Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa

By

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DISSERTATION

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2026

DECLARATION

I, Kay-Lee Elrechia Strauss, hereby declare that the dissertation titled, "**Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa**" submitted to the University of South Africa for the Degree of Master of Science in Life Sciences (Physiology) has not been submitted by me or any other person for any degree at this or any other University, and that it is my own work in design and in execution, and that all material cited herein has been duly acknowledged.

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DEDICATIONS

This study is dedicated to my parents, Elrika Jolene and Stephen Emanual Malgas, for your unwavering support, love, and encouragement, and for raising me in the way of the Lord and Saviour, Jesus Christ. I also dedicate this study to my siblings, Emico Airlia and Giana Emi-Lee Malgas, as well as my late grandmother, Rachel Susanna Strauss, whom I lost during the course of this degree. I know this accomplishment would have made you very proud. Thank you for all your love, and I miss you dearly. Finally, I would like to thank Realeboha Malebo for always supporting and encouraging me.

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Their mentorship has been instrumental in fostering my academic growth and motivating my broader career aspirations, and I am profoundly grateful for their support beyond the confines of this study. The opportunity to learn under their guidance has been an honour, and I am sincerely appreciative of their dedication to my success.

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Sepedi Abstract

Boemo

Basadi ba baimana bao ba phelago le HIV ba lebane le kotsi ye kgolo ya mathata a a amanago le boimana, go swana le pre-eclampsia (PE), ye e lego bolwetši bja kgatelelo ya madi bjo bo amago mekgwa ye mentši ya mmelo bjo bo akaretšago go senyega ga methapo ya madi, go ruruga ga mmele ka kakaretšo, le go senyega ga ditho. Tšoaetšo ya HIV le kalafo ya di-antiretroviral (ART) ka bobedi di amahanngwa le go senyegega ga sebete; le ge go le bjalo, khuetšo ya tšona ye e kopanego godimo ga mošomo wa sebete sa bomme maemong a pre-eclampsia ga sešo sa hlaloswa gabotse, kudu dinageng tšeo HIV e atilego kudu.

Maikemišetšo

Maikemišetšo a nyakišišo ye e be e le go hlahloba ditlamorago tša tšoaetšo ya HIV le kalafo ya ART godimo ga mošomo wa sebete sa bomme le go rurušega nakong ya boimana, ka go lebanya kudu le khuetšo ya pre-eclampsia.

Mokgwa

Go dirišitšwe mokgwa wa mixed-methods, wo o kopantšego ditlhahlobo tša tsamaiso (systematic reviews) le meta-analyses le dithuto tše mmalwa tša di-cohort. Ditlhahlobo tša tsamaiso le meta-analyses di lekotše khuetšo ya pre-eclampsia, tšoaetšo ya HIV le go pepentšhwa go ART godimo ga di-biomarker tša sebete, e lego aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), le bilirubin ya kakaretšo ya serum. Dithuto tša kliniki di akaretša di-cohort tše mmalwa tšeo di nyakišišitšego go rurušega le mošomo wa sebete go basadi ba baimana bao ba arotšwego go ya ka maemo a PE le HIV. Maemo a plasma a AST, ALT, placental alkaline phosphatase (PLAP), le C-reactive protein (CRP) a lekantšwe le go bapetšwa magareng ga dihlopha go lekola go senyega ga sebete, go tsenela ga placenta, le go rurušega ga mmele ka kakaretšo.

Dipoelo

Dipoelo tša meta-analysis di bontšhitše kgokagano ye kgolo magareng ga pre-eclampsia le koketšego ya di-enzyme tša sebete sa bomme, e lego sešupo sa go senyega ga disele tša sebete. Tšoaetšo ya HIV le go šomišwa ga ART ka bonngwe di amantšwe le maemo a godimo a AST le ALT ge di bapetšwa le basadi bao ba se nago HIV. Ditemogo tša kliniki di netefaditše dipoelo tše, ka go bontšha koketšego ye kgolo ya AST le ALT go basadi bao ba nago le pre-eclampsia, ka maemo a godimo kudu go bao ba nago le tšoaetšo ya HIV le pre-eclampsia ka nako e tee. Maemo a PLAP a fetogile kudu go PE, kudu go basadi ba HIV-positive, se se bontšhago go se šome gabotse ga placenta le tswalano ya placenta le sebete. Maemo a CRP a be a phagame go PE gomme a oketša le go tia ga bolwetši, go basadi ba nang leHIV-

positive ba bontšha maemo a godimo a go rurušega go tšwa mathomong. Go ba gona ka nako e tee ga HIV le PE go lebile go morwalo wo mogolo kudu wa go rurušega.

Mafetšo

Mošomo wo o tšweleditšwego mo porojekeng ye o bontšha gore go se šome gabotse ga sebete go tšwa go tswalano ya tšoaetšo ya HIV, go šomitšwa ga ART, le boimana, gomme go ka amana gape le pre-eclampsia. Dipoelo di bontšha gore diphetogo tša sebete go basadi ba baimana bao ba phelago ka twatši ya HIV di ka se hlaloswe ka HIV, ART goba PE fela, eupša di tšwa go ditlamorago tša tšona tše di kopanego. Ka fao, go tokafatša go hlokomelwa ga mošomo wa sebete le di-marker tša go rurušega go boimana bjo bo lego kotsing go ka thuša go lemoga ka pela go tsenela ga sebete le go lekola kotsi ya bomme, kudu dinageng tšeo HIV e atilego kudu go swana le Afrika Borwa.

Abstract

Background: Pregnant women living with HIV (PWLWHIV) are at increased risk of adverse pregnancy outcomes, including pre-eclampsia (PE), a multisystem hypertensive disorder characterised by endothelial dysfunction, systemic inflammation, and organ damage. Both HIV infection and antiretroviral therapy (ART) are associated with hepatic impairment; their synergistic impact on maternal liver function in the context of pre-eclampsia remains unclear, especially in HIV-endemic regions.

Aim: This study aimed to evaluate the effects of HIV infection and ART exposure on maternal liver function and systemic inflammation during pregnancy, with particular emphasis on the modifying role of pre-eclampsia.

Method: A mixed-methods approach was utilised, combining systematic reviews and meta-analyses with observational cohort studies. Systematic reviews and meta-analyses assessed the impact of PE, HIV infection, and ART exposure on hepatic biomarkers, particularly aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total serum bilirubin. The clinical component included cohorts stratified by PE and HIV status to evaluate inflammatory markers and liver function parameters. Plasma levels of AST, ALT, placental alkaline phosphatase (PLAP), and C-reactive protein (CRP) were measured and compared among groups to evaluate hepatic impairment, placental involvement, and systemic inflammation.

Results: Meta-analytic data revealed a substantial association between PE and increased maternal liver enzymes, indicative of hepatocellular damage. HIV infection and ART exposure were independently associated with elevated AST and ALT levels in comparison to HIV-negative controls. The clinical observations validated these results, demonstrating markedly elevated AST and ALT levels in pre-eclamptic women, with the most pronounced increases noted in those with concurrent HIV infection and pre-eclampsia. PLAP levels were significantly altered in PE, especially in HIV-positive women, indicating placental dysfunction and potential placental-hepatic interplay. CRP concentrations were elevated in PE and further rose with illness severity, with HIV-positive women displaying greater baseline inflammatory levels. The simultaneous presence of HIV and PE led to the greatest inflammatory burden.

Conclusion: These findings demonstrate that liver dysfunction during pregnancy is influenced by the complex interplay between HIV infection, ART exposure, and pre-eclampsia. The results indicate that hepatic abnormalities in PWLWHIV cannot be solely attributed to HIV, ART, or PE, but rather to their combined effects. Therefore, an improved monitoring of liver function and inflammatory markers in high-risk pregnancies may facilitate early identification

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DEFINITION OF TERMS

Pre-eclampsia is defined as gestational hypertension associated with new-onset maternal uteroplacental dysfunction at or after 20 weeks' gestation [1].

Human Immunodeficiency Virus is a virus that infects CD4+ T lymphocytes, leading to a weakened immune system in individuals [2].

Antiretroviral Therapy is a combination of HIV medicines that suppresses the HI-virus, prevents its progression, and reduces the risk of transmission [3].

Liver Function Tests are broadly defined as biochemical tests used to evaluate hepatic dysfunction and disease [4].

Aspartate Aminotransferase plays a key role in the metabolism of amino acids, maintenance of NAD+/NADH ratio in cells, Krebs cycle activity, synthesis of purin/pyrimidine bases, urea and protein synthesis and gluconeogenesis [5].

Alkaline Phosphatase is a membrane-associated enzyme that catalyzes the release of phosphate via cleavage of the phosphate ester bond [6].

Alanine Transaminase is an enzyme that facilitates the interaction between L-alanine and α-ketoglutarate, producing pyruvate and L-glutamate [7].

C-Reactive Protein is an acute inflammatory protein that increases up to 1000-fold at sites of infection or inflammation [8].

Liver Dysfunction is defined as a condition resulting from diseases that cause progressive destruction and regeneration of liver parenchyma, leading to fibrosis, disruption of vascular architecture, and cirrhosis over time [9].

Inflammation is a defense mechanism designed to eradicate microbes or irritants, thus protecting living tissues from infection injuries and enhancing tissue repair [10].

ABBREVIATIONS

ACE: Angiotensin-Converting Enzyme

AIDS: Acquired Immune Deficiency Syndrome

ALP: Alkaline Phosphatase

ALT: Alanine Aminotransferase

ART: Antiretroviral Therapy

AST: Aspartate Aminotransferase

ATP: Adenosine Triphosphate

CI: Confidence Interval

CRP: C-reactive Protein

CVD: Cardiovascular Disease

DBP: Diastolic Blood Pressure

DNA: Deoxyribose Nucleic Acid

DTG: Dolutegravir

ET-1: Endothelin-1

gp120: Glycoprotein 120

GST: Glutathione S-Transferase

GWAS: Genome-Wise Association Study

HAART: Highly Active Antiretroviral Therapy

HBV: Hepatitis B Virus

HCV: Hepatitis C Virus

HELLP: Hemolysis, Elevated Liver Enzymes, Low Platelets

HIV: Human Immunodeficiency Virus

I-FABP: Intestinal Fatty Acid-binding Protein

IL-12p70: Interleukin 12-Protein

IL-1β: Interleukin-1 beta

IL-6: Interleukin-6

ILWHIV: Individuals Living with HIV

INSTI: Integrase Strand Transfer Inhibitor

IP-10: Interferon Gamma-Induced Protein 10

IRIS: Immune Reconstitution Inflammatory Syndrome

LEE: Elevated Liver Enzyme

MeSH: Medical Subject Headings

MOOSE: Meta-analysis of Observational Studies in Epidemiological

MTCT: Mother-to-child transmission

NAFLD: Non-alcoholic Fatty Liver Disease

NASH: Non-alcoholic Steatohepatitis

NNRTI: Non-nucleoside Reverse Transcriptase Inhibitor

NO: Nitric Oxide

NOS: Newcastle-Ottawa Scale

NP: Normotensive Pregnant

NPWLWHIV: Non-Pregnant Women living with HIV

NR: Not Reported

NRTI: Nucleoside Reverse Transcriptase Inhibitor

NtTRI: Nucleotide Reverse Transcriptase Inhibitor

NVP: Nevirapine

PE: Pre-eclampsia

PECOS: Population, Exposure, Comparison, Outcome, Study Design

PGF: Placental Growth Factor

PI: Protease Inhibitor

PICOS: Population, Intervention, Control, Outcome, Study Design

PLHIV: People Living with HIV

PrEP: Pre-exposed Prophylaxis

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analysis

PWLWHIV: Pregnant Women Living With HIV

RIS: Reconstruction Inflammatory Syndrome

ROS: Reactive Oxygen Species

SBP: Systolic Blood Pressure

SD: Standard Deviation

SEM: Standard Error Mean

sFLT-1: fns-like Tyrosine Kinase-1

SMD: Standardised Mean Difference

SOD: Superoxide Dismutase

SSA: Sub-Saharan Africa

TNF-α: Tumour Necrosis Factor-alpha

TSB: Total Serum Bilirubin

VEGF: Vascular Endothelial Growth Factor

VEGFA: Vascular Endothelial Growth Factor A

WHO: World Health Organisation

LIST OF ARTICLES PUBLISHED IN DHET-ACREDITED JOURNALS

1. **Strauss, K.-L. E.,** Phoswa, W. N., & Mokgalaboni, K. The Impact of Antiretroviral Therapy on Liver Function Among Pregnant Women Living with HIV in Co-Existence with and Without Pre-Eclampsia. Viruses, 2025, 17 (1), 28. https://doi.org/10.3390/v17010028

2. **Strauss, K.-L. E**., Phoswa, W. N., Hanser, S., & Mokgalaboni, K. HIV Infection and Antiretroviral Therapy Impair Liver Function in People Living with HIV: Systematic Review and Meta-Analysis. Pharmaceuticals, 2025, 18 (7), 955. https://doi.org/10.3390/ph18070955

3. **Strauss, K.-L.E**.; Phoswa, W.N.; Mokgalaboni, K. Pre-Eclampsia-Induced Maternal Liver Dysfunction: Systematic Review, Meta-Analysis and Meta-Regression of Observation Studies. Life, 2025, 16 (2), 223. https://doi.org/10.3390/life16020223

CONFERENCE PRESENTATION

1. **UNISA Research & Innovation Postgraduate Student Showcase**

Endothelial dysfunction, a predictor of cardiovascular disease in HIV patients on antiretroviral therapy: A systematic review and meta-analysis. 25 – 29 November 2024

2. **UNISA North Eastern Region 14th Annual Postgraduate Student Conference**

Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa. 1 – 3 October 2025

AWARDS

1. Senate Award for Best Performance in an Honours Degree 2024, College of Natural and Agricultural Science
2. First Runner-up Oral Presentation, UNISA Research & Innovation Postgraduate Student Showcase

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Organization of the Dissertation

Chapter 1: Introduction

Summarises the introduction and problem statement, purpose, objectives, and importance of the study.

Chapter 2: Literature review

Highlights the literature in this study and which is divided into three subsections:

Chapter 2.1: Systemic Review: The Impact of Antiretroviral Therapy on Liver Function Among Pregnant Women Living with HIV in Co-Existence with and without Pre-Eclampsia.

The manuscript reviewed evidence on the pathogenesis of PE and the impact of ART on liver function in PWLWHIV with and without PE, aligning with the dissertation title. This section is presented in the Viruses journal formatting style used in a published version.

Chapter 2.2: Systematic Review and Meta-analysis: Pre-eclampsia-Induced Maternal Liver Dysfunction: Meta-Analysis of Observation Studies

Chapter 2.3: Systematic Review and Meta-analysis: HIV Infection and Antiretroviral Therapy Impair Liver Function in People Living with HIV: Systematic Review and Meta-Analysis.

This chapter aligns with the research theme, expanding the population beyond pregnant women to include males and non-pregnant women. This systematic review and meta-analysis examined the effects of HIV infection and ART on liver function in people living with HIV (PLHIV). The results showed an increase in AST and ALT in naïve PLWH compared to HIV-negative individuals. Elevated AST, ALT, and ALP were also observed in ART-exposed PLWH compared to HIV-negative individuals. However, no significant difference was found in ALP between ART-naïve and HIV-negative individuals. The study highlights the dual risk posed by HIV infection and ART exposure on liver function.

Chapter 3: Study Results

Highlights the results in this study, which are divided into four subsections.

Chapter 3.1: Manuscript 1

Aspartate Aminotransferase in Pre-eclamptic and Normotensive Pregnancies with and without HIV

Chapter 3.2: Manuscript 2

Alanine Aminotransferase Levels in HIV-Positive and HIV-Negative Pregnant Women with and Without Pre-Eclampsia

Chapter 3.3: Manuscript 3

Placental Alkaline Phosphatase (PLAP) Levels in HIV-Positive and HIV-Negative Pregnant Women with and Without Pre-Eclampsia

Chapter 3.4: Manuscript 4

The Interaction Between HIV Infection and Pre-Eclampsia on Maternal Systemic Inflammation: Insights From C-Reactive Protein Levels

Chapter 4: Synthesis of Findings

Chapter 5: Limitation, Conclusion and Recommendation

Chapter 6: Appendices

CHAPTER 1: INTRODUCTION

1.1. Introduction

South Africa has a high-burden Human Immunodeficiency Virus (HIV) epidemic, with women, especially those of reproductive age, being disproportionately impacted [11]. To mitigate the effects of HIV and prevent mother-to-child transmission (MTCT), antiretroviral therapy (ART) is readily provided to pregnant women living with HIV [12]. Since the inception of the South African national ART program in 2004, access to ART has significantly increased. It currently covers over 95% of pregnant women, substantially reducing mother-to-child transmission of HIV [13]. Although ART has markedly diminished HIV-related morbidity and mortality, its prolonged impact on organ function, especially the liver, continues to be a concern [14]. Considering this significant prevalence of HIV in South Africa and the extensive administration of ART to pregnant women, it is essential to comprehend the possible detrimental impacts of these treatments on liver function to enhance maternal and foetal health outcomes.

The liver is essential for drug metabolism [15], and ART, particularly certain classes such as protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), has been associated with hepatotoxicity [16]. The potential hepatotoxic consequences of ART in pregnant women remain concerning, particularly in the presence of pre-eclampsia, which independently affects liver enzyme levels and overall hepatic function [17]. Previous findings have demonstrated that, despite ART exposure, people living with HIV (PLHIV) remain at risk of endothelial dysfunction, increasing their susceptibility to cardiovascular disease [18]. Furthermore, both HIV infection and ART have been independently associated with hepatic complications, evidenced by significantly elevated liver enzyme levels, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in people living with HIV (PLWH) [19]. The use of ART during pregnancy has been linked to several maternal and foetal adverse effects, including hypertension in pregnancy, referred to as pre-eclampsia (PE), gestational hypertension, gestational diabetes, foetal growth restriction, low birth weight, and numerous other disorders [20]. It remains unclear whether pregnancy-induced PE is triggered by the virus itself or by the effects of antiretroviral medication. Although other researchers have reported elevated liver enzymes in PE compared with normotensive individuals [21–23], recent evidence has challenged this finding, showing decreased AST and ALT in PE [24]. This thus makes it difficult to understand the contribution of PE to maternal liver dysfunction. PE is associated with systemic endothelial dysfunction, oxidative stress, and inflammatory responses, all of which may exacerbate liver failure [25]. The onset of PE correlates with a shift from an anti-inflammatory (Th2) to a pro-inflammatory

(Th1) immunological response, characterised by increased concentrations of inflammatory cytokines such as Interleukin-1 (IL-2), Interleukin-6 (IL-6), Interleukin-12 (IL-12), Interferon-gamma (IFN-γ), and Tumour Necrosis Factor-alpha (TNF-α) [26,27]. Maternal comorbidities, including chronic renal disease, hypertension, and obesity, elevate the risk of PE development [28]. These physiological alterations lead to vascular injury, compromised placental perfusion, and multi-organ consequences, including hepatic dysfunction [29]. In extreme instances, PE may advance to HELLP (Haemolysis, Elevated Liver Enzymes, Low Platelets) syndrome, which intensifies liver failure and heightens maternal and foetal morbidity and mortality [30]. Hepatic involvement in PE is clinically significant, as impaired hepatic function may result in elevated liver enzymes, hepatocellular injury, and, in severe cases, hepatic rupture or infarction.

Hepatic function is frequently assessed using various biochemical markers that indicate liver injury or dysfunction. Common biomarkers include bilirubin, ALT, AST, ALP, and the aminotransferase ratio, all of which yield essential insights into the liver's capacity to metabolise and eliminate drugs [31]. An increase in these biomarker levels suggests impaired liver function [32]. Another important marker of liver function and systemic inflammation is C-reactive protein (CRP). The liver produces CRP in response to infection, injury, or inflammation, and elevated levels are widely used as an indicator of systemic inflammation [33]. PLHIV frequently exhibit elevated CRP levels, an indicator of systemic inflammation driven by persistent viral replication [34]. A study by Drain et al. (2007) found that high maternal CRP concentrations were linked to maternal disease progression, mother-to-child transmission, and maternal and child mortality [35]. Chronic immunological activation and hepatic inflammation may accelerate liver fibrosis and elevate the risk of liver-related morbidity and mortality. Thus, monitoring these indicators in PLHIV is crucial for the early identification of liver impairment and enhancing therapeutic care techniques to reduce negative outcomes.

Inflammation plays a central role in the pathogenesis of various diseases, including hypertensive disorders of pregnancy and HIV infection, where immunological dysregulation leads to detrimental maternal and foetal outcomes [36]. CRP has conventionally served as a biomarker for systemic inflammation, owing to its sensitivity in identifying both acute and chronic inflammatory conditions [37]. Cellular markers, including neutrophils, lymphocytes, and the neutrophil-to-lymphocyte ratio (NLR), have recently attracted interest as supplementary indicators of immunological activation and inflammatory burden [38]. These haematological indices are cost-effective and easily obtainable from standard blood counts, offering insight into the balance between innate and adaptive immune responses. When assessed in conjunction with CRP, they provide a more thorough evaluation of inflammatory status, especially in intricate circumstances like pregnancy complicated by HIV and PE.

Biomarkers such as ALT, AST, and ALP provide significant insights into liver function, while CRP offers a picture of inflammation among PLHIV, particularly pregnant women with PE. Elevated levels of these indicators suggest hepatic stress and potential metabolic dysfunction, which may be exacerbated by exposure to ART. By integrating these biomarkers, this study seeks to comprehensively evaluate liver function in pregnant women living with HIV, with and without PE.

1.2. Problem Statement

Sub-Saharan Africa bears the greatest burden of HIV globally, and the infection remains a major public health concern [39]. The management of HIV in pregnant women in South Africa, where HIV prevalence is among the highest in the world, poses particular challenges, especially with regard to the safety and effectiveness of ART [40]. Although ART has greatly lowered HIV-related morbidity and mortality, questions have been raised about its possible hepatotoxicity, particularly in at-risk groups like pregnant women. Pregnancy-related liver dysfunction, aggravated by PE and other disorders, makes the clinical management of HIV-positive women increasingly complex [17,41].

