MEKELLE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PUBLIC HEALTH

Sero-prevalence and *Predictors* of Syphilis and HIV Co-infection among pregnant women attending ANC in Mekelle City, Tigray, Ethiopia: *Evidence from Firth Penalized Regression*

By: SENAIT HADDIS (B.Sc)

A THESIS SUBMITTED TO MEKELLE UNIVERSITY, COLLEGE OF HEALTH SCIENCES, SCHOOL OF PUBLIC HEALTH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER DEGREE IN PUBLIC HEALTH (MPH) REPRODUCTIVE HEALTH

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December 2024

Mekelle University

College of Health Sciences

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Advisor's Approval Sheet

This is to certify that the thesis entitled "**Sero-prevalence and Predictors of Syphilis and HIV Co-infection among pregnant women attending ANC in Mekelle City of Conflict-Affected Tigray Region, Ethiopia: Evidence from Firth Penalized Regression**" is submitted in partial fulfillment of the requirements for the degree of MPH (Master of Public Health) with specialization in "Reproductive Health (RH)" to the Graduate Program of the College of Health Sciences of Mekelle University, and has been carried out by Senait Haddis Abebe, with ID No: CHS/RRH/013/13, under my supervision. Therefore, I recommend that the student has fulfilled the requirements, and hence, hereby can submit the thesis to the Department.

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Name of Major Advisor	**Signature**	**Date**
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Name of Co -Advisor	**Signature**	**Date**

Declaration

I hereby declare that this MPH thesis is my original work and has not been presented for a degree in any other university and all sources of material used for this thesis have been duly acknowledged.

Senait Haddis Abebe		27/05/2025
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This MPH thesis had been submitted for examination with my approval as thesis advisor.

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Examiners' Approval Sheet

We, the undersigned, members of the Board of Examiners of the final open defense by "Senait Haddis Abebe (BSc)" **have read and evaluated his thesis** "Sero-prevalence and Predictors of Syphilis and HIV Co-infection among pregnant women attending ANC in Mekelle City of Conflict-Affected Tigray Region, Ethiopia: Evidence from Firth Penalized Regression" **and evaluated the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the MPH Degree in RH.**

_____	_____	_____
Name of Chairperson	**Signature**	**Date**
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Name of Internal Examiner	**Signature**	**Date**
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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the candidate's Department through the office of the Department Graduate Program Coordinator.

Thesis Approved by

_____	_____	_____
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Certification of the Final Thesis

I hereby certify that all the corrections and recommendations suggested by the Board of Examiners are incorporated into the final thesis entitled “Sero-prevalence and Predictors of Syphilis and HIV Co-infection among pregnant women attending ANC in Mekelle City of Conflict-Affected Tigray Region, Ethiopia: Evidence from Firth Penalized Regression” by “Senait Haddis Abebe (BSc)”

Thesis Approved by

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Department Head	**Signature**	**Date**

List of Abbreviations/Acronyms

ADIS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
HIV	Human Immunodeficiency Virus
ML	Maximum Likelihood
MLE	Maximum Likelihood Estimation
MSM	Men who have Sex with Men
MTCT	Mother-To-Child Transmission
PMTCT	Prevention of Mother-To-Child Transmission
STI	Sexually Transmitted Infection
WHO	World Health Organization

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Abstract

Background: Sexually transmitted infections (STIs), particularly syphilis and HIV, significantly impact pregnant women in resource-limited countries, complicating treatment and increasing maternal and neonatal risks. In Mekelle City, Tigray, Ethiopia, barriers including limited antenatal care (ANC) access, physiological vulnerability, behavioral risks, socio-economic constraints, and conflict further exacerbate the burden of these infections, underscoring the critical need for accurate prevalence data and identification of predictive factors.

Objective: This study aimed to determine the sero-prevalence of HIV/syphilis co-infection and identify associated predictors among pregnant women attending ANC in Mekelle City.

Method: A facility-based cross-sectional study was conducted among pregnant women attending ANC services in selected health facilities across Mekelle City, Tigray, Ethiopia. A two-stage stratified sampling procedure was utilized to draw representative pregnant women under ANC from a health facility in each sub city. Data collection involved an interview-administered questionnaire and pregnant women's registration books. Due to the relatively small sample size and rarity of co-infection events, Firth penalized logistic regression was employed for statistical analysis to mitigate bias and ensure model stability. Adjusted odds ratios (AOR) with 95% confidence intervals (CI) were computed to identify significant predictors.

Result: Out of 438 pregnant women enrolled, the sero-prevalence of HIVsyphilis co-infection was 3.7% (95% CI: 1.87–5.43%). Significant predictors included sexual violence (frequency: 13.7%; AOR=2.2, 95% CI: 1.27–3.81, p=0.001), displacement (frequency: 41.1%; AOR=1.6, 95% CI: 1.10–2.32, p=0.043), frequent alcohol consumption (most frequent: 8.9%; AOR=2.1, 95% CI: 1.27–3.46, p=0.004), and limited ANC visits (frequency: 47.5%; AOR=1.8, 95% CI: 1.13–2.87, p=0.014).

Conclusion & Recommendation: The high prevalence of HIV and syphilis co-infection rate underscores the need for targeted sexual violence prevention, improved ANC access, and integrated STI screening within ANC services in Mekelle City. Enhanced education on substance abuse and sexual health is essential.

Keywords: *ANC, Co-infection, Firth Penalized Regression, HIV, Predictors, Pregnant Women, Sero-Prevalence, Syphilis*

1. Introduction

1.1. Background

1.1.1. **Context:**

Sexually transmitted infections (STIs) remain a significant public health concern(1), particularly in developing nations(2,3). Among these, women often bear the burden more heavily yet are less likely to access necessary medical care for treatment(4). Among the most concerning STIs are syphilis and human immunodeficiency virus (HIV), both of which are primarily spread through sexual contact(5,6).

HIV, a viral infection, targets CD4 cells within the immune system, progressively weakening the body's defenses and leaving it vulnerable to opportunistic infections and certain cancers(7–9). The virus remains incurable but manageable through treatments that reduce its impact on the immune system(5). Acquired immunodeficiency syndrome (AIDS) is the disease's most advanced stage. HIV transmission can occur through sexual contact, sharing of needles, blood transfusions, and from mother to child during pregnancy, delivery, or breastfeeding [15]. Both infections can be mitigated by consistent condom use, safe sexual practices, screening of blood products, and regular testing(8)(10).

Syphilis is an infectious disease caused by the bacterium *Treponema pallidum*, a spirochete known for its ability to invade the human body and cause systemic infections. The disease progresses through distinct clinical stages—primary, secondary, latent, and tertiary—each characterized by unique signs and symptoms. If left untreated, syphilis can result in severe complications, including neurological, cardiovascular, and organ system damage, particularly during the late stages. These progressive stages underscore the importance of early diagnosis and treatment to prevent long-term health consequences (11,12). Transmission occurs during oral, vaginal and anal sex, in pregnancy and through blood transfusion. Syphilis in pregnancy may lead to stillbirth, newborn death and babies born with syphilis (congenital syphilis)(5). HIV symptoms typically start with flu-like signs, followed by a long asymptomatic period and eventual progression to AIDS, marked by severe infections and complications. For both HIV and

syphilis, early diagnosis and treatment are critical to managing symptoms and preventing severe health outcomes (13–15).

Co-infections involving HIV are particularly challenging as they can complicate disease progression and treatment(16). HIV and syphilis co-infection is especially problematic, with HIV increasing the risk of syphilis treatment failure and leading to heightened neurocognitive impairment when both infections are present(17). HIV may also heighten susceptibility to various co-infections, and, conversely, certain co-infections can increase the risk of HIV acquisition(18). Studies have shown that individuals co-infected with HIV and syphilis are at a higher risk of neurosyphilis, an infection of the central nervous system by *Treponema pallidum*. This increased risk is attributed to the immunosuppressive effects of HIV, which facilitate the progression of syphilis to the nervous system (19). Consequently, co-infected individuals may experience more severe neurocognitive impairments compared to those with either infection alone(19).

Moreover, HIV infection can complicate the treatment of syphilis. The immunocompromised state induced by HIV may reduce the efficacy of standard syphilis treatments, leading to higher rates of treatment failure and persistent infection (20). This underscores the importance of vigilant monitoring and potentially more aggressive treatment strategies in co-infected patients.

The bidirectional relationship between HIV and syphilis highlights the need for integrated screening and management approaches. Early detection and treatment of both infections are crucial to prevent the progression to neurosyphilis and to mitigate the compounded neurocognitive impairments associated with co-infection(12,21).

1.1.2. Epidemiology

Globally, STIs remain prevalent, with syphilis and HIV contributing significantly to morbidity and mortality, especially in low-income regions (22,23). According to the World Health Organization (WHO), it is estimated that over 1 million sexually transmitted infections (STIs) are acquired each day globally(24), including nearly a million pregnant women affected by syphilis, many of whom lack access to antenatal care (ANC), leading to adverse outcomes (25).

Sub-Saharan Africa (SSA) is disproportionately affected by both HIV and syphilis, with approximately 70% of all HIV cases occurring in the region, largely affecting women and pregnant populations(26). Syphilis and HIV co-infection further complicates health outcomes, as syphilis increases the risk of HIV entry by disrupting mucosal barriers. Syphilis infection significantly increases the risk of HIV acquisition in high-risk populations. A systematic review and meta-analysis found that individuals with syphilis had a 2.67 times higher risk of acquiring HIV compared to those without syphilis(27). In SSA, syphilis prevalence among pregnant women varies by region. The pooled prevalence of syphilis among pregnant women in SSA is estimated at 2.9% (95% CI: 2.4%-3.4%)(28). However, this prevalence varies significantly across different regions: East Africa: 3.2% (95% CI: 2.3%-4.2%)(28), Southern Africa: 3.6% (95% CI: 2.0%-5.1%)(28) and overall range: 0.1% to 10.3%(29).

Despite international and national efforts, both syphilis and HIV remain significant public health issues in Ethiopia. The Ethiopian government launched the Prevention of Mother-to-Child Transmission (PMTCT) acceleration plan in 2012, followed by a national strategy in 2013. However, despite these efforts, gaps in service coverage and implementation persist, especially in antenatal care (ANC) access and awareness(30,31). Studies in Ethiopia have shown syphilis prevalence rates of 1% to 11.2% among pregnant women, with HIV/syphilis co-infection rates ranging from 0.5% to 2.2% (31). A meta-analysis conducted in 2021 found that the pooled prevalence of syphilis among pregnant women in Ethiopia was 2.32% (95% CI, 1.68–2.97). This figure is higher than the 1.1% reported in recent nationwide HIV/syphilis sentinel surveys among ANC attendees. Regional variations in syphilis prevalence were observed, with the highest rates in the Southern Nations Nationalities and Peoples (SNNP) region at 4.06% (95% CI, 2.86–5.26), followed by Amhara at 2.16% (95% CI, 1.57–2.75), and Oromia at 1.46% (95% CI, 0.69–2.23)(32). The pooled syphilis-HIV co-infection rate among pregnant women was found to be 0.80% (95% CI, 0.60–1.01%)(32). A more recent study conducted in 2024 in Amhara regional state referral hospitals reported that 1.3% (95% CI: 0.6, 2.6) of pregnant women were co-infected with HIV and syphilis(33).

The Tigray region mirrors the national prevalence, with HIV affecting approximately 1.1% of the population, and prevalence rates among pregnant women reaching 2.4% (34). Despite Ethiopia's initiatives under the PMTCT program, issues such as limited ANC access, social

stigma, and economic constraints continue to delay effective prevention and management efforts. In Tigray, these challenges are further compounded by socio-economic disparities and regional instability, posing additional barriers for combatting HIV in pregnant women (34).

1.2. The Impact of Conflict on Maternal Health Services

The conflict escalated in November 2020 in Tigray has had a devastating impact on maternal health services, significantly increasing the risk of syphilis and HIV co-infection among pregnant women(35–37). The healthcare system in the region was largely collapsed, with only 3% of health facilities fully functioning. Furthermore, 85% of health centers and 70% of hospitals were partially or completely non-functional, while the critical Health Extension Program, essential for maternal health, had entirely been ceased operations(38,39). Antenatal care (ANC) coverage, which was at 94% before the war, has been severely affected(40). The maternal mortality ratio has risen dramatically, from 186 to 840 per 100,000 live births(41).

