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REPEAT HIV TESTING AND SEROCONVERSION DURING PREGNANCY IN THE CONTEXT OF PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV IN SUB-SAHARAN AFRICA: SYSTEMATIC REVIEW AND META-ANALYSIS

BY

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DECLARATION

I, David Owiredu, do hereby declare that except for cited references that have been duly acknowledged, this work is the result of my own original research done under supervision, and that this work, either in whole or in part has not been presented elsewhere for another degree.

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ABSTRACT

Background: Pregnant women may consistently be at risk of acquiring HIV infection during pregnancy. In sub-Saharan Africa where the prevalence of HIV is high, this only presents implications on vertical transmission of the virus during this critical period. Detecting seroconversions that occur during pregnancy is critical to preventing vertical transmission thus the need for repeat HIV testing. The objective of this systematic review and meta-analysis was to estimate the proportion of repeat HIV testing and the incidence rate of HIV seroconversions detected as a result. The review also sought to compare the risk of MTCT during pregnancy in seroconverted pregnant women receiving ART to seroconverted pregnant women naïve of ART.

Methods: PubMed, Cochrane Central, HINARI, Google Scholar and African Journals Online databases were searched between January 2007 and 10th October 2020 for articles and abstracts reporting on repeat HIV testing and seroconversion during pregnancy in any sub-Saharan African Country. The eligibility criteria were studies conducted in sub-Sahara Africa with data on the conduction of repeat HIV testing during pregnancy. Random effects models were constructed to pool the proportion of repeat HIV testing and the incidence rate of seroconversion during pregnancy. 1725 studies were identified, out of which 22 studies met the inclusion criteria and contributed data for the meta-analysis.

Results: The pooled proportion of repeat HIV testing during pregnancy in sub-Saharan Africa was estimated at 78.4% (CI: 66.7% to 90.1%) with Western Africa having the highest pooled estimate of 90.8% (95% CI: 72.7% to 100%). The pooled cumulative incidence of HIV seroconversion during pregnancy detected through repeat HIV testing was 1.5% (95% CI: 0.9% to 2.1%) the

pooled incidence rate of seroconversion during pregnancy pegged at 3.9 per 100 person years (95% CI: 1.4 to 6.5 per 100 person years). In the only one study that assessed vertical transmission in women who seroconverted during pregnancy, none of the six women who seroconverted transmitted the infection to their babies.

Conclusion: The pooled estimates of HIV seroconversion during pregnancy are high which re-echoes the importance of continuing the implementation of repeat HIV testing especially in areas the prevalence of HIV is considerably high as this would set the stage for detecting seroconversions to enable the timely initiation of ART which is critical reducing the risks of vertical transmission.

DEDICATION

I dedicate this dissertation to God Almighty, my mother, Theresah Lolo, my siblings and all of my big happy family. They have been an unending source of inspiration and support.

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AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Care
ART	Antiretroviral Therapy
CS	Caesarean Section
CT	Counselling and Testing
GDHS	Ghana Demographic and Health Survey
GHS	Ghana Health Service
HIV	Human Immunodeficiency Virus
HSS	HIV Sentinel Survey
MTCT	Mother-to-Child Transmission
NACP	National AIDS Control Program
PMTCT	Prevention of Mother-to-Child Transmission
UNAIDS	United Nations Commission on AIDS
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Human Immuno-Deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) pandemic still remains a global burden notwithstanding the extensive effort and investments that have been made to control it. According to the World Health Organization (WHO), 37.9 million people were estimated to be living with HIV globally at the end of 2018, with about 1.7 million people newly infected and with 770,000 HIV-related deaths (WHO, 2020).

With the global estimate of 160,000 new infections in children in 2018, this translates to roughly 438 new infections each day (UNAIDS, 2018). An estimated 1.7 million children below the age of 15 years were reported to be living with HIV in 2018, representing about 4.5% of the global estimate for people living with HIV (UNAIDS, 2018). In the previous year (2017), an estimated 180,000 new paediatric infections were recorded despite the adoption and implementation of highly effective preventive interventions (UNAIDS, 2018). The vast majority (about 90%) of these cases occurred in sub-Saharan Africa (Drake et al., 2014a) where incidence during pregnancy, childbirth and breastfeeding is high - more than 3 cases per 100 person-years.

Transmission of HIV from infected mothers to their children (vertical transmission) contributes to about half of all paediatric infections in setting with high burden of the infection (Drake et al., 2014b; Ishikawa et al., 2016). In the absence of antiretroviral treatment (ART), the risk of transmission of HIV from seropositive mothers to their babies may be as high as 15-30% during pregnancy and labour, with an further transmission risk between 10-20% linked with extended breastfeeding (Teasdale et al., 2011). Globally, vertical transmission of HIV remains a major

challenge, especially in Sub-Saharan Africa and other low- and middle-income countries (LMICs). The rate of vertical transmission of HIV associated with seroconversion is high (Keating et al., 2012), and coupled with numerous challenges faced by Prevention of Mother-to-Child Transmission (PMTCT interventions in LMICs, puts children at higher risks of infection. This is because, missed opportunities to test and retest women during pregnancy and enrol those who test positive onto ART, conventionally puts the unborn child at an even higher risk of infection and presents a huge challenges towards achieving the goal of eliminating MTCT of HIV (Dako-Gyeke et al., 2016a). The World Health Organization recommends that all pregnant women be tested for HIV during the first trimester and upon testing negative, the test should be repeated in the third trimester by 34 weeks of gestation (Drake et al., 2019; WHO, 2015).

Despite the adoption of these testing strategies, reports indicate prevailing high rates of maternal incidence of HIV during pregnancy in Africa. Incidence rate of HIV as high as 10.8% has been reported in Kenyan couples (Brubaker et al., 2011). The rate of seroconversion during pregnancy in Malawi is reportedly lower (1%) (Keating et al., 2012), although in a meta-analysis, Drake et al (2014) reported a combined seroconversion rate of 3.8 per 100 person-years in African countries.

The current recommendations by the WHO are that all women diagnosed with HIV infection during pregnancy be initiated onto a life-long ART irrespective of the clinical or immunological thresholds such as CD4 counts (WHO, 2013). The advent of these interventions and recommendations have been met with several challenges such as slow uptake and acceptability. At the centre of these challenges are reports of adverse outcomes associated with some ART regimen and the lack of public education that would otherwise inform and encourage uptake rates. Further details of the tests and treatments (ARTs) available, as well as

the repeat testing strategy have been described in sufficient details as part of the conceptual framework and literature review.

Transmission of HIV from an infected mother to a child can occur during pregnancy when the virus in the mother's blood crosses the placenta into the foetal blood (Scarlatti, 2006). There is a 5-10% chance of MTCT during pregnancy if a seropositive pregnant woman is not given an intervention such as Antiretroviral Therapy (ART) (FMOH, 2007). The risk of transmission of the virus from an infected mother to a child however, is highest during labour and delivery (Jourdain et al., 2007). The risk of MTCT during pregnancy and postpartum varies. Without PMTCT intervention, the risk of transmission is between 5-10% during pregnancy, 10-15% during labour and delivery, 5-10% during breastfeeding after birth but between 15-25% without breastfeeding (FMOH, 2007). By 18-24 months postpartum, the overall risk of transmission is between 20-35% and the total risk of MTCT is between 20-40% (Scarlatti, 2006).

Routine HIV testing of pregnant women is imperative to achieving successful PMTCT of HIV (MOH/GHS, 2014; WHO, 2013). Studies have suggested that, pregnant women are at higher risk of acquiring HIV infection compared to the general population (De Schacht, Mabunda, et al., 2014; Feldblum et al., 2014) yet, diagnosing of new HIV infections at the programmatic level, undoubtedly is challenging. Thus, the realistic approach to carry out repeat tests after an initial negative test during pregnancy is more desirable (Mandala et al., 2019). Undoubtedly, this will provide information on the serostatus of pregnant women through counselling and testing (CT) and be enrolled onto PMTCT intervention programs to reduce transmission to babies if they test positive for HIV (Amy et al., 2010; WHO, 2012).

Babies born to women with known HIV-seropositive status are usually closely monitored as HIV-exposed infants, and are given ART regimens (HIV prophylaxis) at delivery and for a period of time postpartum. They are tested through early infant diagnosis programs and in the

incidence of HIV, ART is initiated immediately (MOH/GHS, 2014; WHO, 2010). Collectively, these protocols help reduce the risk of MTCT of HIV both during pregnancy and postpartum. Undoubtedly, identification of pregnant and postpartum women who have seroconverted is key to achieving the UNAIDS 90:90:90 goals (UNAIDS, 2014) that is likely to be missed by several countries in Africa. As a result, many countries have adopted maternal HIV testing as part of routine antenatal care services in order to identify HIV positive pregnant women and initiate ART. However, there is also the need for strategies to identify and test women who miss ANC testing or test negative during pregnancy and acquire the virus in latter trimesters or postpartum.

Since 2006, the World Health Organization has recommended repeat testing for HIV in pregnant women especially in countries profiled as having high prevalence. The guidelines recommended the repeat testing in the third trimester but since 2015, it has extended the recommendation to include repeat testing during pregnancy, at labour or delivery and postpartum (WHO, 2015; World Health Organization, 2007). In countries where the prevalence is low or has areas with considerably higher prevalence (concentrated epidemics), maternal repeat testing is recommended for women in serodiscordant relationships or members of key HIV populations such as female sex workers (FSWs) (WHO, 2015). However, the lack of clarity on the required frequency and specific time points to carry out these testing during pregnancy and postpartum has led to confusion for programme implementers regarding how and when to conduct repeat testing. There is also the possibility of wastage of limited public health resources in situations where programme implementers may conduct more tests as is necessary. The effects of which may be more pronounced in sub-Saharan Africa where there is high prevalence of maternal incidence of HIV and limited health resources.

1.2 Problem Statement

Available evidence indicates that the incidence of HIV among pregnant and postnatal women is high, with a pooled incidence rate of about 4.7 per 100 person-years prepartum and of about 2.9 per 100 person-years during the postpartum period (Drake et al., 2014a). The incidence has been attributed to several factors and physiological changes that arise during pregnancy. Conditions such as disturbances in the vaginal flora, sexual behavioural changes, or prevalence of STDs have been suggested to influence the incidence of HIV in women, especially, pregnant women (Cohen et al., 2010; Eastment & McClelland, 2018; Santelli et al., 2013; Soudeyns, 2015; Wheeler et al., 2012).

High viral loads following maternal HIV acquisition, low levels of passively transferred maternal antibodies, and lack of ART when infections are undetected can contribute to increased incidence of MTCT of HIV (Moodley et al., 2011). Incident maternal HIV infections are invariably linked with increased MTCT risk (Drake et al., 2014a) and the risks even higher in women who seroconvert during pregnancy or breastfeeding (Humphrey et al., 2011; Liang et al., 2009; Scarlatti, 2006). Available information suggests a high incidence of MTCT due to maternal seroconversion in women who initially tested negative during pregnancy (Humphrey et al., 2011; Yeganeh et al., 2014). Unless routine maternal testing and counselling are conducted, pregnant and postpartum women who test negative for HIV in their first antenatal screening but become or seropositive infected later, will have an undetected infection and consequently miss the benefits of PMTCT interventions.

Data to guide repeat testing, frequency and timing of repeat testing, and infections detected as a result of repeat testing are requisite to maximize resources for HIV prevention. Effort to address these gaps is critical in order to prevent new paediatric infections or achieve eradication of MTCT.

1.3 Justification

The prevalence of HIV among pregnant women presents a risk of infection to babies before and after they are born. Transmission of the virus from an infected mother to a child can occur either during pregnancy, labour and delivery or during breastfeeding (Amy et al., 2010; Kinuthia et al., 2015; Mofenson, 2012; Anna J. Rogers et al., 2017; Teasdale et al., 2011).

Data from reports suggests a higher prevalence of HIV in women than in men in sub-Saharan Africa (Hegdahl et al., 2016). Nearly all the incidence of HIV in infants the region is attributed to vertical transmission from an infected mother occurring during pregnancy, labour or breastfeeding (Kharsany & Karim, 2016). The Ghana AIDS commission (2010), reported that about 3% of child mortality in Ghana was as a result of HIV infection. Studies have shown that the higher the viral load in mothers at delivery, the higher the risk of transmission of HIV from the mother to the child (Bulterys, 1998.; Kourtis, Bulterys, Nesheim, & Lee, 2001).

PMTCT interventions are likely to focus on women with established chronic infections and therefore likely to miss acute maternal infections. However, some studies have reported high incidence of MTCT due to maternal seroconversion in women who initially tested negative during pregnancy (Humphrey et al., 2011; Yeganeh et al., 2014). Therefore, repeat testing of HIV is central to PMTCT as it is through that, incident maternal infections, can be detected to allow for timely ARV treatment.

Countries, especially in sub-Saharan Africa need reliable (for example, systematically synthesized) data on the impact of maternal repeat testing for HIV during pregnancy, labour or delivery and postpartum to inform decisions concerning the allocation of HIV resources, especially within the context of preventing vertical transmission of HIV. This systematic review sought to pool the data available on the implementation of repeat HIV testing and the rate of seroconversion detected in women during pregnancy in sub-Saharan Africa. The review

also assessed the data available on the rate of mother-to-child transmission during pregnancy in seroconverted pregnant women to inform evidence-based implementation policies.

1.4 Review Questions

1. What proportion of women were tested for HIV at least twice during pregnancy in sub-Saharan Africa?
2. What is the rate of HIV seroconversion (cumulative incidence and incidence rate of HIV) during pregnancy in sub-Saharan Africa?
3. What is the rate of MTCT of HIV in women who seroconverted during pregnancy and enrolled onto antiretroviral treatment (ART) compared with those who seroconverted but did not enrol onto ART?

1.5 Objectives

1.5.1 General Objective

To conduct a systematic review of studies on repeat HIV testing to detect seroconversion and the rate of vertical transmission during pregnancy in the context of PMTCT of HIV in sub-Saharan Africa.

1.5.2 Specific Objectives

1. To estimate the proportion of women who receive repeat test of HIV during pregnancy.
2. To estimate the incidence rate and cumulative incidence of HIV seroconversion detected through repeat testing during pregnancy.
3. To evaluate the rate of MTCT of HIV in women who seroconverted during pregnancy and enrolled onto antiretroviral treatment (ART) compared with those who seroconverted but did not enrol onto ART.

1.6 Conceptual Framework

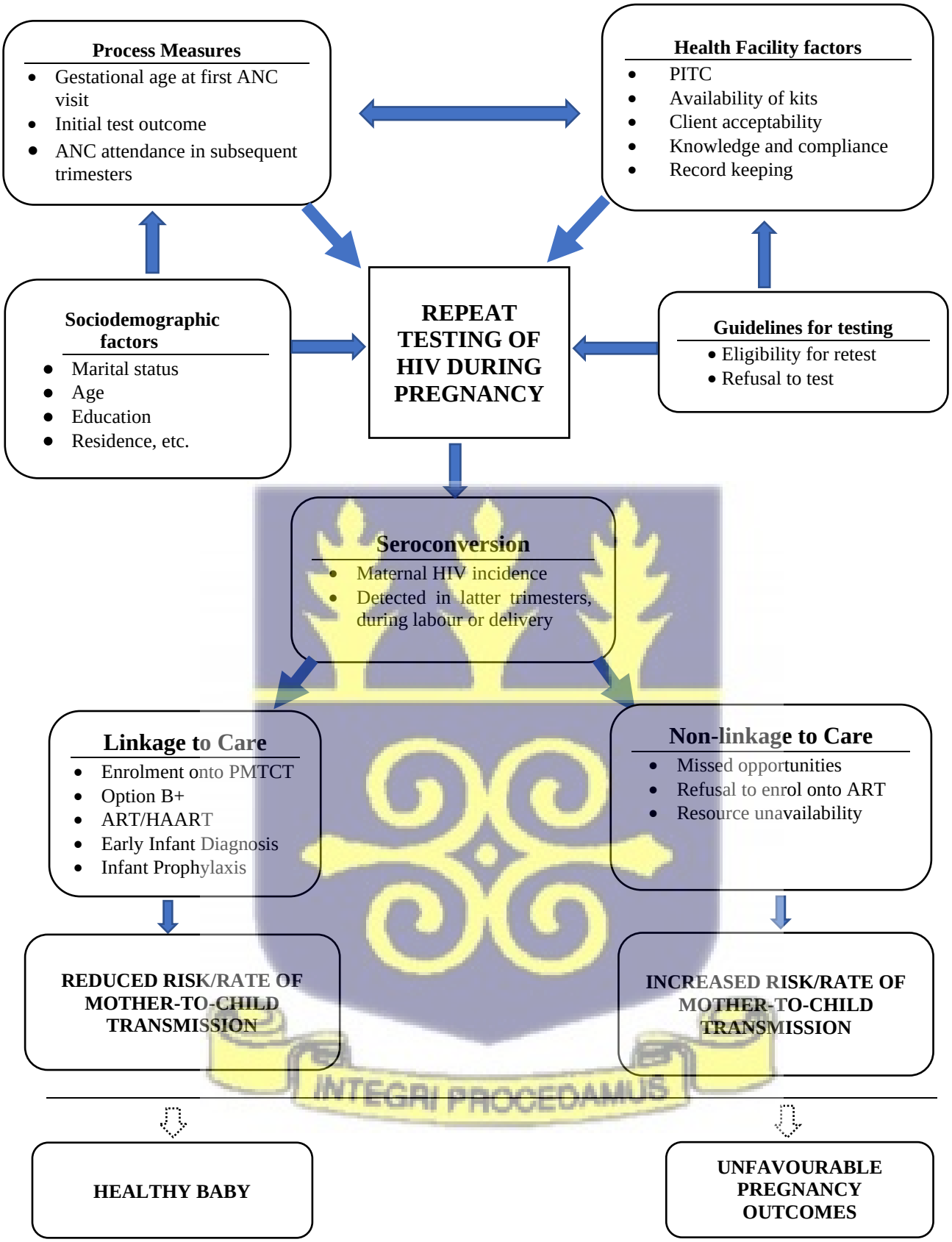

Figure 1: Conceptual framework

Socio-demographic Factors

Reports from studies indicate varying relationships between some sociodemographic factors of pregnant women and health-seeking behaviour, particularly antenatal care (Nketiah-Amponsah et al., 2013; Oladapo & Osiberu, 2009a). Factors such as age, marital status and level of education have all been shown to influence pregnant women's health seeking behaviour of antenatal care at health facilities (Nketiah-Amponsah et al., 2013). Pregnant adolescents for example, have been reported to find it difficult to seek ANC care due to fear of stigmatization, lack of knowledge and guidance on the general need for ANC. Some older pregnant women, especially those who have been pregnant before may feel they are experienced or rather too old to seek antenatal care (Anna Joy Rogers et al., 2016). Married women on the other hand have been reported to be more likely to access antenatal care than unmarried women. Generally, level of education has been associated with health-seeking behaviour of individuals. Educated people are more likely to visit health facilities for medical care than those with low level of education. This translates to low tendency to antenatal clinic during pregnancy among women who are less educated and not well-informed. Accessibility to ANC emanating from distance of health facility from place of residence may affect the timing and frequency of visits to the antenatal clinic, especially in rural communities.