PE remains a major cause of maternal morbidity and mortality. It is a hypertensive pregnancy disorder marked by multi-organ involvement, including liver dysfunction [42]. HIV, ART, and PE may interact synergistically, raising concerns about their combined effects on liver function. While studies have looked at the differential effects of ART in HIV-infected pregnant women with and without PE, most of the current research points to possible hepatotoxicity from some ART regimens [43,44]. While the presence of PE may aggravate ART-induced liver dysfunction, the degree and clinical relevance of this interaction are still not well characterised. Though ART is commonly used during pregnancy, there is little research on how various ART regimens affect liver function in pregnant women with PE relative to those without the condition [45]. Given that liver dysfunction can cause major problems like hepatic failure, preterm birth, and higher maternal and neonatal mortality, this knowledge gap has important consequences for maternal and foetal health [46,47]. The lack of thorough studies evaluating the combined effects of ART and PE on liver function hinders healthcare professionals' capacity to optimise treatment plans for this high-risk group. Given the urgent need for evidence-based guidance, this study aims to evaluate and compare the effects of ART on liver function and inflammatory markers among HIV-infected pregnant women with and without PE in South Africa. This study will offer important insights into the safety of ART in pregnancy by means of liver enzyme profiles, indicators of hepatic damage, and possible risk factors. The findings may contribute

to improved clinical management strategies and ultimately support better maternal and neonatal outcomes in South Africa's HIV-positive population.

1.3. Aim of the Study

This study aims to investigate the potential impact of ART on liver function and systemic inflammation in HIV-infected pregnant women with preeclampsia in South Africa.

1.4. Research Objectives:

a) To conduct a systematic review and meta-analysis to evaluate the effect of pre-eclampsia on maternal liver function in pregnant women.
b) To compare, through meta-analysis, liver function test abnormalities among pregnant women with HIV infection receiving ART and those not receiving ART.
c) To conduct a systematic review exploring the effect of ART on liver function in HIV pregnant women with HIV and preeclampsia.
d) To compare the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), C-reactive protein (CRP), and the AST/ALT ratio between HIV-positive pregnant women with pre-eclampsia receiving ART and normotensive pregnant women in South Africa.
e) To compare systemic inflammation, as measured by C-reactive protein (CRP) levels, among pregnant women with pre-eclampsia, pregnant women with HIV infection, pregnant women with both pre-eclampsia and HIV infection, and normotensive pregnant controls in South Africa.

1.5. Research Question:

What is the impact of ART on liver function and inflammation in HIV-positive pregnant women with pre-eclampsia versus those without pre-eclampsia in South Africa?

1.6. Research Hypotheses:

ART is associated with increased maternal levels of ALT, AST, ALP, and CRP in pregnant women with HIV and pre-eclampsia compared to normotensive pregnant women.

Pregnant women with PE, HIV infection, or both exhibit markedly increased systemic inflammation, as seen by raised CRP levels, in comparison to normotensive pregnant controls.

1.7. Significance of the study

The research examines the impact of HIV, ART, and PE on liver function during gestation in South Africa. It elucidates the interactions among these conditions, which may enhance risk assessment, optimise ART regimens, and improve liver function outcomes in pregnant women. The findings may improve maternal health outcomes, reduce the risk of adverse birth outcomes, and enhance the safety of therapeutic interventions. The study integrates clinical pharmacology, obstetrics, and infectious disease, aligning with contemporary research objectives. Connecting clinical information with public health initiatives, enhancing care for vulnerable populations, and educating health researchers to improve HIV and maternal health outcomes in real-world settings are essential.

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Prologue

The next chapter provides a critical review of current evidence on liver function in individuals living with HIV, pregnant women with pre-eclampsia (PE), and pregnant women without PE. It also examines the role of antiretroviral therapy (ART) and the contribution of HIV to liver dysfunction.

We conducted a systematic review and meta-analysis to synthesize evidence on the effects of HIV, ART, and PE on liver function. Our first systematic review and meta-analysis (published) outline the contribution of PE to maternal liver dysfunction, independent of HIV status or ART exposure. This work provided the foundation for a subsequent systematic review, which examined the effect of PE and ART on liver function in pregnant women with HIV, with or without preeclampsia (accepted, pending APC payment). This was followed by a systematic review and meta-analysis that evaluated the effect of HIV and ART on liver function in the HIV population (published). Our findings showed that HIV, ART, and PE contribute to liver dysfunction irrespective of the HIV, ART or PE.

CHAPTER 2: LITERATURE REVIEW

Chapter 2.1: Systematic Review

This section is presented in the form of a published systematic review titled **"**The Impact of Antiretroviral Therapy on Liver Function Among Pregnant Women Living with HIV in Co-Existence with and without Pre-Eclampsia".

This manuscript is published in a DHET-accredited journal, "Viruses" with the following link:

https://doi.org/10.3390/v17010028

The manuscript reviewed the evidence aimed to examine the pathogenesis of pre-eclampsia and the impact of antiretroviral therapy on liver function in pregnant women living with HIV with and without preeclampsia, which is in alignment with the title of the dissertation. The review presented in its Viruses formatting style used in a published version.

Student Contributions: methodology, investigation, writing – original draft preparation, writing – review and editing, visualization.

viruses

MDPI

Review

The Impact of Antiretroviral Therapy on Liver Function Among Pregnant Women Living with HIV in Co-Existence with and Without Pre-Eclampsia

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Abstract: Pregnant women living with HIV (PWLWHIV) are at an increased risk of developing obstetrics complications such as pre-eclampsia (PE). Antiretroviral therapy (ART) remains the standard treatment for PWLWHIV and non-pregnant women. However, its use has been associated with adverse liver conditions, particularly hepatotoxicity, often marked by elevated liver enzymes (LEEs) as demonstrated by an increased aspartate transferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in PWLWHIV on ART. Morever, there is limited evidence about the effect of ART on liver function among PWLWHIV and PE. Therefore, this review examines the pathogenesis of PE and the impact of ART on liver function in PWLWHIV with and without PE. With the evidence gathered in this review, it is still unclear whether liver dysfunctions in PWLWHIV in co-existence with orwithout PE result from HIV infection or ART administration or are exacerbated by the presence of PE. Among those without PE, there was an increase in liver enzymes, a decrease, and no effect in other studies in ART-treated PWLWHIV compared to the control group. Additionally, among those with PE, the impact of ART remains unclear due to contradicting results. The notable trend was that nevirapine was associated with a reduced risk of liver dysfunction among PWLWHIV without PE. Therefore, more studies are needed in this area, especially in HIV endemic regions, to understand the exact cause of liver dysfunction in this population. This knowledge is crucial for improving liver function and PE management among PWLWHIV.

Keywords: antiretroviral therapy; liver function; human immunodeficiency virus; pre-eclampsia; pregnancy

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1. Introduction

In 2020, the World Health Organisation (WHO) reported that about 800 women lost their lives daily due to avoidable factors associated with pregnancy and childbirth [1]. The primary factors responsible for about 75% of all maternal fatalities include excessive haemorrhage, postpartum infections, complicated delivery, botched abortion, pre-eclampsia (PE), and eclampsia [1]. One of the significant and potentially life-threatening pregnancy-related complications is PE, affecting approximately 2–10% of pregnancies worldwide [2]. Despite regional variations in PE rates, sub-Saharan Africa (SSA) reports an overall prevalence of 13%, above the global average [3]. In South Africa, the prevalence of PEis reportedly standing at 5.7% [4]. These rising rates warrant a need for enhanced maternal healthcare systems, especially in areas with higher prevalence rates, to reduce the complications associated with PE.

PE is a gestational condition characterised by proteinuria and hypertension, thrombocytopenia, and organ damage after 20 weeks of gestation [5]. While treatment options are available for pregnant women with hypertension, there is still no definitive cure for PE [6]. The early delivery of the faulty placenta and baby before the gestational period is frequently used as a method to remedy the issue [7]. This, however, can lead to severe complications for the foetus, including reduced birth weight, restricted foetal growth, and smaller for gestational age [8]. Moreover, inadequate blood flow and impaired placental function can result in intrauterine growth restriction, premature birth [9], and, in severe cases, stillbirth [10]. These factors contribute to chronic health complications for the child [11]. On the other hand, the maternal complications of PE can be severe, including prolonged hypertension, which subsequently leads to eclampsia; HELLP syndrome (haemolysis, elevated liver enzymes, and low platelet count); and organ damage [12]. Therefore, it is important that healthcare providers closely monitor and manage PE to prevent these severe complications for both the mother and the infant. Early detection and appropriate treatment of PE can prevent eclampsia and subsequent complications thereof.

The cause of PE is multifaceted [13]. Other researchers have suggested its pathogenesis may be associated with inadequate placentation due to an impaired immune system [14]. Moreover, genetic variations have also been reported as contributing factors that can impact different physiological pathways, including those involved in regulating blood pressure, endothelial function, and the inflammatory response [15]. The complex and multifaceted aetiology of PE makes it difficult to manage effectively. This becomes worse when the pregnant woman is also infected with HIV. A South African study conducted in 2013 revealed that 26.4% of women with PE were living with HIV when compared to 36.6% in the control group [16]. As most PWLWHIV rely on ART, it is worth noting that some of these ART regimens may interfere with uteroplacental blood flow, resulting in insufficient placental perfusion, a central feature of PE [17–19]. Tooke et al. 2016 showed that ART duration (4 weeks and above) is associated with the development of severe PE [20]. Notably, PE impairs liver function, resulting in elevated liver enzymes (LEE), such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). This elevation is modulated by endothelial dysfunction, which induces hepatic hypoxia and necrosis of hepatocytes [21,22]. Therefore, monitoring liver function frequently in PWLWHIV in co-existence with PE is crucial to improving the health of the foetus and mother during pregnancy. ART is associated with placental alterations, including increased placental oxidative stress and vascular resistance, which may predispose pregnant women to PE. This review examines the pathogenesis of PE and the effect of ART on liver function in PWLWHIV in co-existence with or without PE.

1.1. Inflammatory Response and the Pathogenesis of Pre-Eclampsia

The widespread inflammation and impaired endothelium function is a well-known characteristic of PE [23,24]. The inflammatory response is a crucial factor in the progression of the disease and has an impact on multiple organs, including the liver [25]. Immunological imbalances in mothers, especially related to innate immunity, play a role in initiating the immune response linked to the development of PE. Alterations in the mononuclear phagocyte system substantially impact the inflammatory response [26]. Due to the hypoxic nature of the placenta, pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), and anti-angiogenic factors, like soluble fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin, are released into the maternal bloodstream [27]. Oxidative stress, peripheral blood mononuclear cells, macrophages, and endothelial and vascular smooth muscle cells could cause sFLT-1 levels to rise in PE cases [28]. Research indicates that generalised endotheliosis in the systemic,

renal, cerebral, and hepatic circulation may reduce the production of vasodilators such as nitric oxide, prostacyclin, and the hyperpolarisation factor [29]. This decrease can cause vasoconstrictors like endothelin-1 (ET-1) and thromboxane A2 to increase, resulting in vasoconstriction and hypertension [30]

Moreover, the imbalance between vasodilators and vasoconstrictors can decrease blood flow to the placenta, which can then cause the release of sFLT-1 into the mother's bloodstream [31]. The excess sFLT-1 attaches to and hinders the action of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), intensifying the vasoconstriction and endothelial dysfunction observed in PE [31,32]. Overall, the intricate interaction of multiple elements results in the clinical symptoms of PE.

Due to its extensive blood supply and crucial role in the body's metabolism and immune response, the liver is vulnerable to inflammation and impaired function of the blood vessels lining the liver in PE [24]. PE causes systemic endothelial dysfunction, which affects the hepatic vasculature [33]. Inflammation-inducing cytokines and substances that inhibit the growth of blood vessels damage the cells that line the hepatic sinusoids, resulting in reduced blood flow and a lack of oxygen in liver tissues [34]. Oxidative stress caused by the hypoxic conditions in PE leads to liver cell damage [35]. Hepatocyte injury and death can occur due to the presence of reactive oxygen species (ROS) resulting from hypoxia and inflammation [36]. This leads to the release of liver enzymes, including ALT and AST, into the bloodstream [37]. Pro-inflammatory cytokines such as TNF-α can directly trigger the death of liver cells through apoptosis or necrosis [38]. The cellular damage causes liver enzymes to flow into the maternal bloodstream, indicating hepatic injury [39].

PE triggers an inflammatory response that causes widespread malfunction of endothelial cells throughout the body, impairing multiple organs, including the liver [18,40]. Specific ART regimens, especially those incorporating protease inhibitors (PIs), have been linked to metabolic and vascular alterations, such as endothelial injury, insulin resistance, and dyslipidaemia, risk factors for PE [41]. The increased activity of liver enzymes in PE is caused by damage to the liver's endothelial cells, oxidative stress, and a direct impact on liver cells caused by inflammation. Hence, understanding this relationship is essential for efficient control and management of PE and its associated complications.

1.2. Genetic Factors and the Pathogenesis of Pre-Eclampsia

Genetic predisposition is a significant factor in the onset of PE, and all the components associated with the development of PE have hereditary variables that may contribute to the pathological manifestations [42]. A study conducted by Bezerra et al. (2010) found that having a family history of hypertensive disorders increases the chance of developing eclampsia and HELLP syndrome [43]. Focusing on genetic aspects such as familial clustering, candidate genes, and genomic investigation is important when studying PE. Incorporating genetic factors into the study of PE is important to understand and monitor maternal and foetal health.

PE has familial patterns, indicating a genetic contribution to its aetiology [44]. A previous study found that maternal and paternal early-onset chronic hypertension and paternal early-onset myocardial infarction were independent risk factors for severe PE [45]. A positive family history of cardiovascular disorders before the age of 50 increased the risk of early-onset PE by 5.05-fold compared to the control group. The results suggest that familial early-onset cardiovascular disorders are a predisposing factor for severe PE [45]. Another study found that PE in daughters is associated with increased risks of cardiovascular disease (CVD) in parents [46]. The study showed that parents having one daughter with PE were 1.19 times more likely to develop CVD at age under 55 years. The study suggests that PE and CVD share common heritable mechanisms [46]. These findings

emphasise the significance of considering familial history when evaluating the likelihood of getting severe PE. Additional investigation into the common heritable processes underlying PE and CVD may result in novel understandings and viable therapies for these disorders.

Several genes have been studied for their potential involvement in the development of PE. These genes are associated with pathways linked to angiogenesis, immunological response, oxidative stress, and blood pressure regulation [47]. For example, the vascular endothelial growth factor A (VEGFA) gene encodes VEGFA, which is involved in angiogenesis [48]. Therefore, dysfunction in the VEGFA gene may contribute to the development of PE. Additionally, the expression of placental sFLT-1, an inhibitor of VEGF and placental growth factor, is raised in cases of PE [31]. This results in elevated levels of sFLT-1 in the bloodstream, which decreases after delivery. On the other hand, the angiotensin-converting enzyme (ACE) gene encodes an enzyme that controls blood pressure and electrolyte balance by converting angiotensin I into angiotensin II, which narrows blood vessels and stimulates aldosterone production [49]. Genetic variations substantially affect ACE levels [50]. Thereby resulting in impaired production of angiotensin II, contributing to hypertension and related cardiovascular-related complications [51]. Conversely, TNF genes encode tumour necrosis factor-alpha, a crucial pro-inflammatory cytokine [52]. An increased level of TNF-α is associated with inflammation and oxidative stress, both of which contribute to the development of PE [25]. These genetic variations play a significant role in the development and progression of PE.

Genetic predispositions can amplify inflammatory reactions, impairing endothelium and organ damage, especially in the liver [53]. The variations in genes encoding pro-inflammatory cytokines can make individuals more susceptible to increased inflammatory reactions [54]. For instance, TNF-α and IL-6 can enhance inflammatory reactions and result in adverse pregnancy complications, including PE [55]. The changes in genes associated with oxidative stress can also impair the body's capacity to regulate ROS, resulting in cellular damage and inflammation [56]. It has been shown that the glutathione S-transferase (GST) and superoxide dismutase (SOD) genes can result in an imbalance between enzymes that remove ROS, resulting in oxidative stress and inflammation [57]. On the other hand, the genes involved in angiogenesis can shift the balance between molecules that promote angiogenesis and those that inhibit angiogenesis [58]. This can affect the development of the placenta and, further, promote inflammation. The polymorphism of VEGFA and FLT1 changes the expression of the VEGF gene, resulting in hypoxia and inflammation in PE [32]. FLT-1 gene variants also raise the soluble FLT-1, which leads to endothelial dysfunction and inflammation in PE [59].

A multicentric meta-analysis of 20,064 cases and 703,117 control individuals revealed the presence of 18 independent loci associated with PE, eclampsia, and gestational hypertension [60]. These genetic locations emphasise the importance of natriuretic peptide signalling, angiogenesis, renal glomerular function, trophoblast development, and immune dysregulation. Another genome-wide association study (GWAS) that examined the relationship between PE and maternal hypertension in pregnancy identified at least 19 significant connections, 13 of which were previously unknown [15]. Genes related to blood pressure features were connected to seven new loci. The analysis discovered new risk locations. These findings offer a detailed understanding of the mechanisms behind pregnant hypertensive disorders.

Genetic factors substantially impact the development of PE and can affect the activity of liver enzymes by inducing endothelial dysfunction, inflammatory responses, oxidative stress, and immunological modulation [61]. Therefore, gaining a comprehensive understanding of these genetic associations is essential for enhancing the management and results

of PE. It is also crucial to identify individuals at high risk of secondary complications of PE and further develop focused therapeutic approaches.

1.3. The Role of HIV Infection and Antiretroviral Therapy in the Development of Pre-Eclampsia

The co-occurrence of HIV infection and PE is a rising maternal health concern driven by the shared occurrence of these conditions, especially in South Africa [62]. More recently, Modjadji et al. (2023) have shown that PWLWHIV on ART has an increased risk of cardiometabolic disorder [63]. Altogether, this, with PE, may compromise maternal and foetal health. Therefore, understanding the relationship between HIV and PE includes examining the immunological contribution, as shown in Figure 1. Poor placentation due to placental hypoxia, characterised by oxidative stress, impairs the angiogenic system, T-helper, and immune cells and causes vascular injury. These can be due to increased ET-1, a critical vasoconstrictor, sFLT-1, Th 1, and -7. Altogether, these contribute to endothelial dysfunction; in response, the body promotes platelet activation, further resulting in inflammation, organ damage, and hypertension or PE (Figure 1).

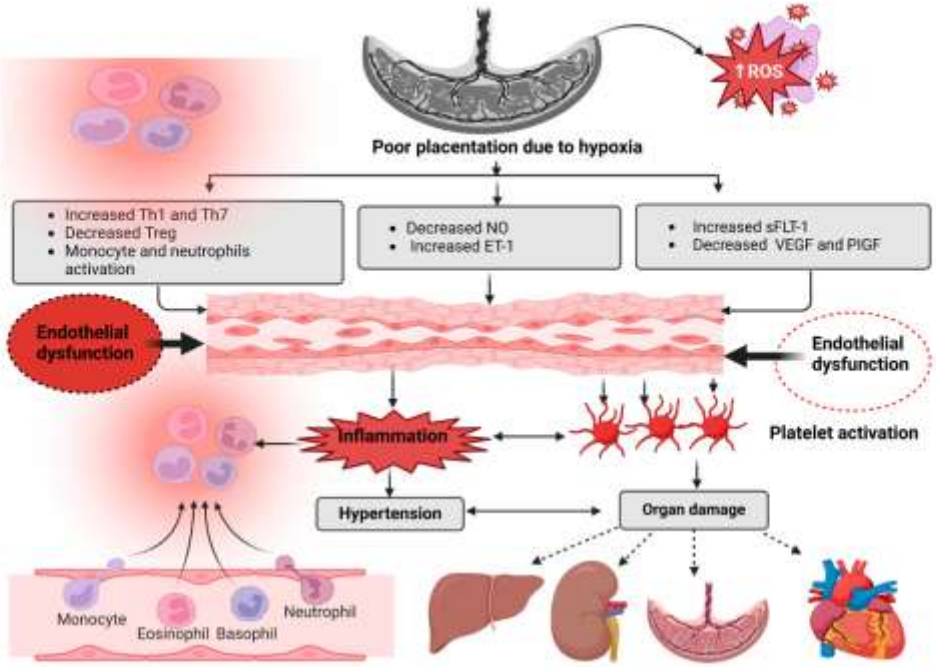

Figure 1. The contribution of immune and angiogenic system dysregulation plays a significant part in the pathophysiology of PE. ROS: reactive oxygen species, ET-1: endothelin-1, Th: T-helper cells, Treg: regulatory T-cell, sFLT-1: soluble fms-like tyrosine kinase, VEGF: vascular endothelial growth factor, PlGF: placental growth factor, and NO: nitric oxide. Adapted from [64]. Created in BioRender, https://BioRender.com/j02h242 (accessed on 5 November 2024).

HIV infection alters immune systems and promotes a pro-inflammatory environment by producing TNF-α, IL-6, and IL-1β, thus resulting in inflammation and contributing to the pathophysiology of PE [65,66]. These cytokines are crucial in the inflammatory processes associated with HIV infection and PE. They worsen endothelial dysfunction and vascular abnormalities, which are important characteristics of PE [67]. The virus targets CD4+ T lymphocytes, resulting in their depletion and weakened immune function [68]. HIV plays a significant role in the pathogenesis of PE; ART-naïve individuals living with HIV

(ILWHIV) have a reduced risk of developing PE due to the suppressed immune system's necessary inflammatory response promoting PE development [62]. Several researchers have demonstrated the undesirable effect of ART and its contribution to the development of PE. Notably, these researchers reported that ART induces oxidative stress and endothelial dysfunction, both of which contribute to the pathogenesis of PE [69,70]. Therefore, it is important to understand the correlation between HIV, ART, and PE to improve maternal and newborn health outcomes in populations with a high prevalence of HIV. This information can improve the prevention of associated complications and management strategies for PWLWHIV.

