hsCRP levels at six months postpartum

Nearly one half (81, 47%) of all the participants had high (>3mg/L), compared to intermediate (1-3 mg/L, 56 [32.3%]) and low (<1 mg/L, 34 [19.9%]) concentrations of hsCRP. The median hsCRP levels were significantly higher in women with MetS compared to those without MetS (4.7 [interquartile range (IQR) 1.8-5.9) versus 2.2 (1.1-4.4) mg/L) (Figure 2a). Significantly more women with MetS (24, 70.6%) compared to those without MetS (57, 41.6%) had higher hsCRP levels associated with increased CVD risk (>3 mg/L) (Table 2). In unadjusted generalized linear regression model, MetS was associated with 70% greater risk of having high hsCRP level (Relative Risk [RR] 1.70 95% Confidence interval [CI] 1.26,2.28, p<0.001) (Table 2). In an adjusted analysis, the risk remained statistically significantly higher (RR 1.40 [1.04,1.88], p=0.03) when adjusted for body mass index and age.

The proportion of women with high concentration of hsCRP levels were higher in women with elevated waist circumference and triglycerides when compared to those with normal waist circumference and low triglycerides (Table 3). However, the association was not statistically significantly when adjusted for body mass index and age or for but other components of MetS.

hsCRP levels after gestational hypertension and preeclampsia

In subgroup analysis, the association between MetS and hsCRP was different between women with versus those without gestational hypertension and preeclampsia. Specifically, in

hypertensive group, compared to those without MetS, women who had MetS had significantly higher median hsCRP ((4.9 [2.9-6.4] versus 1.7 [0.9-4.2] mg/L) (Figure 2b) and more women with MetS compared to those without MetS had elevated hsCRP > 3mg/L (15, 55.6% versus (5, 17.9%), RR 2.16 CI 1.01, 4.62, p=0.04) compared to not having MetS (Table 4). In the normotensive women, the median and high levels of hsCRP were not statistically significantly different between women who had and those who did not have MetS. Although the median hsCRP was higher ((4.3[1.3-5.7] versus 2.3 [11.1-4.6] (Figure 2c). Although more normotensive women with MetS had higher hsCRP (9, 16.7% versus 5, 8.1%, compared to those without MetS, this increase was not statistically significantly different in crude (RR 1.46 (0.93-2.28) and adjusted models RR 1.21 (0.76-1.93)p=0.41(Table 4).

4.6 DISCUSSION

In this study of the association between inflammation and metabolic syndrome (MetS) following pregnancies with and without new onset hypertension in Kenya and sub-Saharan Africa (SSA), we found that women with MetS at six months postpartum, were more likely than those without MetS to have a high median and higher relative cardiovascular disease (CVD) risk level of high sensitivity C-reactive protein (hsCRP). We also found the association between hsCRP and MetS was more likely to be observed among women with gestational hypertension (GH) or preeclampsia (PE) than those without these conditions in their most recent pregnancy. Thus, hsCRP may be a marker of individuals at heightened risk for future CVD and might be an important target for prevention strategies, particularly less resource rich-settings.

In previous studies conducted in resource-rich countries, the association between postpartum hsCRP and prior GH or PE has been inconsistent, with some studies showing

enrichment in elevated hsCRP among those with GH/PE, and others showing reduced or no difference in hsCRP levels[29]. However, compared to the current study, most of the previous studies did not assess the relationship between MetS and hsCRP and how this varied with exposure to GH or PE. Our findings are consistent with other studies conducted in high income countries showing that postpartum MetS is associated with elevated levels of hsCRP[29-31]. Vallejo et al reported the mean hsCRP to be higher in women with (4.7 mg/L) versus those without (3.0 mg/L) MetS (P <0.001) at 15 weeks postpartum[30]. Their study population comprised only those with hypertensive disorders in pregnancy which was similar to our sub-group of those with GH or PE. Smith et al found that the hsCRP level among women exposed or not exposed to preeclampsia were similar and within the intermediate CVD risk group (2.5 mg/L), however, they did not assess whether these associations varied by diagnosis of MetS[25]. Studies on postpartum hsCRP after pregnancies complicated with GH, PE or MetS however, have not been conducted in SSA. These previous studies and our findings demonstrate that low-grade chronic inflammation occur in pregnancies complicated with GH or PE and postpartum MetS.