All the above factors, and possibly others, may variably affect repeat testing of HIV. This is because, these factors directly or indirectly influence attendance to antenatal clinic which determines whether or not a pregnant woman would receive counselling and testing for HIV.

Process Measures

Repeat testing of HIV in pregnant women who present at the antenatal clinic would primarily comprise three components: 1) All pregnant women visiting the antenatal clinic should be counselled and tested for HIV, 2) The test should be confirmed and antiretroviral treatment

initiated for those that test positive, and 3) Those that test negative should be eligible for a repeat test in the third trimester. This makes gestational age at first antenatal visit an important factor (Rogers et al., 2017). Pregnant women who first attend the clinic late in pregnancy are less likely to receive repeat tests because a repeat test is carried out usually during the third trimester. Therefore, for a woman who first visits the clinic in the third trimester, no other test will be carried out before delivery if she tested negative in her first test. Pregnant women who are eligible for repeat tests also have to attend the antenatal clinic in the third trimester in other to receive repeat tests. This is critical because of the possibility of seroconversion and the increased risk of MTCT of the virus associated with the high viral load characteristic of acute maternal infection.

Guidelines for Testing

Current WHO guidelines with respect to HIV testing during and after pregnancy are that; the main mode of testing be the provider initiated testing strategy (PIT), where HIV counselling and testing shall be routinely offered to all pregnant women as part of initial and subsequent antenatal care (ANC) services; pregnant women who test negative initially shall be offered the test again in the third trimester; women with an unknown HIV status at the time of labour be offered the test (but not when delivery is imminent) and a woman with an unknown HIV status postpartum shall be offered HIV testing and in a situation when she is unavailable, rapid testing of the new born initiated as soon as possible (within 48 hours postpartum).

The guidelines also recommend that women who initially decline to be tested for HIV be offered the test again in subsequent visits. But in the event of persistent refusal, her decision should be respected and documented but her refusal shall not compromise the quality of care she receives.

Health Facility Factors

As with all health procedures and dispensation, majority of the effort comes from the input of health facilities and providers. The mode of HIV testing in pregnant women as per national guidelines is the Provider-initiated Counselling and Testing (PITC). This means that Health professionals are mandated to counsel pregnant women seeking ANC on HIV and MTCT and offer them the test. Since some clients may decline the test, it is recommended that counselling be exhaustive, making sure that the pregnant woman is fully informed of the benefits of testing for themselves and their unborn child. Health providers' input is therefore key to pregnant women receiving repeat tests of HIV since they have to initiate the procedure. Availability of resources is also a contributing factor for the implementation of repeat tests. Test kits and other laboratory resources are required to be available if routine testing is to be feasible. Shortage of test kits would mean reduced or no repeat testing as health professionals are likely to reserve the kits for initial HIV screening rather than repeat testing a woman who tested negative during her initial screening. Health officers' knowledge of these guidelines and their compliance is central to the conduction of repeat testing of HIV in pregnant women. Follow-up reviewing of records is particularly important for pregnant women that are eligible for repeat testing. Their ANC records need to be reviewed in the third trimester so that they are counselled and offered the HIV test again.

Seroconversion during pregnancy

Pregnant women who are seronegative at the first ANC routine testing may later test positive. This is because, there is the possibility that they were in the window period or had recently contracted the infection. When the test is repeated in subsequent ANC visits (within 3 months since the last documented negative test), these infections are diagnosed making it possible for timely initiation of ART. Detection of seroconversion during pregnancy is imperative as it has

now been suggested that the risk of vertical transmission may be higher in women who seroconverted than women with established seropositivity before pregnancy.

Linkage to Care/Non-linkage to Care

Seroconverted pregnant women at any point (pregnancy, labour, and delivery) are recommended to be initiated on ART on the same day of the confirmed serostatus. This is to allow a timely reduction in the associated risk of vertical transmission which can be achieved by suppression of the infection or reduction of viral load in the mother. In recent times, there have been the introduction of several interventions targeted at preventing mother-to-child transmission of HIV occurring both pre-and postpartum. These interventions focus on treating HIV positive mothers with a range of antiretrovirals (ARVs) in the drive to suppress the viral load which reduces the risk of vertical transmission. WHO's Option B+ is one example of the numerous designed interventions which recommends the initiation of all HIV-positive pregnant women on a lifelong ART regimen. Early Infant Diagnosis (EIDs) are interventions targeted at testing all HIV-exposed babies (babies born to HIV-infected mothers) using less evasive and highly sensitive testing protocols such DNA/PCR testing. This helps in detecting HIV infections in babies to inform initiation of ART.

There are reports of missed opportunities and gaps in the coverage on PMTCT interventions from several sub-Saharan African countries. Largely, this has been attributed to inconsistent ANC visits and service providers failing to implementing PMTCT actions such as HIV counselling and testing (HCT). Instances where HIV-positive women are not initiated on ART or deferred results in increased risk of transmitting the virus to babies.

Risk of Mother-to-Child Transmission of HIV

The relevance of repeat testing for HIV during pregnancy is to detect seroconversions to allow for timely PMTCT interventions to reduce the risk of vertical transmission to babies. There is a wealth of reports that suggests that timely initiation of ART and other prophylactic regimen significantly reduces the risk of vertical transmission. Initiation of ART is particularly important in women who get the infection during pregnancy. This is because, the viral load of HIV in the first three to four months have been found to be high which increases the risk of vertical transmission in the absence of treatment. For this subgroup (seroconverted pregnant women), reports on the extent or risk of vertical transmission are generally lacking since almost always these seroconversions are detected in healthcare settings where ART is initiated after a confirmed positive test. That notwithstanding, the risk of vertical transmission in seroconverted pregnant women is arguably higher as seen in pregnant women with chronic HIV infection but naïve of ART.

CHAPTER TWO

LITERATURE REVIEW

2.1 HIV Testing Strategies

A range of testing strategies have been described and recommended by the World Health Organization. While these strategies are continually reviewed and revised, the choice of testing strategy is heavily dependent on a range of precedent and prevailing factors of which consent is imperative. The testing strategies include; 1) Client-initiated (Opt-in) test which entails a client requesting or initiating to know his or her serostatus, 2) Provider-initiated (Opt-out), in which a qualified health worker initiates the process of HIV counselling and testing but a client is at liberty to decline consent, 3) Diagnostic test, which is a test recommended by a medical professional whenever a person shows signs and symptoms that are consistent with an HIV related infection or AIDS and must be done to help with clinical diagnosis and treatment, and 4) Mandatory test; which is carried out for all blood intended for transfusion or production of blood products such as serum (WHO). Currently, the Provider-initiated counselling and testing (PICT) is recommended by the WHO for use in testing all pregnant women in the quest to eradicate all vertical transmission of the virus (MTCT) (WHO, 2015).

2.2 Repeat Testing of HIV during Pregnancy

Repeat testing of HIV is a protocol or practice where a person who has tested negative in the past is tested again after some time (usually after three months). It is a recommended HIV testing protocol by the World Health Organization, nested into the guidelines for Prevention of Mother-to-Child Transmission (PMTCT) of HIV (WHO, 2015). This recommendation has been adopted and implemented by several countries globally, especially in settings where HIV prevalence is high. Repeat testing as a testing strategy is aimed at detecting seroconversions during pregnancy (Mandala et al., 2019) which presents a timely opportunity to enrol seroconverted pregnant women onto ART. The enrolment of seropositive mothers have been

reported to minimize the risk of mother-to-child transmission (MTCT) of the virus (Dako-Gyeke et al., 2016a; Drake et al., 2019; Gamell et al., 2017; Kourtis & Bulterys, 2010; Mandala et al., 2019) since their viral load is kept low through antiretroviral therapies.

Despite the daunting implications of an unknown and undocumented serostatus especially during pregnancy, HIV testing and for that matter repeat testing is often refused by a lot of people. In pregnant women, the acceptability of an HIV test has been attributed to several factors. These factors include poor knowledge on MTCT of HIV, stigmatisation, lack of support from spouses, lack of or low level of privacy during counselling and testing (CT) process and questionable ethics of some health workers. Thus, to be able to make gains in reducing vertical transmission during pregnancy, it was necessary to integrate HIV counselling and testing into antenatal care services. Through this module, all pregnant women are to be offered HIV counselling and testing on a PITC basis unless they emphatically refuse acceptance. HIV testing during pregnancy is critical as the risk of infection is two-fold since a mother can transmit the virus to the baby through a number of ways (Mark et al., 2012). Though there continue to be growing knowledge and awareness of MTCT of HIV, reports still indicate that considerable amount of vertical transmissions still do occur (De Schacht, Hoffman, et al., 2014; Drake et al., 2019; Mandala et al., 2019). Though repeat testing has been adopted by many countries, data on its implementation and trends in seroconversion rates is scarce.

Drake et al (2017) reviewed and characterized national guidelines on maternal HIV retesting based on the recommended time (period) and number of tests. In their study, they identified 52 countries representative of variations in HIV prevalence, prioritization of MTCT and geography. They then explored national policies regarding MTCT, HIV testing and treatment published between 2007 and 2017 for guidelines on repeat testing during and after pregnancy as well as during labour or delivery.

They found that most of the countries (31) had guidelines for universal HIV testing on an Opt-out basis upon initiation of ANC beyond which 38 countries had guidelines for repeat testing later in pregnancy following an initial negative test. As far as timing is concerned, there were variations which were sensitive, in some cases, to targeted risk factors, unknown serostatus or an amalgamation of factors. They also reported "a clear relationship" between prevalence of HIV and the guidelines for repeat testing where frequent retests were recommended as the prevalence of HIV of a country increased either during pregnancy, labour/delivery and postpartum.

Increasingly, there is the need of linkage of incident paediatric infections to seroconversion in programmatic settings (Mandala et al., 2019). Of the studies that have extensively researched the implementation rates of repeat testing, majority report low levels with bigger chunk of pregnant women being lost to follow-up (LTFU) or missing opportunities to be retested at all (Dako-Gyeke et al., 2016a; De Schacht, Hoffman, et al., 2014; Drake et al., 2014a; Mandala et al., 2019; Anna J. Rogers et al., 2017). Researching into repeat HIV testing in public health facilities in Zambia, Mandala et al (2019) reported that 32.7% of pregnant women involved in the study was lost to follow up as they were not available for retest when eligible. A qualitative study conducted in Kenya by Rogers et al (2016) also reports low implementation rate of repeat HIV testing during pregnancy. In this cascade of a seemingly failing initiative, seroconversions persist and continue to put children at risk of infection.

Despite its direct implications on achieving elimination of Mother-to-child transmission, repeat HIV testing is reported to be plagued with several challenges. As reported by Mandala et al., (2019), Lost to follow up poses perhaps the greatest challenge to repeat HIV testing in pregnant women. Measures of the repeat testing process requires that a person be documented as seronegative in a test conducted preferably three months earlier. Thus, eligible pregnant women

ought to be available after an initial negative test to be retested at least once more before labour and delivery. Findings from various studies however shows a high number of eligible pregnant women being lost to follow up suggesting that a large proportion of pregnant women do not receive repeat tests. The issue of pregnant women lost to follow up is widely discussed but in the context of the number of visits made out of the required number of ANC visits recommended during pregnancy. In the same manner, substantial proportion of missed opportunities have been reported in studies carried out to assess the implementation rate of repeat HIV testing during pregnancy (Dako-Gyeke et al., 2016b; Heemelaar et al., 2015). This coupled with the fact that some pregnant women do not adhere to the required ANC visiting scheme makes repeat testing difficult to carry out. Rojers et al (2017) characterised missed opportunities in three ways; women who failed to return all to the ANC clinic, women whose visiting dates did not coincide with times at which they were eligible for retesting and pregnant women who were not retested despite being eligible and present at the ANC clinic. When studying the progress and missed opportunities in implementing repeat HIV testing in Kenya, they found that over 73% of eligible pregnant women were not retested. The issue of missed opportunities is perhaps most critical in discussing the implementation of repeat HIV testing during pregnancy and is not limited to developing countries alone. A study carried out in the United States of America also reported that about 72% of women eligible for retesting during pregnancy proceeded to delivery without being retested (Liao et al., 2017).

Eligibility for retesting in itself is challenged by a number of factors. This contributes largely to the characteristic high missed opportunities and lost to follow ups recorded. Factors that influence eligibility to receive repeat test of HIV during pregnancy include initial test outcome, gestational age at first ANC visit, consent to receive HIV counselling and testing (HTC). Other factors that have been linked to repeat testing of HIV during pregnancy include

sociodemographic factors such as age and level of education, parity and gravidity and some health facility factors including unavailability of test kits. These factors are discussed in the subsequent paragraphs.

2.3 Factors Associated with Repeat Testing of HIV

2.3.1 Eligibility to receive a retest

WHO guidelines for repeat HIV testing during pregnancy are that, pregnant women be tested for HIV upon their ANC visit and if they are confirmed seronegative, the test be repeated every three months (WHO, 2015). This is recommended largely for settings with high HIV prevalence such as in some Sub-Saharan African countries. Thus countries such as Kenya and Zambia have adopted these recommendations into their national guidelines for PMTCT interventions, where all pregnant women are routinely tested during pregnancy and postpartum (Heemelaar et al., 2015a; Mandala et al., 2019; A J Rogers et al., 2017; Anna Joy Rogers et al., 2016). In countries where the prevalence is low such as in Ghana, retesting is carried out in the third trimester if a pregnant women had an initial negative test earlier during the same pregnancy (MOH/GHS, 2014). Thus, to be eligible for a repeat test, a pregnant first ought to have tested negative in no less than three months prior. As a result, pregnant women that are missed during their initial ANC visit are less likely to receive repeat HIV testing as Rojers et al (2017) reports.

In many developing countries, substantial proportion of pregnant women have been reported to not engage ANC services during pregnancy (Abor et al., 2011; Kyei et al., 2012; Lincetto et al., 2013; Moller et al., 2017; Oladapo & Osiberu, 2009b). This has implications on several maternal and child health (MCH) outcomes of which HIV testing is included. Women that inconsistently visit the ANC clinic during pregnancy are likely to miss scheduled HIV testing at the time they are eligible (Rogers et al., 2017).

On the matters of eligibility, one key point is the switching of ANC clinics during a single term pregnancy. Some pregnant women switch between ANC clinics which when there is no proper documentation and communication would make the conduction of repeat testing difficult and not cost-effective. This is because, pregnant women changing the health facility at which they had ANC visits earlier during pregnancy might result in a gap of information which are often required for health procedures. Incumbent on the challenges in health information documentation in many developing countries (Awoonor-Williams et al., 2013; Bowman, 2013; Mutale et al., 2013; Saleh, 2012; Sirintrapun & Artz, 2015), this poses a challenge to the monitoring and assessment of key health issues including during pregnancy.

2.3.2 Gestational age at first ANC visit

One other key factor that influences the implementation of repeat testing of HIV pregnancy is the age of the pregnancy at which a pregnant woman is present at the first or initial ANC clinic. This is because, this has an effect on a pregnant woman's eligibility to be retested during that same pregnancy. Despite World Health Organization's and national health services' recommendations for early presentation to the ANC clinic (within 16 weeks of confirmed pregnancy), there are reports globally, of late or no presentation to the ANC clinic of pregnant women (Abor et al., 2011; Arthur, 2012; Hodgins & D'Agostino, 2014).

The reasons that have been attributed to age of pregnancy at which pregnant initiate ANC include parity, gravidity, age, level of education, cost, conceptions about healthcare providers, religion, sociocultural beliefs and several others (Andrew et al., 2014; Banke-Thomas et al., 2017; Hijazi et al., 2018; Pell et al., 2013; Simkhada et al., 2008).

In many countries, the recommended number of ANC visits in a single pregnancy term is between 4 to 5. When pregnant women first visit the ANC early in pregnancy, it impacts their

eligibility for repeat HIV testing and also allows for ample time to fit in the number of recommended visits. Testing negative for HIV during the first ANC visit it the first premise for eligibility to receive a repeat test after three months or in the third trimester. Thus, in cases where the first ANC visit is in the late second or third trimester as has been reported, repeat testing becomes infeasible.

In assessing the implementation of repeat testing during pregnancy in Southwestern Kenya, Rojers et al (2017) found through bivariate analyses that there was a significant association between prior knowledge of serostatus (as positive) and earlier gestational age at first ANC visit. Conducting the same statistical analyses, they reported similar association for older age later year of pregnancy and marital status. Surprisingly, their analyses suggested that women who lived farther away from the health facilities where they accessed ANC services were more likely to have a visit at an earlier gestational age compared to those who live closer.