Immunological systems contribute to the progression of pregnancy and HIV infection [71]. A cohort study conducted in South Africa found that pro-inflammatory cytokines like interferon gamma-induced protein 10 (IP-10) were more notable in PWLWHIV than in HIV-negative women [72]. Additionally, the same study reported significantly higher levels of Th1 cytokines, such as IL-12 and interleukin-12-protein 70 (IL-12p70), Th2 cytokine IL-5, and Th17 cytokine IL-17A in PWLWHIV than the HIV-negative group. The above study suggests that maternal HIV and ART use is associated with distinct systemic cytokine profiles throughout pregnancy, which can exacerbate the progression of PE. Therefore, it is more evident that HIV and PE are associated with increased inflammation [73]. Vyas et al. 2021 reported that PWLWHIV exhibited elevated levels of IL-6, TNFα, soluble CD14, and intestinal fatty acid-binding protein (I-FABP) [74]. Altogether, these results suggest that the presence of HIV in pregnancy promotes inflammation, monocyte activation, and gut barrier dysfunction, potentially contributing to the onset and exacerbation of PE. While HIV predominantly impacts the immune system, it can also invade liver cells, resulting in liver damage and apoptosis [75]. This is partly due to persistent immunological activation and inflammation [76]. Hepatocyte damage and apoptosis can be caused by elevated levels of pro-inflammatory cytokines, such as TNF-α and IL-6 [38,77]. Similar findings have also been reported in animal models of liver disorders [78]. This suggests that inhibiting inflammation can ameliorate liver damage and associated complications. Specific ART treatments, especially those containing PI, can result in hepatotoxicity, which manifests as increased levels of liver enzymes and liver damage [79]. ILWHIV are more susceptible to co-infections, such as hepatitis B and C, which can worsen liver damage and contribute to elevated liver enzyme levels [80]. Therefore, secondary complications among these populations must be curbed to prevent any manifestations of liver dysfunction.

1.4. The Role of HIV and ART in Liver Function

ART is an essential treatment for ILWHIV, since it suppresses the reproduction of the virus and enhances the functioning of the immune system [81]. ART achieves this by reducing inflammation [82]. This is important, because HIV infection is linked to persistent immunological activation and inflammation, which leads to various complications, such as CVD and liver damage [83]. However, ILWHIV on ART can also present with detrimental side effects, specifically on hepatic enzymes, which are markers for liver function [84]. Increased concentrations of these hepatic enzymes may suggest hepatic inflammation or injury, resulting in hepatitis or liver fibrosis [85]. ART categories, including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs), are associated with elevated liver enzyme levels [86,87]. PIs and NNRTIs impair liver enzymes, including ALT and AST. ART can induce hepatotoxicity, which accelerates fibrosis (Figure 2); this condition is characterised by hepatic injury from drug exposure [88,89]. Additionally, as the ART improves, studies show that ILWHIV has elevated incidences of liver conditions such as viral hepatitis, alcohol-related liver disease, drug-induced liver damage, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic

steatohepatitis (NASH) [90–92]. A study by Chwiki et al. (2017) reported an increased level of ALT and AST in males living with HIV on ART [79]. Also, a study by Shiferaw et al. 2016 discovered a significant occurrence of liver enzyme abnormalities, mainly elevated ALT levels in patients on HAART compared to HAART-naïve [93]. The elevated ALT and AST levels were reportedly associated with viral hepatitis, opportunistic infections, CD4 count, and male gender. Drug-induced hepatotoxicity among ILWHIV and ART refers to the inherent toxic effects induced by ART on liver cells, mainly the hepatocytes [89,94]. These ARTs can potentially induce mitochondrial toxicity, altering the normal functioning of hepatocytes and resulting in the accumulation of fat in the liver [95]. For instance, stavudine and zidovudine that belong to NRTIs inhibit the function of DNA polymerase-γ, a crucial enzyme responsible for replicating and repairing mitochondrial DNA [96]. This inhibition leads to impaired functioning of the mitochondria, resulting in oxidative stress and damage to the liver cells [97].

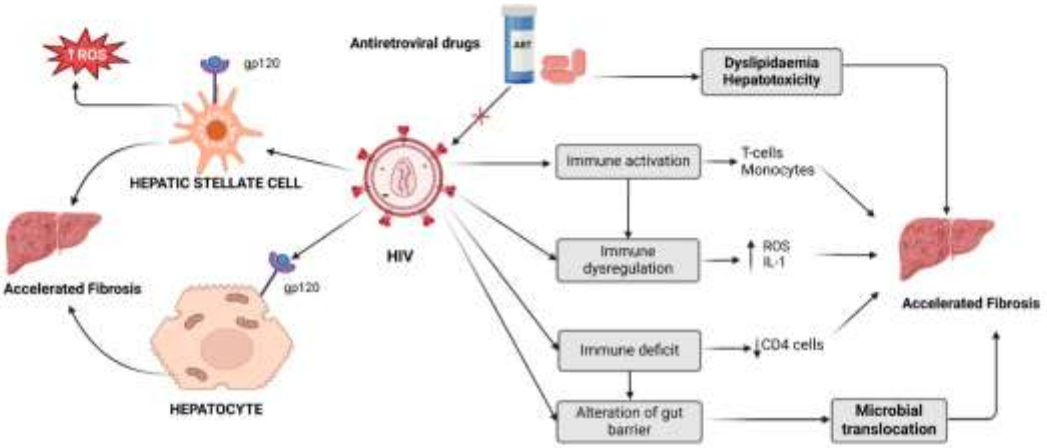

Figure 2. The impact of HIV and ART on the development of liver fibrosis. X: indicates inhibition, ART: antiretroviral therapy, ROS: reactive oxygen species, gp120: glycol-protein 120, CD4: cluster of differentiation-4, and IL-1: interleukin-1. Adapted from [98]. Created in Biorender, https://BioRender.com (accessed on 5 November 2024).

ART contributes to increased liver enzymes through different pathways, including direct impact on the liver, immune system reconstitution, mitochondrial toxicity, and genetic factors [99]. For instance, immune reconstitution inflammatory syndrome (IRIS) occurs when ART improves the immune system, especially in HIV individuals with hepatitis, as the immune response may exacerbate hepatic inflammation and further elevate liver enzymes [100,101]. Additionally, ART in pregnant women with PE seems to have a discordant effect on liver function [102]. The information presented in Table 1 gives an overview of different studies that examine the effects of ART on liver function in PWLWHIV with and without PE. The presented research was conducted in various countries, including the United Kingdom, Ireland, Brazil, France, South Africa, and Nigeria. This provides a wide geographical reach, allowing for an understanding of how these complications may differ across different populations and healthcare systems. The sample size has also varied, with the smallest being 21 individuals to a maximum of 5748. Most studies have reported increased levels of liver enzymes, including AST, ALT, and ALP, in PWLWHIV, especially those actively receiving ART. Additionally, Joy et al. (2019) and Tamuno-Boma et al. (2023) revealed substantial increases in the ALT, AST, and ALP levels in PWLWHIV on ART compared to the ART-naïve group and HIV-negative group [103,104]. In contrast,

Huntington et al. (2015) observed no statistically significant change in ALT levels among PWLWHIV on ART but confirmed the risk of LEE. Other researchers also showed no significant differences in liver function enzyme in PWLWHIV on ART compared to the counterpart group [105,106]. It is worth noting that participants did not have PE in all these studies. Yet, Maharaj et al. (2017) discovered no notable changes in ALT and AST levels between the PWLWHIV with PE and the HIV-negative group with PE [107]. However, the level of gamma-glutamyl transferase significantly increased in PWLWHIV with PE compared to the HIV-negative group. These suggest that the liver dysfunction in this group may be attributed to PE status rather than HIV and ART. On the other hand, Ouyang et al. 2009 reported a significant decrease in baseline liver enzyme elevation in PWLWHIV on nevirapine compared to NPWLWHIV [108]. According to Delicio et al. 2018, the use of NVP, nelfinavir, and atazanavir ART regimens was associated with an increased risk of liver function test abnormalities [109]. These results show that, while ART reduces the viral load, additional factors, including PE status, must be considered in managing liver function-associated complications. The evidence gathered in this study suggests that HIV and ART independently impair liver function (Table 1). Among women living with HIV without PE coexistence, the results highlight the impact of ART on liver enzyme levels. For instance, a study by Tamuno-Boma et al. (2023) revealed that women living with HIV on ART, whether pregnant or not, are more prone to liver dysfunction [103]; this warrants close monitoring and cautious care of this group of patients. There is a lack of evidence investigating the influence of PE on liver function calls for research to focus on this area to assess liver function in PWLWHIV with PE, especially in South Africa, where the prevalence of HIV is very high. This evidence is crucial, since PE can independently impair liver function, irrespective of HIV or ART administration [22]. The complex relationship between HIV, PE, liver function, and ART in pregnancy requires more investigation. The conflicting findings among previous studies emphasise the need for more focused research that longitudinally examines the impact of both PE and ART on liver function in HIV patients. A thorough understanding of these associations is crucial for enhancing ART regimens to minimise liver damage, especially in PWLWHIV with PE.

Table 1. Summary of studies on the effect of antiretroviral therapy in HIV-pregnant women with or without PE.

Authors, Year	Country	Study Design	Range/Mean Age (Years)	Sample and Population	Summary of Findings
Hungtigton et al. 2015 [110]	United Kingdom and Ireland	Cohort	29–39	3815 PWLWHIV on ART.	There was no significant difference in ALT in pregnant women on ART.
Joy et al. 2019 [104]	Nigeria	Cohort	20–40	30 PWLWHIV on HAART 30 HAART-naïve PWLWHIV 30 HIV-negative.	Significant increase in ALT, AST, and ALP in PWLWHIV on HAART compared to the ART-naïve or negative at the 1st, 2nd, and 3rd trimesters.
Maharaj et al. 2017 [107]	South Africa	Cohort	24.8 ± 5.3 28.7 ± 7.3 24.6 ± 6.4 28 ± 6.4	53 HIV-negative with PE 45 PWLWHIV with PE 50 normotensive and HIV-negative 45 normotensive and PWLWHIV.	There is no significant difference in AST and ALT between PE-PWLWHIV and HIV-negative. The gamma-glutamyl transferase increased in PWLWHIV with PE compared to negative.

Table 1. *Cont.*

Authors, Year	Country	Study Design	Range/Mean Age (Years)	Sample and Population	Summary of Findings
Onyeka et al. 2016 [105]	Nigeria	In vivo experimental	30 ± 3.0	21 PWLWHIV on ART 25 NPWLWHIV.	There were no significant differences between the pregnant and non-pregnant groups in the AST, ALP, and ALT levels.
Sibiude et al. 2019 [111]	France	Cohort	<25 25–39 ≥40	5748 PWLWHIV on ART, of which 147 had PE.	Among PWLWHIV on ART at conception, the risk of unexplained LEE was lower with NNRTI compared to PI-based regimens.
Tamuno-Bona et al. 2023 [103]	Nigeria	Cross-sectional	15–60	83 PWLWHIV on ART 82 NPWLHIV on ART 84 PWLWHIV-negative 81 NPHIV-negative.	Significantly higher ALT, AST and ALP levels in PWLWHIV compared to non-PWLWHIV. Lower AST, ALT, and ALP in HIV-negative pregnant women compared to non-pregnant HIV-negative.
Ouyang et al. 2010 [106]	United States	Prospective cohort	27.84 28.02	218 PWLWHIV on nevirapine (NVP) 1011 non-NVP PWLWHIV.	No significant liver elevation was observed in the NVP compared to the non-NVP group.
Ouyang et al. 2009 [108]	United States	Prospective cohort	27.99 35.96	1229 PWLWHIV on NVP 821 NPWLWHIV on NVP.	Significant decrease in baseline in liver enzyme elevation in PWLWHIV compared to NPWLWHIV.
Delicio et al.2018 [109]	Brazil	Cohort	13–46	801 PWLWHIV with 793 on known ART and eight on unknown ART.	NVP, nelfinavir and atazanavir regimens increased the risk of liver abnormalities.

PWLWHIV: pregnant women living with human immune virus; NPWLWHIV: non-pregnant women living with human immune virus; NVP: nevirapine; HAART: highly active antiretroviral therapy; ART: antiretroviral therapy; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.

2. Limitations and Recommendations

Currently, there is limited evidence about the effect of ART on liver function among PWLWHIH with the co-existence of PE. This is evident in the lack of studies from databases such as PubMed and Scopus. This review did not highlight the exact class of ART regimens associated with liver dysfunction. Various forms (PI, NRTI, and NNRTIs) were used in individual studies, with others not specifying the regimen. Integrase strand transfer inhibitors (INSTIs) are currently used as first-line ART due to their superior efficacy, tolerability, minimal drug–drug interaction, and high genetic barrier to resistance [112]. The evidence about the effect of INSTIs such as dolutegravir has been observed among ILWHIV. For instance, the AST level was decreased while GST increased following the dolutegravir regimen in ILWHIV [113]. While dolutegravir reduced the HIV viral load and increased the CD4+ count compared to efavirenz, there was no significant difference in liver function test (AST and ALT) in both groups [114]. Altogether, these studies suggest some potential benefits of INSTI regimens in reducing liver dysfunction. However, there is limited evidence about its effect on liver function in pregnancy and PE. Geographic and population bias also contribute to the inconsistencies in the effect of ART regimens. The generalisability of the findings and conclusions may be limited to other populations, particularly those in regions with distinct healthcare systems, socio-economic conditions, and HIV and PE prevalence rates. Moreover, most of the studies analysed were cohorts, which limits the evaluation of

the long-term impacts of HIV, ART, and PE on the health outcomes of both mothers and the transmission to children.

3. Conclusions and Recommendations

The evidence reviewed in this study showed discordant results about the effect of ART on liver function, especially in PWLWHIV compared to NPWLHIV. Other studies showed a significant increase in LEE or no difference, while others showed a decrease in LEE following ART. Similarly, among those with PE on ART, there were no differences in AST and ALT; however, gamma-glutamyl transferase increased compared to HIV-negative. Lastly, the risk of unexplained LEE was lower with PE on NNRTI than with PI-based regimens. Although HIV is often linked to elevated ALT, AST, and ALP, research on these liver enzymes in PWLWHIV in coexistence with PE remains limited, as evidenced by our finding that only two studies had PE. More importantly, this will enable proper care for PWLWHIV with PE. Therefore, future research should investigate the effect of ART on liver function in PWLWHIV and PE, especially in countries considered HIV endemic, like South Africa. Additionally, longitudinal studies are necessary to comprehend the implications in the long term among this population. Among the studies reviewed, none has explored the INSTI forms on liver function in PWLWHIV in co-existence with or without PE. Therefore, this calls for future studies to explore INSTI on pregnant women, focusing on liver function. Lastly, future studies should incorporate liver function tests in routine assessments during the maternal prevention of mother-to-child transmission program, thus reducing maternal and foetal adverse effects. Conducting future studies in South Africa can improve healthcare professionals' capacity to give comprehensive care to PWLWHIV and PE.

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Chapter 2.2 Systematic Review and Meta-Analysis

Pre-eclampsia-Induced Maternal Liver Dysfunction: Meta-Analysis of Observation Studies

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Systematic Review

Pre-Eclampsia-Induced Maternal Liver Dysfunction: Systematic Review, Meta-Analysis and Meta-Regression of Observation Studies

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Abstract

Introduction: Pre-eclampsia (PE) is a pregnancy-related hypertensive condition defined by the onset of hypertension after 20 weeks of gestation that is associated with proteinuria and maternal organ damage or uteroplacental dysfunction. It continues to be a leading cause of maternal and perinatal morbidity and mortality globally. PE is linked to systemic inflammation, endothelial dysfunction, and oxidative stress, which may compromise hepatic function. **Aim**: This meta-analysis assesses the impact of PE on maternal liver function by evaluating hepatic biomarkers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total serum bilirubin. **Methods**: This meta-analysis of observational studies in Epidemiology (MOOSE) involved a search of PubMed and Scopus and manual screening of studies published between 2000 and 2025. Eligible studies included cross-sectional, case–control, and cohort designs. The quality of the studies was evaluated using the Newcastle–Ottawa Scale. Statistical analysis was conducted using the online meta-analysis, Jamovi version 2.6.44, and IBM SPSS Statistics version 30, and effect estimates were reported as standardized mean differences (SMDs) with 95% confidence intervals (CIs). **Results**: Forty-five studies, comprising 257,929 women 9420 with PE; 248,509 normotensive, were included. Women with PE had elevated AST, MD = 1.81 (95% CI: 1.51 to 2.10; $p < 0.0001$) and ALT, SMD = 1.73 (95% CI: 1.38 to 2.07; $p < 0.0001$); ALP, SMD = 1.43 (95% CI: 0.97 to 1.88; $p < 0.0001$); and total serum bilirubin (TSB), SMD = 0.62 (95% CI: 0.36 to 0.88; $p < 0.0001$) in comparison to normotensive controls. In the meta-regression, maternal age and quality were significant moderators, with older age and high-quality studies associated with smaller and larger effect sizes, respectively, for ALP ($\beta = -0.720$ and $\beta = 1.444$) and TSB ($\beta = -0.304$ and $\beta = 0.761$). For every 1-unit increase in body mass index, there was a significant 0.406-unit decrease in ALT effect size. **Conclusions**: PE is significantly associated with elevated maternal hepatic enzyme levels, indicating hepatocellular damage and impaired liver function. These findings emphasise the necessity for routine liver function monitoring in pregnant women with hypertensive disorders.

Keywords: pre-eclampsia; liver function; aspartate aminotransferase; alanine aminotransferase; alkaline phosphatase; bilirubin; hepatic dysfunction

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1. Introduction

Pre-eclampsia (PE) is a hypertensive disorder occurring during pregnancy, characterised by new-onset hypertension defined as systolic blood pressure (SBP) $\geq$ 140 mmHg

and diastolic blood pressure (DBP) $\geq$ 90 mmHg after 20 weeks of gestation [1–3]. It is accompanied by either proteinuria or maternal organ dysfunction and or uteroplacental dysfunction. It continues to be a significant contributor to maternal and perinatal morbidity and mortality globally, impacting around 2–8% of pregnancies [4]. Pregnancy is associated with substantial metabolic, haemodynamic, and hormonal changes that promote maternal well-being and foetal growth [5]. In most cases, these changes do not result in substantial impairment. However, in the case of PE, these gestational changes may be significant, resulting in compromised liver function. This is attributed to the pathophysiology of PE, which is centred around the abnormal placental growth and function, resulting in systemic inflammation, oxidative stress, and endothelial dysfunction [6–8]. Moreover, inflammation is associated with liver dysfunction, as the liver serves as both a major immunological organ and a target of inflammatory mechanisms [9]. The liver is an essential organ in human physiology, playing a vital role in metabolism, detoxification, protein synthesis, and the regulation of biochemical homeostasis [10,11]. The ability of the liver to maintain stable levels of circulating biomarkers, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin, is essential, as these are sensitive indicators of hepatic integrity and function [12]. Due to its vital role, any impairment in liver function can lead to systemic repercussions, especially during pregnancy, when maternal physiology experiences heightened metabolic and haemodynamic demands [13].

Initiation of an inflammatory response promotes the activation of liver macrophages (Kupffer cells) and neutrophils [14]. These cells secrete pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), as well as reactive oxygen species [15]. This sequence of mediators induces hepatocellular oxidative stress and damage, which presents as elevated liver enzyme levels [16]. Therefore, a timely identification and intervention for PE are crucial to prevent complications and to preserve the health of both the mother and the infant. The hepatic symptoms of PE differ from moderate biochemical anomalies, such as increased liver enzymes, to severe complications such as hepatic rupture, infarction, and in severe cases, HELLP (Haemolysis, Elevated Liver enzymes, and Low Platelets) syndrome [17]. These complications not only compromise the mother's health but also complicate clinical management and affect pregnancy outcomes. Moreover, liver dysfunction exacerbates negative pregnancy outcomes, such as preterm delivery, intrauterine growth restriction, and stillbirth, highlighting the combined risk to mother and foetal health. Therefore, this study aims to examine the effects of PE on maternal liver function by analysing changes in hepatic biomarkers (AST, ALT, ALP, and bilirubin).

2. Methodology

2.1. Study Design

This study is a meta-analysis of observational studies and adheres to the Meta-analysis of Observational Studies in Epidemiological (MOOSE) guideline [18] (Supplementary File S1). This study also followed the PICO framework in designing the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow chart [19]. It also adheres to PECOS criteria as outlined in Table 1.

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Table 1. PECOS criteria.

Population (P)	Pre-eclamptic pregnant women
Exposure (E)	Pre-eclampsia
Comparison (C)	Healthy pregnant women (normotensive)
Outcome (O)	Liver function (AST, ALT, ALP, and bilirubin)
Study design (S)	Cross-sectional, case–control, and cohort

2.2. Search Strategy, Literature Search, and Selection Criteria

Two independent researchers (KES and KM) conducted an extensive literature search using the PubMed and Scopus databases, employing Medical Subject Headings (MeSH) terms to identify relevant published studies, as well as a comprehensive bibliography search. The MeSH terms and Boolean operators used for the search included "Liver Function" OR "Aspartate Aminotransferase" OR "AST" OR "Alanine Aminotransferase" OR "ALT" OR "Alkaline Phosphatase" OR "ALP" OR "Bilirubin" AND "Pre-eclampsia". The review focused on studies published between 2000 and 2025 to identify evidence that reflects current diagnostic criteria, clinical practices, and methodological standards. Studies published before 2000 were excluded because of changes in the definition and management of PE, which could introduce variability.

2.3. Data Extraction and Quality Assurance

Two independent researchers (KES and KM) used a preformatted Excel spreadsheet to extract data from each study. Two researchers evaluated the two sheets, and in cases of disagreement over key items, a third researcher, WNP, was consulted to review the study and the disputed variables before a decision was reached. The primary data obtained from each study comprised the lead author's family name, country of publication, study design, population size, blood pressure (SBP, DBP) and body mass index (BMI) of the participants, and the findings, mean, standard deviation (SD), and sample size of AST, ALT, ALP, and total serum bilirubin. The Newcastle–Ottawa Scale was used to assess the quality of the included case–control, cohort, and cross-sectional studies [20]. This tool focuses on selection, comparability, exposure, and outcome based on the design. Each item was rated with a shaded star or no empty star, and overall quality was judged accordingly. The study was considered high quality (low risk of bias) if it scored 8–9 stars, moderate if it scored 5–7 stars, and low if it scored 0–4 stars.