Compared to prior studies we found higher median hsCRP levels which were at or above the intermediate to high risk range and especially among women with PE or GH in our population suggesting that significant cardiometabolic changes occur in pregnancy and that these changes occur with higher frequency among women with PE or GH. A majority of our study subjects with GH or PE had hsCRP levels ≥ 3mg/L supporting chronic rather than acute inflammation during pregnancy as the most likely pathway to postpartum MetS after new onset of hypertension in pregnancy.

The factors associated with MetS in our study such as weight elevated blood pressure, dyslipidemia, fasting plasma glucose, as well as large hip and waist circumferences are similar to

those reported in multiple studies. When we evaluated each component of MetS, we noted that hsCRP levels were more likely to be elevated if the women had abdominal obesity. Since abdominal obesity has been associated with increased inflammation, this finding suggests that women with abdominal obesity are more likely to have higher prevalence of chronic inflammation during pregnancy and the postpartum period. However, the lack of association with the other individual components of MetS may have been due to smaller sample sizes within these categories.

Our study had the following strengths. It is the first to estimate the risk of postpartum MetS and its association with hsCRP in Kenya and to the best of our knowledge sub-Saharan Africa, a region heavily burdened with GH, PE, and CVD. Our study findings will inform mechanistic, longer prospective observational mechanistic and intervention studies on CVD risk after GH and PE. Secondly, compared to prior studies, our study population included women with GH, making it more representative as both GH have been associated with increased risk of future CVD and GH is more likely to progress to PE.

Our study limitation was that we only performed measurements at one time point for this analysis. Since hsCRP has high variability within subjects, measurements at different times is more representative of the actual levels of hsCRP. Nevertheless, in routine clinical settings only one reading may be used to predict CVD risk among those with MetS. However, we plan to conduct additional analysis of hsCRP and other inflammatory markers as we follow these participants beyond 6 months post-delivery. Also, most of our participants were multiparous because we restricted our age of eligibility to 28 or greater years, yet majority of primiparous women and those with PE are more likely to be younger in this setting. However, our findings remain clinically significant since the CVD risk increases with age. We did not evaluate participants preconception, and therefore we were not able to completely rule out preexisting MetS.

In conclusion, our study demonstrated that postpartum women with MetS are more likely to have elevated hsCRP levels, a marker of chronic inflammation, especially when they have GH or PE. Since elevated levels of hsCRP further predict the risk of CVD, women with MetS and especially after PE or GH may benefit from hsCRP monitoring to target those at highest risk for interventions that may reduce risk for CVD. Multiprong interventions including weight control, dietary advice, increased physical activity, and administration of BP and lipid lowering drugs should therefore be considered in patients with GH and PE and elevated hsCRP.

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Table 7. Characteristics of study participants by metabolic syndrome (MetS)

		MetS N=34	No MetS N=137	P value
Characteristic		**n (%) or mean ± SD**	**n (%) or mean ± SD**	
Age, years		32.6 ± 4.0	33.3 ± 3.7	0.41
	≥ 35	31 (32.4)	123(32.1)	0.98
Married		18(91.2)	32(89.8)	0.81
Secondary or higher education		19(55.9)	100(73.0)	0.05
Employed		4(76.5)	102(74.5)	0.81
Hormonal contraception		4(11.8)	22 (16.1)	0.53
Parity		4 ± 2	3± 1	0.55
Maternal weight, kilograms		83.6 ± 11.7	72.4 ± 13.7	<0.001*
Waist circumference, centimeters		102.2 ±9.5	92.8 ± 11.6	<0.001
Hip circumference, centimeters		114.4 ± 16.2	108.8 ± 10.0	<0.001*
Breastfeeding		20 (58.8)	73 (53.3)	0.56
GH or PE		20 (58.8)	35 (25.5)	<0.001*
SBP, mm Hg		134.8 ± 12.9	122.0 ± 17.1	<0.001*
DBP, mm Hg		90.7 ± 10.2	81.5 ± 12.4	<0.001*
Body mass index (kg/m^2)		34.5 ± 4.9	31.1 ± 6.0	0.003*
	≥30 (obese)	28(82.4)	69(50.4)	<0.001*
Total cholesterol, mg/dl		181.0 ±31.5	174.5±33.9	0.31
Triglycerides, mg/dl		116.8 ± 55.6	76.7 ± 51.9	<0.001*
HDL-cholesterol, mg/dl		44.6 ± 6.9	51.8 ± 11.1	<0.001*
LDL-cholesterol, mg/dl		113.0 ±24.2	107.3±28.4	0.28
Remnant-cholesterol, mg/dl		23.4 ± 11.1	15.3 ±10.4	<0.001*
Fasting plasma glucose, mg/dl		89.2 ± 22.8	76.5 ± 7.1	<0.001*