The conflict has also created substantial barriers to the screening and treatment of syphilis and HIV among pregnant women. The widespread destruction of health facilities has significantly limited access to diagnostic services. Critical shortages of medication and medical supplies have hampered treatment for various conditions, including HIV and Syphilis(42). Many health workers have been displaced or are unable to work due to insecurity and non-payment of salaries(43). Though precise data on syphilis and HIV co-infection among pregnant women in conflict-affected Tigray are limited, the available evidence suggests a potentially high sero-prevalence resulting from the healthcare system's collapse. Addressing this crisis will require urgent efforts to rebuild and strengthen maternal health services, prioritizing the restoration of ANC services, enhancing screening capabilities, and ensuring access to treatment for both syphilis and HIV.

1.3. Statement of the Problem

The dual burden of Syphilis and HIV co-infection among pregnant women poses significant public health challenges, particularly in regions with limited healthcare resources. Globally, the rates of HIV and syphilis co-infection have been rising, with significant increases observed in high-income countries such as the USA, Europe, Canada, and Australia. For instance, the number

of syphilis cases in the USA tripled from 2013 to 2018, and similar trends have been noted in Europe and Australia(44). In low- and middle-income countries (LMICs), syphilis remains endemic, accounting for over 90% of global cases, with pregnant women being a particularly vulnerable group(44).

In sub-Saharan Africa, the prevalence of syphilis among pregnant women attending antenatal care (ANC) clinics is indeed a significant public health concern, with estimates ranging from 2.9% to 4.6% (28,45). This high prevalence underscores the importance of addressing syphilis in the region, particularly given its potential impact on maternal and fetal health. Recent studies have provided more precise estimates of syphilis prevalence among pregnant women in sub-Saharan Africa. A systematic review and meta-analysis found that the pooled prevalence of syphilis among pregnant women in sub-Saharan Africa was 2.9% (95% CI: 2.4%-3.4%)(28). Regional variations were observed, with East and Southern African regions showing higher prevalence rates: East Africa: 3.2% (95% CI: 2.3%-4.2%); Southern Africa: 3.6% (95% CI: 2.0%-5.1%) (28). These figures highlight the ongoing challenge of syphilis in the region and the need for robust screening and treatment programs during antenatal care. The co-infection of HIV and syphilis is indeed a significant concern in sub-Saharan Africa. Several studies have highlighted increased prevalence among PLWH. Syphilis prevalence is notably higher among people living with HIV (PLWH) compared to those without HIV. The relative risk of syphilis among PLWH versus those without HIV is estimated at 3.5 (95% CI: 2.8-4.5)(46).Syphilis prevalence among PLWH varies across sub-Saharan Africa: Southern Africa: 3.1% (95% CI: 2.2%-4.0%); West/Central Africa: 5.5% (95% CI: 2.3%-9.3%); and East Africa: 10.5% (95% CI: 8.0%-13.1%)(46).

Nationally, in Ethiopia, the sero-prevalence of syphilis among pregnant women varies significantly across regions. For example, rates as high as 4.06% have been reported in the Southern Nations, Nationalities, and Peoples' Region (SNNPR), and 1.46% in Oromia (47).The pooled prevalence of syphilis-HIV co-infection among pregnant women in Ethiopia is estimated at 0.80%(47). In the Amhara region, the prevalence of HIV/syphilis co-infection among pregnant women attending ANC was found to be 1.3% (48).

Conflict-affected regions, such as the Tigray region in Ethiopia, face unique vulnerabilities that exacerbate the prevalence and impact of syphilis and HIV co-infection. As highlighted above, the region experienced severe disruptions in healthcare infrastructure, reduced access to antenatal care (ANC), and heightened risk factors for disease transmission, such as sexual violence, displacement, and economic instability. In spite of the critical need for targeted interventions, there is a significant lack of data on the prevalence of syphilis and HIV co-infection for women attending ANC in this conflict-affected region. Limited research especially in the context of recent socio-political crises and surveillance systems on syphilis and HIV co-infection for women attending ANC also make it difficult to quantify the scale of the problem or identify key risk factors in this crises affected region Tigray of Ethiopia.

In terms of approach, most of previous studies conducted in the country were relied on conventional logistic regression models to assess the prevalence and predictors of Syphilis and HIV co-infection. However, these models can produce biased estimates when dealing with risk factors that have very low prevalence, due to sampling imbalances. This methodological gap necessitates the use of advanced statistical techniques, such as penalized regression, which can provide more accurate and reliable estimates by addressing these biases(49).

This study aims to fill these gaps by employing penalized regression techniques to evaluate the sero-prevalence and predictors of Syphilis and HIV co-infection among pregnant women attending ANC clinics in Mekelle city of this conflict-ridden Tigray region. By doing so, it will provide critical understandings into the epidemiological landscape of these infections in the region, identify key socio-economic and healthcare-related predictors, and inform the development of targeted interventions to improve maternal and child health outcomes. This research is particularly timely and relevant, given the unique challenges faced by the Tigray region, and will contribute valuable knowledge to the broader field of maternal health in resource-limited settings.

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1.4. Rational of the study

Despite the high burden of syphilis and HIV co-infection among pregnant women in conflict-affected areas like Tigray, reliable data remain scarce. The crisis has disrupted healthcare systems, limiting access to antenatal care and hindering disease surveillance. Most existing studies use conventional methods that fail to account for rare co-infection patterns. This study

aims to fill the data gap by applying penalized regression techniques to generate accurate estimates and identify key risk factors, providing evidence to guide targeted interventions and improve maternal health outcomes in resource-constrained settings.

1.5. Significance of the Study

This study on Syphilis and HIV co-infection among pregnant women attending ANC clinics in Tigray is highly significant. It will provide vital data on the prevalence and risk factors of these infections, supporting the development of targeted public health interventions to improve maternal and child health. The findings can inform policy decisions, guide the implementation of effective screening and management strategies, and help to modify healthcare services to the region's specific needs, especially given Tigray's unique socio-political challenges.

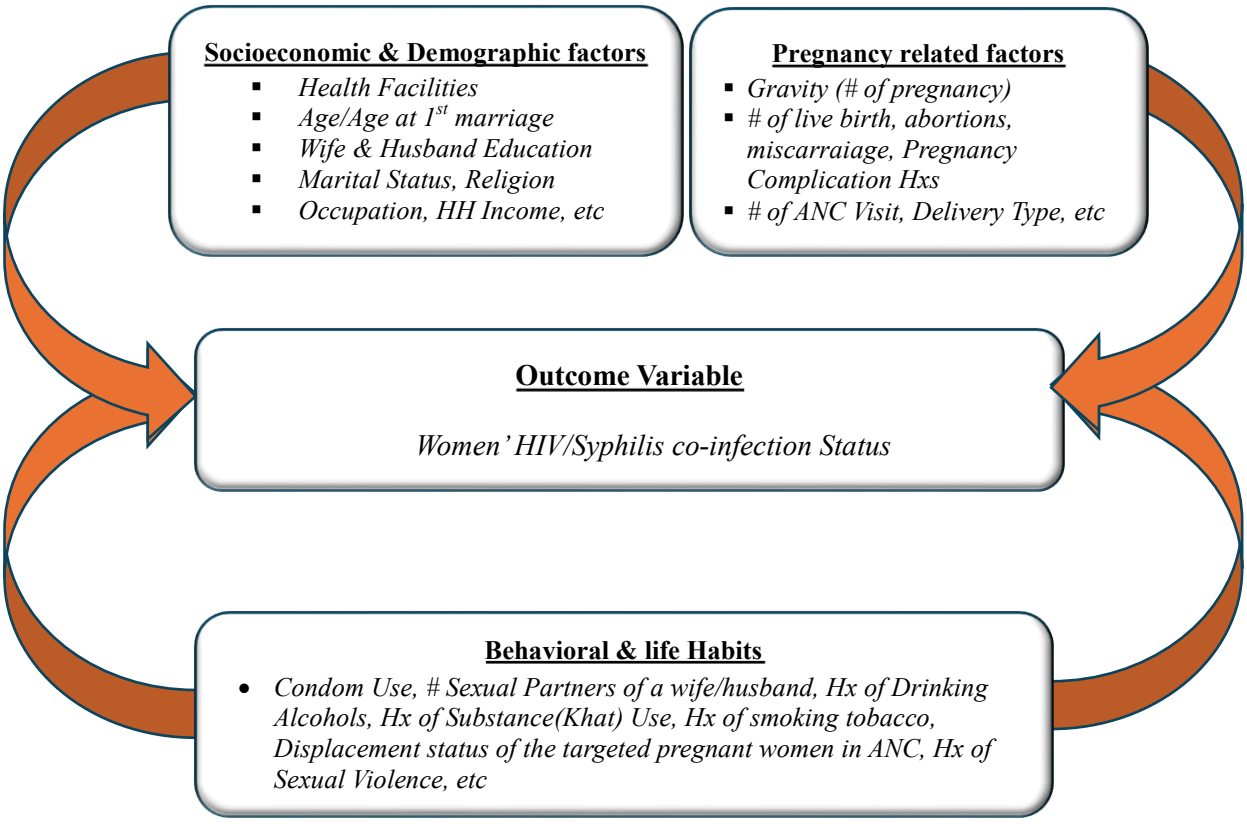


Figure 1: Conceptual Framework

Source: own

Additionally, the study will enhance clinical practices by offering insights into integrating Syphilis and HIV screening within ANC services, leading to better health outcomes. By using advanced statistical methods, the research ensures reliable and valid results. The study will also raise awareness among pregnant women and healthcare providers, promoting preventive behaviors and reducing infection rates. Overall, it is expected to make a meaningful contribution to public health, policy, clinical practice, research methods, and community awareness in Tigray.

1.6. Conceptual Framework:

The conceptual framework that served as a blueprint for the structure and methodology of this thesis study, defining the relationships between the dependent & independent variables, and the rationale behind the investigation, was provided Figure 1.

1.7. Literature Review

1.7.1. Empirical Literature

This empirical literature on the sero-prevalence and risk factors of HIV, syphilis, and their co-infection provides a good understanding of the epidemiology and factors influencing these dual infections, particularly among vulnerable populations such as pregnant women and those attending antenatal care (ANC) clinics.

Sexually Transmitted Infections (STIs) are the most common contagious diseases that disproportionately affect developing countries, particularly in sub-Saharan Africa(50). STIs are among the top five reasons for consultation in general health services in many African countries including Ethiopia(46). It causes substantial productivity loss for individuals and communities, especially where most of the people are less than 40. Nevertheless, both sexes are at high risk for acquiring STIs, however, females are more frequently and severely affected(51).

1.7.2. Syphilis Sero-Prevalence

Published literature reveals syphilis, caused by the bacterium Treponema pallidum, is another common sexually transmitted infection (STI) globally. Its prevalence varies widely, with notable impacts on maternal and child health outcomes. In pregnant women, untreated syphilis can lead to adverse outcomes such as stillbirth, neonatal death, and congenital syphilis(29). Recent systematic reviews and meta-analyses indicates Sub-Saharan Africa reports varying prevalence

rates among pregnant women, underscoring the need for enhanced screening and treatment programs, with the pooled prevalence estimated at 2.9% (95% CI: 2.4%-3.4%); and regional variations of East Africa: 3.2% (95% CI: 2.3%-4.2%), Southern Africa: 3.6% (95% CI: 2.0%-5.1%)(45); and individual studies, however, report varying prevalence rates. In Ethiopia, recent studies show prevalence ranging from 1.4% to 2.9%(47,52); In Somalia, reported prevalence of 4.07% in 2019(53); and other countries prevalence rates also range from 0.3% to 7.3%(54). .