2.4. Statistical Analysis

For the meta-analysis, we used online meta-analysis software [21]. Jamovi version 2.6.44 and IBM SPSS Statistics version 30 were used for meta-regression and subgroup analysis, respectively. We calculated the effect estimates for all indicators by computing the mean, SD, and sample size for each study group. The mean and SD were estimated from the median and range using the protocols reported by Hozo et al. (2005) [22]. When the study reported the standard error of the mean (SEM), SD was estimated as $SD = SEM \times \sqrt{n}$ [23]. If the study reported the median and interquartile range (IQR), the mean was used as the median for a larger sample, and the SD was estimated as SD = IQR/1.35. In studies with multiple PE groups (moderate, mild, or severe), we used the Cochrane method to combine them into a single PE group. We employed the I^2 statistic test to assess statistical heterogeneity [24,25]. I^2 values of $\leq$50% and $\geq$75% were categorised as low and substantial statistical heterogeneity, respectively [26]. Moreover, the Egger regression test, Beggs and Mazumdar's rank correlation, trim and fill, including safe-fail N assessment, were used to assess and adjust for publication bias. Sensitivity analysis was performed using one-

study-exclusion methods to assess the stability of the effect size [27]. Subgroup analyses were conducted by study design, maternal age, gestational age at diagnosis of PE, study quality, BMI, and continent of publication. For meta-regression, different moderators including (maternal age and BMI, duration of gestation at time of diagnosis of PE, study design, and content of publication) were explored. A p-value below 0.05 was considered statistically significant.

3. Results

3.1. Literature Search and Screening

The preliminary search on the PubMed and Scopus databases produced 332 records. Furthermore, we conducted a bibliographic search and identified 33 relevant studies, bringing the total to 365 for review. Initially, we used an Excel spreadsheet to group all records; 2 duplicates were thus identified and excluded. Of the records that underwent initial full screening, 13 were excluded because their titles and abstracts were deemed irrelevant to the topic. Additionally, 305 were excluded for various reasons, including studies in animals, children, irrelevant markers reported, results presented graphically, not containing a control group, article retracted, no liver function test or pre-eclampsia present, the study was conducted after delivery of the baby, study not published in English, articles published before 2000, and review articles. Hence, 45 studies [28–72] were deemed relevant as they satisfied the PECOS criteria outlined in Table 1. To address any discrepancies and prevent bias, a third independent researcher (WNP) participated in the screening and selection process. Refer to Figure 1 for a comprehensive explanation of the screening and selection procedure.

3.2. Characteristics of the Studies Included

We analysed data from 45 studies [28–72] published in peer-reviewed journals between 2000 and 2025, evaluating the effect of PE on liver function in pregnant women. The sample sizes showed significant heterogeneity across research, ranging from small samples [51] to larger population studies [40,42,43]. The overall sample comprised 257,929 pregnant women: 9420 with PE and 248,509 normotensives. The published studies employed various designs, including 26 cross-sectional [28–31,38,39,46,49–53,55–68,71], 11 case–control [32–37,45,48,69,70,72], and 8 cohort designs [40–44,47,54,71]. Studies from 15 countries were analysed (Figure 2), with the majority from India [31,33,55,56,59–61,63,66,69,72], China [32,40,43,47,54,70], Nigeria [28,37,57,67,68], Iraq [34,39,58,62], Pakistan [41,50,52], Ethiopia [29,49], Bangladesh [30,35], Zimbabwe [65], Turkey [36,45], Libya [46], Iran [48], Saudi Arabia [38,64], Egypt [51], Korea [42], Israel [44], Russia [53] and Türkiye [71] (Figure 2). The average blood pressure of the PE group was 150.20 ± 15.14 mmHg systolic and 96.93 ± 9.40 mmHg diastolic. In contrast, the normotensive group had an average SBD and DBP of 115.42 ± 8.95 mmHg and 72.93 ± 6.55 mmHg, respectively. The average maternal age in the PE group reported in 33 studies was 29.65 ± 5.14 years, compared with 32 studies in normotensive women, which reported an average age of 28.75 ± 5.06 years. Moreover, the average BMI of the 17 students in the PE group was 26.77 ± 4.17 kg/m^2, whereas in the normotensive group, it was 24.22 ± 3.64 kg/m^2. The overall features of the included studies are presented in Table 2.

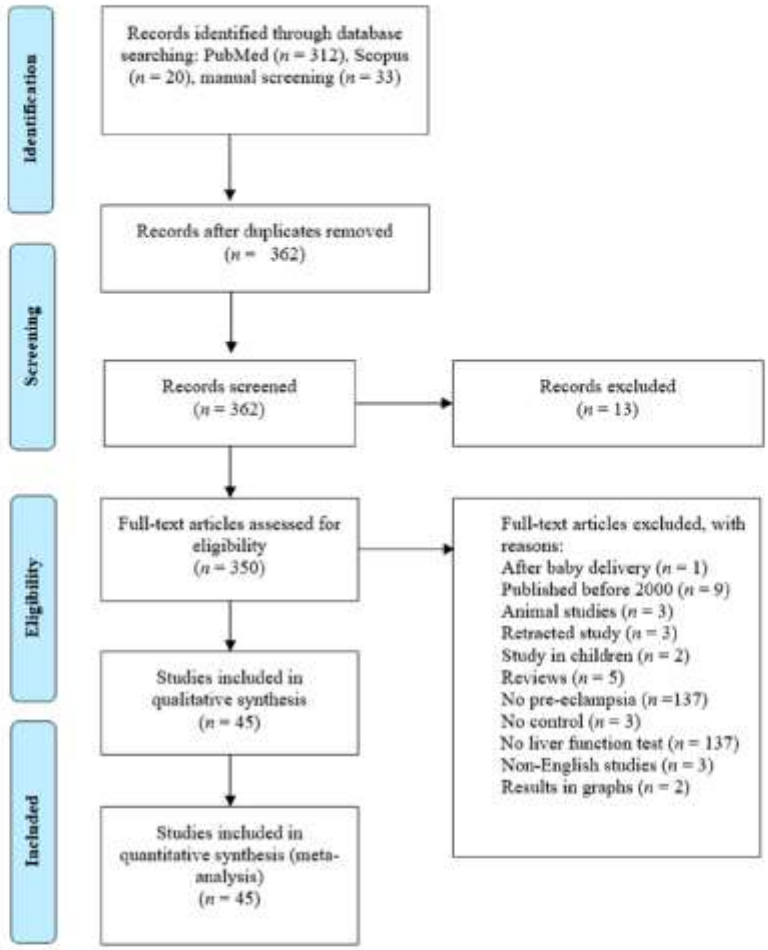

Figure 1. A flowchart illustrating the study selection process.

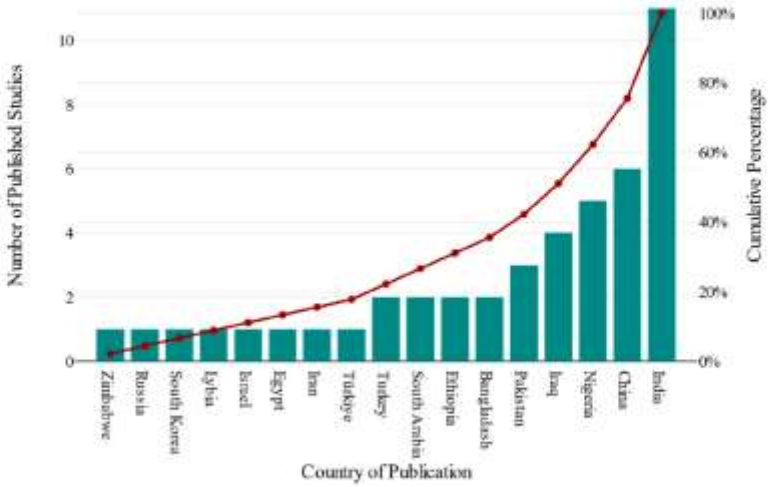

Figure 2. Geographical distribution of publications.

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Table 2. Overview of features of included studies (n = 45).

Author Name and Publication Year	Country	Study Design	PE Group	Normotensive Group	Age (Years) PE Group	Age (Years) Normotensive Group	SBP and DBP (mmHg) PE Group	SBP and DBP (mmHg) Normotensive Group	Markers
Atiba et al., 2016 [28]	Nigeria	Cross-sectional	98	115	28.87 ± 4.33	28.87 ± 6.62	166.15 ± 9.40 99.80 ± 2.66	117.83 ± 13.03 70.87 ± 9.65	AST
Ekun et al., 2018 [68]	Nigeria	Cross-sectional	49	50	33.18 ± 4.55	32.44 ± 4.99	172.35 ± 24.16 112.47 ± 17.73	113.02 ± 9.95 70.20 ± 9.09	AST and ALT
Asha and Varghese, 2017 [69]	India	Case–control	50	30	NR	NR	NR	NR	AST, ALT, and ALP
Chen et al., 2022 [32]	China	Case–Control	73	73	30.17 ± 4.33	29.87 ± 4.14	116.43 ± 10.3 71.96 ± 9.65	106.07 ± 11.91 65.59 ± 9.78	AST and ALT
Cho et al., 2018 [42]	South Korea	Cohort	3973	192,571	30.92 ± 3.75	30.25 ± 3.38	117.60 ± 13.46 74.76 ± 10.00	109.6 ± 10.69 69.00 ± 7.95	AST and ALT
Hamid et al., 2023 [46]	Libya	Cross-sectional	60	40	33.62 ± 6.50	28.77 ± 7.26	NR	NR	ALT and AST
Fang et al., 2024 [47]	China	Retrospective cohort	53	898	29.23 ± 1.65	29.77 ± 1.77	112.3 ± 2.92 72.5 ± 2.92	108 ± 3.45 67.3 ± 2.84	AST, ALT, and ALP
Albayrak and Arslan, 2025 [71]	Türkiye	Retrospective case–control	207	205	31.2 ± 6.89	29.5 ± 5.13	156.5 ± 9.2 96.8 ± 7.5	110.6 ± 7.3 63.5 ± 6.8	AST, ALP, and ALP
Lu et al., 2025 [70]	China	Retrospective case–control	113	217	31.32 ± 5.10	31.53 ± 4.06	140 ± 0.75 90 ± 0.2	140 ± 0.67 90 ± 0.33	AST, ALT, ALP, and bilirubin
Hassanpour and Kazami, 2018 [48]	Iran	Case–control	50	49	NR		NR	NR	ALT, AST, ALP, and bilirubin
Hassen et al., 2022 [29]	Ethiopia	Cross-sectional	51	51	32.9 ± 6.3	29.5 ± 3.3	142.8 ± 6.54 92.8 ± 5.22	NR	ALT, AST, ALP, and bilirubin
Herdawy et al., 2020 [38]	Saudi Arabia	Cross-sectional	100	100	NR	NR	NR	NR	ALT and AST
Ipek et al., 2024 [45]	Turkey	Case–Control	92	91	31.0 ± 10.0	27.0 ± 6.0	NR	NR	ALT and AST
Khan et al., 2023 [31]	India	Cross-sectional	150	150	29.24 ± 3.43	29.09 ± 3.08	154.28 ± 22.31 100.21 ± 12.34	119.08 ± 12.20 74.34 ± 6.22	ALT and AST

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Table 2. Cont.

Author Name and Publication Year	Country	Study Design	PE Group	Normotensive Group	Age (Years) PE Group	Age (Years) Normotensive Group	SBP and DBP (mmHg) PE Group	SBP and DBP (mmHg) Normotensive Group	Markers
Mishra et al., 2021 [33]	India	Case–control	35	25	30 ± 5	NR	151.3 ± 3.0 70.83 ± 3.41	100.0 ± 1.82 111.2 ± 4.94	AST and bilirubin
Mondal et al., 2016 [30]	Bangladesh	Cross-sectional	50	50	26.58 ± 3.97	26.06 ± 5.02	156.56 ± 14.03 104.69 ± 8.88	107.5 ± 12.95 69.68 ± 7.82	ALT and bilirubin
Munazza et al., 2013 [52]	Pakistan	Comparative cross-sectional	50	50	15–45	15–45	166.60 ± 24.04 106.50 ± 13.18	116.80 ± 9.022 73.44 ± 7.29	ALT, AST, and bilirubin
Qasim and Ameen, 2021 [34]	Iraq	Case–control	50	50	16–40	16–40	NR	NR	ALT and AST
Sakr et al., 2019 [51]	Egypt	Cross-sectional	25	25	18–35	18–35	NR	NR	AST and ALT
Singh and Rachna, 2021 [72]	India	Case–control	30	30	18 and above	18 and above	167.33 ± 25.45 103.33 ± 12.41	113.33 ± 7.58 75.00 ± 5.08	ALT, AST, and ALP
Sultana et al., 2021 [35]	Bangladesh	Case–control	50	50	24.66 ± 3.22	24.06 ± 3.71	NR	NR	ALT
Uckan and Sahin, 2018 [36]	Turkey	Case–control	30	30	30.7 ± 6.01	28.5 ± 6.03	175.6 ± 16.82 95.8 ± 7.97	122.5 ± 7.2 72.6 ± 8.73	AST and ALT
Udenze et al., 2014 [37]	Nigeria	Case–control	21	21	31.73 ± 5.5	32.57 ± 5.5	153.4 ± 52 99.1 ± 34.5	116.19 ± 12.00 70.7 ± 1.12	AST, ALT, and ALP
Al Ghazali et al., 2014 [39]	Iraq	Cross-sectional	53	21	25.23 ± 6.23	27.28 ± 6.14	168.58 ± 20.54 110.39 ± 9.025	117.4 ± 9.95 76.7 ± 9.13	AST and ALT
Nie et al., 2025 [40]	China	Retrospective cohort	1200	11,499	30.09 ± 5.40	29.19 ± 4.64	134.76 ± 7.95 84.53 ± 7.56	115.28 ± 12.95 71.36 ± 6.57	ALT, AST, and bilirubin
Shahid et al., 2019 [41]	Pakistan	Prospective cohort	63	17	27.03 ± 5.89	30.63 ± 9.40	125.44 ± 16.23 83.95 ± 7.6	108.22 ± 5.89 79.55 ± 8.45	ALP and bilirubin
Taimoor et al., 2017 [50]	Pakistan	Comparative cross-sectional	50	50	25.92 ± 5.56	25.92 ± 5.56	NR	NR	AST, ALP, and bilirubin
Walle et al., 2022 [49]	Ethiopia	Comparative cross-sectional	63	63	28.1 ± 4.61	27.5 ± 4.77	145.4 ± 8.6 94.5 ± 6.1	103.7 ± 10.2 69.5 ± 7.5	AST, ALT, and bilirubin

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Table 2. Cont.

Author Name and Publication Year	Country	Study Design	PE Group	Normotensive Group	Age (Years) PE Group	Age (Years) Normotensive Group	SBP and DBP (mmHg) PE Group	SBP and DBP (mmHg) Normotensive Group	Markers
Haggai et al., 2022 [44]	Israel	Prospective cohort	36	37	31.17 ± 7.60	28.59 ± 4.77	156.0 ± 14.2 94.25 ± 12.1	117.92 ± 10.8 70.0 ± 8.4	AST and ALT
Zhang et al., 2025 [45]	China	Prospective cohort	1588	35,838	32.4 ± 4.2	32.4 ± 4.2	NR	NR	AST and ALT
Zhestkova et al., 2023 [53]	Russia	Cross-sectional	73	50	30.63 ± 2.39	31.75 ± 2.01	150.23 ± 6.73 93.43 ± 3.06	108 ± 3.45 65 ± 2.9	ALP and bilirubin
Singh et al., 2017 [35]	India	Cross-sectional	70	70	25.6 ± 3.7	25.1 ± 3.9	156.5 ± 18.4 102.0 ± 16.5	117.2 ± 8.9 76.7 ± 4.5	AST, ALT, ALP, and bilirubin
Zhang et al., 2022 [54]	China	Cohort	244	5281	31.9 ± 4.5	30.9 ± 4.0	NR	NR	AST, ALT, and ALP
Narnani and Bhargava, 2019 [36]	India	Comparative cross-sectional	39	100	20–45	NR	NR	NR	AST, ALT, ALP and bilirubin
Obutu et al., 2023 [37]	Nigeria	Cross-sectional	35	35	NR	NR	169.56 ± 20.02 107.45 ± 8.14	117.42 ± 6.01 75.36 ± 8.20	AST, ALT, and ALP
Salman, 2016 [38]	Iraq	Comparative cross-sectional	40	40	NR	NR	158.5 ± 8.92 111.75 ± 7.1	106.75 ± 7.9 73.0 ± 5.16	AST, ALT and bilirubin
Das et al., 2015 [60]	India	Cross-sectional	50	50	NR	NR	164.38 ± 22.04 104.48 ± 12.16	114.72 ± 8.01 72.41 ± 6.28	ALT, AST, ALP, and bilirubin
Roy and Lodhi, 2019 [61]	India	Cross-sectional	30	30	27.53 ± 5.15	29.23 ± 6.08	NR	NR	AST, ALT, and bilirubin
Al-Sultan et al. [62]	Iraq	Comparative cross-sectional	60	35	NR	NR	NR	NR	AST, ALT, and ALP
Afroz et al., 2021 [63]	India	Cross-sectional	50	50	28.0 ± 5.95	26.20 ± 5.32	153.4 ± 16.7 91.7 ± 5.2	113.4 ± 4.5 74.1 ± 6.6	AST and ALT
Saha et al., 2022 [39]	India	Cross-sectional	60	60	32.42 ± 6.45	26.44 ± 8.14	146.32 ± 8.21 94.46 ± 6.84	110.86 ± 12.54 79.44 ± 6.47	AST, ALT, ALP, and bilirubin
Al-Jameil et al., 2015 [64]	Saudi Arabia	Cross-sectional	40	40	31.55 ± 6.14	31.20 ± 5.84	167.0 ± 24.43 98.51 ± 11.16	113.56 ± 13.93 67.66 ± 9.38	AST, ALT, ALP, and bilirubin

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Table 2. Cont.

Author Name and Publication Year	Country	Study Design	PE Group	Normotensive Group	Age (Years) PE Group	Age (Years) Normotensive Group	SBP and DBP (mmHg) PE Group	SBP and DBP (mmHg) Normotensive Group	Markers
Makuyana et al., 2002 [65]	Zimbabwe	Cross-sectional	38	72	27 ± 6	25 ± 6	165 + 20 109 + 13	118 + 11 74 + 8	AST, ALT, ALP, and bilirubin
Hazari et al., 2014 [66]	India	Cross-sectional	40	40	32.42 ± 6.45	25.13 ± 2.34	146.32 ± 8.21 94.46 ± 6.84	110.9 ± 10.4 67.4 ± 4.8	AST, ALP, ALP and bilirubin
Edebiri et al., 2025 [67]	Nigeria	Comparative cross-sectional	40	20	NR	NR	NR	NR	ALT, AST, and ALP

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PE: Pre-eclampsia; NR: Not Reported; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase. Age, SBP, and DBP are reported as means ± SD or range.

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3.3. AST Levels in Pregnant Women with Pre-Eclampsia Versus Normotensive Pregnant Women

A total of 42 studies examined AST levels, including 9256 participants in the PE group and 248,386 subjects in the normotensive group. The analysis employed a random-effects model, revealing a statistically significant difference between the two groups (Figure 3), with an overall SMD of 1.81 (95% CI: 1.51 to 2.10). The overall effect estimates were statistically significant ($p < 0.0001$). However, significant heterogeneity was noted ($p = 0$), with an $I^2 = 99.1\%$.

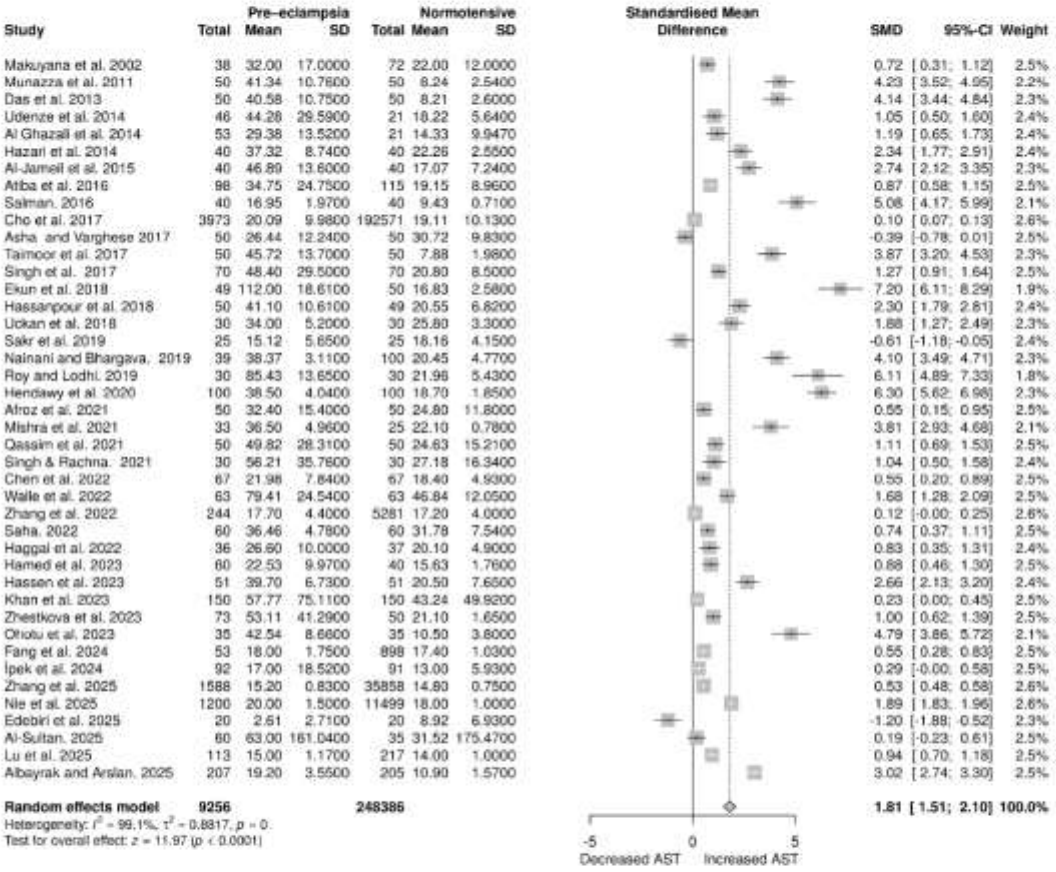

Figure 3. Random effects meta-analysis assessing the impact of PE on aspartate aminotransferase levels [28,29,31–34,36–40,42–72].