Values are in n (%); Abbreviations: Mets, Metabolic syndrome; GH, Gestational hypertension, hypertension occurring after twenty weeks of pregnancy or within 12 weeks postpartum without symptoms or signs of end organ damage such as proteinuria, renal, liver, cardiac or neurological complications; PE, Preeclampsia, hypertension and symptoms or signs of end organ damage; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BP, Blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein *p<0.05

Table 8. Metabolic syndrome and high relative CVD risk levels of hsCRP

MetS or components	hsCRP >3 mg/L N=81	hsCRP ≤3 mg/L N=90	RR	95% CI	P	aRR	95% CI	P
MetS (≥3 components)	24 (29.6)	10 (11.1)	1.70	1.26- 2.28	0.03*	1.40	0.85-2.30	0.18
BP ≥130/85 mm Hg	20 (24.7)	26 (28.9)	0.91	0.55-1.52	0.73	0.88	0.53-1.47	0.63
TG ≥150 mg/dl	11 (13.6)	5 (5.6)	1.52	0.81-2.87	0.20	1.30	0.68-2.48	0.42
HDL-C ≤40 mg/dl	42 (51.9)	42 (46.7)	1.12	0.72-1.72	0.62	1.00	0.64-1.56	0.99
FPG ≥100 mg/dl	4 (4.9)	2 (2.2)	1.43	0.53-3.90	0.49	1.44	0.53-3.97	0.48
WC ≥88 centimeters	70 (86.4)	54 (60.0)	2.71	1.284.55	0.01*	1.63	0.79-3.37	0.19

Values are in n (%); hsCRP-high-sensitivity C-reactive protein, MetS, Metabolic syndrome; BP, Blood pressure; TG, triglycerides, HDL-C, high density lipoprotein cholesterol; FPG-fasting plasma glucose; WC, waist circumference; RR, relative risk; * P<0.05

Table 9. Metabolic syndrome and high relative CVD risk levels of hsCRP stratified by gestational hypertension and preeclampsia status

	Gestational hypertension and preeclampsia					No Gestational hypertension or preeclampsia				
Metabolic syndrome	¶hsCRP >3 mg/L N=27	hsCRP ≤3 mg/L N=28	RR	95% CI	P	¶hsCRP >3 mg/L N=54	hsCRP ≤3 mg/L N=62	RR	95% CI	P
MetS (≥3 components)	15 (55.6)	5 (17.9)	2.18	1.02-4.67	0.04*	9 (16.7)	5 (8.1)	1.46	0.71-2.99	0.30
BP ≥130/85 mmHg	14 (51.9)	12(42.9)	1.20	0.56-2.56	0.63	6 (11.8)	14(22.6)	0.62	0.26-1.45	0.27
TG ≥150 mg/dl	6 (22.2)	3 (10.7)	1.46	0.58-3.62	0.41	5 (9.3)	2 (3.2)	1.58	0.63-3.99	0.32
HDL-C ≤40 mg/dl	16 (59.3)	11 (39.3)	1.50	0.70-3.25	0.29	26 (48.1)	31(50.0)	0.96	0.56-1.64	0.88
FPG ≥100 mg/dl	3 (11.1)	2 (7.1)	1.25	0.38-4.15	0.72	1 (1.9)	0 (0.0)	2.20	0.31-15.69	0.44
WC ≥88 cm	26 (96.3)	15 (53.6)	8.88	1.20-65.42	<0.03*	44(81.5)	39 (62.9)	1.75	0.88-3.48	0.11

Values are in n (%); hsCRP-high-sensitivity C-reactive protein, MetS, Metabolic syndrome; BP, Blood pressure; TG, triglycerides, HDL-C, high density lipoprotein cholesterol; FPG-fasting plasma glucose; WC, waist circumference; RR, relative risk; * P<0.05