Late presentation at the ANC clinic was found to be one of the key challenges of general ANC and implementation of repeat HIV testing in Kenya (Rogers et al., 2016). In their qualitative study, they found that, late presentation at the ANC was a common practice in their study population as they tend to have their first ANC visit after the first the trimester. This affected repeat testing because if a pregnant was tested after 28 weeks' gestation, they become ineligible for retesting during prepartum or delivery. They further reported that, factors such as unawareness of pregnancy due to failed tracking of menstrual cycle and some cultural issues were some of the reasons study participants gave.

2.3.3 Sociodemographic factors

Across almost all health research, sociodemographic and socioeconomic characteristics have been attributed to various study interests. Characteristics of people such as age, sex, level of education, marital status, place of residence, religious affiliation and the like have been researched to determine their association to many study outcomes.

Age for example, is perhaps by far the most reported sociodemographic variable that has been associated to many study outcomes. In relation to repeat testing, age of pregnant women has been reported to be associated to some key process measures such as gestational age at first ANC visit and returning to the ANC clinic when eligible to be retested. Investigating missed opportunities for repeat testing during pregnancy in the USA, Liao et al (2017) found that older women were more likely to miss the opportunity to be retested compared to younger women. This finding is not entirely surprising as several studies have suggested that older women are likely to be inconsistent with ANC visits during pregnancy (Andrew et al., 2014; Dairo & Owoyokun, 2011; Mathole et al., 2004) though there are other studies that have reported otherwise (Abor et al., 2011; Asundep et al., 2013; Lori et al., 2014). A study involving Kenyan, Ghanaian and Malawian women reported that primigravidae (women who are pregnant for the first time) and much younger were more likely to seek medical and professional assistance with pregnancy. Age as a determinant of ANC attendance has direct implications on likelihood of repeat testing being carried out as the procedure is invariable tied to ANC visits, particularly in the third trimester in Ghana.

Level of education has also been reported to be intrinsically linked to health-seeking behaviours of people in all settings, more so in developing countries. The association however has been described differently and have been found to weigh in opposing directions. When years of education and intended doctor consultation for some selected clinical symptoms were assessed

in some European provinces, Frie (2010) reported that people with fewer years of education were more likely to seek professional medical help compared to people with more years of education (adjusting for age and gender). This might appear absurd but a possible reason for this could be the likelihood of more educated people to be able to be more abreast with selfcare. However, some other studies have reported that, people with little or no formal education were more reluctant to seek medical help. With antenatal care seeking behaviour in perspective, a study conducted in the Northern region of Ghana found that, about 85% of the study participants who have had some formal education had at least four or more ANC visits before delivery compared to those who had no formal education. Thus they, through statistical analyses found a positive association between higher educational level of pregnant women and greater ANC attendance.

The linkage of formal education to health seeking behaviour and consequently special care such as ANC, poses viable premise for paying more attention to implementing health sector interventions especially in rural areas of developing countries. This is because, formal education is comparatively low compared to urban communities, attributable to disparities in girl-child education. The level of awareness of pregnant women on pertinent health conditions has an implication on their behaviour towards accessing ANC services including PMTCT interventions.

Socioeconomic determinants of health seeking behaviour of people is a widely discussed topic. Financial indices such as expenses made on health as a fraction of total household expenditure is increasingly being used as an indicator of Universal Health Coverage (UHC) and significantly considered in health financing. In Ghana, and most sub-Saharan African countries, there have been the advent of several interventions in response to recommendations

for the abolition of user fees to facilitate the reduction of maternal and neonatal mortality as is enshrined in the Millennium Development Goals (MDG) and the more current Sustainable Development Goals (SDGs) (Abubakari et al., 2017). Since 2008, Ghana has been implementing a policy that provides free maternal health care service to women during pregnancy provided they are registered with the national health insurance scheme (F. A. Johnson et al., 2016). This intervention notwithstanding, there is evidence suggesting that many women are unable to take full benefit of the exclusion of user fees to access ANC services optimally (Mills et al., 2008).

Financial constraints or barriers defined in terms of geographical location, capability to pay and the likes have been attributed to the issue of pregnant women not being able to make the required 4-5 ANC visits before delivery (Abubakari et al., 2017). Socio-economic variables such as woman's level of education, husband's occupation and income level, household wealth status as well as financial stress have been reported to be associated with the patronage of maternity services and are indicative of economic resources (Moore et al., 2011).The Ghana Statistical Service reports that, women in the middle and highest wealth quintile are more likely to seek professional ANC services compared to those in the lowest quintile (Ghana Statistical Service, Ghana Health Service, 2015). Within such a context where there are no user fees, the persistence of sub-optimal ANC visits could be attributed to inability of the poor to take full benefit as a result of some other out-of-pocket payments that might be required (such as purchase of medications unavailable at the primary health facility and transportation cost) and health providers' discrimination (Ataguba & McIntyre, 2012).

2.3.4 Parity and Gravidity

Parity is the number of pregnancies that a woman has carried beyond 20 weeks gestation. In counting parity, both live births and pregnancies lost after 20 weeks (such as stillbirths) are included. Gravidity on the other hand, is an indicative measure that represents the number of confirmed pregnancies a woman has had regardless of the outcome.

Parity has been reported to be associated with ANC presentation in pregnant women. Pell (2013) reported that a complex association existed between parity and ANC initiation. In his study, he found that, women who were pregnant for the first time (primigravidae) were more likely to seek help and advice and commence ANC earlier. He also recounted that; such women were more likely to delay ANC initiation as a result of uncertainty prompted by unfamiliarity with the signs of pregnancy. Similar studies have also found that, adolescents and single younger women in some cases veiled their pregnancies and delay presentation at ANC to avoid the probable social repercussions such as dismissal from school, expulsion from home, stigmatization and even desertion by partner (Banke-Thomas et al., 2017; Black et al., 2012; Hackett et al., 2019; Owolabi et al., 2017; Ziblim et al., 2018).

Older multiparous (women who have more than one pregnancy viable beyond 20 weeks of gestation) on the hand, have been reported to initiate ANC late in pregnancy. Reportedly, they are more familiar with the pregnancy experience and as such are less anxious about monitoring the advancement of the pregnancy (Pell et al., 2013).

2.4 Seroconversion/Incidence of HIV during Pregnancy

Seroconversion is the advancement in the count of antibodies to detectable amounts in the blood aimed at destroying an infectious agent. Incident HIV during pregnancy in this context, can be defined as a positive test in a woman who previously tested negative earlier in pregnancy (Mandala et al., 2019).

Altogether, seroconversion during pregnancy constitute one of the key health issues aggressively pursued in modern times. This is because of its two-fold implications for both the mother and the foetus. Seroconversion during pregnancy is so important now as it has been proposed that the risk of MTCT maybe be higher compared to women who were seropositive before pregnancy (Pizzo & Butler, 1991). Buttressing this claim is the finding that the levels of HIV on circulation in an infected person is considerably higher in the first few weeks of infection and is likely to remain so for the first three to four months post infection (Daar et al., 1991; Streeck & Nixon, 2010). Other propositions also speak of the implications of the sheer volume of maternal blood that a foetus is exposed to via the placenta as it increases during the advancement of the pregnancy (Dinh et al., 2015). Put together, the risk of MTCT could be attributed to the timing of infection with HIV, the viral load in the mother's blood and the amount of blood that a foetus is exposed to. Over the years, studies have been interested in postulating a such a clear association (Burgard et al., 2010; Chappell & Cohn, 2014; Shaffer et al., 1999) but were challenged with determining the exact period during pregnancy that the incident infection occurred.

Incident HIV during pregnancy increasingly is being targeted and included in PMTCT interventions hence the rigorous advocation for repeat testing, especially in high prevalence settings. Due to fact that HIV is transmissible even during its window period and the

insensitivity of rapid antibody test during this period (Olowe et al., 2016), repeat testing is crucial especially during pregnancy.

More recently, studies have been carried out to determine seroconversion rates in several high prevalence settings as it forms a basis for implementation of repeat testing of HIV during pregnancy. One of such recent studies was carried out in Zambia by Mandala et al (2019). In their prospective study, they reported that upon retesting, 55 pregnant women out of 11, 282 (0.49%) who previously tested negative for HIV were found to be positive. Though the seroconversion rate reported by the study is comparably low, they hinted that a sizable proportion of their study participants were lost to follow up hence the reported value could have been higher. However, they reported an HIV incidence rate of 1.06 per 100 person-years which was slightly higher than the national incidence rate of 0.80 per 100 person-years of 2012.

In a similar study conducted in Kenya, two women were reported to have seroconverted out of the 132 (Cumulative Incidence = 1.5% and Incidence rate = 4.4 per 100 person-years) that were retested during the same pregnancy (Rogers et al., 2017). In this study also, a significant amount of missed opportunities was recounted and the authors hinted that, if they were to extrapolate the rates to the target population of the study and thus if all pregnant women eligible for repeat testing were retested, an additional 18 women could have been identified to have seroconverted by delivery (Rogers et al., 2017).

The findings of a study conducted in Lesotho which also is a high HIV prevalence setting were not so different compared to the aforementioned studies in Zambia and Kenya. The national prevalence of HIV in the adult population of Lesotho is estimated at 25% (National Institute of Statistics et al., 2015). According to the same source, the prevalence is even considerably higher in women aged 25-29 years, nearly 37% and exceeding 40% among those between 30-

39 years. Machekano et al., (2018) discussed that, identification of the risk factors and the timing of incident HIV during pregnancy and after delivery nested with sociodemographic and behavioural factors attributed to the acquisition of HIV is essential for designing and implementing effective interventions. In their study aimed at doing just that, they enrolled 941 pregnant women between September 2013 and August 2015. Out of 850 women who initially tested negative for HIV, 28 seroconverted during the study period. Taking their study period into consideration (average follow-up per participant was 25 ± 7 months), they reported to have estimated an overall incidence rate of 1.58 per 100 person-years. One highlight finding of the study is the recorded seroconversion when charted against age groups. The authors found the overall incidence rate to be significantly higher in adolescents (2.19 per 100 person-years) and younger women (2.26 per 100 person-years). Evaluation of sociodemographic and behavioural characteristics also produced interesting findings. They found that, women 24 years or younger had higher risk of HIV infection when compared to women above 24 years. Women who initiated ANC after 20 weeks gestational age had in excess of six times the risk of HIV infection when compared to women who initiated ANC earlier (before 20 weeks gestational age) and the risk of HIV acquisition was also found to be higher in women who reported to have more than one sexual partner.

Drake et al (2014) conducted a systematic review and metanalysis of literature on incident HIV during pregnancy and postpartum.

They found that the incidence rate of HIV during pregnancy ranged from 10 to 16.8 per 100 person-years. The pooled incidence rate however, was reported to be 4.7 per 100 person-years (95% CI 3.3 to 6.1) which is considerably higher than the incidence rates reported by some current studies (Heemelaar et al., 2015a; Kinuthia et al., 2015; Liao et al., 2017; Mandala et al., 2019). The pooled incidence rate during postpartum was estimated at 2.9 per 100 person-

years (95% CI 1.8 to 4.0) which was not statistically different from the pooled incidence rate during pregnancy.

The systematic review and meta-analysis shed light on the pertinent risk of MTCT and provided sound grounds for intensifying PMTCT interventions in hopes of identifying women who needed to be linked to ARTs to reduce the risk of MTCT. The pooled incidence rate of HIV during pregnancy (4.7 per 100 person-years) was quite high which perhaps supports the fact that pregnant women are at higher risks of HIV infection.

2.5 Mother-To-Child Transmission of HIV

Vertical transmission of HIV to a child can occur during pregnancy when the virus in the mother's blood crosses the placenta into the foetus's blood. Toth et al (2001) upon conducting a DNA Polymerase Chain Reaction test reported the presence of similar viral loads of HIV in babies born to HIV positive mothers within 48 hours postpartum. The similarity of viral loads in babies and mothers after such a short time after delivery suggests that the babies were infected during pregnancy. This is because, it usually takes some weeks for an HIV infection to be detectable, that is for enough antibodies to be produced by the body indicative of the infection. This is often referred to as the window period and varies from person to person but likely to fall between 3 to 12 weeks. Thus it could be concluded that these babies were infected during pregnancy as a result of their considerably high viral loads (Kourtis & Bulterys, 2010). During pregnancy, there is a 5-10% chance of MTCT during pregnancy if a seropositive pregnant woman is not given an intervention such as Antiretroviral Therapy (ART) (FMOH, 2007).

The risk of transmission of the virus from an infected mother to a child is highest during labour and delivery, attributable to a range of reasons. During labour, the baby is likely to sustain some bruises on the skin which increases the risk of infection as the baby is exposed to the virus in the mother's body fluids and blood. This increases the risk of infection in the baby by

10-15% if the mother has not been enrolled on an intervention prior to labour and delivery (Jourdain et al., 2007). Such babies are likely to be seronegative when tested within 48 hours postpartum but may seroconvert if the test is repeated after a few weeks postpartum.

During labour, conditions such as prolonged labour, premature rapturing of membranes, pre-term delivery, or difficult labour in which episiotomy is given can increase the risk of mother-to-child transmission (MTCT) of the virus (Short et al., 2014). To reduce of transmission of the virus during labour and delivery, some studies recommend that a caesarean section be performed before the inception of labour free of antiretroviral use (Chappell & Cohn, 2014).

The risk of transmission of the virus from an infected mother during twin birth has been reported to be disproportionate with the first twin at a greater risk compared to the second (Scavalli et al., 2007). Lactating mothers who are seropositive risk transmitting the virus to their children through breastfeeding. Detectable loads have been reported in children merely after 6 weeks postpartum indicative of infection though breastfeeding in the first few days postpartum (FMOH, 2007). Maternal risk factors of infection are invariably linked to risk of infection in children. Though the linkage is non-linear, an increase in risk of infection in a mother may also increase the risk of infection in a child. It has been reported that, high viral load in a mother at the time of delivery increases the risk of transmission to the child (Mark et al., 2012). In general, enrolment of seropositive women unto PMTCT interventions are likely to reduce viral loads and consequently reduce risk of MTCT both during and after pregnancy.

The risk of MTCT during pregnancy and postpartum varies. Without PMTCT intervention, the risk of transmission between 5-10% during pregnancy, 10-15% during labour and delivery, 5-10% during breastfeeding after birth but between 15-25% without breastfeeding (FMOH,

2007). By 18-24 months postpartum, the overall risk of transmission is between 20-35% and the total risk of MTCT is between 20-40%.

2.6 Prevention of Mother-To-Child Transmission of HIV Intervention

The commencement of PMTCT intervention in Ghana since 2001 has gone through numerous changes, in tune with the WHO recommendations and protocols. When the program started, seropositive pregnant women were the focal point. At the onset of labour, a single dose of Nevirapine was offered to the pregnant woman and after delivery, the baby was put on syrup Nevirapine medication for a month. In 2007, a better option was made available where two tablets of Zidovudine and Lamivudine were given to seropositive pregnant women after 28 weeks gestational age. Babies born to seropositive mothers were then given the same medication for a week postpartum.

The World Health Organization in 2010 revised the protocols and recommended that all pregnant women be offered an ARV Prophylaxis to prevent Mother-to-Child transmission of HIV which would capture incident HIV infections during pregnancy. The prophylaxis was to be initiated at about 14 weeks gestational age and continued through pregnancy to after one year postpartum.

In recent times, all pregnant women are enrolled unto ART program upon testing positive for HIV and the treatment is carried out for life. However, babies born to seropositive mothers are given ARV prophylaxis for up to 6 weeks postpartum, in which case they can be breastfed till their first birthday after which they must be weaned.

To achieve these targets, it is requisite that all pregnant women seeking ANC services be tested for HIV and upon a confirmed positive serostatus be initiated unto Highly-Active Antiretroviral

Therapy (HAART) (WHO, 2013). This recommendation has been adopted by Ghana and several other and being implemented. As such all seropositive pregnant women are offered a lifetime ART and upon delivery, their babies are given a daily ARV prophylaxis till they are six weeks old. Such exposed babies are monitored and given daily dose of Cotrimoxazole prophylaxis till a final HIV testing is carried out when they are 18 months old. Provided that both mother and child are on ARTs, the child can be exclusively breastfed for the first six months but must be weaned completely when they are a year old. This strategy incorporates a family-based approach for PMTCT services in Ghana where the key service providers are trained health professionals such as Midwives, Pharmacists, Doctors and Nurses.

PMTCT is a globally accepted and implemented intervention that has been instituted in response to risk of MTCT and the goal to completely prevent new infections of HIV. In the United States of America for example, PMTCT is considered as one of the key and highly effective Public Health Interventions (Roth et al., 2016) where the PMTCT intervention package include HIV testing and counselling, provision of Antiretrovirals, delivery by caesarean and discouraged breastfeeding. The successful implementation of this strategy has resulted in the reduction of the risk of MTCT from 25%-30% in the absence of an intervention to less than 2% (Marino, 2015).

2.7 Knowledge of Pregnant Women on MTCT of HIV

The availability of sufficient information, education and communication coupled with community mobilization are considered as key fundamentals for ensuring the utilization of PMTCT and associated services. However, reports suggest that there is lack of knowledge about MTCT of HIV regarding prevalence, points and risk of transmission during pregnancy, delivery and breastfeeding (Abajobir & Zeleke, 2013; Abtew et al., 2016; Adeleke et al., 2009; Egbe et al., 2016; Falnes et al., 2010; Malaju & Alene, 2012).

In-depth knowledge on MTCT of HIV at the community level is often lacking, characterised with sparse overview of the transmission mechanisms of the virus, even in settings where PMTCT interventions are active. Several authors have claimed that despite the extensive information, education and communication campaigns as well as the scale-up of PMTCT interventions, women's knowledge on risk factors and the periods of transmission, and this has been attributed to the ineffectiveness of PMTCT interventions observed in some settings (Barker et al., 2015; Birhane et al., 2015; Kibao, 2017). A study conducted in Ethiopia found that out of 386 participants (pregnant women), only 67 were abreast of knowledge of possible preventive methods of MTCT. The authors further reported that, there is an association between general knowledge of HIV/AIDS and MTCT and PMTCT, in that, pregnant women with sufficient knowledge of HIV/AIDS had fair knowledge of MTCT and PMTCT of HIV (Abtew et al., 2016). The authors concluded that, knowledge on MTCT and PMTCT was low in the study site and recommended the more efforts be targeted at proficiently educating women on the subject.