3.4. ALT Levels in Pre-Eclampsia Compared to Normotensive

A total of 45 studies examined ALT levels, comprising 9419 participants in the PE group and 248,503 participants in the normotensive group. The analysis employed a random-effects model due to high heterogeneity ($I^2 = 99.3\%$; $p = 0.0$). The results revealed a statistically significant difference between the two groups, with an SMD of 1.73 (95% CI: 1.38 to 2.07), $p < 0.0001$, as shown in Figure 4.

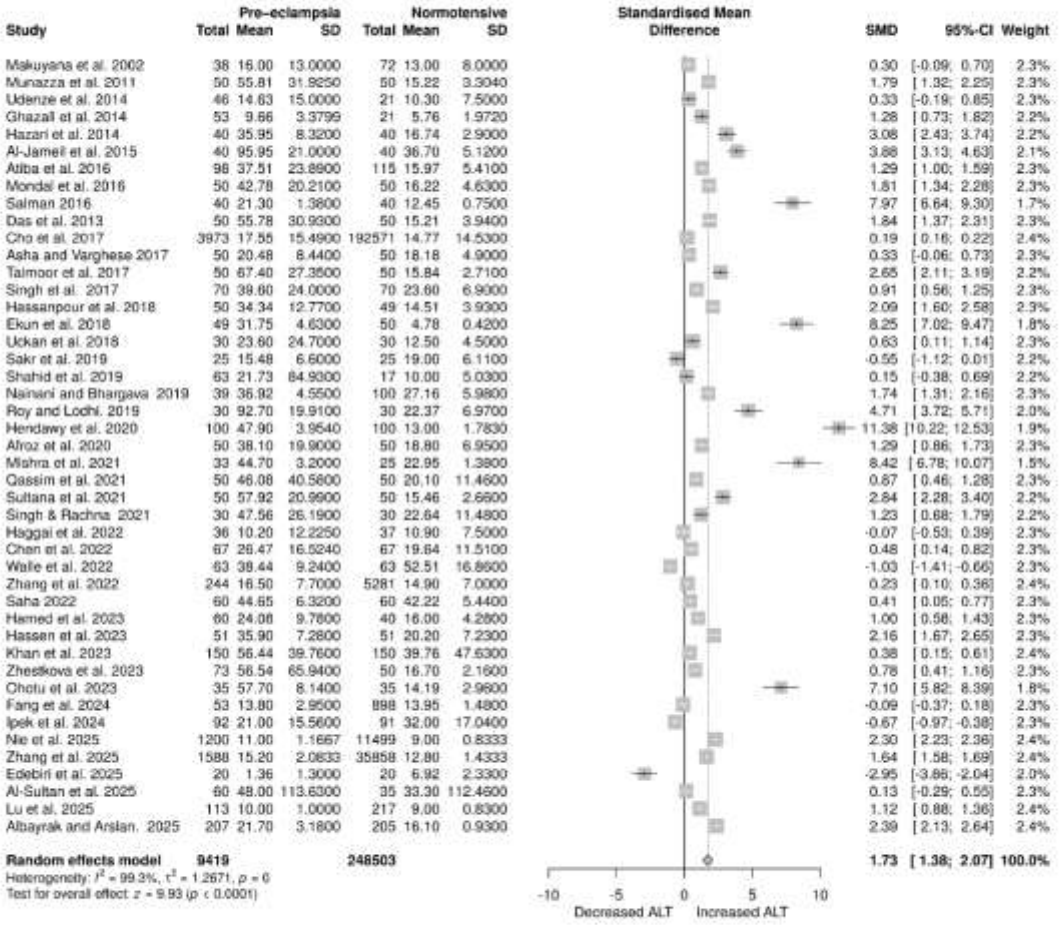

Figure 4. Random effects meta-analysis assessing the impact of PE on alanine aminotransferase levels [28–72].

3.5. ALP Levels in Pre-Eclampsia Compared to Normotensive Pregnant Women

Twenty-four studies analysed ALP levels, comprising 3069 participants in the PE group and 43,318 subjects in the normotensive group. The random effects model meta-analysis revealed an increase in ALP, with an SMD of 1.43 (95% CI: 0.97 to 1.88), as shown in Figure 5. The overall effect results demonstrated a statistically significant difference between the groups ($p < 0.0001$). However, substantial heterogeneity was observed ($p < 0.01$), with an I^2 value of 98.3%.

3.6. Total Bilirubin Levels in Pre-Eclampsia Versus Normotensive Pregnant Women

In total, 24 studies examined total serum bilirubin (TSB) levels, including 2392 pregnant women with PE and 13,612 normotensive women. A random-effects meta-analysis was employed, and the effect estimates revealed an increased TSB (SMD = 0.62, 95% CI: 0.36 to 0.88). The overall effect was statistically significant ($p < 0.0001$). Significant heterogeneity was observed ($p < 0.0001$), with an I^2 value of 93.6% (Figure 6).

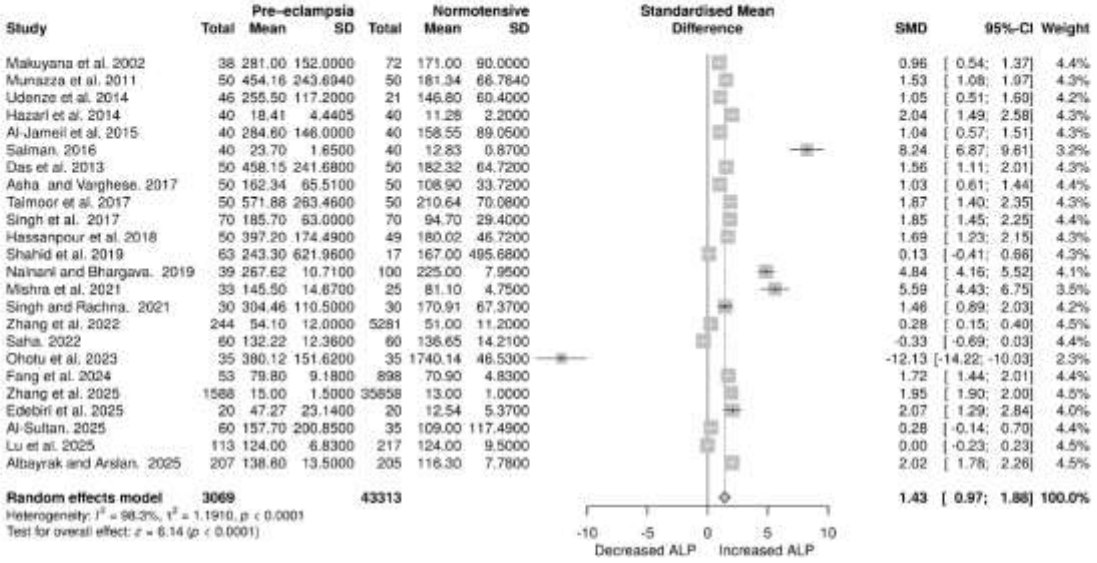

Figure 5. Random effects meta-analysis assessing the impact of PE on alkaline phosphatase levels [33,37,41,43,47,48,50,52,54–60,62,64–67,69–72].

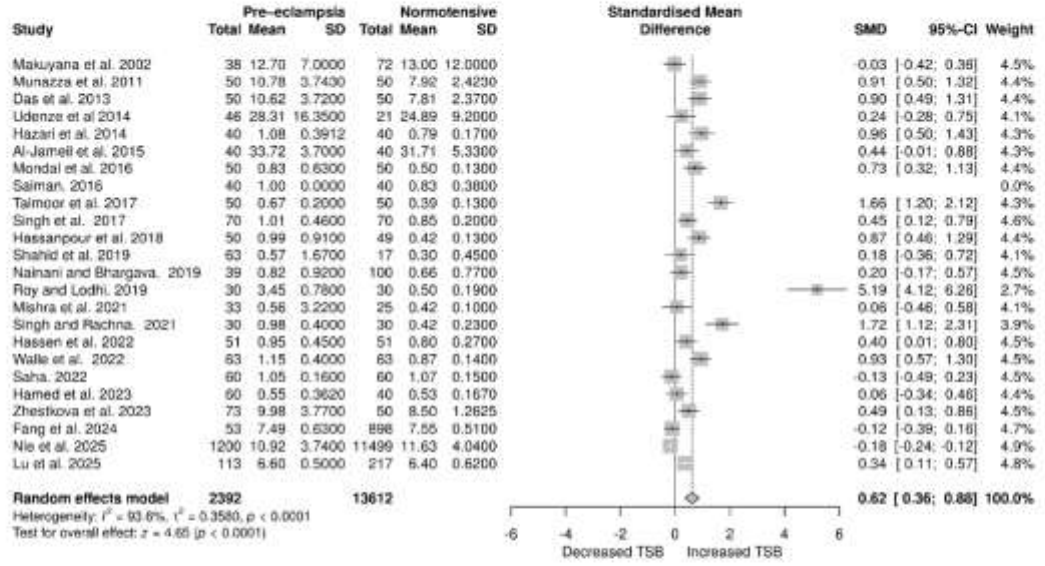

Figure 6. Random effects meta-analysis assessing the impact of PE on total serum bilirubin levels in pregnant women [29,30,33,37,40,41,46–50,52,53,55,56,58–61,64–66,70,72].

3.7. Publication Bias Assessments

This meta-analysis included more than 10 studies, allowing for the assessment of publication bias. Funnel plot inspection, Egger's and Beggs' tests indicated potential publication bias for AST levels (Figure 7A) (E value = 9.596, $p < 0.001$) and Begg and Mazumdar's rank correlation (value = 0.422, $p < 0.001$). Moreover, the trim-and-fill value was 0.00, with a fail-safe N of 42,802 and $p < 0.001$. Similarly, for ALT (Figure 7C), the Egger regression test suggested evidence of publication bias (value = 10.430, $p < 0.001$),

and the Beggs' value was 0.335 ($p = 0.001$). The Fail-Safe N value was 51,532, $p < 0.001$, whereas the trim and fill value was 0.00. Likewise, for TSB levels, funnel plot inspection (Figure 7B), Egger regression (value = 6.172, $p < 0.001$), and Beggs's test (value = 0.297, $p = 0.044$) all indicated publication bias. Moreover, the evidence showed a significantly high fail-safe N value (215,000; $p = 0.001$) and a trim-and-fill value of 0.00. In contrast, for ALP, visual inspection of the funnel plot suggested no evidence of publication bias (Figure 7D). This is confirmed statistically by the Egger regression test ($p = 0.05$, regression coefficient = −0.2009) and the Beggs test ($p = 0.131$, test statistic = 0.225). The fail-safe N value was 3965, with a trim-and-fill of 0.000. These results altogether suggest that the pooled effect size from the ALP meta-analysis is robust and unlikely to be substantially influenced by publication bias.

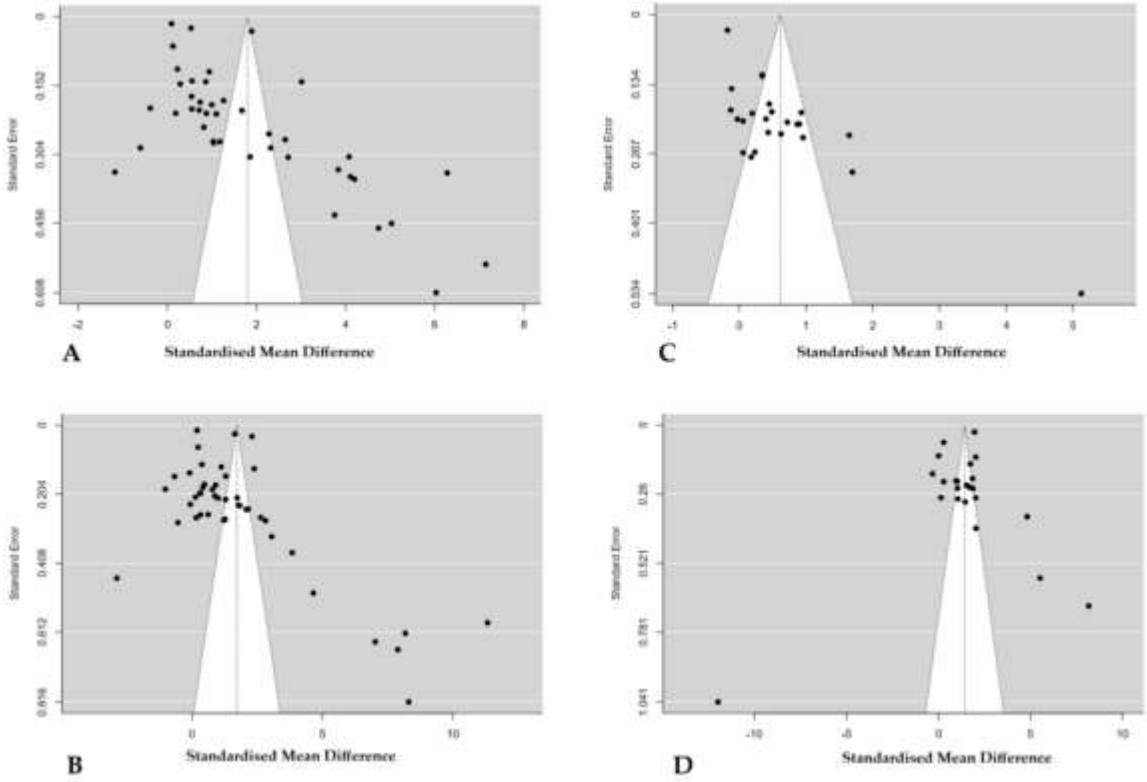

Figure 7. Funnel plots illustrate the possible publication bias among the studies included in the meta-analysis. (**A**) Studies that analysed AST levels in pregnant women with PE and normotensive pregnant women. (**B**) Studies that analysed total bilirubin levels in pregnant women with PE and normotensive pregnant women. (**C**) Studies that analysed ALT levels in pregnant women with PE and normotensive pregnant women. (**D**) Studies that analysed ALP levels in pregnant women with PE and normotensive pregnant women. The black dot indicates an individual study. A gray colour region indicates a pseudo-confidence interval region.

3.8. Subgroup Analysis

A subgroup analysis was conducted to identify sources of heterogeneity in AST, ALT, ALP, and bilirubin levels by study design, maternal age, period of gestation during diagnosis of PE, continent, study quality, and BMI, as presented in Supplementary File S2, Table S1. For the AST subgroup, factors including study design, quality, continent, BMI,

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gestational duration, and maternal age did not account for the observed heterogeneity, as they did not reduce heterogeneity (Supplementary File S2, Table S1). Similarly, for ALT and ALP, none of these factors altered heterogeneity. In contrast, in the TSB subgroup, we found that study design, particularly cohort ($I^2 = 0\%$) and gestational age at diagnosis ($I^2 = 51.1\%$), were potential sources of observed heterogeneity.

3.9. Meta-Regression

The meta-regression output is presented in Table 3. For AST, among these moderators, study design ($\beta = -0.630$, $p < 0.001$) was the significant moderator. Additionally, the significant positive regression coefficient ($\beta = 1.084$, $p < 0.001$) indicates that for every 1-unit increase in the quality score, the effect size increases by 0.761 on average (Table 3). Therefore, there was no evidence that maternal age, gestational age at diagnosis, continent, or BMI explained the heterogeneity observed for AST. Similarly, we found that study design ($\beta = 1.084$, $p < 0.019$) and quality ($\beta = 0.729$, $p = 0.039$) were significant moderators of the ALT effect size. Moreover, the significant negative regression coefficient on ALT BMI ($\beta = -0.406$, $p = 0.006$) suggests that for every 1-unit increase in BMI, the effect size decreases by 0.406 units. For ALP, maternal age ($\beta = -0.720$, $p = 0.010$), quality ($\beta = 1.444$, $p = 0.003$), and continent ($\beta = 2.07$, $p < 0.0001$) were significant moderators. For TSB, study design ($\beta = -0.384$, $p = 0.004$), quality ($\beta = 0.761$, $p < 0.001$), and maternal age ($\beta = -0.304$, $p = 0.019$) were significant moderators.

Table 3. Meta-Regression Examining the Influence of Moderator Variables on the Effect Estimates.

Outcomes	Moderators	β	se	p	Lower CI	Upper CI
AST	Intercept	2.827	0.335	<0.001	2.170	3.484
	Study design	−0.630 *	0.183	<0.001	−0.989	−0.270
	Intercept	1.7535	0.424	<0.001	0.923	2.584
	Maternal age	0.0198	0.172	0.909	−0.318	0.358
	Intercept	2.306	0.322	<0.001	1.675	2.936
	BMI	−0.281	0.157	0.075	−0.589	0.028
	Intercept	2.078	0.2617	<0.001	1.565	2.591
	Gestation age	−0.120	0.0910	0.187	−0.298	0.058
	Intercept	0.182	0.470	0.698	−0.739	1.103
	Quality	1.084 *	0.299	<0.001	0.498	1.670
	Intercept	2.131	0.566	<0.001	1.021	3.241
	Continent	−0.179	0.293	0.542	−0.753	0.395
ALT	Intercept	3.01	0.573	<0.001	1.884	4.131
	Study design	−1.08 *	0.459	0.019	−1.979	−0.180
	Intercept	1.443	0.451	0.001	0.558	2.327
	Maternal age	0.137	0.203	0.499	−0.260	0.534
	Intercept	2.454	0.318	<0.001	1.831	3.078
	BMI	−0.406 *	0.147	0.006	−0.694	−0.117
	Intercept	1.340	0.363	<0.001	0.628	2.052
	Gestation age	0.228	0.190	0.231	−0.145	0.601
	Intercept	0.653	0.547	0.232	−0.419	1.725
	Quality	0.729 *	0.353	0.039	0.037	1.421
	Intercept	1.8034	0.650	0.006	0.529	3.078
	Continent	−0.0452	0.338	0.894	−0.709	0.618

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Table 3. *Cont.*

Outcomes	Moderators	β	se	p	Lower CI	Upper CI
ALP	Intercept	1.816	0.583	0.002	0.673	2.958
	Study design	−0.231	0.297	0.435	−0.813	0.350
	Intercept	3.027	0.673	<0.001	1.709	4.345
	Maternal age	−0.720 *	0.278	0.010	−1.266	−0.175
	Intercept	1.689	0.539	0.002	0.633	2.745
	BMI	−0.177	0.286	0.535	−0.737	0.383
	Intercept	0.993	0.490	0.043	0.032	1.954
	Gestation age	0.220	0.226	0.330	−0.223	0.663
	Intercept	−0.705	0.753	0.349	−2.181	0.772
	Quality	1.444 *	0.488	0.003	0.488	2.400
	Intercept	−2.14	0.938	0.022	−3.982	−0.305
	Continent	2.07 *	0.528	<0.001	1.032	3.104
Bilirubin	Intercept	1.189	0.234	<0.001	0.730	1.647
	Study design	−0.384 *	0.134	0.004	−0.647	−0.122
	Intercept	1.237	0.294	<0.001	0.660	1.814
	Maternal age	−0.304 *	0.129	0.019	−0.558	−0.050
	Intercept	0.856	0.277	0.002	0.312	1.399
	BMI	−0.142	0.145	0.328	−0.427	0.143
	Intercept	0.5900	0.248	0.017	0.104	1.075
	Gestation age	0.0151	0.135	0.911	−0.250	0.280
	Intercept	−0.607	0.338	0.073	−1.269	0.056
	Quality	0.761 *	0.206	<0.001	0.358	1.164
	Intercept	−0.0808	0.662	0.903	−1.379	1.217
	Continent	0.3815	0.354	0.282	−0.313	1.076

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; CI: confidence interval; se: standard error; *: shows statistically significant effect.

3.10. The Sensitivity Analysis for Robustness and Stability of Effect Size

For AST, excluding four studies individually resulted in a change in effect size. Briefly, the exclusion of Salman [58] led to SMD = 1.74, 95% CI (1.44 to 2.03, $p < 0.0001$), Ekun [68], SMD = 1.70, 95% CI (1.41 to 1.99, $p < 0.0001$), Roy and Lodhi [61], SMD = 1.73, 95% CI (1.43 to 2.02, $p < 0.0001$) and Hendawy [38], SMD = 1.70, 95% CI (1.41 to 1.99, $p < 0.0001$). For ALT, six studies changed the effect size. The exclusion of Ohotu [57] changed effext to SMD = 1.65, $p < 0.0001$; Edebiri [67], SMD = 1.82, $p < 0.0001$; Mishra [33], SMD = 1.62, $p < 0.0001$; Hendawy [38], SMD = 1.54, $p < 0.0001$; Ekun [68], SMD = 1.060, $p < 0.0001$; and Salman [58], SMD = 1.61, $p < 0.0001$. We also noted that excluding only 4 of 25 studies for ALP changes the effect size. For instance, the effect changed for the exclusion of Salman [58] SMD = 1.21 (0.76 to 1.65, $p < 0.0001$), Mishra [33], SMD = 1.28, 95% CI (0.82 to 1.73, $p < 0.0001$); Ohotu [57], SMD = 1.73, 95% CI (1.30 to 2.17, $p < 0.0001$); Nainani [56], SMD = 1.28, 95% CI (0.83 to 1.73, $p < 0.0001$). For TSB, only excluding the Roy and Lodhi (2019) study [61] changed the effect size to SMD = 0.49 (0.26 to 0.72, $p < 0.0001$), a 21% decrease from the initial effect in the same direction.