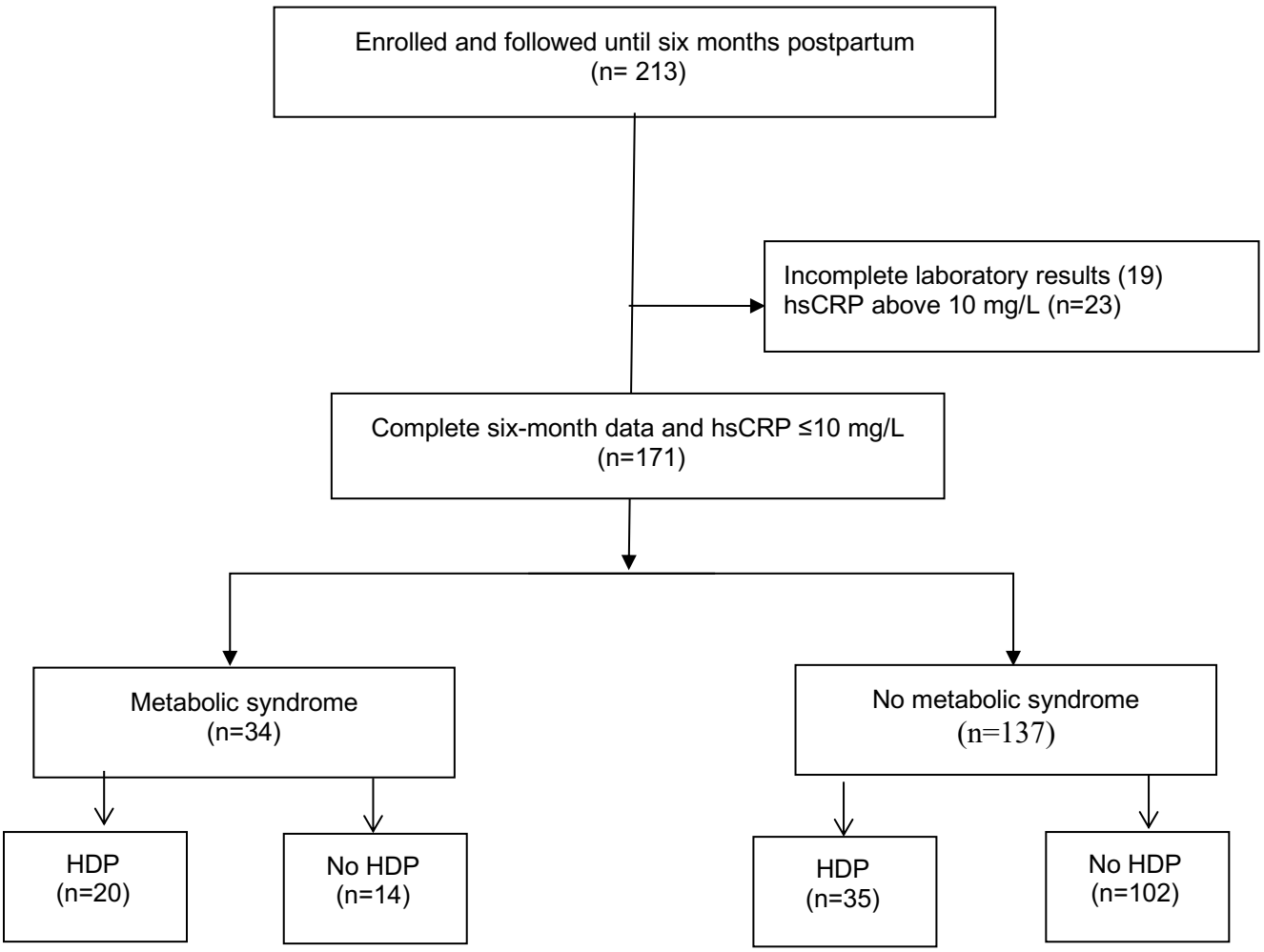

Figure 4. Enrollment and follow-up of study participants

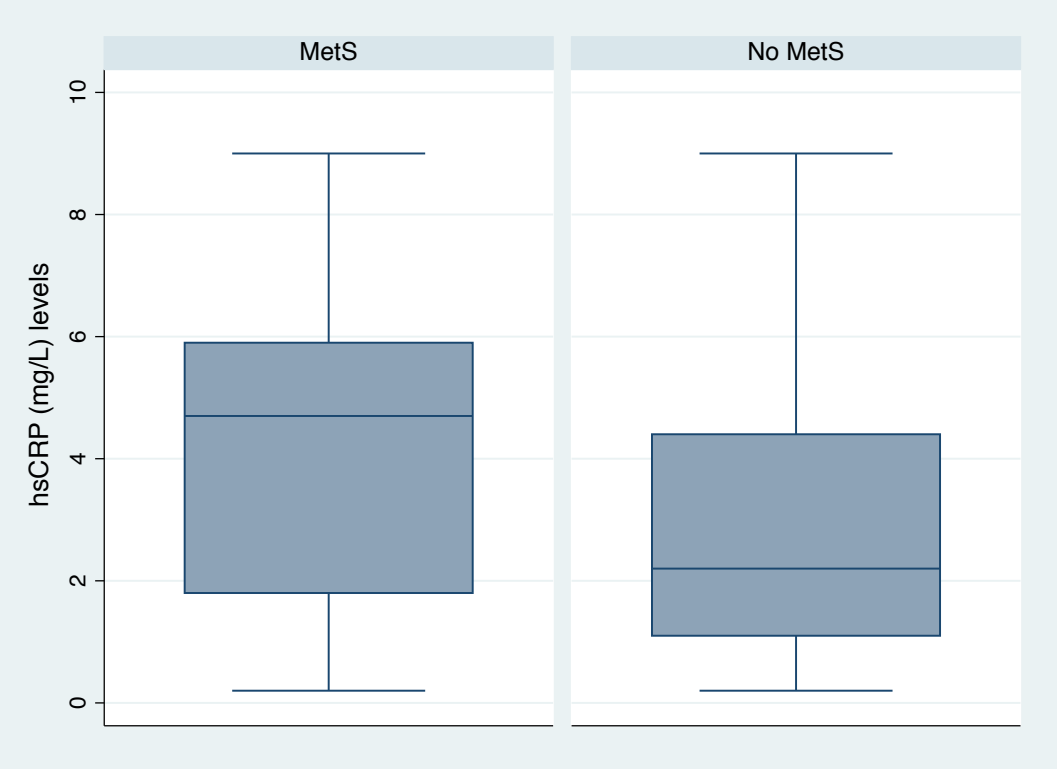
MetS