A similar cross-sectional study conducted in Southwestern Nigeria reported contrasting results when the authors found that pregnant women's knowledge about MTCT and PMTCT was high. In this study, Olugbenga-Bello et al (2013), reported that the participants had about 92.1% knowledge of MTCT and 91.4% knowledge of PMTCT. However, they discovered that despite the high level of awareness, about 71.27% of the study participants had poor attitudes towrads the utilization of PMTCT on several levels.

In 2011, Daniel et al researching into knowledge, perception about ART and PMTCT and adherence to ART in seropositive women in the Ashanti region of Ghana, found that more than 90% of the HIV seropostive women involved in the study had insufficient knowledge about

Antiretroviral therapy and PMTCT. They recounted that, such women had 3.5 times the odds of defaulting ART (OR = 3.5; 95% CI = 1.89, 6.21) but found no significant association between level of education and knowledge of ART and PMTCT.

Various studies have also reported that, there are some health-facility factors that either aid or hinder the implementation and success rate of PMTCT interventions (Deressa et al., 2014; Ekouevi et al., 2012). In a cross-sectional study conducted in Addis Ababa, Ethiopia, though high level of knowledge of risk of MTCT was high in the study participants, the authors reported that, some participants were reluctant to utilize PMTCT services or shunned it completely (Deressa et al., 2014). The authors reported that some participants disclosed that, poor counselling, lack of awareness and knowledge about HIV conselling and testing (HCT), unavailability of counsellors and lack of interest were some of the key reasons why they refused to consent to HCT and consequently, PMTCT services.

Issues such as questionable attidue by health workers towwards clients (particularly, HIV positive patients), health workers' motivation, general service delivery, availability of drug supply and related resources, staff shortages and other logistical problems have been found to udermine health outcomes. In many resource-limited settings, long distances to health facilities coulpled with the incurred costs of transportation serve as a challenge to frequenting ANC clinics and consequently affecting the chances of acessing ART without defaulting. Compounding this with the sub-par referral and tracking system as a result of poor health information system in many devveloping countries, there is often a disconnect between ANC and PMTCT services especially when clients switch health facilities (Gourlay et al., 2013).

CHAPTER THREE

METHODS

This dissertation used systematic review approach in synthesizing evidence on repeat HIV testing and seroconversion during pregnancy in sub-Saharan Africa. It adopts PICOS (P-Patients, I-intervention, C-control, O-Outcomes, S-Study) to describe the population, intervention, comparators, outcome of intervention and study types included in primary studies on the subject. The protocol for this systematic review and meta-analysis has been registered with the International Prospective Register of Systematic Reviews with registration number: CRD42020208300 (a copy of the published report has been attached in the appendices section).

3.1 Criteria for considering studies for this review.

Studies

All studies including RCTs, cohort, case-control and cross-sectional studies, conducted between January 2006 and October 20th, 2020, were eligible for inclusion in the review. The restriction to 2006 is based on the roll-out of comprehensive guidelines on repeat HIV testing during pregnancy (indicated in PMTCT guidelines) (WHO, 2006). Case series and case studies, as well as studies whose data on key outcomes was not accessible even after contacting the author(s) were excluded from the review.

Participants

Pregnant women living in a sub-Saharan African country, accessing antenatal care (ANC) in a health facility or an outreach programme and had at least one HIV-negative test been eligible for inclusion in the review. Pregnant women who had their first test so late and were ineligible for retesting were excluded.

Intervention

Repeat HIV testing during pregnancy as recommended by the WHO guidelines, aimed at preventing mother-to-child transmission by providing ARTs to pregnant women with incident infections upon retesting during pregnancy, labour or delivery. Repeat HIV testing and Seroconversion studies conducted before the rollout of the WHO repeat testing guidelines (indicated in the PMTCT guidelines) in 2006 were excluded from the review. Also, ART interventions for pregnant women with established (chronic) HIV infections were excluded from this review.

Comparison/control

Women who seroconverted during pregnancy and received no ART till delivery were considered as comparators to those who seroconverted and were enrolled onto ART.

Outcomes

Primary outcome

Conduction of a repeat HIV test during pregnancy, labour or delivery in women who tested negative earlier during the same pregnancy period.

Secondary outcomes

1. Incidence of HIV in pregnant women with documented HIV negative test earlier in pregnancy upon repeat testing.
2. Vertical transmission from a woman who seroconverted during pregnancy to baby.

Studies lacking primary data to back the reported estimates (proportion of repeat HIV testing and rate of HIV seroconversion or studies whose estimates were generated using mathematical modelling and computer simulations were not eligible for inclusion in the review.

Adverse outcomes

All documented adverse events relating to the intervention or indirect associations related to repeat HIV testing or its implementation in any form reported by selected studies were considered. For the purpose of this review, vertical transmission of HIV despite ART was considered as a primary untoward outcome and not an adverse event.

3.2 Search methods for identification of studies

3.2.1 Electronic database searches

Relevant studies published in English or French on repeat HIV testing or HIV retesting during pregnancy in sub-Saharan Africa were identified through searching PubMed, Cochrane Library, HINARI and Google Scholar databases published between 1st January 2007 and 20th October 2020. Other sources such as African Journals Online and Science Direct were searched for more studies. The key search terms for review questions 1 and 2; were "repeat HIV testing", "HIV retesting", "pregnancy", Sub-Saharan Africa, "HIV seroconversion" and "maternal HIV incidence". The synonyms, plural and singular forms, and British and American spellings of the search terms were included in the search. All the 48 sub-Saharan African countries as listed on the World Bank records (insert the link here) were included individually as search terms. Using the Boolean logic, for example, for PubMed search, the following combination was done; "Repeat HIV testing" OR "HIV retesting" OR "Prenatal HIV testing" OR "Maternal HIV testing" OR "Antenatal HIV screening" AND Seroconversion OR "HIV incidence" OR "HIV seropositivity" OR "Incidence of HIV" AND "pregnancy" OR maternal OR perinatal OR prenatal OR antenatal OR labour OR labour AND all 48 SSA countries separated by 'OR' with the Boolean.

The key search terms for review question 3 were; "pregnancy", "seroconversion", "seroconverted pregnant women", "incident HIV infection", "antiretroviral therapy", "outcome", "mother-to-child transmission" and their applicable terms linked with Boolean operators.

3.2.2 Other sources searched

The bibliographies of all relevant articles were screened for additional studies, and their full texts were accessed for inclusion. The team also reviewed the reference of published systematic reviews on the subject, conducted within the last decade to identify potentially relevant primary studies for inclusion, applying the pre-specified eligibility criteria.

3.3 Selection of studies

All records from the various searches were pooled together and deduplicated. The titles and abstracts of articles were screened independently by two reviewers and those meeting our pre-specified eligibility criteria were selected using pre-tested study selection form (see Appendices). Studies considered for full-text review were those who's abstract or titles mentioned repeat HIV testing, HIV incidence, or seroconversion during pregnancy in any sub-Saharan African country. Research articles on unrelated topics, outside of sub-Saharan Africa or the time limit (1st January 2007 to 20th October 2020) were excluded.

Titles of references of all studies considered for full-text review were assessed for inclusion in the review. Full-texts of studies were excluded when (I) HIV incidence rates or cumulative HIV incidence or proportion of repeat HIV testing were not reported and could not be calculated; (II) women were HIV positive before pregnancy; (III) seroconversion rates or proportion of repeat HIV testing were based on mathematical modelling or computer simulations; (IV) HIV incidence or proportion repeat testing from January 2007 could not be

extracted from studies that started before the set limit but ended after January 2007; (V) prevalence and incidence of HIV could not be disaggregated. In addition, all non-English articles were considered for full-text review but were found to be either unrelated to the review topic or did not have abstracts or full-texts for assessment.

The combined database searches resulted in 1,725 studies for the review. An additional 31 studies were identified through references cited in these articles, contributing to total of 1756 studies. After deduplication, the total records remaining were 1506, out of which 42 were selected for full-text review. Study selection was carried out by David Owiredu and independently verified by Dr Antony Danso-Appiah. Disagreements or discrepancies between the reviewers were resolved through discussion.

3.4 Data extraction and management

Data on the following outcomes Proportion of repeat HIV testing, cumulative incidence of HIV, incidence rate of HIV, and risk/rate of mother-to-child transmission in seroconverted pregnant women were extracted. Also, we extracted data on sample size, number of incident HIV infections, number of pregnant women eligible for repeat tests, person-time follow-up, proportion of repeat HIV tests, test assay used, repeat HIV testing timepoints and intervals, study location and sociodemographic details.

3.5 Assessment of risk of bias in the included studies

The standardized Cochrane guidelines available in Review Manager V5.4 (www.tech.cochrane.org/revman) were applied in assessing the risk of bias in each of the included randomized controlled trial (RCT) across six key domains: sequence generation, allocation concealment, blinding (investigators, outcome assessors and participants), incomplete outcome data, selective outcome reporting and other sources of bias (Higgins et al. 2011). For each domain, a judgment of 'low risk' of bias, 'high risk' of bias or 'unclear' risk

of bias was made. Risk of Bias in observational studies was assessed using the Risk of Bias Tool for Prevalence Studies developed by Hoy et al (2012). The risk of bias was assessed for four domains: selection bias, non-response bias, measurement bias and bias related to data analysis. This was done independently by each of the two members of the team. For each domain, a judgment of 'low risk' of bias, 'high risk' of bias or 'unclear' was made. One reviewer carried out risk of bias assessment which was checked independently by a second reviewer. Any discrepancy was resolved through discussion between the reviewers.

3.6 Data Synthesis

Data was analysed using STATA v16. A meta-analysis was conducted for data extracted from studies in which proportion of repeat HIV testing, cumulative incidence and incidence rate during pregnancy were similar. Firstly, the standard deviations of the estimate of outcomes reported by the various studies were determined from the reported point estimates and the suitable denominators assuming a binomial distribution. After stabilizing the variance of the included studies using the Freeman-Tukey double arc-sine transformation, the study-specific estimates were pooled using a random-effects meta-analysis model, to obtain an overall summary estimate of the prevalence or incidence across studies. Heterogeneity was evaluated by the I^2 test on Cochrane's Q statistic, which is measured by I^2 values assuming that I^2 values of 25%, 50% and 75% represent low, medium and high heterogeneity, respectively. For the detection of the publication bias, funnel plots analysis was performed.

3.6.1 Subgroup analysis

Where the heterogeneity detected was significantly high (>75%), subgroup analysis was performed to detect the possible sources. The subgroups analysed included, study design and geographical area within sub-Saharan Africa (West, East, Central, North and South).

3.6.2 Handling missing and incomplete data

The reviewers attempted to collect complete data on items in the data extraction template. Authors of the included studies were contacted where pertinent information was missing or the reported information was insufficient to calculate the effect estimates. In the two instances where this occurred and no feedback was received from the authors, the affected studies were listed as pending and eventually excluded from the meta-analysis. Missing values were not inputted.

3.6.3 Sensitivity analysis

Sensitivity analyses were performed to assess the impact of outlier studies included in the meta-analysis (for example, studies with large sample sizes, high proportions or rates etc.,) on the resultant estimates. The effects of risk of bias, missing data and unpublished studies were also assessed using sensitivity analysis.

3.7 Grading level of evidence

The GRADE (Grading of Recommendations, Assessment, Development, and Evaluation approach was adopted) in assessing the quality of evidence as presented by included studies. For assessment of the overall quality of evidence for each of the three key outcomes (proportion of repeat testing, incidence rate or cumulative incidence of HIV and risk of MTCT of HIV in seroconverted women) that pooled data from either RCTs, cohort or cross-sectional studies, each study was evaluated against the five postulated GRADE considerations; risk of bias, imprecision, inconsistency, indirectness and publication bias and was graded as high, moderate, low or very low quality accordingly.

3.8 Declaration of conflict of interest

The reviewers and collaborators hereby declare no conflict of interest pertaining to the conduction and dissemination of this systematic review and meta-analysis.

CHAPTER FOUR

RESULTS

4.1 Characteristics of Included Studies

Out of the 1506 unduplicated articles identified, 42 were selected for further review of full-text articles where accessible, and 22 were included in the data synthesis and analyses (Figure 1).

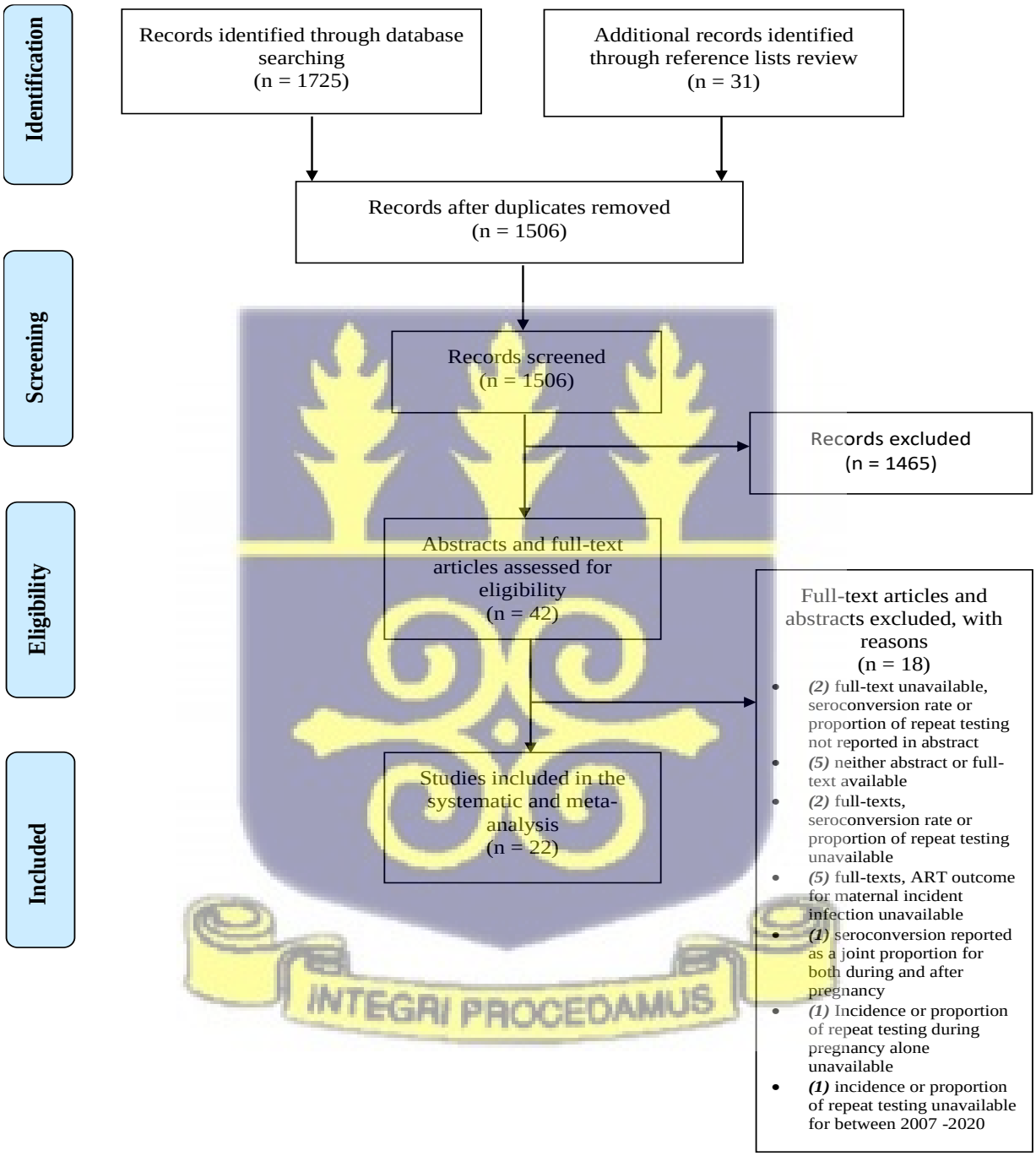

Figure 2: PRISMA flow diagram for study selection

Description of the included studies

Characteristics of the included studies and brief summaries are presented in Table 1. By region, six studies were identified from Wes Africa, five from East Africa, ten from Southern Africa and no studies from North Africa.

By study design, ten studies were prospective cohort, five retrospective cohort, five cross-sectional studies and two randomized controlled trials (RCTs) that were included in the systematic review and meta-analysis. All included studies reported on the number of pregnant women eligible for repeat testing in latter trimesters during the same pregnancy. Thirteen studies (13/23) reported the incidence rate of seroconversion during pregnancy whiles 21 studies reported cumulative incidence of seroconversion during pregnancy.

Only one study reported on the rate of mother-to-child transmission of HIV in women who seroconverted during pregnancy but were on ART. No study was identified reporting on the risk or rate of mother-to-child transmission of HIV in seroconverted pregnant women who were not initiated on ART.