3.11. Quality Assessment of Included Observational Studies

The quality of observational studies is presented in Supplementary File S2, Tables S2–S4. For the seven evaluated cohorts, six studies received 10 stars across all domains, and one study received 8 stars due to poor reporting of the adequacy of follow-up; however, all were rated as high quality (Supplementary File S2, Table S2). In 26 cross-sectional studies, 20 scored 7 stars, and one scored 5 stars [68], all of which were considered of moderate quality. Four studies received 8 stars, and 1 study scored 9 stars; thus, all

were rated as high quality (Supplementary File S2, Table S3). For case–control studies, 11 studies scored 8–10 stars and were regarded as high quality, except for a study by Asha and Varghese (2017) [69], which was rated 6/10, indicating moderate quality (Supplementary File S2, Table S4). Overall, 51% of the studies were rated as high quality and 49% rated as moderate quality.

4. Discussion

This systematic review and meta-analysis evaluated data from 45 observational studies investigating the impact of PE on maternal liver function by analysing four biochemical markers: AST, ALT, ALP, and TSB. The results demonstrate that pregnant women with PE have elevated levels of liver function enzymes when compared to normotensive pregnancies. These results suggest that PE may impair hepatic function and induce hepatocellular stress in pregnancy. Our findings are consistent with evidence from previous studies, which show a consistent trend in the association between PE and liver dysfunction [29,31,32,40,72]. This is also confirmed by HIV patients, irrespective of treatment [73,74]. The overall data demonstrated statistically significant increases in AST, ALT, ALP, and bilirubin levels in women with PE, demonstrating hepatocellular damage and supporting the notion that systemic inflammation and endothelial dysfunction impair hepatocyte function [75].

Although the mechanism by which PE initiates liver dysfunction is complex, emerging evidence distinguishes two primary pathogenic subtypes of PE, with distinct mechanisms promoting liver dysfunction that depend on the stage of PE: early- and late-onset PE [76]. Early-onset PE is associated with defects in trophoblast invasion, resulting in abnormal placentation, placental ischaemia, and endothelial injury [76,77]. It's essential to note that the trophoblast plays a crucial role in the attachment of the developing embryo to the endometrium, providing protection and forming part of the placenta [78]. However, any impairment in its function would disrupt all these delicate functions. On the other hand, defects in the trophoblast cause an imbalance in the production of angiogenic and antiangiogenic factors. Whereby, the release of antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 receptor (sFlt-1), is increased compared to angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PGF) [79–81]. The imbalance between angiogenic and anti-angiogenic factors ultimately leads to endothelial dysfunction, which affects maternal organs, including the heart, liver, and kidneys, and, in severe cases, the brain (Figure 8). On the other hand, late-onset PE is associated with maternal metabolic dysfunction and systemic inflammation. Therefore, these contrasting pathways suggest that liver dysfunction in early-onset PE may arise due to placental ischemia-induced hepatic endothelial damage [74,82–84], whereas in late-onset PE, maternal metabolic abnormalities and chronic inflammation may exert a greater influence.

Endothelial dysfunction suppresses the release of vasodilators, such as prostacyclin, and promotes the release of vasoconstrictors, like thromboxane [85]. The activity promotes vasoconstriction of hepatic blood vessels, thereby inducing hypoxia, necrosis, and hepatocyte degeneration. This subsequently increases the levels of AST and ALT in the blood, both of which reflect liver injury [17]. The significant increase in ALP found in PE women likely indicates both hepatic impairment and placental involvement, as placental isoforms of ALP are physiologically elevated during pregnancy [86,87]. Therefore, PE-induced liver dysfunction is mediated by endothelial injury and inflammation that impair hepatic function [88,89]. Furthermore, vascular damage in PE impairs normal hepatic blood flow, leading to hepatocyte injury and reduced liver function, including bilirubin processing and clearance. For example, other studies have shown reduced haemoglobin levels in PE compared to controls [86,87], suggesting that haemoglobin may have been broken down through haemolysis, as reflected in elevated blood bilirubin levels. Previous reports have

shown a substantial increase in red blood cell breakdown in severe cases, such as HELLP syndrome, resulting in elevated circulating bilirubin levels [90]. Altogether, impaired hepatic clearance and increased haemoglobin breakdown resulted in the accumulation of serum bilirubin in PE [9,17,48,52]. Our results are consistent with previous reports that showed higher bilirubin levels in PE compared with normotensive individuals [50,52,91]. This activity is associated with an increased rate of haemolysis (Figure 9).

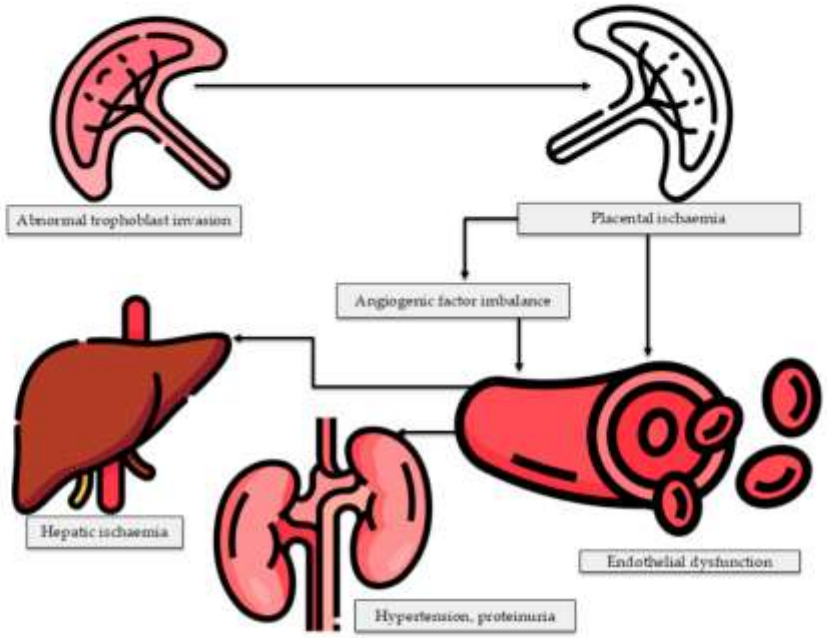

Figure 8. Schematic depiction of the disparity between angiogenic and anti-angiogenic factors resulting in endothelial dysfunction. This malfunction leads to multi-organ involvement in pre-eclampsia, impacting essential maternal organs such as the liver, kidney, and brain. Figure created using FLATICON and Microsoft PowerPoint.

Indeed, elevated AST, ALT, ALP, and bilirubin in PE indicate compromised hepatic clearance or increased haemolysis, both of which suggest liver injury and dysfunction.

This study has several strengths, the main one being that it is the first meta-analysis to examine the impact of PE on liver function in pregnant women. Additionally, it has employed an extensive literature review, with a substantial sample size, and strict adherence to the MOOSE guideline. The studies included were of good quality, as none were rated as poor on the Newcastle–Ottawa Scale. By integrating data from multiple countries (Figure 2), it provides a comprehensive overview of PE's involvement in hepatic health during pregnancy on a global scale. Interestingly 52% and 48% of studies were of higher and moderate quality, respectively. Nevertheless, limitations must also be acknowledged. The evidence presented substantial heterogeneity across all outcomes. However, subgroup analysis and meta-regression were thoroughly conducted to identify sources and their association with the effect size. To some extent, variation in outcomes was attributed to the study design, particularly the cohorts and the gestational age at diagnosis of PE (more than 30 weeks), which were noted as potential sources of heterogeneity for bilirubin. However, for other outcomes (AST, ALT, and ALP), the variation could not be explained through the subgroup. A thorough meta-regression showed an association between moderators and effect sizes across outcomes. We noted publication bias, as evidenced by funnel

plot asymmetry, Egger's regression, Beggs's test, trim-and-fill, and fail-safe n assessment. Not all studies documented baseline data, such as blood pressure, BMI, maternal age, and gestational age at the time of PE diagnosis, which remain critical for PE diagnosis. The information about the assay method used to assess these liver enzyme tests is limited; therefore, it was not used for subgroup analysis or meta-regression. As evidence is gathered from observational studies, it is essential to interpret the results with caution, as they do not establish causality and are susceptible to confounding, selection bias, and measurement bias. Moreover, for cross-sectional studies, while they may suggest an association, they fail to assess incidence or risk.

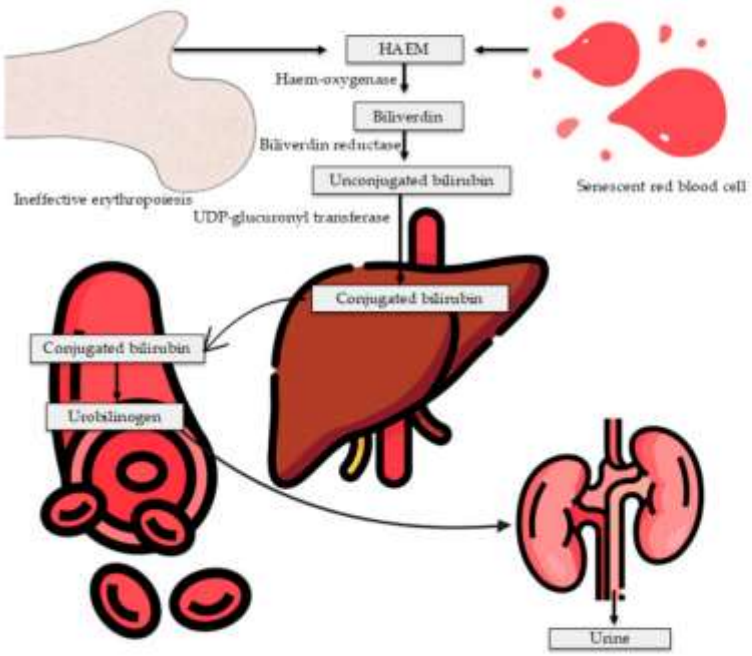

Figure 9. A. Schematic depiction of how ineffective erythropoiesis and aging red blood cells contribute to the elevation of bilirubin. UDP: uridine diphosphate. Figure created using FLATICON and BIOICONS.

5. Conclusions

This meta-analysis reveals that PE induces liver dysfunction during pregnancy, as evidenced by significant elevations in liver function tests, including AST, ALT, ALP, and total bilirubin. These findings highlight the importance of monitoring liver function in pregnancy, especially if this is associated with hypertensive disorders. However, substantial variability and publication bias warrant careful interpretation. Based on evidence from observational studies, we recommend that future research focus on high-quality studies, including clinical trials, to investigate the potential treatment of liver dysfunction in PE during pregnancy and to prevent severe maternal and foetal complications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life16020223/s1, Supplementary File S1: MOOSE Checklist; Supplementary File S2: Table S1: Subgroup analysis showing the effect of different factors on liver function, Table S2: Quality assessment of Cohort Studies, Table S3: Quality assessment of Cross-sectional studies, Table S4: Quality assessment of case–control studies.

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Conflicts of Interest: The authors declare no conflict of interest.

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Chapter 2.3 Systematic Review and Meta-Analysis

This chapter is formatted as a published article entitled, “HIV Infection and Antiretroviral Therapy Impair Liver Function in People Living with HIV: Systematic Review and Meta-Analysis”

Published in Pharmaceuticals: link: https://doi.org/10.3390/ph18070955, and aligned with the theme of the research, expanding the population beyond pregnant women, to also include males, and non-pregnant women. This systematic review and meta-analysis examined the effects of HIV infection and ART on liver function in people living with HIV (PLHIV). The results showed an increase in AST and ALT in naïve PLWH compared to HIV-negative individuals. Elevated AST, ALT, and ALP were also observed in ART-exposed PLWH compared to HIV-negative individuals. However, no significant difference was found in ALP between ART-naïve and HIV-negative individuals. The study highlights the dual risk posed by HIV infection and ART exposure on liver function in PLHIV.

Student contribution: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing – original draft preparation, writing – review and editing, visualization.

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Systematic Review

HIV Infection and Antiretroviral Therapy Impair Liver Function in People Living with HIV: Systematic Review and Meta-Analysis

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Abstract

Background: The use of antiretroviral therapy (ART) has improved the lives of people living with HIV (PLWH). However, its use is associated with secondary complications, notably hepatotoxicity. This systematic review and meta-analysis assess the effects of HIV infection and ART on liver function in PLWH. **Method:** A comprehensive literature search was performed in PubMed, Scopus, and Google Scholar from inception to 12 February 2025. Studies analyzing liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in PLWH undergoing ART, those who are ART-naïve, and HIV-negative individuals were considered. Data analysis was performed using a meta-analysis web tool, and the results were reported as standardized mean differences (SMDs) and 95% confidence intervals (CIs). **Results:** Twenty-six studies were included in the meta-analysis. The findings showed an increase in AST, SMD = 1.85 (0.93 to 2.78, $p < 0.0001$, $I^2 = 93.8\%$), and ALT, SMD = 2.65 (1.25 to 4.04, $p = 0.0002$, $I^2 = 97.8\%$) in PLWH who were naïve compared with those who were HIV negative. Additionally, there was a pronounced elevation in AST, SMD = 1.49 (0.48 to 2.50, $p = 0.0038$, $I^2 = 98\%$); ALT, SMD = 2.30 (1.14 to 3.45, $p < 0.0001$, $I^2 = 98\%$); and ALP, SMD = 1.40 (0.55 to 2.26, $p < 0.01$, $I^2 = 97\%$) in PLWH exposed to ART compared with HIV-negative individuals. However, there was no significant difference in ALP, SMD = 0.53 (–0.92 to 1.98, $p = 0.4726$, $I^2 = 98\%$) between PLWH who were ART-naïve and HIV-negative individuals. **Conclusions:** The results show that HIV infection and ART administration are associated with elevated liver function test enzymes, suggesting that each may contribute to liver dysfunction among PLWH. These results highlight the dual risk posed by HIV infection and ART exposure.

Keywords: antiretroviral therapy; AST; ALT; ALP; hepatotoxicity; HIV; liver function; systematic review; meta-analysis

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1. Introduction

The human immunodeficiency virus (HIV) targets human CD4 cells, thereby impairing the immune system and thus making individuals susceptible to infections [1–3]. If left untreated, HIV can progress into the advanced stage, acquired immune deficiency syndrome (AIDS) [2]. Interestingly, the introduction of antiretroviral therapy (ART) has improved the quality of life among people living with HIV (PLWH) [4]. ART is a group of HIV medications that improves the immune system and reduces morbidity and mortality by suppressing HIV replication [4]. However, many ART regimens are associated with

adverse events, including hepatotoxicity and virologic failure, which can lead to liver injury or dysfunction [5,6]. This, in turn, raises concerns about the impact of prolonged exposure to ART on the overall health of the liver in PLWH.

Existing evidence has highlighted the hepatotoxic effects of a specific ART. For instance, a previous study has shown that the use of nevirapine-based therapy is associated with severe liver abnormalities [7]. It is worth noting that while newer ART regimes such as dolutegravir (DTG) have proven to reduce viral levels and stabilize CD4 count effectively, their overall effect on liver function in PLWH is concerning, especially if there is a co-infection with the hepatitis virus [8–10]. Among those with co-infection, the liver-related mortality rate is increasing compared with HIV-monoinfected individuals [11]. Other researchers suggest that the duration of ART exposure is a key factor, with prolonged exposure contributing to liver damage and dysfunction [12–14].

The liver is a vital organ in the metabolism and excretion of drugs, making it susceptible to the effects of medications, including ART [15]. This can result in liver dysfunction, subsequently leading to secondary complications due to impaired hepatic clearance and metabolic function [16]. Therefore, understanding the relationship between ART use and liver function is crucial for maximizing the treatment of HIV infection and reducing the likelihood of secondary complications. Liver function is evaluated by using biochemical markers, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and their elevation indicates hepatic impairment [17,18]. Previous evidence has reported abnormal liver enzymes in PLWH on highly active antiretroviral therapy (HAART) regimens, even in the absence of hepatitis C virus (HCV) or hepatitis B virus (HBV) co-infections [19]. HAART consists of a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and one drug from another class, such as non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTI), protease inhibitors (PIs), or integrase-nucleoside strand transfer inhibitors (INSTIs) [20,21]. All these ART regimens have different effects on the overall hepatic health [22,23]. For instance, NNRTIs, such as nevirapine, are associated with hepatic toxicity [24,25]. On the other hand, long-term exposure to NNRTIs is not associated with the risk of hepatotoxicity [26]. This provides an unclear picture of the overall effect of these NNRTIs on hepatic health, particularly in PLWH. NRTIs, such as zidovudine and didanosine, inhibit the enzyme mitochondrial polymerase gamma, thus impairing DNA synthesis [27,28]. This activity results in the accumulation of toxic products that present as hepatic steatosis, lactic acidosis, and liver failure [29]. Surprisingly, NRTI taken as pre-exposure prophylaxis (PrEP) even in healthy individuals presents with mitochondrial toxicity [30]. This suggests that NRTI could increase the risk of mitochondrial oxidative stress and hepatic health in those living with HIV.

Notably, while liver enzyme abnormalities are frequently observed in PLWH on ART, other studies have reported increased liver enzymes in PLWH who are ART-naïve [5,31]. Existing evidence has demonstrated a slight increase in liver enzyme abnormality in HAART-naïve individuals compared with PLWH on HAART regimens [5]. This suggests that hepatic dysfunction in PLWH may be attributed to factors other than ART exposure. In 2016, Shiferaw and colleagues reported an association of liver enzyme elevation, mainly AST and ALT, with CD4 count, male sex, opportunistic infections, and viral hepatitis [5]. Although some studies suggest that ART exerts a hepatoprotective effect [32,33], this claim remains inconclusive due to conflicting evidence. Other studies found no effect of HIV or ART on liver function [34,35], while others reported an increase in liver enzymes, suggesting an undesirable effect on hepatic health [36–38]. On the other hand, other researchers reported a reduced activity of ALP, AST, and ALT during the early and late stages of ART treatment [32,33]. This complex relationship makes it difficult to distinguish

between liver dysfunction induced by HIV infection and that induced by ART exposure in PLWH. Given these inconsistencies, it is important to evaluate the overall effect of HIV and ART on liver function among PLWH by focusing on the main liver enzymes such as AST, ALT, and ALP.

- Aim and Objectives

This study aimed to evaluate the overall effect of HIV and ART on liver function among PLWH.

- Objectives

To determine AST, ALT, and ALP levels in HIV individuals on ART and those who are ART-naïve compared with HIV-negative individuals.

2. Materials and Methods

2.1. Information Sources, Search Strategy

This study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [39] (Supplementary File S1). The protocol was registered through the Open Science Framework (OSF) (https://doi.org/10.17605/OSF.IO/B8JHF) to ensure transparency. A thorough literature search was conducted independently by K.E.S. and K.M. on PubMed and Scopus utilizing Medical Subject Headings (MeSH) terms. The MeSH terms employed for the search included "HIV", "Human Immunodeficiency Virus", "ART", "Antiretroviral Therapy", and "Liver Function Test." In addition, the relevant Boolean operators "OR" and "AND" were used to build the search. Additionally, Google Scholar was also used to search for relevant studies.

2.2. Eligibility Criteria and Selection Process

Studies were considered relevant and included if they satisfied the following PICOS criteria. The study population (P) consisted of PLWH (adolescents and adults); intervention groups (I) were either HAART/ART or ART-naïve; the control group (C) was HIV negative; and the outcome of interest (O) was liver function, measured using surrogate markers such as AST, ALT, and ALP. The study design (S) included cross-sectional, case control and cohorts. Studies were excluded if they were conducted on HIV patients without ART exposure, without an HIV negative group as a control, studies in animals, or those not reporting any marker of liver function. This process was undertaken by two independent researchers (K.-L.E.S. and K.M.) by screening the title and abstract, followed by full text screening. To resolve any disagreement and ensure accuracy, a third independent researcher (W.N.P.) participated in the screening and selection process.

2.3. Data Items and Extraction

Two independent researchers (K.-L.E.S. and K.M.) used an Excel spreadsheet to extract data from individual studies. The two spreadsheets were compared, and if there was disagreement about variables and a study, a third independent researcher, W.N.P., was invited to assess the study and the variables in question before making a conclusion. The primary data items extracted from each study included the main author's family name, country of publication, study design, population size, age of participants, gender (number of males and proportion) in the ART group, ART and duration of intervention, class of ART, CD4 counts, findings, mean, standard deviation (SD), and sample size of AST, ALT, and APL.

2.4. Quality Assessment and Risk of Bias

The methodological quality was assessed following the Newcastle–Ottawa Scale (NOS) [40]. The scale comprises four main domains, such as selection, comparability, and exposure. Within each domain, a couple of items were considered, and these were rated with stars. Studies that received 7–9 stars were regarded as high quality (low risk of bias), 4 to 6 stars were classified as moderate quality (moderate risk of bias), and those between 0 and 3 stars were considered low quality (high risk of bias).

2.5. Synthesis Method and Statistical Analysis

Meta-analysis was performed when more than two studies reported the same outcomes (AST, ALT, and ALP). An online meta-analysis web tool, freely accessible, was utilized for data analysis [41]. We determined the effect estimates for all liver enzymes by computing the mean, SD, and sample size from each group. Where median and range were reported in the original study instead of mean and SD, we estimated the mean and SD from the median and range following the guidelines established by Hozo et al., 2005 [42]. In instances where the original study provided the standard error of the mean (SEM) instead of SD, we calculated the SD using the formula SD = SEM $\times \sqrt{n}$, where n is the sample size of the specific group (ART, ART-naïve, or HIV negative) [43]. In studies with several ART arms, we adopted the Cochrane method to combine the data sequentially into one treatment group (accessed 12 February 2025) (https://www.statstodo.com/CombineMeansSDs.php). For effect size, the standardized mean difference (SMD) was estimated using Hedges g or Cohen's D approach based on the number of studies. When the number of studies was $\leq$20, Hedges g was used due to its reliability. For analysis with more than 20 studies, Cohen's d was preferred. The SMD was then interpreted as follows: 0.2 and 0.5 were considered small and medium effects, respectively. Meanwhile, 0.8 and above were considered a large effect size. We utilized the I^2 statistic test to evaluate statistical heterogeneity [44]. The I^2 values of $\leq$50% and $\geq$75% were classed as low and substantial statistical heterogeneity, respectively. In the case of high heterogeneity, subgroup analysis was performed based on study design, continent of publication, sample size, gender distribution, and class of ART. Publication bias was evaluated graphically through funnel plots and statistically through Egger's test, and an Egger *p*-value of less than 0.05 supported the presence of bias [45]. The trim and fill method was used to adjust for publication bias. Sensitivity analysis was also conducted to evaluate the stability of the effect size by removing one study at once and re-analyzing the overall effect. *p*-Values of less than 0.05 were deemed statistically significant.