No MetS
hsCRP (mg/L) levels
0
2
4
6
8
10

Figure 5 a

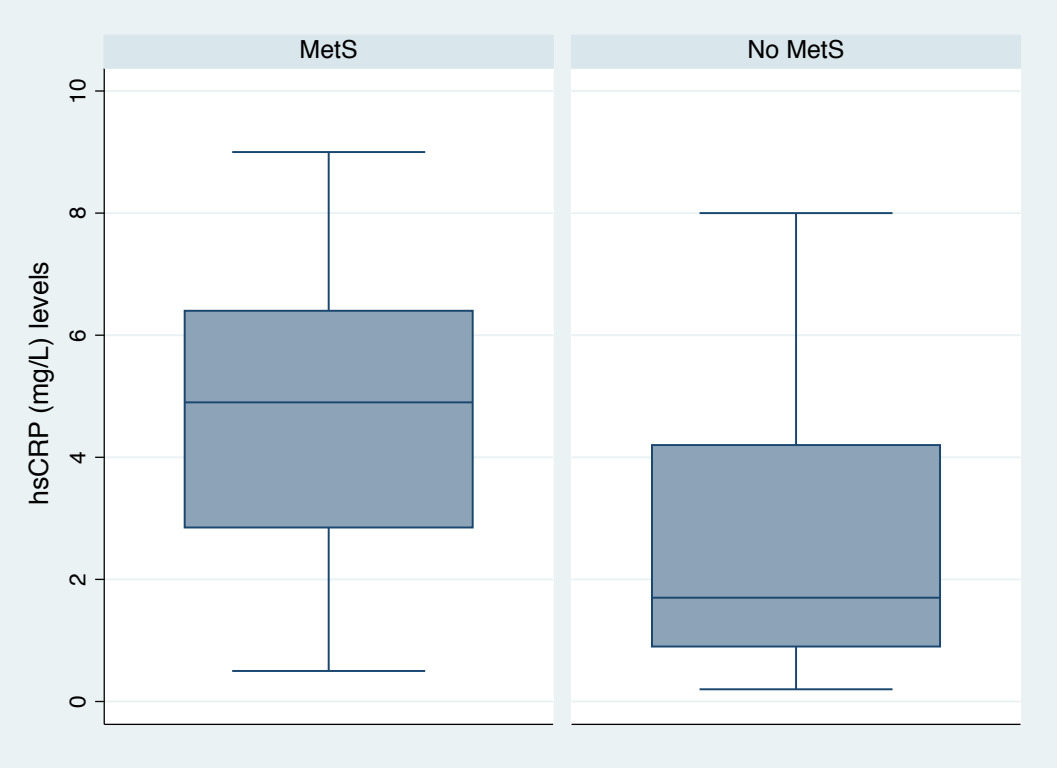
MetS
No MetS
hsCRP (mg/L) levels
0
2
4
6
8
10