Syphilis is one of the oldest known STIs caused by the spirochete Treponema pallidum subspecies pallidum that can inflict very serious complications when left untreated(55). Despite the existence of effective preventive and treatment measures, annually 12 million people are infected, and a considerable proportion of these infections occur in countries with limited resources of sub-Saharan Africa and Asia (56,57). In pregnant women, this teratogenic pathogen crosses the placenta and infects the fetus during the early period of gestation. Published literature indicates that syphilis is considered as the second most leading cause of stillbirth worldwide and results in prematurity, low birth weight, neonatal death, and infections in newborns(58,59).

Recent estimates documented that approximately **2 million pregnant women** are infected with syphilis each year worldwide(60) and 520,905 had adverse pregnancy outcomes, including 212,327 stillbirths or early fetal deaths, 91,764 neonatal deaths, 65,267 preterm or low birth weight infants, and 151,547 infected newborns(61). HIV during pregnancy is also associated with various undesirable consequences for the mother, fetus, and neonates such as maternal death, abortion, stillbirth, and low birth weight(30,62,63). According to a systematic review and meta-analysis, the pooled prevalence of syphilis among pregnant women in sub-Saharan Africa is 2.9% (95% CI: 2.4%-3.4%). This figure is based on 44 studies comprising 175,546 subjects(28,45,64). The prevalence varies by region within sub-Saharan Africa: East Africa: 3.2% (95% CI: 2.3%-4.2%); and Southern Africa: 3.6% (95% CI: 2.0%-5.1%). These regional prevalences are higher than the overall sub-Saharan African pooled prevalence(28,45).

According to recent studies, the prevalence of syphilis among pregnant women in Ethiopia has indeed shown an upward trend, but the figures vary across different regions and studies: ANC-based sentinel surveillance data shows an increase in syphilis prevalence from 1% in 2012 to 1.2% in 2014 for Ethiopia overall, and 1.4% in the Oromia region(65). The seroprevalence of

syphilis among pregnant women in different parts of Ethiopia ranges from 1.1% to 5.1%: Buno Bedele zone, southwest Ethiopia: 1.1%(65); Yirgalem Hospital, Southern Ethiopia: 5.1%(66); Sede Muja District, Northwest Ethiopia: 1.9%(67); Gondar, Ethiopia: 2.6% to 3.7%(67). Some studies have reported even higher prevalence rates: up to 5.1% in Debre Berhan town, Ethiopia(67) and as high as 7.3% to 9.8% among HIV patients in different parts of Ethiopia(66). These findings indicate that syphilis remains a significant public health concern among pregnant women in Ethiopia, with prevalence rates varying by region and population group. The upward trend and relatively high prevalence rates underscore the need for strengthened screening, prevention, and treatment efforts in antenatal care settings across the country(54,66)..

1.7.3. HIV Sero-Prevalence

HIV continues to be a major global health challenge, with significant regional disparities in prevalence. Sub-Saharan Africa remains the epicenter of the HIV/AIDS epidemic, despite housing only 11% of the world's population(68). The situation in Ethiopia reflects this broader regional context, with notable variations in HIV prevalence across different areas and populations.

Ethiopia's overall HIV prevalence has shown a declining trend over the years. According to the Ethiopian Demographic and Health Survey (DHS), adult HIV prevalence decreased from 3.3% in 2000 to 0.9% in 2016(68). However, this national average masks significant regional disparities. The HIV prevalence in Ethiopia is unevenly distributed among its regions:High Prevalence Areas: Gambella (4.4%), Addis Ababa (3.48%), Dire Dawa (2.72%), and Harari (2.67%)(68); Low Prevalence Areas: Somalia (0.12%) and Southern Nations, Nationalities, and People's Region (SNNPR) (0.35%)(68) and Certain zones have been identified as HIV hotspots, including Nuer and Agnuak in Gambella, West Wollega and Illubabor in Oromia, and parts of the Afar and Amhara regions(68). Contrary to the general trend, the odds of HIV infection were found to be higher in rural areas compared to urban areas(68). This finding challenges previous assumptions and highlights the need for targeted interventions in rural settings. Several factors contribute to the varying HIV prevalence across Ethiopia: *Education*-individuals with secondary-level education were more likely to be infected with HIV(68); *Contraceptive Use*-those using contraceptive methods showed higher odds of HIV infection(68); and *Sexually Transmitted Infections (STIs)*-People with STIs were at increased risk of HIV infection(68). Ethiopia has

adopted the global 95-95-95 strategy to combat HIV/AIDS. As of 2023, the country's progress varies by region. National Level figure shows 84% of people living with HIV (PLHIV) know their status, 98% of those are on antiretroviral therapy (ART), and 98% of those on ART are virally suppressed(69). Ethiopia faces several challenges in its fight against HIV/AIDS.The spatial disparities in HIV prevalence necessitate strategic targeting of resources and policy interventions at the zonal level(68). HIV testing and counseling services (HTC) coverage has fluctuated, with the highest coverage recorded in 2023(69). CDC supports evidence-based interventions to reduce TB incidence and mortality among PLHIV, including TB preventive treatment and scaled-up use of shorter regimens(70). In conclusion, while Ethiopia has made progress in reducing overall HIV prevalence, significant regional disparities persist. Targeted interventions, improved resource allocation, and continued efforts towards the 95-95-95 goals are crucial for further reducing HIV transmission and improving outcomes for those living with HIV in the country.

1.7.4. HIV/Syphilis Co-Infection Sero-Prevalence

HIV and syphilis co-infection presents significant public health challenges due to their synergistic effects on transmission and health outcomes. The sero-prevalence of HIV/syphilis co-infection varies considerably across regions and populations, with sub-Saharan Africa experiencing a particularly high burden. Studies worldwide have reported HIV and syphilis co-infection rates ranging from 6.21% to 46.5%(71). Some notable examples include: Singapore: 6.21%; Turkey: 8-25%; Mexico: 25%; and Taiwan: 46.5%. A meta-analysis of studies in sub-Saharan Africa found a pooled syphilis prevalence of 7.3% among people living with HIV (PLWH). However, significant regional variations were observed: Southern Africa: 3.1%; West/Central Africa: 5.5%; and Eastern Africa: 10.5% (72). In Addis Ababa, Ethiopia, a study found that the sero-prevalence of syphilis among HIV-infected individuals was 9.8%, compared to 1.3% among HIV-uninfected individuals(73). This represents an odds ratio of 8.01 (95% CI: 2.4 to 26.6), indicating a strong association between HIV and syphilis infection.

HIV/syphilis co-infection sero-prevalence among pregnant women attending antenatal care (ANC) varies across different regions and studies. In Amhara regional state, northern Ethiopia, HIV/syphilis co-infection sero-prevalence rate: 1.3% (95% CI: 0.6, 2.5)(33). In Luanda, Angola,

13% of among HIV-positive pregnant women had co-infections(74); and in a study conducted in Tanzania, HIV/syphilis co-infection sero-prevalence rate 0.66%(50,75).

1.7.5. Risk Factors of HIV/Syphilis Co-infection

HIV/syphilis co-infection in pregnant women attending antenatal care (ANC) is a significant public health concern globally, with varying risk factors across different regions. Globally, demographic factors such as older age, lower education levels, and low socioeconomic status are associated with increased risk. Behavioral factors, including multiple sexual partners, unsafe sexual practices, and sex work, contribute significantly to co-infection rates. In sub-Saharan Africa (SSA), a meta-analysis revealed varying prevalence rates, with Eastern Africa showing the highest at 10.5% (95% CI: 8.0%-13.1%)(46). More specifically, the relationship of those risk factors with the co-interaction can be given as follows:-

Socioeconomic and Demographic Factors

(a). **Age:** Ewunetie et al. reported that pregnant women younger than 25 years were significantly protected from HIV-infection [AOR, 0.43 (95% CI, 0.20-0.91), P = .026](74). However, Kengne-Nde et al. found that co-infection was more common among those aged 25 or older(75).

(b). **Education:** Lower education levels are associated with increased risk of co-infection(75).Kengne-Nde et al. found that having no/some primary education (compared to secondary education and above) increased the risk for HIV and syphilis co-infection [OR: 3.29; 95% CI (1.60, 6.74)](76). Another Ethiopian study reported that completing secondary school education (AOR = 0.15; 95% CI: 0.04-0.53) and graduating from college (AOR = 0.03; 95% CI: 0.01-0.22) were protective factors against maternal HIV infection(77).

(c). **Marital Status:** Marital status also played a role, with divorced, separated, or widowed individuals showing an increased risk (adjusted odds ratio [AOR] range: 1.5–2.7) (78). Kengne-Nde et al. found that single pregnant women were three times more likely to have HIV/syphilis co-infection than those who were married, in a stable relationship, widowed or divorced(75).

(d). **Household Income:** A study in Ethiopia found that pregnant women with a family monthly income greater than 8001 ETB (1 USD = 56 ETB) had lower odds of

HIV infection (AOR = 0.19; 95% CI: 0.04-0.87)(77). This suggests that higher income may be protective against HIV infection.

Behavioral Factors

(a). Alcohol Consumption: Alcohol consumption has been identified as a significant risk factor for HIV/syphilis co-infection. A study by Yeganeh et al. found that pregnant women with co-infection were significantly more likely to consume alcohol(75). Additionally, Kengne-Nde et al. reported that alcohol consumption increased the risk of HIV/syphilis co-infection [OR: 1.87; 95% CI (1.16, 3.03)](76). Other study indicates that Pregnant women who reported drinking alcohol during pregnancy had higher odds of HIV/Syphilis co-infection (AOR: 1.5, 95% CI: 1.1-2.3)(79).

(b). Substance Use: Substance use has been identified as a risk factor for co-infection. Ewunetie et al. found that pregnant women who used substances were more likely to have syphilis infection [AOR = 3.39, 95%CI (1.31–8.77)](80).

(c). Number of Sexual Partners: Having multiple sexual partners significantly increases the risk of co-infection. Ewunetie et al. reported that women with more than one lifetime sexual partner had higher odds of developing maternal syphilis infection [AOR = 3.59, 95% CI (1.09–11.71)](80).

(d). **History of Sexually Transmitted Infections (STIs):** A history of STIs significantly increases the risk of co-infection. Ewunetie et al. reported that pregnant women with a history of STIs had higher odds of syphilis infection [AOR = 3.46, 95%CI (1.32–9.08)] (80). Another study also reveals Women with a history of STIs were at increased risk of HIV/HBV co-infection (AOR: 4.6, 95% CI: 1.4-14.9) and HBV/Syphilis co-infection (AOR: 6.3, 95% CI: 1.2-15.9) (81,82).

(e). **Unsafe Sex:** Women with a history of unsafe sex practices were at significantly higher risk of HIV/Syphilis co-infection (AOR: 8.2, 95% CI: 1.5-16.7) (81,82).

Pregnancy Related Factors

(a). **History of Abortion**: Pregnant women with a history of previous abortion had significantly higher odds of HIV infection (AOR = 7.73; 95% CI: 3.33-17.95) (77).

(b). Gravidity and Parity: While specific AORs were not provided for these factors, studies have indicated that multiparity and multigravidity may be associated with increased risk of HIV/Syphilis co-infection(77,81).

These findings highlight the need for targeted interventions, comprehensive screening programs, and integrated HIV and STI management strategies in antenatal care settings across Ethiopia(48), (82).

1.7.6. Firth Penalized Logistic Regression

Models of binary dependent variables often encounter situations where events (ones) are much rarer than non-events (zeros)(83,84). This scenario presents challenges in accurately estimating probabilities and effects using standard logistic regression(85). Examples of phenomena that do not occur very often can be found in all areas, where the percentage of cases of interest falls below 10 or even 5%. In socio-economic surveys, model rare phenomena could include the estimation of the proportion of workers who changed their job in the week prior to the interview. In health surveys, responses to the use of certain drugs or diseases can also be quite infrequent(85).