3. Results

3.1. Literature Search and Study Selection

Our initial search across the PubMed and Scopus databases yielded 278 records. Additionally, we searched for studies on Google Scholar, and 26 relevant studies were identified. Initially, using an Excel sheet, we identified and removed 5 duplicate records from both databases. After screening titles, abstracts, and keywords, we found 2 records irrelevant to our research question.

Consequently, 299 records remained and underwent independent screening by K.-L.E.S. and K.M. Of the latter records, 271 were excluded for various reasons, including studies in animal models, studies in children, graphical presentation of data, hepatitis co-infection, no ART regimens, no HIV diagnosis, no liver function tests as outcomes of interest, study not published in English, and review studies. Only 26 studies [32,34–38,46–65] were found to be relevant to this study (Figure 1).

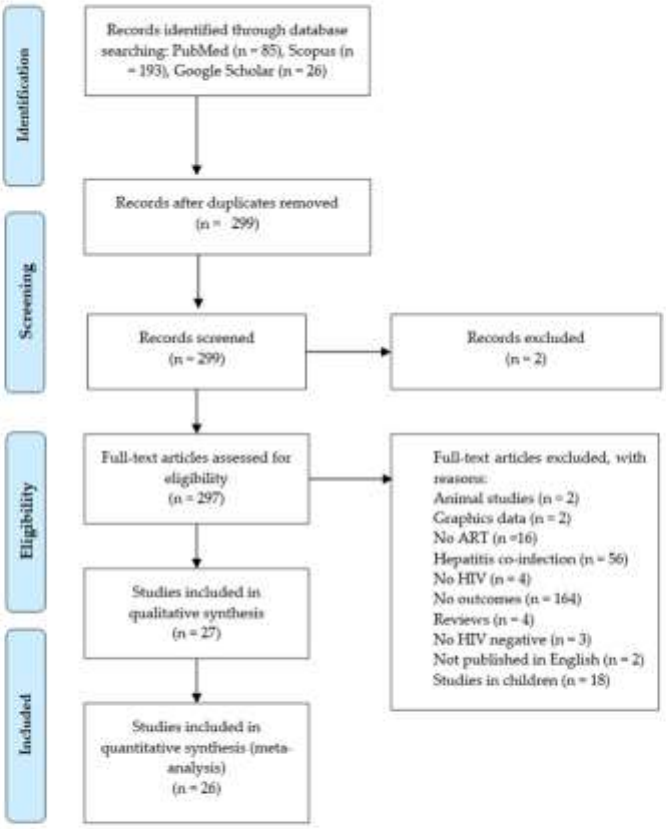

Figure 1. A PRISMA flow diagram showing the study selection procedure.

3.2. The Demographic Features of the Studies

We examined data from 26 studies [32,34–38,46–65], published from 2007 to 2024, assessing the impact of ART on liver function in PLWH (Table S1). The sample sizes exhibited considerable variability across studies, ranging from small samples [62] to larger cohorts [38]. The total sample size comprised 4139 individuals, where all cohorts within the research comprised PLHIV on ART, ART-naïve people, and HIV-negative individuals. The research investigated the effects of ART on many demographic groups, including PLWH, ART-naïve individuals, HAART-naïve individuals, HIV-negative controls, pregnant women, and children affected by HIV. The published studies employed diverse research methodologies, such as case control [34,52,58,62,63] and cohorts [46,48,51], with most studies fulfilling the requirements of a cross-sectional design [32,34–38,47,49,50,53–57,59–61,64,65]. The evidence was scattered across six countries, with the majority carried out in Nigeria [32,34,37,38,47–49,51,54,55,57,60–62,64,65], succeeded by four in India [35,50,53,63], and one each in Brazil [46], Cameroon [36], Ghana [56], Kenya [59], and Libya [58]. The substantial quantity of Nigerian studies signifies a considerable emphasis on ART-related studies in the country. The age of the participants of the included studies ranged between 16 and 70 years, and the duration of ART treatment differed among studies, with some being short-term [48] and others being long-term [37].

3.3. Quality Assessment and Risk of Bias

The result of quality assessment and risk of bias among the included studies is presented in Supplementary File S2, Tables S2–S4. All cohort studies received 6 stars and were thus rated to be of moderate quality and risk of bias (File S2, Table S2). Cross-sectional studies varied in terms of quality and risk of bias, with 10 studies scoring 7 stars and thus classified as high quality and low risk of bias (File S2, Table S3), while case control studies received scores of 7 to 8 stars, reflecting high quality and low risk of bias (File S2, Table S4).

3.4. The Effect of HIV and ART on Aspartate Aminotransferase

Seventeen studies [32,34–36,47,49–52,54–56,58–62] with 970 PLWH who were ART-naïve and 1022 HIV-negative individuals reported sufficient data on AST. The results from a random effects model indicate a significant increase in AST in ART-naïve PLWH compared with HIV-negative individuals, with an SMD of 1.85, 95% CI (0.93 to 2.78, $p < 0.0001$), as shown in Figure 2A. The evidence revealed significant heterogeneity, with an I^2 of 93.8%. Additionally, to explore the effect of ART on AST, we found that 24 studies [32,34–38,46–49,51–56,58–65] had sufficient data on AST, of which 1649 PLWH were on ART and 1409 were HIV-negative individuals. The results revealed a significantly higher AST in the ART group compared with the HIV-negative group, SMD = 1.49, a 95% CI (0.48 to 2.50, $p = 0.0038$) (Figure 2B). The study reveals a significant level of heterogeneity ($I^2 = 98.2\%$).

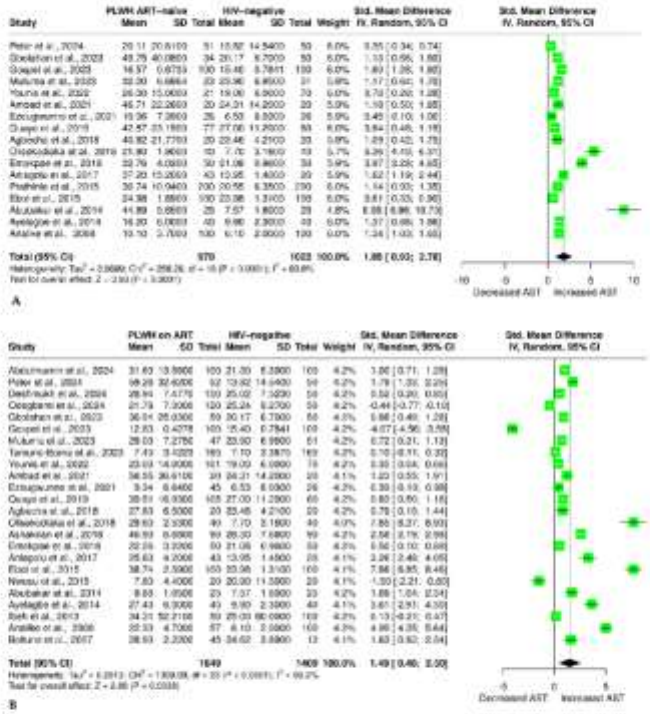

Figure 2. (**A**) Random effect meta-analysis evaluating the effect of HIV on aspartate aminotransferase in ART-naïve PLWH compared with the HIV-negative group [32,34–36,47,49–52,54–56,58–62]. (**B**) Random effect meta-analysis evaluating the effect of ART on aspartate aminotransferase in PLWH on ART compared with the HIV-negative group [32,34–38,46–49,51–56,58–65]. The solid line shows the line of no effect, the dashed line shows the effect size, the green block shows the weight of the study, the horizontal line crossing the green block shows the confidence intervals, and the diamond plot shows the combined effect size.

3.5. Effect of HIV Infection and ART on ALT

Evidence on the level of ALT from 18 studies [32,34–36,47,49–52,54–62] with 1000 PLWH who were ART-naïve and 1052 HIV-negative individuals was analyzed. The results showed a significant elevation in ALT in the ART-naïve group compared with the HIV-negative group (SMD = 2.65 (1.25 to 4.04, p = 0.0002)), as shown in Figure 3A. However, evidence showed a significant heterogeneity (I^2 = 97.8%). We also found that 25 studies [32,34–38,46–49,51–65] had sufficient data on ALT, in 1679 PLWH on ART and 1439 HIV-negative individuals, and the data were subjected to random-effect model meta-analysis. The results revealed a significant increase in ALT levels in PLWH on ART compared with HIV-negative individuals (SMD = 2.30 (1.14 to 3.45, p < 0.0001)), as shown in Figure 3B. Consistently, the analyzed evidence revealed a significant heterogeneity (I^2 = 98%).

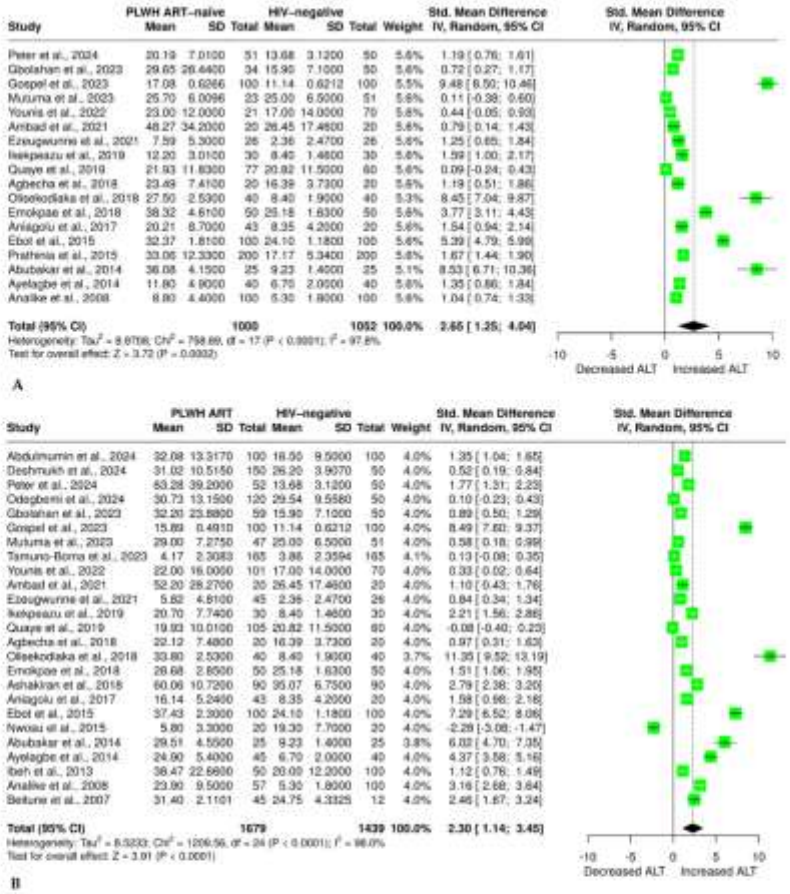

Figure 3. (**A**) Random effect meta-analysis evaluating the effect of no ART on alanine aminotransferase in ART-naïve PLWH compared with the HIV-negative group [32,34–36,47,49–52,54–62]. (**B**) Random effect meta-analysis evaluating the effect of ART on alanine aminotransferase in PLWH on ART compared with the HIV-negative group [32,34–38,46–49,51–65]. The solid line shows the line of no effect, the dashed line shows the effect size, the green block shows the weight of the study, the horizontal line crossing the green block shows the confidence intervals, and the diamond plot shows the combined effect size.

3.6. The Effect of HIV Infection and ART on Alkaline Phosphatase

The effect of HIV infection on ALP was evaluated in 13 studies [32,34–36,47,49,52,55–57,59,60,62] comprising 662 ART-naïve PLWH and 672 HIV-negative individuals. The analysis of a random effects model revealed no significant difference in ALP levels between ART-naïve and HIV-negative individuals [SMD = 0.53 (–0.92 to 1.98, $p = 0.4726$)], as shown in Figure 4A. The evidence revealed a significant heterogeneity ($I^2 = 98\%$). Furthermore, the effect of ART on ALP was evaluated in 18 studies [32,34–38,47–49,52,53,55–57,59,60,62,65], with 1121 PLWH on ART and 1147 HIV-negative individuals. The results showed a statistically significant increase in ALP levels in PLWH on ART compared with HIV-negative individuals [SMD = 1.40 (0.55 to 2.26, $p < 0.01$)], as shown in Figure 4B. However, the accumulated evidence showed a significant level of heterogeneity ($I^2 = 97\%$).

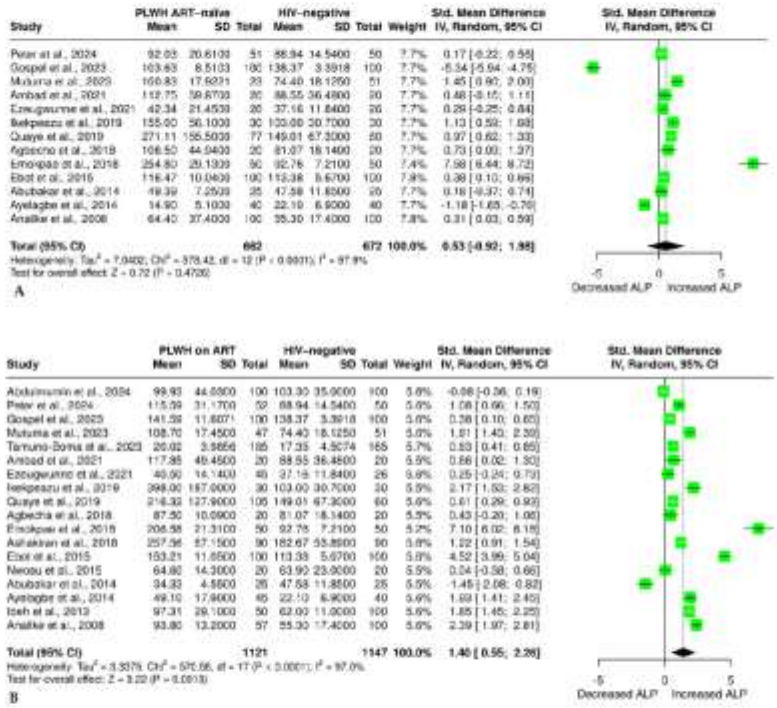

Figure 4. (**A**) Random effect meta-analysis evaluating the effect of no ART on alkaline phosphatase in ART-naïve PLWH compared with the HIV-negative group [32,34–36,47,49,52,55–57,59,60,62]. (**B**) Random effect meta-analysis evaluating the effect of ART on alkaline phosphatase in PLWH on ART compared with the HIV-negative group [32,34–38,47–49,52,53,55–57,59,60,62,65]. The solid line shows the line of no effect, the dashed line shows the effect size, the green block shows the weight of the study, the horizontal line crossing the green block shows the confidence intervals, and the diamond plot shows the combined effect size.

3.7. Publication Bias and Sensitivity Analysis

Publication bias was assessed through visual inspection of the funnel plot and Egger's regression test. Briefly, when assessing the effect of HIV infection on AST, the funnel plot indicated a potential publication bias (Figure 5A). Egger's test supported the presence of funnel plot asymmetry (intercept: 5.38, 95% CI: 1.45 to 9.31, $p = 0.017$). The result of the trim and fill method showed a change in effect size, SMD = 1.47, $p < 0.05$ (File S2, Figure S1A), while the effect was still large. The overall results suggest that there was moderate publication bias. For those PLWH on ART, the AST funnel plot showed potential

publication bias (Figure 5B). Egger's test supported the presence of funnel plot asymmetry (intercept: 9.46, 95% CI: 2.15 to 16.77, $p = 0.019$). However, the trim and fill method showed a decrease in effect size, SMD = 0.22, $p > 0.05$ (File S2, Figure S1C), compared with the initial SMD = 1.49. These discrepancies suggest that the initial effect size may have been increased due to unpublished studies. The sensitivity test revealed that the exclusion of a study by Gospel [60] was an outlier, and Nwosu [65], due to a small sample size, increased the effect size (1.73 and 1.62). In contrast, the exclusion of Ebot [36] as an outlier and Olisekodiaka [54] due to lower CD4 count in ART-naïve reduced the effect size (1.22 and 1.23, respectively) (File S2, Table S5). For studies on HIV infections on ALT, the funnel plot indicated a potential publication bias (Figure 5C). Egger's test supported the presence of funnel plot asymmetry (intercept: 8.54, 95% CI: 2.23 to 14.86, $p = 0.0017$). However, the results of the trim and fill method did not alter the original effect size, suggesting that the effect size reflects the true effect, and therefore, the results are more reliable (File S2, Figure S1B). For the PLWH on ART on ALT, the funnel plot indicated a potential publication bias (Figure 5D). Egger's test supported the presence of funnel plot asymmetry (intercept: 11.59, 95% CI: 6.43 to 16.75, $p = 0$). Additionally, a trim and ill test showed a decrease in effect size, SMD = 0.62, $p > 0.05$ (File S2 Figure S1D). This suggests that the initial effect size might have been attributed to unpublished studies. A sensitivity analysis also showed a minor change in effect size when some studies were excluded. However, the effect remained large, ranging from 1.93 to 2.48 (File S2, Table S6). Interestingly, no evidence of publication bias was observed on ALP from the evidence on HIV infection and those who were ART-naïve. The funnel plot did not indicate a potential publication bias (Figure 5E). Egger's test did not support the presence of funnel plot asymmetry (intercept: 1.97, 95% CI: –9.59 to 13.53, $p = 0.745$). The funnel plot did not indicate a potential publication bias for studies on ART versus HIV negative (Figure 5F). This was supported by Egger's test (intercept: 6.81, 95% CI: –0.11 to 13.74, $p = 0.072$).

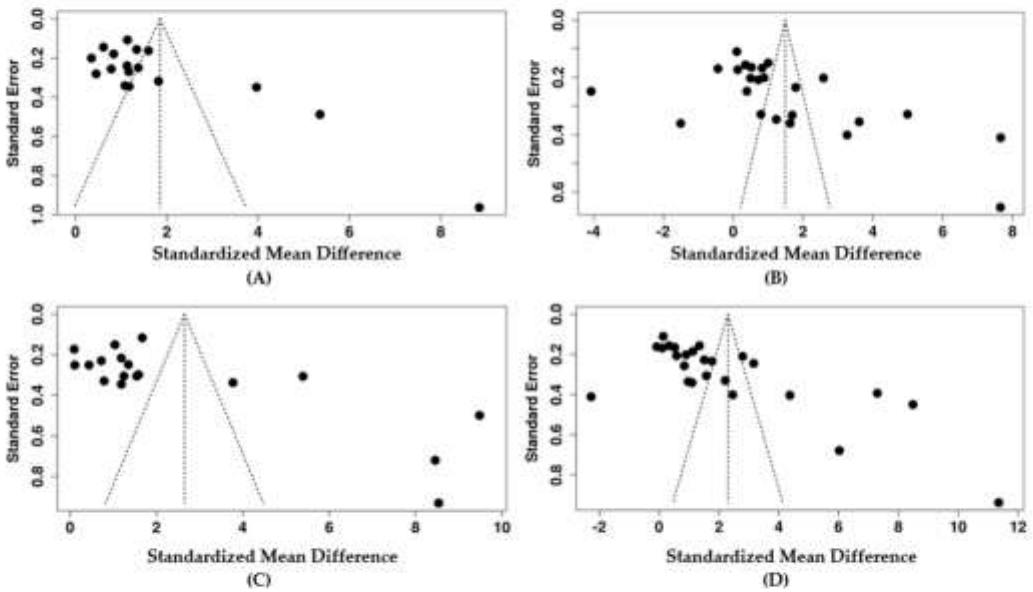

Figure 5. *Cont.*

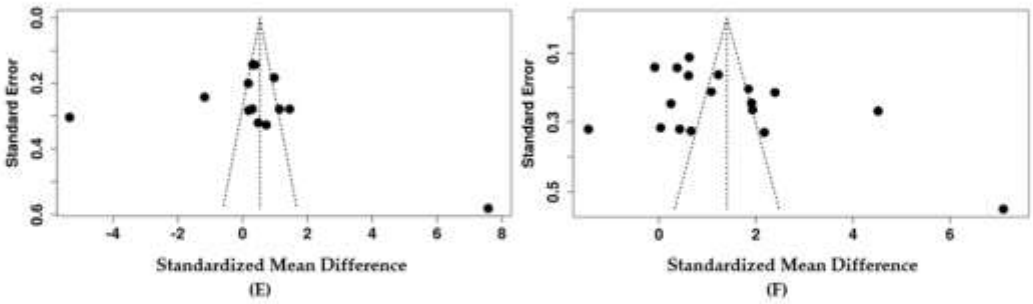

Figure 5. Funnel plots depicting the evidence of potential bias across the included studies in the meta-analysis. (**A**) Studies that assessed AST in ART-naïve PLWH compared with HIV-negative individuals. (**B**) Studies on the effect of PLWH on ART compared with HIV-negative individuals on AST. (**C**) Studies on ALT in ART-naïve PLWH compared with that in HIV-negative individuals. (**D**) Studies on the effect of ART on ALT in PLWH compared with that HIV-negative individuals. (**E**) Studies on ALP in ART-naïve PLWH compared with that in HIV-negative individuals. (**F**) Studies on the effect of ART on ALP. The individual dot shows the study included.