Table 1a: Characteristics of studies included in this review

Location	First Author, Year	Country	Study Design	Study duration	Sample size[1]	Avg. Age of participants/yrs	Gestation age at 1st ANC visit (IQR*)	Incidence measure**	MTCT measure
Western Africa	**Traore, 2012**	Burkina Faso	Cohort, prospective	June 2010 - June 2011	8632	24.9	14 weeks	rate & proportion	-
	Egbe et al., 2016	Cameroon	Cohort, prospective	Sept. 12- Dec.4, 2011	477	-	-	rate & proportion	-
	Imade et al., 2013	Nigeria	Cross-sectional	March 2010 - Jan. 2012	727	28	-	rate & proportion	-
	Nyoyoko & Umoh, 2016	Nigeria	Cross-sectional	July -Sept. 2013	502	-	-	proportion	-
	Afolabi et al., 2013	Nigeria	Cohort, prospective	April and March 2010	197	-	-	proportion	-
	Ejikunle, 2019	Nigeria	Cohort, prospective	4 months	163	29.1	-	proportion	proportion
Eastern Africa	**Mushamiri et al.,**	Kenya	Cohort, prospective	Jan. 2013 - Nov. 2015	1228	21	-	proportion	-
	Rojers et al., 2017	Kenya	Cohort, retrospective	2011 - 2014	1375	23.5	-	rate & proportion	-
	Adelene, 2018	Kenya	Cross-sectional	Nov. 2017 -Jan. 2018	380	-	-	-	-
	Lawi, 2015	Tanzania	Cross-sectional	Jan.- Mar 2012	373	25	-	proportion	-
	Mbena et al., 2014	Tanzania	Cross-sectional	Jan. - March 2013	400	-	-	proportion	-
	Homsy et al., 2019	Uganda	RCT	22 Feb 2013 - 22 April 2016	820	24	-	rate & proportion	-

[1]number of pregnant women eligible for repeat testing **IQR*** Interquartile range **RCT** Randomized Controlled Trial
** measure of seroconversion either cumulative (proportion) or incidence rate
# Measure of vertical transmission as either a rate or proportion

Table 1b: Characteristics of studies included in this review cont'd

Location	First Author, year	Country	Study design	Study duration	Sample size	Avg. age of participants	Gestation age at first ANC visit (IQR*)	Incident measure**	MTCT measure
Southern Africa	**Machekano et al., 2018**	Lesotho	Cohort, prospective	Sept. 2013 - August 2015	941	-	-	rate & proportion	-
	Keating et al., 2012	Malawi	Cohort, retrospective	Jan.- October 2009	1087	26	-	rate & proportion	-
	Moodley et al., 2009	South Africa	Cohort, prospective	July 2006- April 2007	2377	24	22 weeks (6 to 41)	rate & proportion	-
	de Beer 2020	South Africa	Cohort, retrospective	1 July 2014 -31 Dec. 2016	7222	-	21 weeks (15 to 29)	proportion	-
	Fatti et al., 2017	South Africa	Cohort, prospective	01 March 2013- 31 May 2015	1356	-	16 weeks (12-16)	rate & proportion	-
	Yapa et al., 2020	South Africa	RCT	15 July 2015 - 30 Jan. 2017	1149	25	-	rate & proportion	-
	Chetty et al., 2017	South Africa	Cohort, retrospective	2010-2015		-	-	rate & proportion	-
	Kieffer et al., 2011	Swaziland	Quasi experimental	Oct. 2008- Jan. 2009	-	-	-	rate & proportion	-
	Mandala et al., 2019	Zambia	Cohort, prospective,	Feb. -Dec. 2013	16838	23	22 weeks (18-26)	rate & proportion	-
	Heemelaar et al., 2015	Zambia	Cohort, prospective	May -June, 2012	275	-	-	proportion	-

[1]number of pregnant women eligible for repeat testing **IQR*** Interquartile range **RCT** Randomized Controlled Trial
** measure of seroconversion either cumulative (proportion) or incidence rate
# Measure of vertical transmission as either a rate or proportion

4.2 Risk of Bias of Included Studies

The risk of bias in the included observational studies was assessed using the Hoy et al (2012) Risk of Bias tool which presents a guide to assessing the methodological quality of observational studies. Across the four key domains, the scale and interpretation of total risk scores are; 0-3 (low risk), 4-6 (moderate risk) and 7-9 (high risk). Most studies (21 out of 23) were scored as having low risk of bias as they reported detailed methods regarding the target population, sampling frame, sampling, validity of outcome measurements, response rate and data analysis. One study was found to have moderate risk of bias. Another was rated as unclear risk due to the unavailability of full-text of the research article for assessment and some pertinent information regarding methods were not reported in the abstract. See the Appendix for a sample of the risk of bias tool by Hoy et al (2012).

Details of the risk of bias assessment for the two randomized controlled trials included in the review are presented in Table 2. A third study (Kieffer et al 2011) which adopted a quasi-experimental study design was adjudged as having moderate risk of bias when it was assessed using Cochrane's Risk of Bias In Non-randomized Studies-of Interventions (ROBINS-I).

Table 2: Risk of Bias Assessment of included RCT studies

Homsy et al., 2019		
Risk of Bias Domain	**Judgement**	**Support for Judgment**
Random Sequence Generation	Low risk	Numbered randomization lists were computer-generated
Allocation Concealment	Low risk	allocation done sequentially by the study coordinators
Selective Reporting	Unclear risk	Study protocol unavailable to inform judgment
Blinding of Participants and personnel	Low risk	No blinding of participants or personnel but unlikely that the outcome be influenced as a result
Blinding of Outcome Assessment	Unclear risk	No description communicated by authors
Incomplete Outcome Data	Low risk	More than 80% participants completed follow-up
Other Bias	High risk	(1). Condom use was assessed through participant recall (recall bias). (2) STI testing was done with Rapid Tests only.
Yapa et al., 2020		
Random Sequence Generation	Low risk	Sequence generation was randomized
Allocation Concealment	Low risk	Both participants and investigators were not aware of their allocation until later
Selective Reporting	Unclear risk	Study protocol unavailable to inform assessment
Blinding of Participants and personnel	Low risk	Investigators and healthcare workers in the clusters were blinded
Blinding of Outcome Assessment	Unclear risk	No information available for judgement
Incomplete Outcome Data	Low risk	No missing outcome data reported
Other Bias	High risk	Endpoints measured as presented by information in maternity case records which had concerns regarding quality of the data

4.3 Proportion of repeat HIV testing during pregnancy

Of the twenty-two included studies, nineteen (19) studies reported on the proportion of repeat HIV testing during pregnancy. The reported proportion of repeat testing ranged between 24.5% (95% CI: 19.4% to 29.6%) in (Heemelaar et al., 2015) in Zambia, to 100% among most of the studies. In studies that reported 100% repeat HIV testing during pregnancy, pregnant women were usually enrolled as a cohort upon a negative HIV test at the first ANC visit. Overall pooled estimate from the meta-analysis showed that more than three quarters (78.4%, 95% CI: 66.7%-90.1%) of eligible pregnant women received at least two HIV tests during pregnancy (labour and delivery included). (Fig. 3)

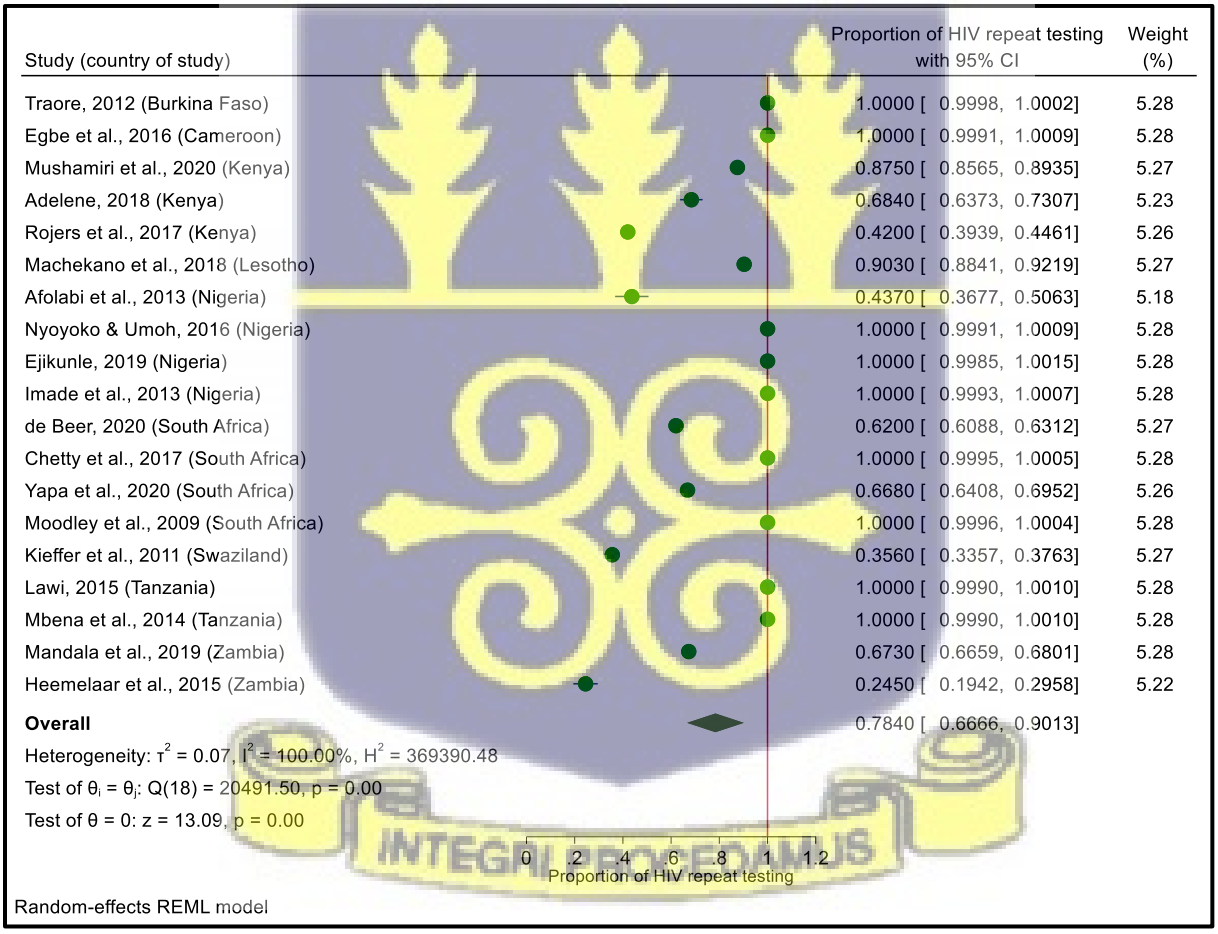

Figure 3: Forest plot of the proportion of repeat HIV testing during pregnancy

4.3.1 Subgroup analysis of proportion of HIV repeat testing by type of study

From the 9 prospective cohort studies included, the pooled proportion of repeat testing was estimated at 79.4% (95% CI: 61.1% to 97.7%) whilst the 3 retrospective cohort studies had a pooled estimate of 68.0% (95% CI: 34.7% to 100%) repeat HIV testing during pregnancy. Five (5) cross-sectional studies on the other hand showed pooled estimate of 93.8% (95% CI: 81.7% to 100.0%) of eligible pregnant women receiving HIV repeat testing during pregnancy. Only one of the two randomized controlled studies (Yapa et al 2020) presented an estimated proportion of repeat testing during pregnancy. The only quasi-experimental study included (Kieffer et al 2017) reported that 66.8% of the 1149 eligible pregnant women received repeat HIV testing during pregnancy. (Fig. 4).

4.3.2 Subgroup analysis of proportion of HIV repeat testing by African regions

Six studies from Western Africa, 8 from southern Africa and 5 from Eastern Africa reported on the proportion of repeat HIV testing in eligible pregnant women during pregnancy. The pooled estimate of the proportion of repeat HIV testing was highest in Western Africa with 90.8% (95% CI: 72.7% to 100%) followed by East Africa with 79.6% (95% CI: 58.0% to 100%) and then southern Africa with 78.4% (95% CI: 66.7% to 87.8%). (Fig. 5).

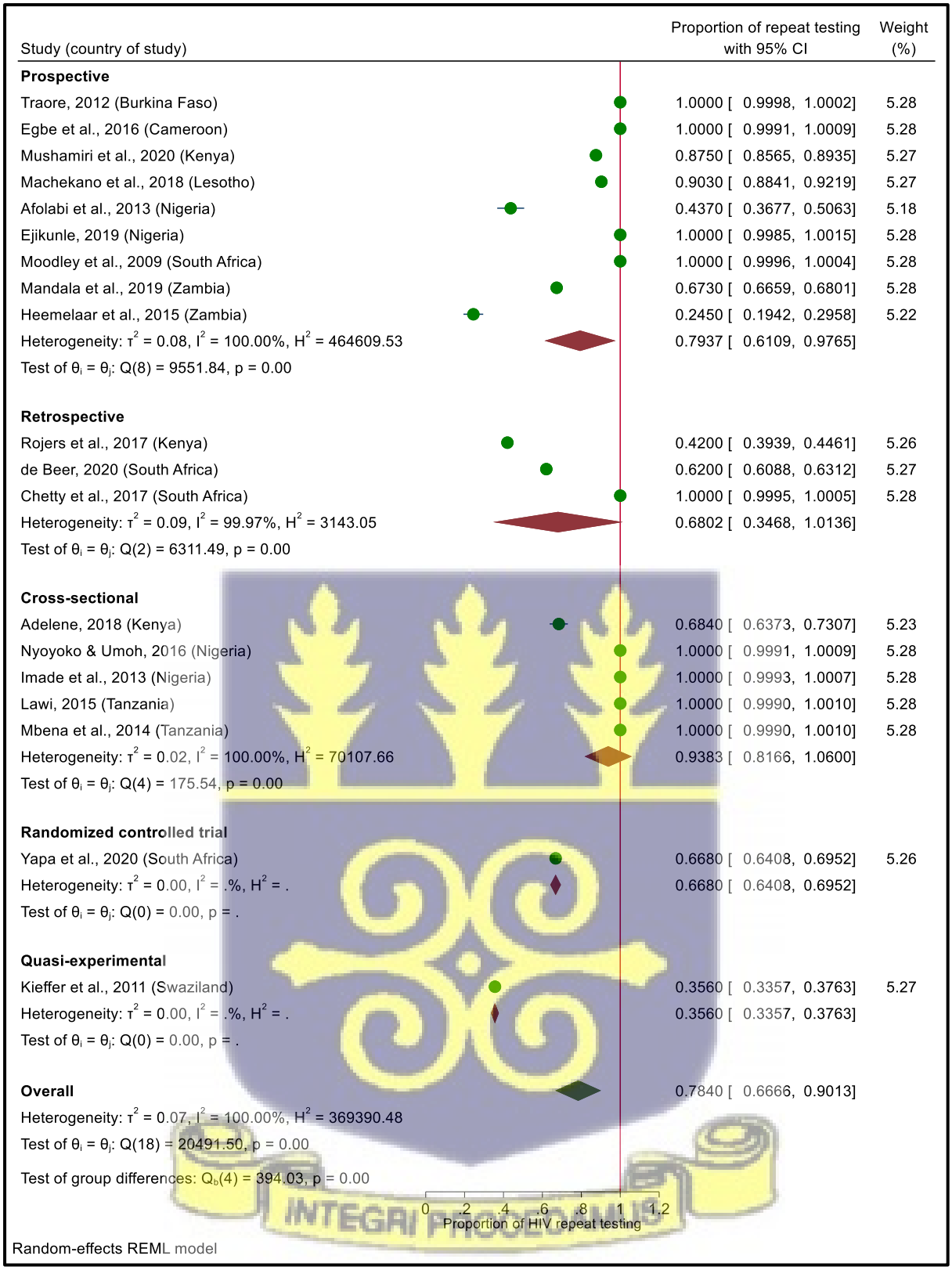

Figure 4: Forest plot of subgroup analysis of proportion of HIV repeat testing among pregnant women by study design

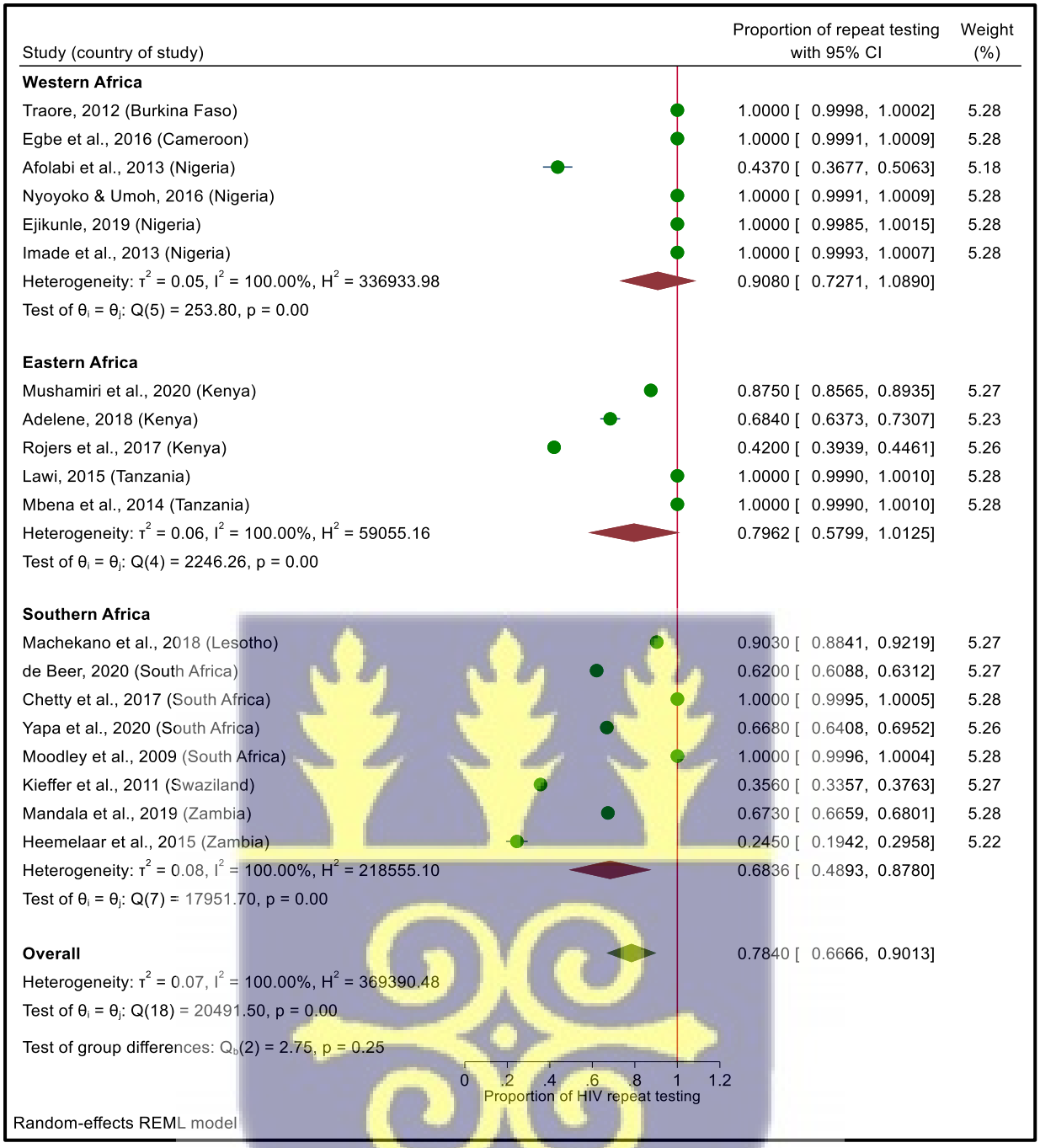

Figure 5: Forest plot of subgroup analysis of proportion of HIV retesting among pregnant women by African region

Age of Study participants

From all studies included in the review, participants enrolled were pregnant women between the reproductive age group (15-49 years). The mean or median ages reported in the studies ranged between 21 and 29.1 years. Whiles some studies reported a significant association between participants' age group and the likelihood of having an HIV retesting during pregnancy, others found that age was not a significant predictor of the outcome. Mandala et al., 2019 however reported that, about 38.1% of older women (≥35 years) in their cohort was lost to follow-up and thus did not receive repeat HIV tests during pregnancy. For the current study however, data available for extraction from the included studies did not permit age-group stratification in order to obtain pooled proportion of repeat test from a sub-group analysis. Moodley

Gestational age at first ANC visit

Four studies reported on the gestational at which pregnant women first presented at the ANC and tested negative for HIV. The reported average gestational age at first ANC visit ranged from 14 to 22 weeks. Moodley et al (2009) reported that in their cohort, pregnant women booked ANC at 28 weeks gestation or less and were likely to be retested for HIV between 30-36 weeks gestation and in some cases even after 36 weeks.