Challenges with Low Prevalence Risk Factor

Logistic regression via maximum likelihood (ML) estimation is indeed a commonly used approach for analyzing binary outcomes in epidemiological studies, including those assessing the prevalence and risk factors of Syphilis, HIV, and their co-infection among pregnant women attending antenatal care (ANC). However, this method can face significant challenges when dealing with risk factors that have very low prevalence, leading to biased estimates due to sampling imbalance(*i.e.*, an extremely larger proportion of zeros than ones) (86,87). When estimating risk factors with extremely low prevalences for a binary outcome, classical techniques like logistic regression often fail to provide meaningful results(86). This is because: *the likelihood function of the logistic regression model may not converge when prevalences are very low; low prevalences can lead to numerical problems in the estimation process; and coefficient estimates may become biased, or estimation may not be possible at all.* The consequences of these issues include: *increased bias in estimates, higher variability in results, wider confidence*

intervals; and loss of statistical power. In essence, lower prevalences lead to a significant loss in both accuracy and precision of risk estimates(86).

Overestimation of Odds Ratios

One particularly concerning issue is that logistic regression tends to overestimate odds ratios in studies with small to moderate sample sizes(87). This bias is systematic and shifts away from the null hypothesis, meaning that: *regression coefficient estimates move away from zero*; and *odds ratios move away from one*. This overestimation can be especially problematic in epidemiological studies, as it may lead to erroneous interpretations of the results and potentially misguided public health interventions(87).

Potential Solutions

To address these challenges, several methods have been proposed but the two common approaches are *Penalized Regression Methods* (87) and Bayesian Approaches(88). Thus, while logistic regression via maximum likelihood remains a popular tool for analyzing binary outcomes in epidemiological studies, researchers must be aware of its limitations when dealing with low-prevalence risk factors. The use of alternative methods, such as penalized regression techniques or Bayesian approaches, should be considered to obtain more accurate and reliable estimates in these scenarios. Additionally, sensitivity analyses using multiple methods can help researchers understand the robustness of their results when analyzing sparse data sets(88)

This thesis employed Firth penalized regression techniques to assess the prevalence and predictors of Syphilis, HIV, and their co-infection among pregnant women attending ANC clinics in Tigray, Ethiopia.

Firth penalized regression models are increasingly recognized as effective tools for predicting outcomes in scenarios involving rare events (*i.e.*, an extremely larger proportion of zeros than ones), particularly in the context of avoiding overfitting issues common in traditional maximum likelihood estimation (MLE). It is a robust alternative to conventional logistic regression, particularly for small samples (*i.e.*, more predictors, having no at least 10 cases for each predictors), rare events, or data separation. It achieves more reliable and less biased parameter estimates by adding a penalty to the likelihood.

When assessing sero-prevalence and identifying predictors of co-infection (such as Syphilis and HIV among pregnant women), researchers often confront methodological challenges, especially if the sample size is small or if some events (e.g., HIV/Syphilis co-infections) are relatively rare.In conventional logistic regression, estimation difficulties arise in scenarios of data separation—when one or more predictors perfectly predict the outcome—or when dealing with limited sample sizes and rare outcomes. Such issues can lead to inflated estimates, wide confidence intervals, or even non-convergence. Firth penalized logistic regression provides a robust alternative in this context. Specifically, in the analysis of predictors of HIV/Syphilis co-infection (such as sexual violence, multiple sex partners, khat consumption, low education, low household income, lack of ANC visits, and early initiation of sexual activity), Firth's method stabilizes parameter estimates, reduces estimation bias, and offers improved inference reliability compared to conventional logistic regression.This thesis work endeavors to illuminate the superior efficacy of Firth's method in managing small datasets within scientific research and advocates for its more widespread application(89).

2. Research Question and Objective of the Study

1.7.1. Research Questions

Question 1: What is the prevalence of co-infection of Syphilis and HIV among pregnant women under ANC follow-up in the studied health facilities in Mekelle City, Tigray, Ethiopia?

Question 2: What are the socio-economic, demographic, maternal, conflict and behavioral predictors of Syphilis and HIV co-infection among pregnant women attending ANC clinics in Mekelle City, Tigray, Ethiopia?

1.7.2. Objective of the Study

3.3.1. General Objective

The overall objective of this thesis is to assess the prevalence and predictors of Syphilis and HIV co-infection among pregnant women attending antenatal care (ANC) in selected health facilities in Mekelle City, Tigray, Ethiopia.

3.3.2. Specific Objectives

More specifically, this proposed study aims to:

(a). Determine the prevalence of HIV and Syphilis co-dynamics among pregnant women attending ANC in the study area in Mekelle City, Tigray, Ethiopia.

(b). Explore the socio-demographic, maternal, conflict and behavioral characteristics of HIV and Syphilis co-interaction among pregnant women attending ANC in the study area in Mekelle City, Tigray, Ethiopia.

(c). Identify the relative significance of socio-demographic, maternal, conflict and behavioral predictors of HIV and Syphilis co-dynamics among pregnant women attending ANC in the study area in Mekelle City, Tigray, Ethiopia.

3. Materials and Methods

3.1. Study Area and Period

The thesis study was conducted in Mekelle, the regional capital of Tigray, Ethiopia. Mekelle is located approximately 783 km north of Addis Ababa, at an elevation of 2,254 meters above sea level in a mid-altitude agro-ecological zone. Geographically, the city is situated between 13°24'30" to 13°36'52" North Latitude and 39°25'30" to 39°38'33" East Longitude. According to Tigray Meteorology Service Center, Mekelle receives an average annual rainfall of 608 mm in 2024, primarily during the main rainy season from mid-June to mid-September, with additional rainfall occurring from February to March. The mean maximum and minimum temperatures are 27.1°C and 13.4°C, respectively. The city covers a total area that ranges between 109-200 km²; and serves as the economic and political capital of the Tigray regional state(90,91).

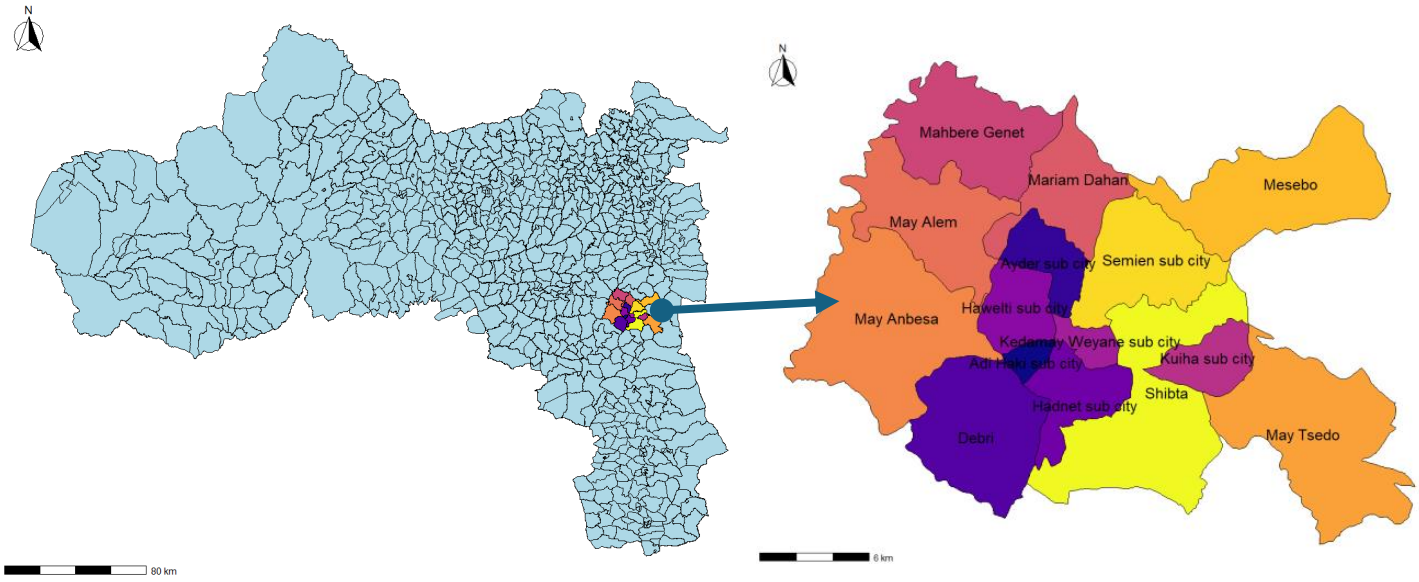


Figure 2: Map of Study Area: Mekelle with its sub cities & new tabia boundaries

There is no exact population figure of Mekelle. Even though, its population was counted as 215914 by the 2007 Ethiopian population and housing census, the city's demography has been rapidly changing since the last census. As a result different studies estimated different population size of Mekelle,233,000(119,800F)(92), 340,859 (51%F) (93), 556,127 (94) and 587,000(95). According to the Tigray regional health bureau profile report, Mekelle has three public hospitals and eleven health centers.

The study was conducted from December 1, 2024, to April 1, 2025, in 15 randomly selected public hospitals of the seven sub cities: Quiha G/Hospital, AEWO G/Hospital & Quiha H/Center in Quiha Sub city; Aynalem H/Center, Debrie H/Center & Kasech H/Center in Hadnet Sub city; Mekelle H/Center in K/Weyane Sub city; Semen H/Center, Mekelle Hospital & Lachi H/Center in Semen Sub city; Adishinduhun H/Center & Serawat/Momona H/Center in Hawlti Sub city; Ayder Comprehensive Referral Hospital & Adiha Primary Hospital in Ayder Subcity and Lekatiti Primary Hospital in Adihaki Sub city. According to the department of Reproductive, Maternal, Newborn and Child Health (RMNCH) and data obtained from DHIS2, Tigray Regional Health Bureau, there are various services currently offered in each of those facilities in Mekelle even though their services (quality & magnitude) are disproportionately differ from one facility to the other, potentially due to their resources (manpower, equipment, etc) and budget as well. VCT, ART, family planning, abortion, immunization, Fistula, laboratory services (HIV, Hepatitis, Syphilis, BG, Urine, etc) are to mention some among the several services undertaken in each of those health facilities.

3.2. Study Design

A facility-based cross-sectional study design was used to assess the sero-prevalence and predictors of Syphilis and HIV co-infection among pregnant women attending ANC clinics in Mekelle.

3.3. Population

3.3.1. Source Population

The source population included all pregnant women attending antenatal care (ANC) in the selected public hospitals in Mekelle.

3.3.2. Study Population

The study population was a subset of this group, consisting specifically of pregnant women in the reproductive age (15-49), attending ANC in the selected public hospitals in Mekelle during the study period who agreed to participate.

3.3.3. Study Units

The study units were those pregnant women in the reproductive age attending ANC chosen using systematic random sampling (with sampling interval-*k*: Average Monthly Pregnant women per HFs-N_i/ sample size of each facility-n_i) from those selected public hospitals in Mekelle during the study period.

3.4. Eligibility Criteria

3.5.1. Inclusion Criteria

This work consisted of the following study participants

- Pregnant women of age 15-49 attending antenatal care (ANC) in the selected seven public hospitals from each of the seven sub cities (One HFs from each sub city).

3.5.2. Exclusion Criteria

This work, on contrary, excluded those pregnant women of reproductive age attending ANC

- Who had duplicate records, where the same pregnant woman attended ANC multiple times during the study period. Only the first entry was retained.