Figure 5b

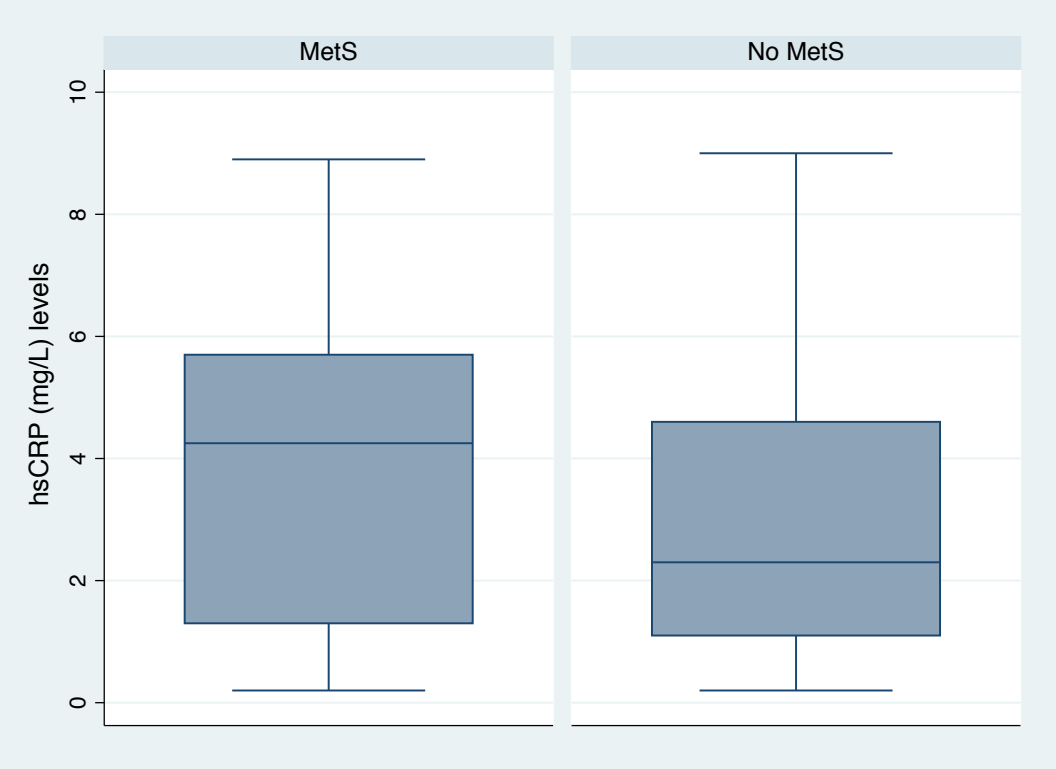

Figure 5c

Figure 5. Box plots comparing median high-sensitivity C-Reactive Protein(hsCRP) levels by metabolic syndrome at 6 months postpartum

Box plots comparing median high-sensitivity C-Reactive Protein(hsCRP) levels by metabolic syndrome at 6 months postpartum among: (a) all women (b) women with gestational hypertension or preeclampsia (c) women with normal blood pressures

Abbreviations: hsCRP-high-sensitivity C-reactive protein; MetS, Metabolic syndrome

Chapter 5. CONCLUSIONS AND WAY FORWARD

5.1 CONCLUSIONS

These studies demonstrate high prevalence of cardiovascular risk among individuals exposed to HIV (Chapter 2) and women exposed to hypertensive disorders in pregnancy, specifically preeclampsia and gestational hypertension (Chapters 3 and 4). In Chapter 2, we show that while the risk of MetS was similar among adults on versus those not on antiretroviral therapy (ART), ART in general was associated with dyslipidemia, low HDL levels and elevated blood glucose which are risk factors for CVD. On the other hand, HDP was a risk factor for MetS and therefore CVD. This increased CVD risk is most likely due to increased inflammation as evidence by the higher levels of hsCRP and higher prevalence of high relative CVD risk levels of hsCRP (>3mg/L) in women with MetS and among women with MetS and have HDP. Therefore, additional studies and interventions to improve care are needed.