Parity and Gravidity

Three studies (Traore 2012; Yapa et al 2020 and Mandala et al, 2019) reported the mean parity of pregnant women that participated in their respective studies. For Traore (2012), the mean parity was 1.27 while both Yapa et al (2020) and Mandala et al (2019) both reported a mean parity of 1 (one). Mandala et al (2019) further reported a 71.3% proportion of repeat testing in primiparous women but 55.3% in women in the ≥4 parity group.

4.4 Cumulative Incidence of HIV Seroconversion during pregnancy

In all, twenty-one (21) studies contributed data for the analysis of the cumulative incidence of HIV seroconversion among during pregnancy in sub-Saharan Africa. The seroconversion proportion ranged from 0.0% in Traore et al (2012), Homsy et al (2019) and Heemelaar et al (2015) to 5.3% in Mbena et al (2014) in Tanzania. The pooled cumulative incidence of HIV seroconversion among pregnant women detected as a result of repeat HIV testing was 1.5% (95% CI: 0.9% to 2.1%). (Fig.6).

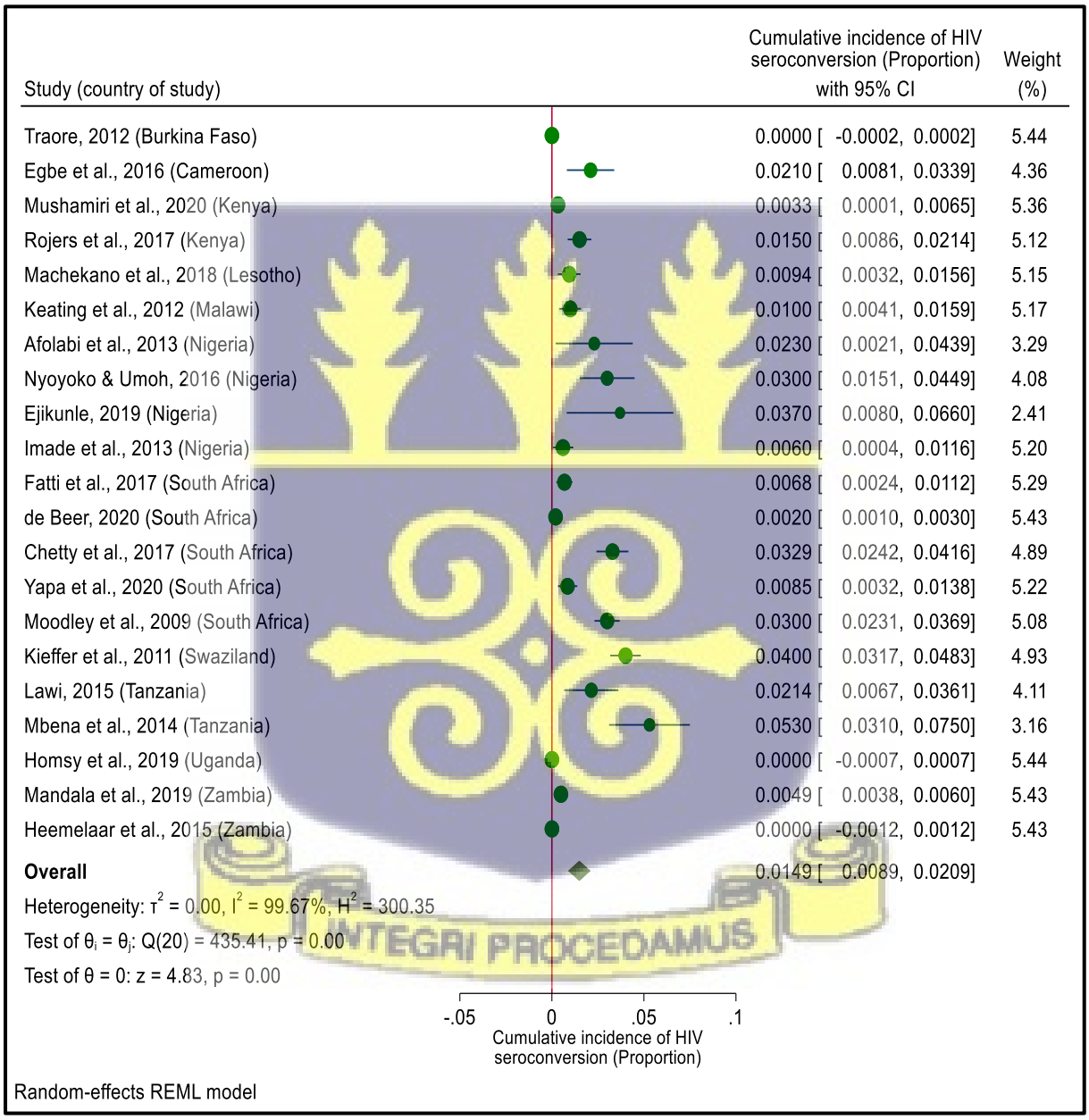

Figure 6: Forest plot of the cumulative incidence of HIV seroconversion among pregnant women

The funnel plot analysis of the 21 studies used in estimating the pooled cumulative incidence of HIV seroconversion during pregnancy showed the presence of publication bias as majority of the included studies were outside the funnel plot window. (Fig. 7).

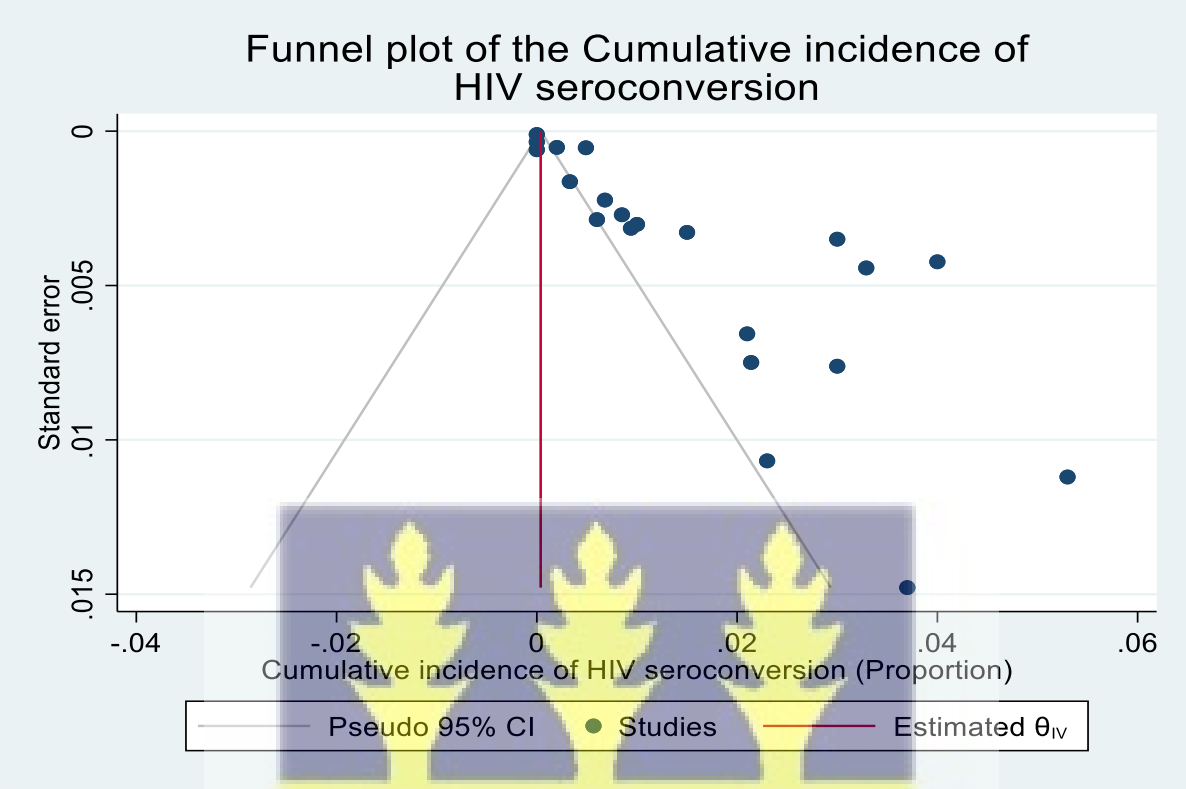

Figure 7: Funnel plot of the cumulative incidence of HIV seroconversion among pregnant women

4.4.1 Subgroup analysis of Cumulative Incidence of HIV Seroconversion during pregnancy by study design

Ten prospective studies reviewed provided data to estimate the cumulative incidence of HIV seroconversion during pregnancy. The pooled cumulative incidence of HIV seroconversion from the prospective studies were 1.1% (95% CI: 0.4% to 1.8%) whereas the pooled cumulative incidence of HIV seroconversion four retrospective studies was 1.5% (95% CI: 0.2% to 2.7%). Among the four cross-sectional studies, the pooled cumulative incidence of HIV seroconversion was 2.6% (95% CI: 0.7% to 4.4%). However, from the two randomized controlled studies that were included, the pooled cumulative incidence of HIV seroconversion was 0.4% (95% CI: -0.5% to 1.2%). (Fig. 8).

4.4.2 Subgroup analysis of Cumulative Incidence of HIV Seroconversion during pregnancy by African region

In the six studies from West Africa, the pooled cumulative incidence of HIV seroconversion during pregnancy was estimated at 1.6% (95% CI: 0.5% to 2.8%). The pooled estimate of cumulative of HIV seroconversion among pregnant women from the five studies conducted in East African countries was 1.6% (95% CI: -0.0% to 3.3%). In the Southern part of Africa, the pooled HIV seroconversion rate among pregnant women from 10 studies was estimated at 1.4% (95% CI: 0.5% to 2.3%) (Fig. 9).

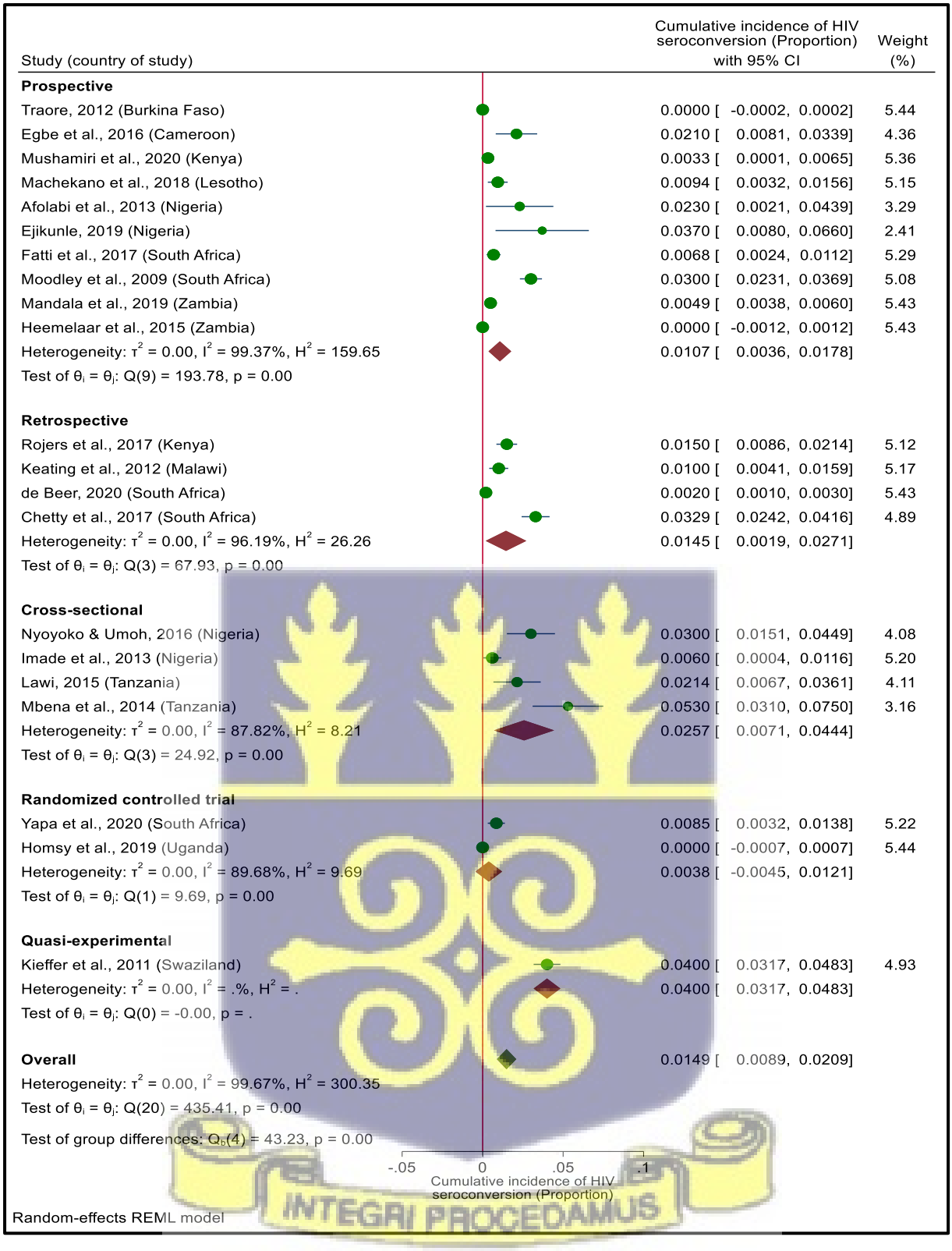

Figure 8: Forest plot of subgroup analysis of cumulative incidence of HIV seroconversion during pregnancy by study design

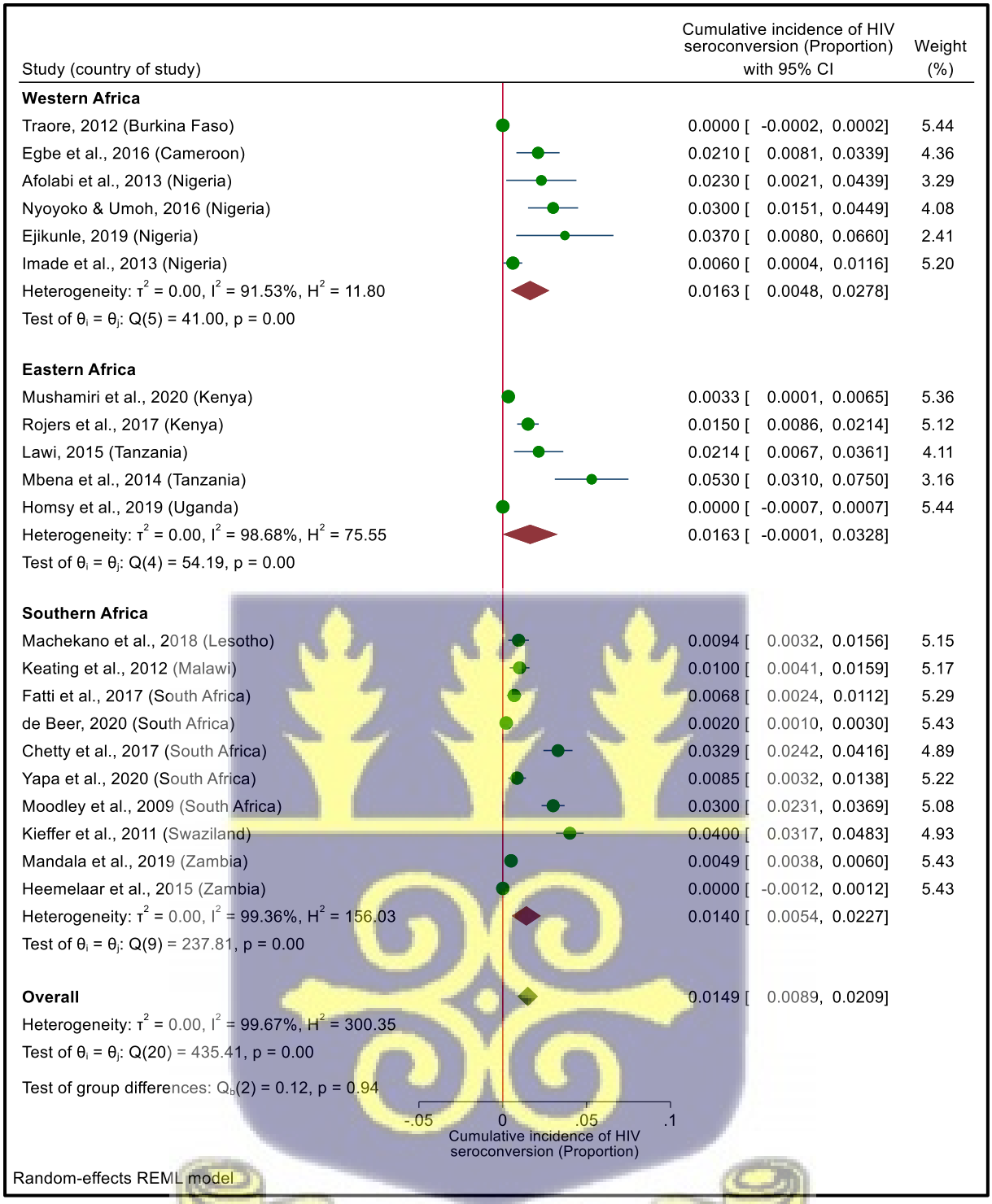

Figure 9: Forest plot of subgroup analysis of cumulative incidence HIV seroconversion during pregnancy by African region