3.5. Study Variables

3.6.1. Dependent/Outcome Variables

The primary interest to determine facility-based prevalence and the predictors of HIV and Syphilis co-infection among pregnant women attending ANC in Mekelle, Tigray's capital was the women's **Syphilis and HIV Co-infection Status** (denoted as: Y_{ij}), defined as a binary

variable indicating whether a pregnant woman attending ANC was co-infected with both syphilis and HIV. It was coded as follows

- $Y_{ij} = \begin{cases} 1 & \text{if a pregnant woman attending ANC had infected with both Syphilis and HIV} \\ 0 & \text{otherwise (infected with none or either)} \end{cases}$

3.6.2. Independent Variables

The predictor variables (X_{ij}) were, on the other hand, categorized into three major groups. Socio-demographic variables, which included *age, marital status, wife/husband's education status, wife/husband's occupational status, age at marriage, etc*; maternal characteristics, which encompassed *total number of births, live births, stillbirths, abortions, ANC visits, etc*; and lastly, behavioral and conflict-related variables such as *previous sexually transmitted infections (STIs), history of drinking alcohols, history of smoking cigarette, history of consuming Khat, history of previous sexual partner, condom usage, displacement status, history of sexual violence, etc*. These factors potentially influence the prevalence and predictors of syphilis, HIV, and their co-infection. Understanding these variables allows for the identification of at-risk groups and the development of targeted interventions to reduce infection rates and improve maternal and child health outcomes. These include, but are not limited to, the following:

3.6. Sample Size Determination and Sampling Procedure

3.6.1. Sampling Technique

The study employed a two-stage stratified cluster sampling strategy, utilizing Primary Sampling Units (PSU) and Secondary Sampling Units (SSU). In the first stage, sub-cities in Mekelle served as strata, and health facilities within each sub-city acted as the PSU. In the second stage, pregnant women visiting these health facilities were selected as the SSU. The sample size was allocated proportionally to the average number of pregnant women in each health facility, ensuring that larger facilities had greater representation. For example, Yekatit 11 Health Center, with an average of 472 pregnant women, had the largest sample size (120), while smaller facilities, like Mekelle Health Center with 80, had a sample size of 20. The total population of

pregnant women across all facilities was 2,692, and 438 participants are selected for the study using probability proportional to size (PPS). This approach ensures proportional representation, geographic diversity, and efficiency, while minimizing sampling bias.

3.6.2. Sample Size Determination

The sample size for this study was determined using a single population proportion formula(96), considering the assumptions that the average prevalence for all HIV/Syphilis co-infection among pregnant women was 5% (73). The calculation assumed a 95% confidence level ($Z_a/_2$ = 1.96), a margin of error of 2.5% (d = 0.025), and used the following formula:.

$$n = \frac{\left(Z_{\frac{\alpha}{2}}\right)^2 \times p \times (1-p)}{d^2}$$

Plugging in the values:

$$n = \frac{\left(Z_{\frac{\alpha}{2}}\right)^2 \times p \times (1-p)}{d^2} = \frac{(1.96)^2 \times (0.05) \times (1-0.5)}{(0.025)^2} \approx 292$$

To adjust for the effect of cluster sampling, a design effect (DEFF) of 1.5 was applied:

$$n_{adjusted} = 292 \times DEFF = 292 \times 1.5 = 438$$

Therefore, the final estimated sample size for this study was 482 pregnant women (Compensation about 10% for non-response rate was omitted since ODK was used to avoid non-response rate). The following table summarizes ***Health Facilities*** drawn using cluster sampling as PSU, and their corresponding ***Pregnant Women under ANC's*** sample size as SSU, calculated with probability proportional to size (PPS), where size refers to the number of pregnant women per health facility in each sub city as shown in table below.

Table 1: Sampling and Sample Size Determination

Sub cities (Stratum)	Health Facilities (cluster)	Average Monthly Pregnant women per HFs	HFs selected using Cluster Sampling	Average Monthly Pregnant women per HFs	Sample Size (PPS)
Ayder Sub city	Ayder CRH	228	Ayder CRH	228	58
	Adiha H/C	114			
Hadinet Sub city	Aynalem H/C	78	Kasech H/C	300	76
	Debrie H/C	98			
	Kasech H/C	300			
Hawlti Sub city	A/Shinduhum H/C	238	A/Shinduhum H/C	238	60
	Serawat H/C	94			
Adihaki Subcity	Lekatit P/H	472	Lekatit P/H	472	120
Semen Sub city	Semen H/C	230	Mekelle Hospital	322	82
	Mekelle Hospital	322			
	Lachi H/C	30			
K/Weyane	Mekelle H/C	80	Mekelle H/C	80	20
Quiha Sub city	Quiha G/H	88	Quiha G/H	88	22
	HEWO G/H	66			
	Quiha H/C	254			
Total		2692		1728	438

3.7. Data Collection and Laboratory Diagnosis

To comprehensively study the relationship between HIV and syphilis co-infection among pregnant women, two primary tools were employed for data collection: ***an interview-administered questionnaire*** and ***pregnant women's registration books***.

The mobile interview-administered questionnaire was prepared using Open Data Kit (ODK) tool and utilized to gather detailed data on various socio-demographic factors; maternal characteristics; conflict, sociocultural and behavioral practices such as age, marital status, educational level, income, unprotected sexual behaviors, history of sexually transmitted infections (STIs), substance use, the frequency of antenatal care visits, etc. This information is

crucial for understanding the contextual factors that may predispose pregnant women to HIV/syphilis co-infection or affect their treatment outcomes.

Pregnant women's registration books served as a secondary data source to provide accurate clinical information. These records were reviewed to extract laboratory test results, including HIV status, syphilis test results (e.g., rapid diagnostic tests or confirmatory assays), and other related laboratory findings. By examining these records, the study ensured that critical diagnostic data on HIV and syphilis co-infection was included and accurately linked to socio-demographic and behavioral data collected from the questionnaires.This dual approach allowed the study to bridge self-reported information with objective clinical data, providing a holistic understanding of HIV/syphilis co-infection dynamics. It also supported the identification of key risk factors, enabling targeted interventions to reduce the burden of co-infections and improve maternal and fetal health outcomes in the study population.

3.8. Data Quality Control

The questionnaire designed in ODK was checked and pretested before the actual data collection begins. The questionnaire in ODK tool was initially be developed in English and then translated into the local language (Tigrigna) for data collection. Two supervisors and seven data collectors, all with experience in maternal health surveys, were recruited to oversee and facilitate the data collection in those selected health facilities of Mekelle city. A pre-test was conducted on 5% of the study participants in *Adishinduhun Health Center*, which were not be included in the main study. Modifications were made to the questionnaire based on the pre-test results. Intensive training was provided to the supervisors and data collectors on how to facilitate data collection. Supervision ensured the completeness and consistency of the data, with the overall data collection process controlled by the principal investigator.

3.9. Statistical Method and Analysis

In many public health and epidemiological studies, logistic regression based on maximum likelihood estimation (MLE) is commonly employed to examine factors associated with a binary outcome and to estimate the corresponding regression coefficients. However, when the outcome is rare or data are sparse, conventional logistic regression using MLE can encounter several issues. These include biased or even infinite estimates of regression coefficients, failure of the

likelihood function to converge due to data separation, and inaccurate Wald tests and confidence intervals. To address these limitations, Firth's penalized logistic regression (FPLR), also known as penalized likelihood regression, has been proposed as an alternative. FPLR modifies the likelihood function by introducing a penalty term that reduces small-sample bias and ensures finite, stable coefficient estimates even under separation conditions (97–100).

In the present study, the overall prevalence of HIV and syphilis co-infection among pregnant women attending antenatal care (ANC) was 3.6%, qualifying it as a rare event. As such, Firth logistic regression was used to assess the associations between various predictors and the binary co-infection outcome (coded as 1 for co-infected, 0 otherwise). The choice of FPLR was further justified by the small number of events (~16 co-infected cases) relative to the number of predictors (23), resulting in an events-per-variable (EPV) ratio of approximately 0.69. This is far below the conventional threshold of EPV ≥ 10 required for reliable estimates in standard logistic regression. Under such conditions, MLE may produce unstable, biased, or undefined estimates due to issues such as perfect separation, inflated standard errors, and non-convergence. Firth's method corrects for these challenges by applying a bias-reducing penalty to the log-likelihood function, effectively mitigating the inflation of odds ratios and ensuring convergence. The general form of the Firth penalized logistic regression model for a binary outcome, Y_i, can be expressed as follows:

$$\text{logit}(P(Y_i = 1|X_i)) = \text{logit}(p) = \log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi}$$

where Y_i is the binary outcome for the i^{th} individual, X_{ij} represents the j^{th}predictor for the i^{th} individual, and β_j are the regression coefficients. Unlike standard logistic regression, which maximizes the conventional likelihood, $l(\beta)$, FPLR maximizes a penalized likelihood:

$$l^*(\beta) = l(\beta) + \frac{1}{2}\lfloor I(\beta) \rfloor$$

Here, $l^*(\beta)$ is the log-likelihood from the standard logistic model, and $I(\beta)$ is the Fisher information matrix. The additional term $\frac{1}{2}\lfloor I(\beta) \rfloor$ serves to shrink extreme estimates and reduce bias, particularly when the outcome is rare or when predictors exhibit complete or quasi-complete separation. Adjusted odds ratios (AORs), 95% confidence intervals, and p-values were

calculated and interpreted. Model fit was evaluated using penalized likelihood ratio tests and pseudo R-squared values, and multicollinearity was checked using Variance Inflation Factors (VIFs). The FPLR analysis was conducted using the “logistf()” function in R and the “firthlogit” command in Stata. On top of this, patients' characteristics were summarized through univariate descriptive and spatial analyses. The univariate analysis provided a detailed overview of the demographic and clinical variables using frequencies, percentages, means, and standard deviations. Additionally, a spatial distribution map was employed to visualize the prevalence of HIV and syphilis co-infection across sub-cities and associated health facilities, offering geographic insights into the burden and clustering of cases within the study area. This approach ensured the generation of stable and unbiased estimates, thereby providing a more accurate understanding of the predictors of HIV and syphilis co-infection among pregnant women in the conflict-affected setting of Mekelle.

3.10. Ethical Statement

The study received ethical approval with a **RE.:MU-IRB 2442/2025** from the Institutional Review Board of College of Health Sciences, Mekelle University. Before the data collection, a signed informed consent to produce evidence based result from Tigray Region Health Bureau, and written consent were secured from study participants after a thorough explanation and understanding of the purpose of the study. The information obtained was made anonymous and de-identified prior to analysis to ensure confidentiality, and used only for the intended purpose. The study was also conducted in accordance with the Helsinki Declaration.

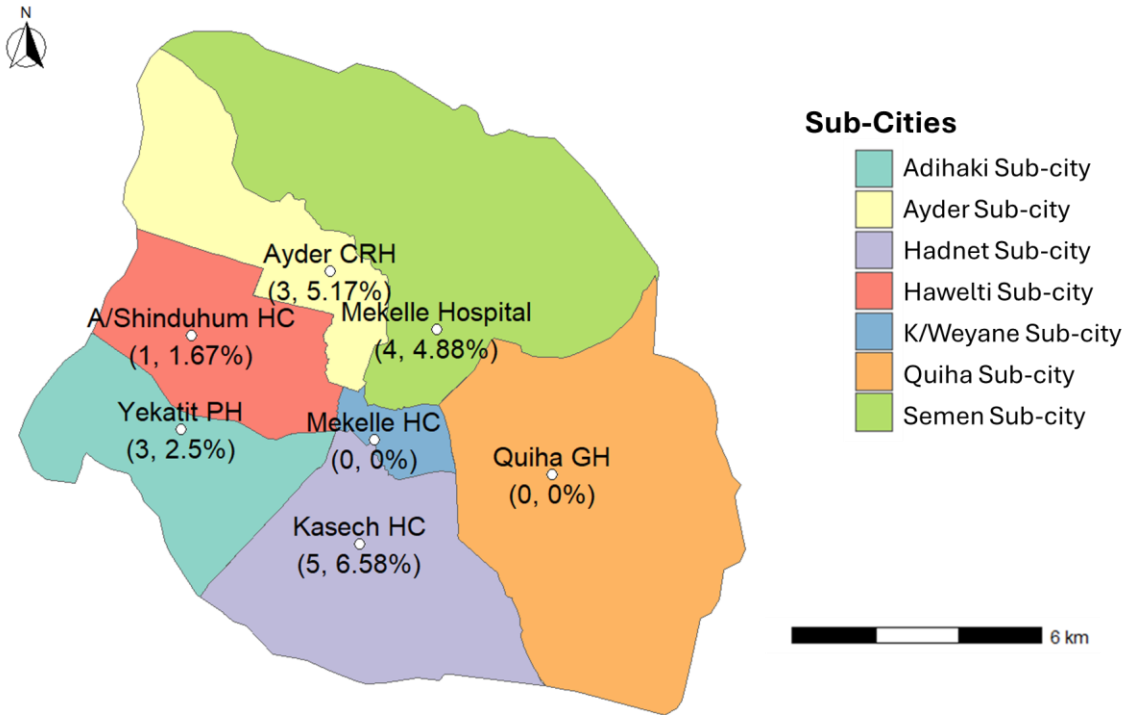
4. Main findings/Results

This section presents the findings of the study on the sero-prevalence and predictors of HIV and syphilis co-infection among pregnant women attending antenatal care (ANC) services in selected health facilities in Mekelle City, Tigray, Ethiopia. The analysis includes descriptive statistics that summarize the socio-demographic, behavioral, and clinical characteristics of the participants, followed by bivariate analyses to identify variables potentially associated with co-infection. Finally, the results of the multivariable Firth Penalized Logistic Regression model are reported, highlighting the independent predictors of HIV and syphilis co-infection. All findings are presented with appropriate statistical measures, including adjusted odds ratios (AOR), 95% confidence intervals (CI), and p-values to assess the strength and significance of associations.