5.2 WAY FORWARD

In general, CRP is considered as a downstream biomarker for atherothrombosclerosis and is therefore not a cause but consequence of chronic inflammation. Inflammatory markers like interleukin 6 (IL-6) have both upstream and downstream effects and may be more specific to etiopathogenesis of chronic inflammation related to CVD. Other biomarkers of inflammation such as Interleukin 1 and tumor necrosis factor may also be critical in the etiology of MetS, CVD following GH or PE and among HIV infected individuals and warrant further evaluation. In the HIV infected group, these studies should have HIV negative control groups to evaluate whether HIV and its treatment or traditional risk factors are the responsible for CVD risk in this population.

Thus, we need to demonstrate the association between these inflammatory markers and other markers of insulin resistance or metabolic syndrome in our study populations.

There is a need to evaluate interventions that can lower CVD risk in our study populations. For example lipid lowering drugs, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, or statins ability to reverse the angiogenic imbalance, endothelial or endovascular damage, inflammatory state, and oxidative stress associated with cardiovascular disease in non-pregnant patients may be of benefit to patients with or those who have recently had HDP[1-4]. Secondly, we can explore early implementation of lifestyle interventions that lower cardiovascular disease risk[5]. Smoking cessation can reduce cardiovascular events and all-cause mortality by up to one-third[6]. Screening, monitoring and reduction of blood pressure through lifestyle changes such as diet modification, weight loss, and increasing physical activity, and pharmacotherapy are effective in reducing cardiovascular disease risk from hypertension[5,7]. Similarly, screening, diagnosis, monitoring, lifestyle changes and medical management of diabetes may also lower the risk of cardiovascular disease among diabetics. The American Heart Association recommended physical activity reduce cardiovascular disease risk by targeting low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), and blood pressure lowering[8]. Finally, dietary recommendations to reduce the risk of hypertension and cardiovascular disease such as Dietary Approaches to Stop Hypertension (DASH) Dietary pattern, rich in fruits, vegetables, whole grains, and low fat dairy foods, reduced dietary sodium intake, the Mediterranean dietary pattern, rich in fruits, vegetables, legumes, nuts, fish, and low fat dairy with moderate consumption of olive oil as the primary source of fat, the Nordic diet, or the AHA recommended diet low in saturated fats and high in fruits, vegetables, fish, whole grains, and high-fiber foods and reduction in alcohol consumption especially among

heavy drinkers are evidence based effective interventions[9-11]. Other pharmacological management options include metformin, an insulin-sensitizing agent, fibrates and thiazolidinediones, niacin, aspirin[12-17].

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VITA

Alfred Osoti earned his medical degree and Masters in Obstetrics and Gynaecology from University of Nairobi in Kenya and Master of Public Health (Epidemiology) at the University of Washington. As an MPH student he was awarded the UW School of Public Health MPH Outstanding Student of the Year in the Department of Epidemiology. Prior to joining the MPH program, Dr. Osoti was a Fogarty Scholar and Fogarty Fellow within the Kenya Research and Training Center. Prior to that, he conducted cohort studies on incident HIV infection risk among couples in stable partnerships and a randomized clinical trial on male partner testing during pregnancy. He is currently a Senior Lecturer, Department of Obstetrics and Gynecology at the University of Nairobi in Kenya. He has conducted and published several collaborative studies with the World Health Organization and other partners on maternal health. He is also a recipient of the 2018 Word Congress of Cardiology and Cardiovascular Health and American Heart Association's International Young Investigators Award. He is an awardee of the inaugural National Institutes of Health Emerging Global Leader K43 training grant to evaluate the risk and correlates of metabolic syndrome after delivery in a 3-year prospective cohort. The focus of this dissertation is on risk of metabolic syndrome among HIV infected adults and the risk of metabolic syndrome six months postpartum after hypertensive disorders in pregnancy. It uses data from the K43 grant.