4.5 Incidence Rate of HIV Seroconversion during pregnancy

Thirteen (13) studies provided data for the estimation of the incidence rate of HIV seroconversion during pregnancy. The seroconversion rate ranged from 0.0 per 100 person years in Traore et al (2012), Yapa et al (2020) and Homsy et al (2019) to 16.8 per 100 person years in Kieffer et al (2011) in Swaziland. The pooled incidence rate of HIV seroconversion during pregnancy was 3.9 per 100 person years (95% CI: 1.4 to 6.5 per 100 person years). (Fig. 10)

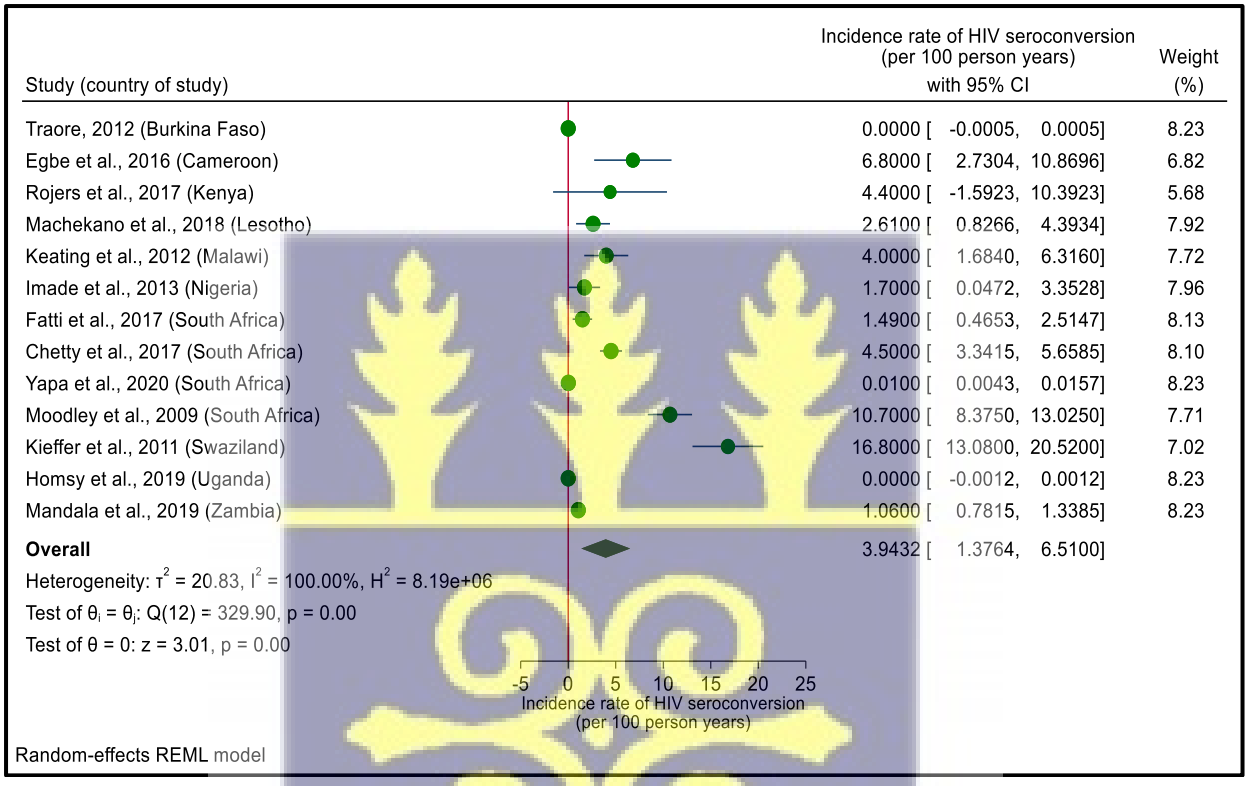

Figure 10: Incidence rate of HIV seroconversion during pregnancy per 100 person years

4.5.1 Subgroup analysis of Incidence rate of HIV seroconversion during pregnancy by study design

Six prospective studies reviewed contributed data to estimate the pooled incidence rate of HIV seroconversion during pregnancy. The pooled incidence rate of HIV seroconversion from the prospective studies was 3.6 per 100 person years (95% CI: 3.1 to 6.8 per 100 person years). On the other hand, the pooled incidence rate of HIV seroconversion during pregnancy from three

retrospective studies was 4.4 per 100 person years (95% CI: 3.4 to 5.4 per 100 person years). In the two randomized controlled studies that provided data, the pooled incidence rate of HIV seroconversion was 0.005 per 100 person years (95% CI: -0.005% to 0.014 per 100 person years) with one study (Homsy et al 2011) recording no seroconversion in their study.

4.5.2 Subgroup analysis of Incidence rate of HIV seroconversion per 100 person years by African region

From studies conducted in West African countries, the pooled incidence rate of HIV seroconversion during pregnancy was estimated at 2.3 per 100 person years (95% CI: -1.2 to 5.9 per 100 person years). The pooled incidence rate of HIV seroconversion during pregnancy from two studies in Eastern Africa was 1.1 per 100 person years (95% CI: -2.6 to 4.9 per 100 person years). In the Southern part of Africa, the pooled incidence rate of HIV seroconversion during pregnancy from 8 studies was 4.97 per 100 person years% (95% CI: 1.2 to 8.8 per 100 person years) (Fig. 11)

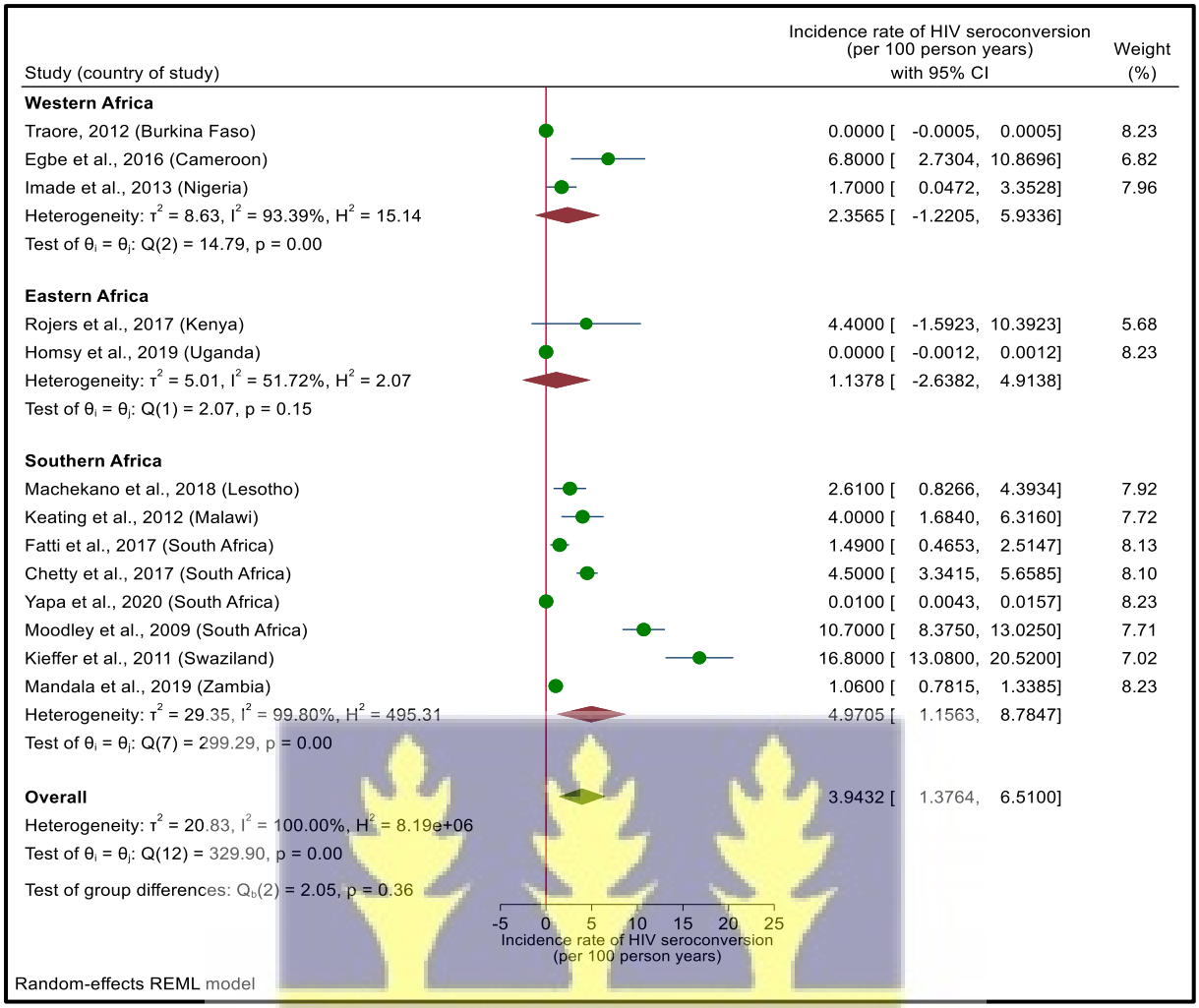

Figure 11: Incidence rate of HIV seroconversion during pregnancy per 100 person years by African region

4.6 Mother-to-Child Transmission of HIV in seroconverted women during pregnancy

Rates of vertical transmission in seroconverted women detected antepartum was presented in one study which was conducted in Nigeria. The study identified 6 seroconversions during pregnancy out of the 163 eligible women screened during labour. Five out of the 6 seroconverted women had live birth deliveries with the sixth resulting in an intrauterine fetal death. All the five HIV-exposed babies tested negative for HIV at six weeks postpartum when an HIV DNA PCR test was carried out indicating no vertical transmission. All six of the seroconverted women were enrolled onto ART at point of the confirmed HIV positive test.

None of the identified studies reported on the rate of vertical transmission in seroconverted women not initiated on ART during pregnancy. As a result, no comparison was assessed to determine the association and risk of MTCT in seroconverted pregnant women who initiated ART.

4.7 Level of Evidence presented by studies included in this review

Nineteen of the twenty-two included studies were observational studies, one quasi-experimental study and two RCTs. All but one of the observational studies included were scored as low quality when graded individually using the GRADE approach. Though all of these studies had low risks of bias, the other three criteria for upgrading the evidence presented by observational studies (large effect, dose-response effect, or plausible confounding) were inapplicable. To support the judgement, it was inferred that, the true proportion of repeat testing and seroconversion may differ significantly from the reported estimates in the various studies. One study was not graded because of the unavailability of the full-text article which would have otherwise provided the needed information was assessing risk of bias and the other GRADE criteria.

The two RCTs and the quasi-experimental study were each graded as moderate because of the low risk of bias and precision of the reported estimates. The reported use of randomization and blinding in the RCTs, directness and consistency were some of the key criteria assessed.

CHAPTER FIVE

DISCUSSION

The proportion of repeat HIV testing during pregnancy in this meta-analysis was found to be high at a pooled proportion of 78.4% (CI: 66.7% to 90.1%). The least proportion was reported by Heemelar et al (2019), where they estimated that 24.5% of the 322 pregnant women who tested negative at their first ANC visit were retested during pregnancy. Conversely, Egbe et al (2016) reported a repeat testing proportion of 100% in their study of assessing HIV incidence during pregnancy and postpartum in Cameroon. A similar study conducted in Lesotho which is a high HIV prevalence setting by Machekano et al (2018) reported that 90.3% of their enrolled cohort had at least one repeat HIV test.

By region, the pooled estimate of the proportion of repeat HIV testing from six studies conducted in West Africa was 90.80% and was the highest of the three African regions where studies were identified from. The pooled estimate from five studies conducted in eastern Africa was 79.62% (CI: 57.99 to 100%) with two studies reporting a 100% rate of repeat HIV testing implementation. In southern Africa where 8 studies were included in the meta-analysis, the pooled estimate of proportion of repeat HIV testing was 68.36% (CI: 48.93 to 87.80%). Routine HIV testing during pregnancy has long been recommended as a key component of PMTCT. Repeat testing as an advanced HIV testing strategy ensures the capturing of pregnant women as well as their partners under the PMTCT. In the reportage of these considerably high uptake of the recommendation, it is likely that majority of incident HIV infections are identified to ensure the timely initiation of ART. This is exceptionally important as the risk of vertical transmission of the virus in the first 10-12 weeks is high as a result of the significantly higher viral loads (Johnson et al., 2012). Majority of studies included in this review reported high implementation of repeat testing during pregnancy (labour and delivery included) as recommended by WHO and individual country health ministries.

Perhaps, the integration of PMTCT interventions with the existing antenatal and postnatal healthcare services is the major factor of the high implementation levels. Despite this, reports of missed opportunities for repeat HIV testing during pregnancy and postpartum are not uncommon. One of the included studies, Mandala et al (2019) reported that a sizeable proportion of eligible Zambian women (32.7%, n=5499/16838) were lost to follow-up and thus missed the opportunity for receiving repeat tests later in pregnancy or postpartum. Another included study conducted in Kenya by Rogers et al (2017) reported that out of the total of 1375 pregnant women who should have had a repeat test in latter trimesters, only 132 did receive HIV test. This included (1) eligible pregnant women who did not return to the ANC clinic; (2) eligible pregnant women whose visits to the ANC were not spaced out in a way that met the repeat test protocol (at least three months since previous negative test) and (3) eligible pregnant women who did not receive repeat tests despite being present at an ANC visit.

From the studies reviewed, several themes applicable to improving the implementation rate of HIV retesting was apparent. For example, at the patient level, Rogers et al (2017) reported that variant forms of stigmas may have influenced the ANC-seeking choices of pregnant women. At the clinic level, some identifiable factors were health providers' ability to remember when a pregnant woman is eligible for retest, clinic workload on day of presentation at the ANC and availability of sufficient HIV test kits have been reported to affect repeat testing rates, providers knowledge on repeat HIV testing guidelines, among others.

The current review and meta-analysis found that the incidence of HIV during pregnancy in sub-Saharan Africa is considerably high. From the meta-analysis, the pooled cumulative incidence of seroconversion detected as a result of repeat HIV testing was 1.5% (CI: 0.9%-2.1%). Individual studies reported cumulative incidence of seroconversion ranging from 0.0%

to the highest 5.3% in Tanzania. The incident rate of seroconversion detected through repeat HIV testing during pregnancy was also high with a pooled estimate of 3.9 per 100 person-year (CI: 1.4 to 6.5 per 100 person years). A single quasi experimental study conducted in Swaziland reported an estimated incidence rate of seroconversion at 16.8 per 100 person-years which was the highest rate reviewed in this analysis.

A systematic review conducted in 2014 by Drake et al reported a slightly higher global estimate of the rate of seroconversion during pregnancy at 4.7 per 100 person-years and about half the rate (2.9 per 100 person-years) during the postpartum period. In their meta-analysis, the authors did not find a significantly higher risk of HIV infection when they compared pregnant or postpartum women to non-pregnant women. However, the pooled risk of mother-to-child transmission was estimated at 22.7% among pregnant and postpartum women. In this review, the highest rate of seroconversion during pregnancy per 100 person years found to be from Swaziland is similar to what Drake et al (2014) also reported. In a subgroup analysis by African region, the current meta-analysis recorded a pooled estimate of the incidence rate of HIV during pregnancy as 4.97 per 100 person-years in Southern Africa countries (Lesotho, Malawi, South Africa, Swaziland and Zambia which presented included studies). These countries are known areas with high prevalence of the infection especially in non-pregnant women (United Nations, 2012).

In all of these seroconverted pregnant women, there is an increased risk of transmitting the virus to baby either during pregnancy, delivery or postpartum. Along these three pathways, intrauterine transmission of the virus constitutes one of the prime ways of vertical transmission. This meta-analysis sought to compare the rate and risk of vertical transmission of the infection in seroconverted pregnant women who initiated ART and those who did not. Though no study describing this outcome was identified, a recent study (Ejikunle, 2019) reported that 5

seroconverted women enrolled on ART did not transmit the virus to their babies as they were seronegative when tested at 6 weeks postpartum. Vertical transmission rate is likely to be dissimilar in seroconverted pregnant women initiated unto ART, compared to those not initiated. The pooled MTCT risks in that regard would have informed the reinforcement of WHO recommendations for prompt initiation unto lifelong ART in infected pregnant women.

The risk of vertical transmission has been described profoundly across several planes. One of such key angles is serodiscordant couples where the woman is seronegative. In such situations, a pregnant woman with a seropositive partner is at high risk of contracting the virus if the partner is not on active ART. However, studies into this particular situation reports that, being aware of their positive serostatus, discordant couples upon reception of reinforced information regarding practicing safe sex and access to ART led to a decrease in the risk of HIV incidence.