4.1. Facility-based Sero-Prevalence of HIV/Syphilis in Mekelle

In this study, the overall sero-prevalence of HIV/Syphilis co-infection was found to be 3.7%, based on 16 positive cases out of a total sample size of 438 pregnant women (95% CI: 1.87%, 5.43%). Despite the low overall burden, substantial variation was observed across health facilities. Kasech Health Center reported the highest prevalence at 6.58% (95% CI: 1.01%, 12.15%), followed by Ayder Comprehensive Referral Hospital (5.17%, 95% CI: 0.00%, 10.87%) and Mekelle Hospital (4.88%, 95% CI: 0.22%, 9.54%). Yekatit Primary Hospital and A/Shinduhum Health Center reported lower rates of 2.50% (95% CI: 0.00%, 5.29%) and 1.67% (95% CI: 0.00%, 4.91%) respectively. No co-infection cases were detected at Mekelle Health Center and Quiha General Hospital. These findings underscore the importance of localized facility-based surveillance and targeted prevention efforts to address potential hotspots of HIV/Syphilis co-infection among pregnant women in the region.

Figure 3: HIV/Syphilis Prevalence by Health facilities, Mekelle, Tigray



4.2. Demographic Characteristics of Study Participants

Table-2 presents the socio-demographic characteristics of the 438 pregnant women attending ANC who participated in the study, with specific reference to their syphilis and HIV testing status. The distribution of testing uptake across health centers shows that Yekatit Primary Hospital contributed the highest proportion of tested participants for both syphilis (25.1%) and HIV (25.8%), followed by Mekelle Hospital (18.0%) and Kasech Health Center (16.9% and 17.1% respectively). The majority of tested women were within the age range of 25–34 years, representing 58.7% for syphilis and 58.9% for HIV, followed by the 15–24 year group. Orthodox Christianity was the predominant religion (over 86% for both infections), and most participants were married (85.4% syphilis, 86.8% HIV). Occupational data indicated that a substantial number of women (70.5% syphilis, 71.9% HIV) were unemployed, while among husbands, nearly half were also unemployed (47.0% syphilis, 46.6% HIV). In terms of education, most women had primary education (49.3% syphilis, 49.8% HIV), and a considerable number of their husbands had tertiary education (31.5% syphilis, 32.6% HIV). Household income was relatively diverse, with a significant proportion earning above 10,000 birr per month (26.9% syphilis, 26.5% HIV). These socio-demographic patterns suggest differential testing uptake across

facilities and demographic groups, highlighting the need for targeted public health strategies that consider age, occupation, education, and income disparities.

Table 2: Socio-Demographic Characteristics of Study Participants (n=438)

Variables	Parameters	Syphilis Testing		HIV Testing	
		No: n(%)	Yes: n(%)	No: n(%)	Yes: n(%)
Health Center	A/Shinduhum Health Center	3(0.7%)	57(13.0%)	3(0.7%)	57(13.0%)
	Ayder Comprehensive RH	4(0.9%)	54(12.3%)	3(0.7%)	55(12.6%)
	Kasech Health Center	2(0.5%)	74(16.9%)	1(0.2%)	75(17.1%)
	Mekelle Health Center	2(0.5%)	18(4.1%)	1(0.2%)	19(4.3%)
	Mekelle Hospital	3(0.7%)	79(18.0%)	3(0.7%)	79(18.0%)
	Quiha General Hospital	1(0.2%)	21(4.8%)	1(0.2%)	21(4.8%)
	Yekatit Primary Hospital	10(2.3%)	110(25.1%)	7(1.6%)	113(25.8%)
Age Group	15-24	7(1.6%)	111(25.3%)	2(0.5%)	116(26.5%)
	25-34	12(2.7%)	257(58.7%)	11(2.5%)	258(58.9%)
	25-45	6(1.4%)	45(10.3%)	6(1.4%)	45(10.3%)
Religion	Orthodox	22(5.0%)	379(86.5%)	16(3.7%)	385(87.9%)
	Muslim	3(0.7%)	29(6.6%)	3(0.7%)	29(6.6%)
	Other	0(0.0%)	5(1.1%)	0(0.0%)	5(1.1%)
Marital Status	Married	25(5.7%)	374(85.4%)	19(4.3%)	380(86.8%)
	Unmarried	0(0.0%)	39(8.9%)	0(0.0%)	39(8.9%)
Husband's Occupation	Employed in Organizations	11(2.5%)	156(35.6%)	6(1.4%)	161(36.8%)
	Employed in Own Business	11(2.5%)	51(11.6%)	8(1.8%)	54(12.3%)
	Unemployed	3(0.7%)	206(47.0%)	5(1.1%)	204(46.6%)
Wife's Occupation	Employed in Organizations	5(1.1%)	73(16.7%)	3(0.7%)	75(17.1%)
	Employed in Own Business	0(0.0%)	31(7.1%)	2(0.5%)	29(6.6%)
	Unemployed	20(4.6%)	309(70.5%)	14(3.2%)	315(71.9%)
Wife's Level of Education	No formal education	0(0.0%)	14(3.2%)	0(0.0%)	14(3.2%)
	Primary	15(3.4%)	216(49.3%)	13(3.0%)	218(49.8%)
	Secondary	5(1.1%)	95(21.7%)	4(0.9%)	96(21.9%)
	Tertiary	5(1.1%)	88(20.1%)	2(0.5%)	91(20.8%)
Hasband's Level of Education	No formal education	1(0.2%)	14(3.2%)	1(0.2%)	14(3.2%)
	Primary	12(2.7%)	146(33.3%)	10(2.3%)	148(33.8%)
	Secondary	2(0.5%)	115(26.3%)	3(0.7%)	114(26.0%)
	Tertiary	10(2.3%)	138(31.5%)	5(1.1%)	143(32.6%)
Household Income	Less than 2500	1(0.2%)	67(15.3%)	1(0.2%)	67(15.3%)
	2500 to 5000	4(0.9%)	110(25.1%)	1(0.2%)	113(25.8%)
	5001 to 7500	3(0.7%)	45(10.3%)	1(0.2%)	47(10.7%)
	7501 to 10000	5(1.1%)	73(16.7%)	2(0.5%)	76(17.4%)
	Above 10000	12(2.7%)	118(26.9%)	14(3.2%)	116(26.5%)

4.3. Maternal Characteristics of Study Participants

Table-3 also presents the maternal characteristics of the 438 pregnant women attending ANC included in the study in Mekelle City, Tigray, Ethiopia, with a focus on their syphilis and HIV testing status. Regarding gravidity, the majority of participants had 1–2 pregnancies, accounting for 49.8% of syphilis-tested and 50.7% of HIV-tested women, followed by those with 3–4 pregnancies. A small proportion (7.5%) had more than four pregnancies. In terms of parity, women with no previous live births represented 28.3% of those tested for syphilis and 28.5% for HIV, while those with one or two live births comprised a considerable share of the tested group. Women with more than two live births were less represented (12.1% syphilis, 11.6% HIV). Notably, a high percentage of participants reported no history of abortion (82.4% syphilis, 84.0% HIV) or miscarriage (71.5% syphilis, 72.1% HIV), indicating generally favorable reproductive histories.

Table 3: Maternal Characteristics of Study Participants (n=438)

Variables	Parameters	Syphilis Testing		HIV Testing	
		No: n(%)	Yes: n(%)	No: n(%)	Yes: n(%)
Number of Pregnancy	1-2 Preg	10(2.3%)	218(49.8%)	6(1.4%)	222(50.7%)
	3-4 Preg	11(2.5%)	162(37.0%)	9(2.1%)	164(37.4%)
	>4	4(0.9%)	33(7.5%)	4(0.9%)	33(7.5%)
Number of live births	0	7(1.6%)	124(28.3%)	6(1.4%)	125(28.5%)
	1	5(1.1%)	140(32.0%)	1(0.2%)	144(32.9%)
	2	7(1.6%)	96(21.9%)	4(0.9%)	99(22.6%)
	>2	6(1.4%)	53(12.1%)	8(1.8%)	51(11.6%)
No.Children_Aborted	0	22(5.0%)	361(82.4%)	15(3.4%)	368(84.0%)
	1 & above	3(0.7%)	52(11.9%)	4(0.9%)	51(11.6%)
No.Children_Miscarriage	0	18(4.1%)	313(71.5%)	15(3.4%)	316(72.1%)
	1 & above	7(1.6%)	100(22.8%)	4(0.9%)	103(23.5%)
ANC follow-up during pregnancy	1 Visit	11(2.5%)	208(47.5%)	10(2.3%)	209(47.7%)
	2 Visits	4(0.9%)	56(12.8%)	2(0.5%)	58(13.2%)
	3 Visits	6(1.4%)	69(15.8%)	1(0.2%)	74(16.9%)
	4+ Visits	4(0.9%)	80(18.3%)	6(1.4%)	78(17.8%)

The study also revealed that Antenatal care (ANC) follow-up was low, with 47.5% and 47.7% of participants undergoing only one visit for syphilis and HIV testing, respectively.

4.4. Behavioral and Conflict related Characteristics of Study Participants

Table-4 outlines the behavioral and conflict-related characteristics of pregnant women enrolled in the study in relation to their syphilis and HIV testing status.

Table 4: Behavioral and Conflict related Characteristics of Study Participants (n=438)

Variables	Parameters	Syphilis Testing		HIV Testing	
		No: n(%)	Yes: n(%)	No: n(%)	Yes: n(%)
Age at Marriage	15-24	22(5.0%)	297(67.8%)	14(3.2%)	305(69.6%)
	25-34	3(0.7%)	71(16.2%)	5(1.1%)	69(15.8%)
	25-45	0(0.0%)	45(10.3%)	0(0.0%)	45(10.3%)
Displacement Status	No	15(2.4%)	233(23.5%)	10(2.3%)	238(54.3%)
	Yes	10(2.3%)	180(41.1%)	9(2.1%)	181(41.3%)
Age at first sexual intercourse	15-24	20(4.6%)	341(77.9%)	13(3.0%)	348(79.5%)
	25-34	3(0.7%)	39(8.9%)	5(1.1%)	37(8.4%)
	25-45	2(0.5%)	33(7.5%)	1(0.2%)	34(7.8%)
Previous experience of sexual partners in the wife	No	20(4.6%)	331(75.6%)	17(3.9%)	334(76.3%)
	Yes	5(1.1%)	82(18.7%)	2(0.5%)	85(19.4%)
Tobacco consumption in the past 12 months	No	23(5.3%)	409(93.4%)	19(4.3%)	413(94.3%)
	Yes	2(0.5%)	4(0.9%)	0(0.0%)	6(1.4%)
Khat consumption in the past 12 months	No	18(4.1%)	376(85.8%)	14(3.2%)	380(86.8%)
	Yes	7(1.6%)	37(8.4%)	5(1.1%)	39(8.9%)
Alcohol consumption rate in the past 12 months	Never	4(0.9%)	126(28.8%)	4(0.9%)	126(28.8%)
	Least Frequent	14(3.2%)	156(35.6%)	9(2.1%)	161(36.8%)
	Occasional	3(0.7%)	92(21.0%)	2(0.5%)	93(21.2%)
	Most Frequent	4(0.9%)	39(8.9%)	4(0.9%)	39(8.9%)
Condom usage in the past 12 months	No	21(4.8%)	378(86.3%)	17(3.9%)	382(87.2%)
	Yes	4(0.9%)	35(8.0%)	2(0.5%)	37(8.4%)
Another sexual partners' availability in the husband	No	17(3.9%)	240(54.8%)	11(2.5%)	246(56.2%)
	Yes	8(1.8%)	173(39.5%)	8(1.8%)	173(39.5%)
Any form of sexual violence experience in the past	No	23(5.3%)	353(80.6%)	18(4.1%)	358(81.7%)
	Yes	2(0.5%)	60(13.7%)	1(0.2%)	61(13.9%)

The majority of participants were married between the ages of 15–24 (67.8% syphilis-tested, 69.6% HIV-tested), and similarly, most had their first sexual experience within this age range (77.9% and 79.5% respectively), suggesting early sexual debut and marriage as common experiences in this population. Displacement due to conflict was also notable, with 41.1% of syphilis-tested and 41.3% of HIV-tested women reporting displacement, highlighting a potential vulnerability factor. Most participants (93.4% for syphilis and 94.3% for HIV) had not used tobacco in the past year, though a small proportion reported khat (8.4% syphilis, 8.9% HIV) and

alcohol use. Condom use was low, with only 8.0% and 8.4% reporting usage in the past 12 months for syphilis and HIV, respectively. Additionally, 39.5% reported that their husbands had other sexual partners, and 13.7%–13.9% of women had experienced some form of sexual violence, underscoring critical risk behaviors and gender-based vulnerabilities. These findings point to the importance of integrating behavioral health education, sexual and reproductive rights advocacy, and conflict-sensitive programming within ANC services to address risk factors for HIV and syphilis co-infection.

4.5. Parameter Estimates from Firth Penalized Logistic Regression:

4.5.1. Risk factors of HIV/Syphilis Co-infection

Table 2 presents the findings of the Firth penalized logistic regression model, which was used to address issues of small-sample bias and separation in the binary outcome of HIV/Syphilis co-infection among pregnant women. The analysis revealed several significant predictors at the 5% significance level. Women with primary (AOR=1.7, 95% CI: 1.32–2.19, p=0.021) and no formal education (AOR=1.5, 95% CI: 1.19–1.90, p=0.014) had higher odds of co-infection compared to those with tertiary education. Similarly, lower household income was associated with elevated risk, with women earning between 2500–5000 birr (AOR=1.5, 95% CI: 1.03–2.18, p=0.012), 5001–7500 birr (AOR=1.3, 95% CI: 1.05–1.61, p=0.017), and above 10,000 birr (AOR=2.1, 95% CI: 1.45–3.05, p=0.003) showing significantly higher odds than the reference group earning less than 2500 birr. The multivariate analysis of maternal characteristics such as number of ANC visits revealed that women with only 1–2 ANC visits had significantly higher odds of co-infection (AOR = 1.3; 95% CI: 1.09–1.55; p = 0.038) compared to those with more than four visits (AOR = 1.3, 95% CI: 1.09–1.55, p = 0.038) compared to those with more than four visits, while attending 3–4 visits was associated with a lower, though not statistically significant, risk (AOR = 0.2, 95% CI: 0.06–0.72, p = 0.297).

Behavioral characteristics also showed strong associations. Women who had their first sexual intercourse before age 25 were significantly more likely to be co-infected (AOR=1.6, 95% CI: 1.12–2.28, p=0.003) compared to those who initiated at age 34 or above. Additionally, women with a history of previous sexual partners (AOR=1.4, 95% CI: 1.04–1.88, p=0.045),

displacement experience (AOR=1.6, 95% CI: 1.10–2.32, p=0.043), and any form of sexual violence exposure (AOR=2.2, 95% CI: 1.27–3.81, p=0.001) were more likely to be co-infected.

Risk behavior-related variables such as frequent alcohol consumption showed a strong dose–response relationship, where those drinking occasionally (AOR=1.7, p=0.031) or most frequently (AOR=2.1, p=0.004) had increased odds of co-infection compared to never users. Likewise, *non-use of condoms* in the past 12 months was a significant predictor (AOR=1.9, 95% CI: 1.16–3.10, p=0.021).

4.5.2. Model Comparison

To assess the robustness of the findings (potential issues of rare events and address potential issues of small sample bias and data separation) in modeling HIV/Syphilis co-infection among pregnant women, both conventional logistic regression and Firth penalized logistic regression were compared using AIC and BIC. The Firth penalized model yielded lower AIC (132.76) and BIC (196.86) values compared to the conventional logistic regression model (AIC = 153.19, BIC = 217.29), indicating superior model fit. The conventional model showed signs of instability due to sparse event data, such as inflated standard errors and fitted probabilities close to 0 or 1. In contrast, the Firth model provided more reliable and robust estimates by applying penalization that effectively corrects for bias associated with small sample sizes and rare outcomes. These results suggest that the Firth penalized logistic regression is a more appropriate approach for drawing valid inferences in this conflict-affected population, where data sparsity and quasi-complete separation are prevalent challenges.

4.5.3. Model fitting

The penalized likelihood ratio test comparing the full Firth logistic regression model to the null model yielded a test statistic of 22.5 ($df = 8, p = 0.004$), indicating that the included predictors significantly improved model fit. Pseudo R-squared values were also calculated to assess the explanatory power of the model, with a McFadden's pseudo R-squared of 0.78, that approximately 78% of the variation in the outcome was explained by the predictors, reflecting a

Table 4: Finding of Firth Penalized Logistic Regression of each Predictor

Variable	AOR	SE	95% LCI	P-value
1.Age Group				
15-24	1.0	-	-	-
25-34	0.6	0.34	0.03-1.13	0.573
25-45	0.1	0.76	0.02-0.35	0.352
2.Religion				
Orthodox	1.0	-	-	-
Muslim	0.8	1.37	0.10-6.43	0.897
Other	7.4	0.89	1.36-4.24	0.438
3.Marital Status				
Unmarried	1.0	-	-	-
Married	0.4	1.58	0.02-9.14	0.745
4.Husband's Occupation				
Work in GOs	1.0	-	-	-
Work in Own B	0.4	2.13	0.04-3.41	0.455
Unemployed	0.4	0.93	0.06-2.15	0.302
5.Wife's Occupation				
Work in GOs	1.0	-	-	-
Work in Own B	2.4	0.73	0.57-9.86	0.559
Unemployed	0.3	1.00	0.05-2.41	0.445
6.Wife's Level of Education				
Tertiary	1.0	-	-	-
Secondary	1.9	0.67	0.5-6.89	0.734
Primary	1.7	0.13	1.32-2.19	0.021
No FE	1.5	0.12	1.19-1.9	0.014
7. Husband's Level of Education				
Tertiary	1.0	-	-	-
Secondary	0.7	1.51	0.04-13.81	0.917
Primary	0.8	1.44	0.05-13.15	0.954
No FE	0.2	1.43	0.01-2.63	0.526
8. Household Income				
Less than 2500	1.0	-	-	-
2500 to 5000	1.5	0.19	1.03-2.18	0.012
5001 to 7500	1.3	0.11	1.05-1.61	0.017
7501 to 10000	1.7	0.31	0.93-3.12	0.055
Above 10000	2.1	0.19	1.45-3.05	0.003
8. Number of Pregnancy				
1-2 Pregnancy	1.0	-	-	-
3-4 Pregnancy	0.5	0.96	0.08-3.57	0.68
>4 Pregnancy	0.4	1.10	0.05-3.63	0.70
9. Number of live births				
No Live Birth	1.0	-	-	-
1+ Live Birth	0.7	1.03	0.09-4.89	0.73
10. Number of Children Aborted				
0	1.0	-	-	-
1 & above	1.1	1.37	0.08-16.81	0.854
11.Number of Children Miscarriage				
0	1.0	-	-	-
1 & above	0.5	0.65	0.13-1.65	0.432
12. ANC follow-up during pregnancy				
#ANC Visits	**AOR**	**SE**	**95% LCI**	**P-value**
>4 Visits	1.0	-	-	-
3-4 Visits	0.2	0.65	0.06-0.72	0.297
1-2 Visits	1.3	0.09	1.09-1.55	0.038
13.Displacement Status				
Non-Displaced	1.0	-	-	-
Displaced	1.6	0.19	1.1-2.32	0.043
14. Age at first sexual intercourse				
34 & above	1.0	-	-	-
25-34	1.2	0.23	0.76-1.88	0.051
15-24	1.6	0.18	1.12-2.28	0.003
15. Previous sexual partners' experience of the wife				
No	1.0	-	-	-
Yes	1.4	0.15	1.04-1.88	0.045
16.Tobacco consumption in the past 12 months				
No	1.0	-	-	-
Yes	2.2	0.75	0.5-9.56	0.784
17. Khat consumption in the past 12 months				
No	1.0	-	-	-
Yes	1.9	1.87	0.05-72.26	0.219
18. Alcohol consumption rate in the past 12 months				
Never	1.0	-	-	-
Least Frequent	0.4	1.01	0.06-3.11	0.053
Occasional	1.7	0.17	1.23-2.39	0.031
Most Frequent	2.1	0.13	1.63-2.71	0.004
20. Condom usage in the past 12 months				
No	1.0	-	-	-
Yes	1.9	0.25	1.16-3.1	0.021
21Another sexual partners experience in husband				
No	1.0	-	-	-
Yes	1.1	0.01	1.08-1.12	0.06
22. Any form of sexual violence experience in the past				
No	1.0	-	-	-
Yes	2.2	0.28	1.27-3.81	0.001

strong model fit. These results demonstrate that the selected risk factors-such as sexual violence, multiple sex partners, condom use, khat consumption, low education, early age at first sex, low household income, and lack of ANC visits-collectively provide a statistically significant and meaningful prediction of HIV/Syphilis co-infection risk in the study population. Additionally, multicollinearity among predictors was assessed using Variance Inflation Factors (VIFs), all of which were below the commonly accepted threshold of 5, indicating no significant multicollinearity and that the predictors contribute independently to the model. This combination of fit statistics and diagnostic checks supports the robustness and reliability of the model in identifying key risk factors for HIV/Syphilis co-infection in the study population.

5. Discussion

5.1. Demographic Characteristics and Sero-Prevalence

The present study assessed the sero-prevalence and predictors of HIV/Syphilis co-infection among pregnant women attending ANC in Mekelle City, a conflict-affected area of the Tigray region in northern Ethiopia. The HIV/syphilis co-infection prevalence of 3.7%(95% CI:1.87-5.43%) found among pregnant women in Mekelle City is notably higher than the pooled national estimate for Ethiopia, which is 0.80% (95% CI, 0.60–1.01%); and that of Amhara region, which is 1.3% (95% CI: 0.6, 2.6), highlighting the impact of local context-particularly conflict and socioeconomic disruption-on HIV/syphilis co-infection rates among pregnant women(101,102). Conversely, it was lower than the studies done in Angola (6.3%) (81). The variation in prevalence across health facilities, with Kasech Health Center reporting the highest rate 6.58%(95%CI:1.01-12.15%), underscores the heterogeneity of infection risk within urban centers, likely influenced by localized social determinants and access to healthcare services(50,64,103).

The demographic profile of participants reveals that the majority were aged 25–34 years (more than 55%), married (more than 86%), and predominantly followers of Orthodox Christianity(more than 85%), consistent with the population structure of the Tigray region (104) and a similar study conducted in Amhara region(82). The high proportion of unemployed women (70.5%) and nearly half of their husbands being unemployed (47.0%) reflects the socioeconomic challenges in the area, potentially exacerbated by the conflict and aftereffect, a pattern also reported in other humanitarian settings(105). Similar demographic patterns have been reported in other Ethiopian studies, where unemployment and low socioeconomic status are linked to increased vulnerability to sexually transmitted infections (STIs) including HIV and syphilis(103).