Strength and Limitations of this review

One of the notable strengths of this systematic review and meta-analysis is the use of a broad search strategy which included both peer-reviewed studies in addition to completed but unpublished studies. Also, two independent reviewers assessed abstracts and full-texts for relevance, eligibility and extraction of data.

This review and meta-analysis are limited by the pooling of data from studies with differing research design and methods. Although subgroup analyses were performed to ascertain the effect of heterogeneity, the studies even when grouped by design were significantly heterogenous. However, the absence of data on some key variables in most of the included studies that would have otherwise permitted further subgroup analyses, limited the power of the meta-analysis. Also, differences in the sensitivity of the various tests used across the included studies may have led to the overestimation of HIV seroconversion. Again, timing of seroconversion is linked to the timing of repeat testing in that, women accessing ANC at an

earlier gestational age have more opportunity to be detected as seroconversion compared to those whose first ANC visit is during late pregnancy.

CHAPTER SIX

CONCLUSION

The proportion of repeat HIV testing during pregnancy was high in this systematic review and suggests a high coverage of PMTCT interventions and implementation of WHO guidelines. Similarly, the cumulative incidence and incidence rate of HIV seroconversion was high which is indicative of an increased risk in vertical transmission. However, the rate of vertical transmission in seroconverted pregnant women all of who were receiving ART was 0 out of 5 HIV-exposed babies reported by one study.

RECOMMENDATIONS

Recommendations for Public Health practice

The results of this systematic review have implications for PMTCT interventions in sub-Saharan Africa. Firstly, pregnant residing in high prevalence areas should be offered repeat HIV testing consistently in order to detect seroconversion. This should be extended to the postpartum period in order to capture women who were either lost to follow-up or did not seek ANC care as they are likely to attend postnatal care (PNC). This approach would continually ensure the prompt linkage of infected women to care. Presently, the lack of specific ARV regimen for pregnant with incident HIV suggests that maternal ART is the best treatment for suppressing the viral load despite sparse reports of some adverse events.

Recommendations for Policy

It is imperative that pregnant women as well as postpartum women be continually educated on the benefits of practicing safe sex as they are likely to transmit HIV and possibly other STIs to their babies. It is therefore recommended that existing public education policies be revisited and intensified in order to sensitize key populations on the associated risks of HIV infection.

Recommendations for Research

There is the need for the conduction of more studies with primary focus on the risk of MTCT of HIV attributable to seroconversion during pregnancy as it would help in better establishing the cost-effectiveness of repeat HIV testing during pregnancy as an intervention to eliminate MTCT.

Also, future studies into repeat HIV testing during pregnancy should report on the timeline during pregnancy where repeat tests are conducted at the number of seroconversions detected as a result. This would help in establishing the timepoints at which seroconversions normally occur to allow for refocusing of testing efforts.

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APPENDICES

APPENDIX I

Report of protocol registration on the International Prospective Register of Systematic Reviews (PROSPERO)

Systematic review

To edit the record click *Start an update* below. This will create a new version of the record - the existing version will remain unchanged.

1. * Review title.

Give the title of the review in English

Repeat HIV testing and seroconversion during pregnancy in the context of prevention of mother-to-child transmission of HIV in sub-Saharan Africa: systematic review and meta-analysis

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

15/09/2020

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

22/09/2021

5. * Stage of review at time of this submission.

Tick the boxes to show which review tasks have been started and which have been completed.
Update this field each time any amendments are made to a published record.

Reviews that have started data extraction (at the time of initial submission) are not eligible for inclusion in PROSPERO.
If there is later evidence that incorrect status and/or completion date has been supplied, the published PROSPERO record will be marked as retracted.

This field uses answers to initial screening questions. It cannot be edited until after registration.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No

Review stage	Started	Completed
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

6. * Named contact.

The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.

Anthony Danso-Appiah

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:
Dr Danso-Appiah

7. * Named contact email.

Give the electronic email address of the named contact.

tdappiah@yahoo.co.uk

8. Named contact address

PLEASE NOTE this information will be published in the PROSPERO record so please do not enter private information, i.e. personal home address

Give the full institutional/organisational postal address for the named contact.

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

+447958721346

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

University of Ghana

Organisation web address:
www.ug.edu.gh

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong.
NOTE: email and country now MUST be entered for each person, unless you are amending a published record.

Mr David Owiredu. University of Ghana
Dr Anthony Danso-Appiah. University of Ghana
Dr Forzia Osman. University of Ghana

12. * Funding sources/sponsors.

Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

None

Grant number(s)
State the funder, grant or award number and the date of award

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.

State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

1. What proportion of women were tested for HIV at least once during pregnancy in sub-Saharan Africa?

2. What proportion of women tested positive for HIV after an initial negative test earlier in pregnancy?

3. What is the rate of HIV seroconversion during pregnancy in sub-Saharan Africa?

4. What is the risk of MTCT of HIV in women who seroconverted during pregnancy and enrolled onto antiretroviral treatment (ART) compared with those who seroconverted but did not enroll onto ART?

16. * Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

Relevant studies published in English or French on repeat HIV testing or HIV retesting during pregnancy in sub-Saharan Africa will be identified through searching PubMed, EMBASE and LILAC published between 1 January 2007 and 31 July 2020 without language restrictions. Other sources such as Google Scholar and African Journals Online, HINARI, ScienceDirect or relevant journals will be searched. The key search concepts will be; "repeat HIV testing", "HIV retesting", "pregnancy", Sub-Saharan Africa, "HIV seroconversion" "maternal HIV incidence", and their applicable synonyms. All the 48 sub-Saharan African countries (World Bank records) will be included individually in the searfch. Reference list of retrieved studies will be manually searched and experts in the field will be contacted for addtional studies.

17. URL to search strategy.

Upload a file with your search strategy, or an example of a search strategy for a specific database, (including the keywords) in pdf or word format. In doing so you are consenting to the file being made publicly accessible.

Or provide a URL or link to the strategy. Do NOT provide links to your search **results**.

https://www.crd.york.ac.uk/PROSPEROFILES/208300_STRATEGY_20200909.pdf

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

Repeat testing of HIV is a strategy or practice where a pregnant woman who has tested negative in the past is tested repeatedly (usually after three months). It is a recommended HIV testing strategy by the World Health Organization, nested into the guidelines for Prevention of Mother-to-Child Transmission (PMTCT) of HIV. This strategy has been adopted and implemented by several countries globally, especially in settings where HIV prevalence is high. Repeat testing is aimed at identifying seroconversions during pregnancy for timely enrollment onto antiretrovrial treatment (ART) to prevent vertical transmission of the infection to the baby.

19. * Participants/population.

Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

Pregnant women living in a sub-Saharan African country, as defined by the World Bank, accessing antenatal care (ANC) in a health facility or an outreach programme and has at least one HIV test. Pregnant women who had their first test so late and were ineligible for retesting would be excluded from the study.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

Repeat HIV testing during pregnancy as recommended by the WHO guidelines aimed at preventing mother-to-child transmission. Antiretroviral treatment (ART) strategies for women who seroconvert during pregnancy aimed at preventing vertical transmission of HIV. Studies on repeat HIV testing and seroconversion conducted before the rollout of the WHO repeat testing guidelines (indicated in the PMTCT guidelines) in 2006 will be excluded from the review.

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

Women who seroconverted during pregnancy but were not enrolled onto any ART regimen will be used as comparators to those who seroconverted during pregnancy and were enrolled onto ART.

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

All studies including RCTs, cohort, case control studies and cross-sectional studies, conducted between January 2007 and October 31st 2020 will be eligible for inclusion in the review. The specified period is based on the roll-out of comprehensive guidelines on repeat HIV testing during pregnancy (indicated in PMTCT guidelines) by the WHO in 2006. Case series and case studies, as well as studies whose full data will not be accessible even after requesting from the author(s) will be excluded from the review.

23. Context.

Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

Studies conducted in sub-Saharan African countries between January 2007 when WHO rolled-out repeat testing guidelines and October 2020 when database search will be carried out.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

1. Proportion of repeat HIV testing during pregnancy (HIV retesting in latter trimesters after an initial negative HIV test during the same pregnancy).

2. Proportion of women who tested positive for HIV after an initial negative test earlier in pregnancy.

3. Incidence of HIV (seroconversion) during pregnancy described as pregnant women who tested positive for HIV upon retesting in later trimester after an initial HIV negative test.

4. Risk of MTCT of HIV in women who seroconverted during pregnancy and were enrolled onto antiretroviral treatment (ART) compared with those who seroconverted but did not enroll onto ART.

* Measures of effect

Binary outcomes will be presented using either relatives risks (RR) or odds ratios and for continous outcomes, mean differences will be used as the effect measure, all presented with their respective 95% confidence intervals (CIs).

25. * Additional outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main outcomes. Where there are no additional outcomes please state 'None' or 'Not applicable' as appropriate to the review

None.

* Measures of effect

Not applicable.

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

A pretested data extraction template will be used to extract information on the country, publication details, study design, number and type of participants, population characteristics (relevant sociodemographic details), HIV diagnostic protocol, proportion of women tested more than once during pregnancy, rate of seroconversion during pregnancy and risk of MTCT of HIV. In studies where the primary estimates are not reported and also lack the information that would allow for their calculation, the lead authors will be contacted to provide the required the information. Results from multinational studies will be disaggregated and profiled nationally to show the dynamics in individual countries. Where it is impossible to do this, such studies will be presented as one accompanied with citations of the included countries. Two reviewers will extract data independently and any disagreement will be resolved through discussion between the authors.

27. * Risk of bias (quality) assessment.

State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.

The standardized Cochrane guidelines available in Review Manager V5.4 (www.tech.cochrane.org/revman) will be used to assess risk of bias in each of the included studies in six domains: sequence generation, allocation concealment, blinding (investigators, outcome assessors and participants), incomplete outcome data, selective outcome reporting and other sources of bias (Higgins et al. 2011). For each domain, we will make a judgment of 'low risk' of bias, 'high risk' of bias or 'unclear' risk of bias. The Cochrane guidelines will be used in addition to the Risk of Bias Tool for Prevalence Studies developed by Hoy et al (2012). The risk of bias will be assessed for four domains: selection bias, non-response bias, measurement bias and bias related to data analysis. This shall be done independently by each of the two members of the team. For each domain, a judgment of 'low risk' of bias, 'high risk' of bias or 'unclear' will be made. Any discrepancy will be resolved through discussion between the two team members.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data.
If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

Data will be analyzed using Stata software (Stata Corp V.15, Texas, USA). A meta-analysis will be conducted for data extracted from studies in which proportion of repeat HIV testing and HIV seroconversion during pregnancy were similar. Firstly, standard deviations for the study-specific estimates will be determined from the point estimate and the appropriate denominators assuming a binomial distribution. Then, the study-specific estimates will be pooled through a random-effects meta-analysis model, to obtain an overall summary estimate of the prevalence/incidence across studies, after stabilizing the variance of individual studies using the Freeman-Tukey double arc-sine transformation. Heterogeneity will be assessed by the χ^2 test on Cochran's Q statistic, which is quantified by I^2 values assuming that I^2 values of 25%, 50% and 75% represent low, medium and

high heterogeneity respectively. For the detection of the publication bias, funnel plots analysis and Egger's test will be performed. Results of the statistical analysis will be by geographic region (central, eastern, northern, southern and western Africa).

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.

Where the heterogeneity detected is significantly high, a subgroup analysis will be performed to detect the possible sources using these variables: age, study setting (hospital or community-based), and geographical area (central, eastern, northern, southern and western Africa).

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness	No
Diagnostic	No
Epidemiologic	Yes
Individual patient data (IPD) meta-analysis	No
Intervention	Yes
Meta-analysis	Yes
Methodology	No
Narrative synthesis	No
Network meta-analysis	No
Pre-clinical	No
Prevention	Yes
Prognostic	No
Prospective meta-analysis (PMA)	No
Review of reviews	No
Service delivery	No
Synthesis of qualitative studies	No
Systematic review	Yes
Other	No

Health area of the review

Alcohol/substance misuse/abuse	No

Blood and immune system	No
Cancer	No
Cardiovascular	No
Care of the elderly	No
Child health	No
Complementary therapies	No
COVID-19	No
Crime and justice	No
Dental	No
Digestive system	No
Ear, nose and throat	No
Education	No
Endocrine and metabolic disorders	No
Eye disorders	No
General interest	No
Genetics	No
Health inequalities/health equity	No
Infections and infestations	No
International development	No
Mental health and behavioural conditions	No
Musculoskeletal	No
Neurological	No
Nursing	No
Obstetrics and gynaecology	No
Oral health	No
Palliative care	No
Perioperative care	No

Physiotherapy	No
Pregnancy and childbirth	Yes
Public health (including social determinants of health)	Yes
Rehabilitation	No
Respiratory disorders	No
Service delivery	No
Skin disorders	No
Social care	No
Surgery	No
Tropical Medicine	No
Urological	No
Wounds, injuries and accidents	No
Violence and abuse	No

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.

English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

England
Ghana

33. Other registration details.

Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them.
If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

No I do not make this file publicly available until the review is complete

35. Dissemination plans.

Do you intend to publish the review on completion?

Yes

This systematic review and meta-analysis is expected to serve as a basis for evaluating the guidelines for repeat HIV testing during pregnancy as outlined in WHO's PMTCT guidelines. The final report will be submitted for the academic award of a Master of Public health degree and published as a scientific paper in a peer-reviewed journal. Findings from this present study will also be submitted to relevant health authorities. The review will also be updated in the future to monitor changes and trends to shape health service delivery and policies.

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

Repeat HIV testing; HIV seroconversion; pregnancy; antenatal care; Prevention of Mother-to-Child Transmission of HIV; sub-Saharan Africa

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

38. * Current review status.

Update review status when the review is completed and when it is published.
New registrations must be ongoing.

Review_Ongoing

39. Any additional information.

Provide any other information relevant to the registration of this review.

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint.
List authors, title and journal details preferably in Vancouver format.

APPENDIX II

Risk of Bias Tool Adapted from Hoy et al., 2012

Name of author(s):

Year of publication:

Study title:

Risk of bias items	Risk of bias levels	Points scored
1.Was the study's target population a close representation of the national population in relation to relevant variables, e.g. age, sex, occupation?	**Yes (LOW RISK)**: The study's target population was a close representation of the national population.	0
	No (HIGH RISK): The study's target population was clearly NOT representative of the national population.	1
2. Was the sampling frame a true or close representation of the target population?	**Yes (LOW RISK)**: The sampling frame was a true or close representation of the target population.	0
	No (HIGH RISK): The sampling frame was NOT a true or close representation of the target population.	1
3.Was some form of random selection used to select the sample, OR, was a census undertaken?	**Yes (LOW RISK)**: A census was undertaken, OR, some form of random selection was used to select the sample (e.g. simple random sampling, stratified random sampling, cluster sampling, systematic sampling).	0
	No (HIGH RISK): A census was NOT undertaken, AND some form of random selection was NOT used to select the sample.	1
4. Was the likelihood of non-response bias minimal?	**Yes (LOW RISK)**: The response rate for the study was ≥75%, OR, an analysis was performed that showed no significant difference in relevant demographic characteristics between responders and non- responders	0
	No (HIGH RISK): The response rate was <75%, and if any analysis comparing responders and non-responders was done, it showed a significant difference in relevant demographic characteristics between responders and non-responders	1
5. Were data collected directly from the subjects (as opposed to a proxy)?	**Yes (LOW RISK)**: All data were collected directly from the subjects.	0
	No (HIGH RISK): In some instances, data were collected from a proxy.	1
6.Was an acceptable case definition used in the study?	**Yes (LOW RISK)**: An acceptable case definition was used.	0
	No (HIGH RISK): An acceptable case definition was NOT used	1
7. Was the study instrument that measured the parameter of interest (e.g. prevalence of low back pain) shown to have reliability and validity (if necessary)?	**Yes (LOW RISK)**: The study instrument had been shown to have reliability and validity (if this was necessary), e.g. test-re- test, piloting, validation in a previous study, etc.	0
	No (HIGH RISK): The study instrument had NOT been shown to have reliability or validity (if this was necessary).	1
8. Was the same mode of data collection used for all subjects?	**Yes (LOW RISK)**: The same mode of data collection was used for all subjects.	0
	No (HIGH RISK): The same mode of data collection was NOT used for all subjects.	1
9.Were the numerator(s) and denominato r(s) for the parameter of interest appropriate	**Yes (LOW RISK)**: The paper presented appropriate numerator(s) AND denominator(s) for the parameter of interest (e.g. the prevalence of low back pain).	0
	No (HIGH RISK): The paper did present numerator(s) AND denominator(s) for the parameter of interest but one or more of these were inappropriate.	1
10. Summary on the overall risk of study bias	**LOW RISK**	0-3
	MODERATE RISK	4-6
	HIGH RISK	7-9

APPENDIX III

Risk of Bias Assessment of Included studies		
Observational Studies		
Study ID	**Risk Score**	**Interpretation**
Ejinkunle et al., 2019	1	Low risk
de Beer, 2020	1	Low risk
Imade et al., 2013	1	Low risk
Keating et al., 2012	1	Low risk
Moodley et al., 2009	0	Low risk
Mandala et al., 2019	1	Low risk
Afolabi et al., 2013	2	Low risk
Mbena et al., 2014	2	Low risk
Rojers et al., 2017	1	Low risk
Kieffer et al., 2011	2	Low risk
Adelene, 2018	4	Moderate risk
Mushamiri et al., 2020	2	Low risk
Heemelaar et al., 2015	1	Low risk
Lawi, 2015	0	Low risk
Traore, 2012	**	Unclear risk
Chetty et al., 2017	3	Low risk
Egbe et al., 2016	0	Low risk
Fatti et al., 2017	0	Low risk
Machekano et al., 2018	1	Low risk
Nyoyoko & Umoh, 2016	1	Low risk