5.1.1. Maternal and Behavioral Characteristics

Most pregnant women had favorable reproductive histories, with many reporting no prior abortions or miscarriages. Unlike to those attributes, their ANC attendance was low, with 47.5% and 47.7% of pregnant women attending only one visit for syphilis and HIV testing, respectively. Women with 1–2 visits had significantly higher odds of co-infection (AOR = 1.3, 95% CI: 1.09–

1.55%; p = 0.038), while those with 3–4 visits had lower, non-significant odds (AOR = 0.2, 95% CI: 0.06–0.72%; p = 0.297) compared to those with ≥4 visits. This aligns with findings from (106) in Tigray of northern Ethiopia, where conflict led to a drop in ANC coverage and a rise in maternal mortality.

Behavioral factors identified in this study, such as early age at first sexual intercourse (15-24 years: 77.9%), low condom use (8.0%), and high rates of reported sexual violence (13.7%), reflect significant risk behaviors and gender-based vulnerabilities for the co-infection. These findings are consistent with previous research in Ethiopia and other conflict-affected settings, where early sexual debut and low condom use are common and contribute to the spread of STIs (107). The reported displacement of over 40% of participants due to conflict further compounds vulnerability, as displacement disrupts social networks and access to healthcare, increasing exposure to risky sexual behaviors and violence.

5.1.2. Predictors of HIV/Syphilis Co-infection

The Firth penalized logistic regression model identified several significant predictors of HIV/Syphilis co-infection. Lower educational attainment (primary or no formal education) was associated with higher odds of co-infection, with primary education (AOR=1.7, 95% CI: 1.32–2.19, p=0.021) and no formal education (AOR=1.5, 95% CI: 1.19–1.90, p=0.014), supporting the evidence that education enhances knowledge and adoption of preventive behaviors against STIs(76). Interestingly, higher household income was also linked to increased co-infection risk (AOR=2.1, 95% CI: 1.45–3.05, p=0.003), which may appear counterintuitive but could reflect complex social dynamics, such as increased mobility or engagement in transactional sex in certain income groups, as noted in some African contexts (Masha et al., 2017; Wondimu et al., 2021).

Behavioral predictors, including Early sexual debut (<25 years) (AOR=1.6, 95% CI: 1.12–2.28, p=0.003), history of previous sexual partners (AOR=1.4, 95% CI: 1.04–1.88, p=0.045), and non-use of condoms (AOR=1.9, 95% CI: 1.16–3.10, p=0.021) were all significant predictors, strongly influenced co-infection risk, consistent with global literature emphasizing these as key drivers of HIV and syphilis transmission (108,109). The association of displacement (AOR=1.6, 95% CI: 1.10–2.32, p=0.043) and **experience of sexual violence** (AOR=2.2, 95% CI: 1.27–3.81, p=0.001) with co-infection highlights the intersection of conflict-related vulnerabilities and STI

risk, echoing findings from humanitarian settings where conflict exacerbates exposure to sexual exploitation and limits access to protective services (Vu et al., 2014; Spiegel et al., 2010).

Frequent alcohol consumption showed a dose-response relationship (occasional: AOR=1.7, p=0.031; most frequent: AOR=2.1, p=0.004), another significant predictor, aligning with studies that link substance use to risky sexual behaviors and reduced condom negotiation, thereby increasing STI risk(110,111). The Firth penalized logistic regression model provided a better fit (AIC=132.76, BIC=196.86) than the conventional model, which is consistent with recommendations for rare event data analysis(112). The model explained approximately 78% of the variation in co-infection (McFadden's pseudo *R-squared=0.78*), indicating strong explanatory power.

6. Study Limitations

6.1 Strength of the study

This study's strength lies in its timely focus on a conflict-affected region, addressing a critical gap in HIV/syphilis co-infection data among pregnant women in Tigray. The use of Firth penalized logistic regression enhanced the reliability of estimates in the context of rare events and a small sample size. The two-stage stratified sampling ensured representativeness across sub-cities, while combining clinical records with interview-administered questionnaires improved data accuracy. Additionally, the inclusion of diverse predictors—ranging from socio-demographic to conflict-related factors—provided a comprehensive understanding of the co-infection risk landscape.

6.2 Limitation of the study

Though the study provides valuable insights, it is not free of limitations in that it is cross-sectional design, which may preclude causal inference, and may also be exposed to potential reporting bias in sensitive behavioral variables such as history of drinking alcohol, Khat usage, condom experience, etc. The conflict context may also limit generalizability to more stable settings.

7. Conclusion

This study revealed a 3.7% sero-prevalence of HIV and syphilis co-infection among pregnant women attending ANC in Mekelle City, notably higher than national estimates, reflecting the impact of conflict-related vulnerabilities. Key predictors of co-infection included low educational attainment, early sexual debut, limited ANC visits, displacement, sexual violence, low condom use, and frequent alcohol consumption. The Firth penalized logistic regression model provided robust estimates, underscoring the need for targeted interventions addressing behavioral, socio-economic, and conflict-related risk factors to improve maternal health outcomes in crisis-affected regions like Tigray.

8. Recommendation

These findings emphasize the need for integrated, context-sensitive interventions in conflict-affected areas like Tigray. Strengthening ANC services to include routine screening for both HIV and syphilis, combined with behavioral counseling targeting early sexual initiation, condom promotion, and substance use reduction, is critical. Additionally, addressing structural factors such as education and economic empowerment, alongside protection against sexual violence and support for displaced populations, should be prioritized (WHO, 2016; Spiegel et al., 2010). The use of Firth penalized logistic regression in this study provided robust estimates despite the rarity of co-infection events, demonstrating a methodological strength that can inform future research in similar low-prevalence, conflict-affected settings (Heinze & Schemper, 2002).

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Annex A: Information and consent form

A.1. Information

01. ***Name of health facility:*** _____

02. ***Health facility code:*** _____

03. ***Questionnaire Identification Number:*** _____

04. ***Date:*** _____

Introduction:

Greetings! My name is_____. I am here on behalf of **Senait Haddis Abebe** from *Department of Reproductive Health (RH)*, ***College of Health Sciences***, **Mekelle University** in the post graduate program to interview you for the study ***Prevalence and Predictors of HIV and Syphilis Co-infection*** in Mekelle, Tigray.

Purpose:

I am interviewing pregnant women under ANC aimed to study ***Prevalence and Predictors of HIV and Syphilis Co-infection*** in Mekelle,Tigray which is helpful to solve the problems detected and at the end hoped that the information you give me could help to strengthen or to design or to plan the appropriate intervention.

Confidentiality:

I would like to assure you that this privacy should be strictly secured throughout. All your information will be numbered; your name will be secured; not pass to a third person. Your answer to any of the questions will not be given to anyone else and no report of the study will ever identify you.

Risk/discomfort:

By participating in this study, you may feel that it has some discomfort especially on wasting your time to respond this self-administration questionnaire but this may not be too much as you are one of the members of the communities; your response will help as important input to determine the prevalence of HIV & Syphilis co-dynamics and associated factors among pregnant women under ANC. However, there is no physical or psychological risk expected being involved in the study.

A.2. Consent to participate in the study

Participation: You have to know that your participation is largely based on your willingness and approval. There are questionnaires to be answered by you. You are expected to answer all of the questions but you have the right to say **"No"** and/or **"Not participate"** in the study (you can choose **"No"** to respond to some or all of the questions). You have also a full right to withdrawal from this study at any time you wish without losing any of your right and without any penalty.

- **Address the principal investigator:** *Senait Haddis Abebe*
- **Cell Phone:** *+251913626023*
- **E-mail:** haddissenait@gmail.com

Now, is it clear? Are you willing to participate in responding to the questions in this study, please?

- **Yes [ ]** ⇒Continue to the next page **2**
- **No [ ] Thank You!**

- Name of the data collector_____ Signature_____ Date_____
- Name of the supervisor_____ Signature_____ Date_____

Annex B: Questionnaire

Introduction:

HIV and syphilis are significant health concerns for pregnant women, especially in low-resource settings like Ethiopia. Co-infection can lead to severe health risks, including maternal complications and increased risk of mother-to-child transmission. Identifying predictors of HIV, syphilis, and their co-infection among pregnant women in antenatal care (ANC) is essential for targeted screening and preventive measures. This study aims to assess socioeconomic, behavioral, pregnancy-related, and maternal health factors associated with infection risk among ANC attendees in Mekelle, Tigray. This study is committed to maintaining strict confidentiality and ethical standards. All personal information collected from participants will be anonymized, and data will be securely stored to prevent unauthorized access. Participation is voluntary, and participants can withdraw at any time without any impact on their ANC services. The findings will be reported in aggregate form, ensuring no individual is identifiable. Ethical approval has been obtained from the relevant institutional review boards, and informed consent will be obtained from each participant prior to data collection.

Section 1: Socioeconomic & Demographic Factors

1.1.What is your age?_____ (in year)

1.2.At what age did you get married for the first time?_____(in year)

1.3.What is your highest level of education?

□ No formal education

□ Primary school

□ Secondary school

□ College or higher

1.4.What is your marital status?

□ Single

□ **Married**

□ **Divorced**

□ **Widowed**

1.5.What is your average household monthly income in Birr?_____

Section 2: Behavioral & Life Habits

2.1.How often have you used condoms in the past one year?

□ **Always**

□ **Sometimes**

□ **Never**

2.2.How many sexual partners have you had in the past one year?_____

2.3.How often have you consumed alcohol in the past one year?

□ **Daily**

□ **Weekly**

□ **Monthly**

□ **Rarely**

□ **Never**

2.4.How often have you used substances (e.g., tobacco, khat, etc.) in the past one year?

S/No.	Types of Substances	Daily	Weekly	Monthly	Rarely	Never
1.	Tobacco					
2.	Khat					

2.5.How often have you had an STI diagnosis or symptoms in the past one year?

□ **Never**

□ **Once**

□ **More than once**

Section 3: Pregnancy-Related Factors

3.1.How many times have you been pregnant (Gravidity)?_____

3.2.How many children have you given birth to (Parity)?_____

3.3.Have you experienced any pregnancy complications?

□ Yes
□ No

3.4.How many antenatal care (ANC) visits have you had during this pregnancy?_____

3.5. What type of delivery are you planning?

□Natural/Vaginal
□C-Section
□Not sure

Section 4: Maternal Characteristics

4.1. What is your current BMI (Body Mass Index)?

□Underweight
□Normal
□Overweight
□Obese

4.2. How would you describe your nutritional status?

□Poor
□Fair
□Good
□Excellent

4.3. Have you ever been diagnosed with any of the following health conditions?

S/No.	Types of Substances	Yes	No
1.	TB		
2.	DM		
3.	Hypertension		
4.	Other		

4.4. Are you up-to-date with vaccinations recommended during pregnancy?

☐ **Yes**
☐ **No**

Section 5: Conflict-Related Factors

5.1 Have you experienced displacement due to the conflict?

☐ **Yes**
☐ **No**

5.2 Have you ever experienced sexual violence?

☐ **Yes**
☐ **No**

5.3 Do you live in a crowded environment, such as an IDP camp ?

☐ **Yes**
☐ **No**

5.4 Do you have access to clean water and sanitation facilities?

☐ **Yes**
☐ **No**

5.5 How far is the nearest healthcare facility from your home?

☐ **Less than 5 km**
☐ **5–10 km**
☐ **More than 10 km**

5.6 Have you faced any of the following barriers to accessing ANC services? (Check all that apply)

☐ **Distance to facility**
☐ **Cost of transportation**
☐ **Fear of stigma or discrimination**
☐ **Lack of awareness about ANC services**
☐ **Other (Specify: _____)**

Section 6: Outcome Variables (*To be completed from Pregnant Women's Book Registration*)

6.1 Have you been tested for HIV during this pregnancy?

☐ **Yes**

□ **No**

6.2 Have you been tested for syphilis during this pregnancy?

□ **Yes**
□ **No**

6.3 If yes, please indicate your test results (confidential and optional)

HIV Status: [ ] Positive [ ] Negative
Syphilis Status: [ ] Positive [ ] Negative
Co-infection (if applicable): [ ] Positive [ ] Negative