3.8. Subgroup Analysis

A thorough subgroup analysis was performed to find the cause of observed statistical heterogeneity on AST, ALT, and ALP in PLWH who were exposed to ART, ART-naïve PLWH, and HIV-negative individuals. The analysis was based on study design, continent, sample size, and gender distribution, as presented in File S2, Figures S2–S7. Results on AST in HIV-ART-naïve when compared with HIV-negative results in the subgroup showed that case control (I^2 = 31%) (File S2, Figure S2A), studies in Asian countries (I^2 = 0%) (File S2, Figure S2B), those that did not state the gender (I^2 was reduced from 94% to 63%) (File S2 Figure S2D), and studies that used NNRTI and NRTI from regimens were all factors contributing to heterogeneity (File S2 Figure S2E). On the other hand, no factors were found to be associated with heterogeneity on AST in PLWH on ART when compared with HIV negative (File S2, Figure S3A–E). This suggests an unexplained heterogeneity in AST in PLWH on ART. For ALT in the ART-naïve group, case control and Asian countries minimally contributed to the observed heterogeneity; post-subgroup I^2 changed in the case control to 55% (File S2, Figure S4A) and 84% in Asian publications (File S2, Figure S4B). The studies that did not report the class of ART used were a source of heterogeneity (I^2 = 0%) (File S1, Figure S4E). Additionally, those that used NNRTI and NRTI might have contributed minimally to the observed variation (I^2 = 72.2%) (File S2 Figure S4E). For ALT in PLWH on ART, only case control studies showed minimal changes in heterogeneity from the original results (I^2 = 86%) (File S2 Figure S5A). For ALP in ART-naïve, a case control changed I^2 to 5% (File S2 Figure S6A) and 49% among studies that had a sample size of less than 100 (File S2, Figure S6C). Moreover, studies that did not report the class or form of ART used seem to have contributed minimally to heterogeneity (File S2, Figure S6E). Likewise, for ALP in ART-exposed individuals, Asia and case control studies and studies that did not specify the form or class of ART were the contributors to the observed heterogeneity, as shown in File S2, Figure S7A,B,E.

4. Discussion

This systematic review and meta-analysis examined data from 26 clinical studies investigating the effects of HIV infection and ART on liver function among PLWH. Our results revealed a significant elevation in AST and ALT among PLWH who were ART-naïve compared with HIV-negative individuals, indicating that HIV infection itself may play a role in hepatic impairment [32,47,49–52,54,55,60]. However, we noted no signif-

icant differences in ALP in ART-naïve individuals when compared with HIV-negative individuals [32,34,35,49,60].

Additionally, we noted a significant increase in all liver enzymes, including AST, ALT, and ALP, in PLWH undergoing ART when compared with HIV-negative individuals. The findings demonstrate that ART, although crucial for HIV infection management, is associated with hepatotoxicity [35–37,46,47,49,51,54,56,59,61,62]. The notable increase in AST and ALT levels among ART–exposed individuals is supported by previous studies [32,66,67]. More recently, Gbolahan and colleagues observed substantial elevations in AST and ALT levels in PLWH undergoing HAART and those who were pre-HAART compared with HIV-negative controls [61]. The same trend was previously reported by another study, which found that individuals using ART had significantly increased AST and ALT and ALP levels [36,62]. Altogether, these results support the correlation between ART exposure and hepatic dysfunction. These suggest that ART may exacerbate liver dysfunction among PLWH.

Other researchers have shown increased ALT in PLWH compared with HIV-negative individuals, and this was reportedly observed during ART exposure [64]. This is supported by recent evidence, which showed that AST and ALT were increased as the duration of ART exposure increased [37]. Others suggest that an increase in the level of the liver enzymes depends on the baseline CD4 count when ART is first initiated. This is supported by Deshmukh et al., 2024 [63], who reported a significant yet negative correlation between CD4 count and elevated liver enzymes, suggesting that the lower the CD4 count, the higher the AST and ALT in PLWH exposed to ART. Previous evidence has also shown an association between liver dysfunction, especially in ART-exposed PLWH, and age, suggesting that as PLWH age, the risk of liver dysfunction also increases [68].

Different findings were reported in another study, showing no significant change in AST, ALT, or ALP levels between ART and ART-naïve individuals, suggesting that specific regimens may have specific effects on liver function, with some promoting safer liver function while others having null effects [69]. This is partly supported by our subgroup analysis, which showed that the class of ART has no effect on the ALP in ART-naïve. Although our findings showed an association between ART exposure and liver dysfunction, this might be exacerbated by HIV infection. The observed elevation in liver enzymes among PLWH who were ART-naïve when compared with HIV-negative individuals further supports the notion that HIV infection itself may induce liver dysfunction. Other studies consistently support this. For instance, Anyanwu et al. (2021) reported a significant increase in AST levels among PLWH not receiving ART [70]. However, the magnitude of elevation in the ART-naïve group was lower than that in the ART-exposed group, highlighting the potential effect of ART on liver health deterioration. Although it is difficult to distinguish the hepatic dysfunction arising from HIV infection or ART exposure, it is crucial in clinical settings to understand this complex interaction to make a concrete distinction. A recent study in liver cancer therapeutics has shown that targeting metabolic pathways and key regulators, such as STAM binding protein-like 1 (STAMBPL1), may offer novel strategies to alleviate the risk of hepatocellular carcinoma among those with chronic liver injury, including those affected by HIV and long-term HIV exposure [71]. In a preclinical study, a 12-month exposure to NRTIs led to liver oxidative damage concomitant with mitochondrial DNA loss [23]. This results in mutation, which impairs the function of the mitochondrial electron transport chain, thus reducing ATP synthesis. This lack of energy impairs hepatocyte function [72,73].

Based on the observed findings, the evidence suggests that the elevations in these liver enzymes can be attributed to HIV infection and the effects of ART exposure. Hepatocyte oxidative stress is reported to be an inducer of hepatic dysfunction in PLWH [19]. Some

of the mechanisms by which ART increases these liver enzymes seem to be associated with immune reconstitution inflammatory syndrome (IRIS) and directly through drug-induced liver damage [74,75]. Chronic HIV infection induces liver dysfunction, and this is mediated through persistent inflammation and a reduction in hepatoprotective factors like interferon gamma (IFN-γ), which results in liver fibrosis, as shown in Figure 6 [64,76]. As PLWH have reduced CD4+ T-cells and dysregulated immune responses, this can suppress IFN-γ, thus resulting in fibrosis [76]. HIV infects the gut lymphoid tissue, damaging the intestinal epithelium and increasing permeability. This allows the translocation of bacterial lipopolysaccharides (LPS) to the hepatocytes, thus activating Kupffer cells [76]. It can also infect Kupffer cells and hepatocytes indirectly through inflammatory mediators, leading to elevated liver enzymes [77,78]. The HIV glycoprotein (gp120) binds to chemokine receptors on hepatic stellate cells, activating metabolic pathways that promote the generation of reactive oxygen species (ROS) and collagen production; altogether, these promote liver fibrosis [64,76].

On the other hand, the ART-associated liver enzyme elevation mechanisms are complex; these encompass mitochondrial toxicity, direct hepatotoxicity from ART, and alterations in bile acid metabolism [79]. NRTI regimens are known to induce mitochondrial dysfunction, resulting in oxidative stress and hepatocellular damage [23]. Similarly, NNRTIs, such as nevirapine, ritonavir, and lopinavir, impair liver enzyme metabolism, resulting in elevated AST, ALT, and ALP levels. Likewise, efavirenz contributes to hepatic steatosis, resulting in liver enzyme elevation [80]. More recently, our team found that ART exposure in pregnant women with pre-eclampsia promotes immune activation and inflammation, resulting in elevated liver enzymes and hepatotoxicity [81].

Our findings imply that there is a need for regular monitoring of liver function among PLWH undergoing ART. Recent evidence suggests that combining tenofovir, lamivudine, and dolutegravir may reduce liver function relative to traditional regimens [64]. A routine evaluation of AST, ALT, and ALP levels should be incorporated into primary and secondary healthcare clinical practice to identify ART-induced hepatic impairment early and develop strategies to curb these dysfunctions. This could assist in providing effective treatment for HIV with fewer hepatic side effects.

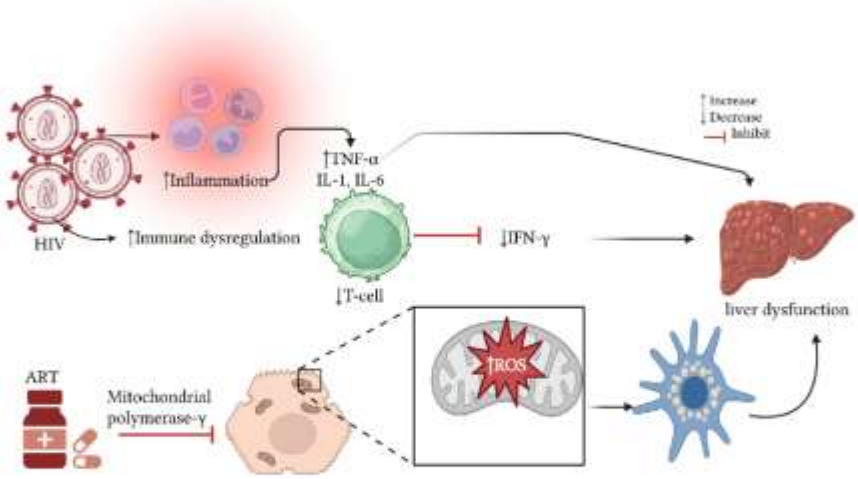

Figure 6. Pathways by which HIV infection and ART contribute to liver dysfunction [81]. Created using Biorender. INF-γ: interferon-gamma; TNF-α: tumor necrosis factor alpha; IL-6: interleukin-6; ROS: reactive oxygen species; ART: antiretroviral therapy; HIV: human immunodeficiency virus.

Strengths and Limitations

Several strengths and limitations should be acknowledged. First, this study adhered to the PRISMA guideline of reporting and is registered with the Open Science Framework registry to ensure transparency. Of the included studies, 15 (58%) were regarded as high quality and low risk of bias, while 11 (42%) were classified as moderate quality and risk of bias. These suggest that the overall quality of the included studies was good. It is worth noting that these studies exhibited significant heterogeneity, which seems to have influenced the overall effect estimates in AST, especially in ART-naïve patients when compared with HIV-negative patients. However, a detailed subgroup analysis was conducted to find the potential sources, showing that larger samples yielded more consistent outcomes. While the form or class of ART used was not reported in all studies, we were able to conduct a subgroup analysis to find the sources of the observed heterogeneity. Many studies were cross-sectional and case studies, with a lack of longitudinal studies to assess the long-term effect of ART on these liver function tests. Although the overall sample size was sufficient, it is important to acknowledge that individual studies' sample sizes ranged from 20 to 400 participants. While HIV is more prevalent in South Africa, among the analyzed studies, none were conducted in this country. Additionally, only 4 studies were conducted on female participants.

5. Conclusions

This study demonstrates that both HIV infection and ART exposure are associated with elevated liver enzymes (AST, ALT, and ALP). ART-exposed patients showed an elevation in AST, ALT, and ALP enzymes. In ART-naïve individuals, there was a pronounced increase in AST and ALT without an effect on ALP compared with HIV-negative individuals.

Recommendation and Future Perspectives

Based on the findings from this study, we recommend that future clinical studies focus on newer ART regimens, increase follow-up, and report the exact form of ART regimens used to ascertain their contribution to liver dysfunction. We also recommend that, in clinical settings, the liver function should be taken into consideration when ART is first initiated and should be regularly monitored in those exposed to ART so that any dysfunction can be identified early and further controlled or prevented. Regular monitoring of liver enzymes through tests alongside CD4 counts and viral load is recommended, especially for those exposed to HAART. This initiation will help in deciding on the discontinuation of ART regimens if it is associated with increased toxicity. Our findings warrant individualized ART selection, considering pre-existing hepatic dysfunctions and drug–drug interactions. Additionally, conducting longitudinal studies to evaluate liver function in ART-naïve and ART-exposed patients may be necessary in HIV-prevalent communities. Therefore, we recommend that future studies focus more on female participants, especially in HIV-prevalent countries like South Africa, to better inform the health care system in improving the health of these populations, as the findings obtained in this study might not be translated to such prevalent populations. Additionally, patient education on liver health and potential ART-related side effects may be important to reduce hepatic complications associated with ART exposure. These measures can improve liver health management, enhance patient outcomes, and improve the quality of life of PLWH.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ph18070955/s1, Supplementary File S1: PRISMA checklist. Supplementary File S2: Figure S1: Result of the sensitivity analysis based on the trim and fill test. A: AST in ART-naïve compared with HIV negative. B: ALT in ART-naïve compared with HIV negative.

C: AST in ART compared with HIV negative. D: ALT in ART compared with HIV negative; Figure S2: Subgroup analysis on AST in ART-naïve compared to HIV-negative individuals. A: The level of AST in PLWH who were ART-naïve versus HIV negative, based on study design. B: ART-naïve versus HIV negative on AST based on continent. C: AST levels in PLWH who are ART-naïve versus HIV negative, based on sample size. D: AST levels in ART-naïve versus HIV negative, based on gender distribution. E: AST levels in ART-naïve versus HIV negative, based on class of ART; Figure S3: Subgroup analysis on AST in PLWH exposed to ART compared with HIV-negative individuals. A: AST in PLWH exposed to ART compared with HIV-negative individuals based on study design. B: AST in PLWH exposed to ART compared with HIV-negative individuals based on the continent of publication. C: AST in PLWH exposed to ART compared with HIV-negative individuals based on sample size. D: AST in PLWH exposed to ART compared with HIV-negative individuals based on gender distribution. E: AST in PLWH exposed to ART compared with HIV-negative individuals based on the class of ART; Figure S4: Subgroup analysis on ALT in ART-naïve compared with HIV-negative individuals. A: ALT level in PLWH on ART-naïve group compared with HIV negative, based on study design. B: Levels of ALT in ART-naïve PLWH compared with HIV negative, based on the continent of publication. C: ALT level in PLWH on ART-naïve compared with HIV negative, based on sample size. D: ALT levels in PLWH on ART-naïve compared with HIV negative, based on gender distribution. E: ALT levels in PLWH on ART-naïve group compared with HIV negative based on class of ART regimens; Figure S5: Subgroup analysis on ALT among PLWH on ART compared with HIV-negative individuals. A: ALT among PLWH on ART compared with HIV-negative individuals based on study design. B: ALT among PLWH on ART compared with HIV negative individuals based on continent. C: ALT among PLWH on ART compared with HIV-negative individuals based on sample size. D: ALT among PLWH on ART compared with HIV-negative individuals based on gender distribution. E: ALT among PLWH on ART compared with HIV-negative individuals based on the class of ART regimens; Figure S6: Subgroup analysis showing the effect of different factors on ALP in PLWH who are ART-naïve compared with HIV negative. A: ALP levels in PLWH who are ART-naïve compared with HIV-negative individuals, based on study design. B: ALP level in PLWH who are ART-naïve vs. HIV negative, based on the continent of publication. C: ALP levels in PLWH who are ART-naïve compared with HIV negative, based on sample size. D: ALP levels in PLWH who are ART-naïve compared with HIV negative, based on gender. E: ALP levels in PLWH who are ART-naïve compared with HIV negative, based on the class of ART regimens; Figure S7: Subgroup analysis showing the effect of different factors on ALP in PLWH on ART compared with HIV negative. A: ALP levels in PLWH on ART compared with HIV-negative individuals, based on study design. B: ALP levels in PLWH on ART compared with HIV-negative individuals in terms of the continent of publication. C: ALP levels in PLWH on ART compared with HIV-negative individuals based on gender. D: ALP levels in PLWH on ART compared with HIV-negative individuals based on sample size. E: ALP levels in PLWH on ART compared with HIV-negative individuals based on class of ART regimens; Table S1: General overview of characteristics of included studies; Table S2: Quality assessment of cohort studies; Table S3: Quality assessment of cross-sectional studies; Table S4: Quality assessment of case control studies; Table S5: Sensitivity analysis using one study exclusion at a time on AST in PLWH compared to HIV negative; Table S6: Sensitivity analysis using one study exclusion at a time on ALT in PLWH compared to HIV negative.

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Abbreviations

AIDS	acquired immunodeficiency virus
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ART	antiretroviral therapy
ARV	antiretroviral
AST	aspartate aminotransferase
CD4	cluster of differentiation 4
CI	confidence interval
DTG	dolutegravir
HAART	highly active antiretroviral therapy
HBV	hepatitis B Virus
HCV	hepatitis C Virus
HIV	human immunodeficiency virus
MeSH	Medical Subject Headings
NNRTI	non-nucleoside reverse transcriptase inhibitors
NOS	Newcastle–Ottawa Scale
NRTI	nucleoside reverse transcriptase inhibitor
PICO	population, intervention, comparator, outcome
PLWH	people living with HIV
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SD	standard deviation
SEM	standard error of mean
SMD	standardized mean difference

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Prologue

The following chapter presents a collection of manuscripts arising from comprehensive research on the biochemical and physiological alterations associated with pregnancy, pre-eclampsia (PE), and HIV infection. These studies investigate the complex relationship between maternal hepatic function, placental activity, and systemic inflammation.

The research explores how pregnancy, compounded by hypertensive disorders, HIV status, and ART exposure, affects maternal physiological adaptation and placental integrity, using key biochemical markers such as alanine aminotransferase (ALT), placental alkaline phosphatase (PLAP), C-reactive protein (CRP), and aspartate aminotransferase (AST). The study aims to elucidate the mechanisms that may underlie variations in hepatic enzyme expression and placental biomarker activity by comparing results from normotensive and PE pregnancies in both HIV-positive and HIV-negative women. The manuscripts provided here are part of a larger effort to link clinical observation with biochemical insights, resulting in a comprehensive understanding of maternal health under complex physiological and pathological conditions. Finally, this collection emphasises not only the varied nature of pregnancy-related disorders but also the potential of biochemical indicators to aid early detection, risk assessment, and clinical management strategies in obstetric care.

CHAPTER 3: Clinical Findings

This section provides an overview of the results from the clinical study and is divided into three manuscripts, presented as such.

Chapter 3.1: Manuscript 1

Aspartate Aminotransferase in Pre-eclamptic and Normotensive Pregnancies with and without HIV

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Student contributions: Conceptualisation, investigation, methodology, laboratory analysis, software analysis, data curation, writing – original draft preparation, and visualisation

Abstract:

Background: Pre-eclampsia (PE) is a hypertensive disease disorder of pregnancy, linked to considerable maternal and perinatal morbidity. The aetiology includes endothelial dysfunction, abnormal placentation, and hepatic involvement. Aspartate aminotransferase (AST), an key enzyme in amino acid metabolism and a marker of hepatocellular damage, may offer insights into hepatic changes during PE. In sub-Saharan Africa, where both PE and human immunodeficiency virus (HIV) infection are widespread, comprehending their synergistic impact on maternal hepatic function is crucial.

Objective: This study aimed to compare plasma AST levels in normotensive and PE pregnancies stratified by HIV status, and to determine whether HIV infection and antiretroviral therapy (ART) influence hepatic enzyme activity in PE.

Methods: Prospective case-control study performed at Charlotte Maxeke Johannesburg Academic Hospital, South Africa, involving 72 pregnant women (48 normotensive and 24 pre-eclamptic), comprising both HIV-positive and HIV-negative individuals. Plasma AST concentrations were measured with a human AST ELISA kit. Data were analysed using GraphPad Prism 5.0, employing both nonparametric and parametric statistical tests, with significance set at $p < 0.05$.

Results: While AST concentrations were typically elevated in the PE group compared to normotensive pregnancies, these variations were not statistically significant ($p = 0.7973$). Likewise, comparisons categorised by HIV status, between HIV-negative and HIV-positive normotensive or PE groups, revealed no significant differences in AST levels ($p > 0.05$). Clinical parameters, including systolic and diastolic blood pressure, exhibited substantial differences between normotensive and PE patients ($p < 0.0001$), hence validating the accuracy of group classification.

Conclusion: The results indicate that PE and HIV infection, either alone or in combination, have no significant effect on plasma AST levels during pregnancy. Despite the established involvement of the liver in PE and the possibility for ART-induced hepatotoxicity in HIV, AST may not be a sensitive solo biomarker for detecting subtle hepatic alterations in both circumstances. Larger studies with broader liver function panels and longitudinal follow-up are needed to elucidate these connections.

Key Words: Aspartate Aminotransferase, Pre-eclampsia, HIV, Liver Function, Pregnancy, Antiretroviral Therapy

1. Introduction

Pregnancy is a dynamic physiological condition marked by substantial metabolic, immunological, and circulatory modifications that safeguard maternal health and facilitate foetal growth [1]. Pre-eclampsia (PE) is one of the most prevalent hypertension disorders during pregnancy, significantly impacting maternal and perinatal morbidity and mortality globally [2] [3]. PE is clinically characterised by the emergence of hypertension and proteinuria after 20 weeks of gestation, frequently associated with systemic complications affecting the liver, kidneys, and coagulation mechanisms [4]. The precise aetiology of PE remains unknown; nonetheless, it is thought to stem from abnormal placental development and insufficient uterine blood flow [5]. This may result in difficulties including placental abruption, foetal growth limitation, and premature birth [6]. Early identification and intervention of PE is essential in preventing severe consequences for both the mother and the infant.

The liver is critical in maternal adaptation throughout pregnancy, and changes in its function may yield significant insights into disease pathogenesis [7]. Aspartate aminotransferase (AST) is an enzyme commonly utilised as a biomarker for hepatocellular damage [8]. AST facilitates the reversible transference of an amino group between aspartate and α-ketoglutarate, yielding oxaloacetate and glutamate, which are essential intermediates in the citric acid cycle and amino acid metabolism. By producing oxaloacetate, AST supports the citric acid cycle essential for adenosine triphosphate (ATP) synthesis [9]. AST is abundant in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it functions as a biomarker of tissue damage in disorders such as hepatitis and myocardial infarction [10]. Increased blood AST concentrations may indicate organ damage, making it a significant diagnostic tool. In cases of liver dysfunction, AST levels may be significantly elevated because the liver plays a crucial role in metabolism.

The scenario is further complicated by the significant prevalence of human immunodeficiency virus (HIV) infection in areas where PE is common, especially in sub-Saharan Africa [11]. HIV infection is linked to chronic inflammation, immunological dysregulation, and possible hepatotoxicity resulting from antiretroviral therapy (ART) [12]. The overlapping risk factors

pose significant clinical questions concerning the cumulative impact of HIV infection and PE on maternal hepatic function. HIV infection can intensify the severity of PE and elevate the likelihood of negative consequences for both the mother and the infant [11]. The administration of ART in HIV-positive pregnant women with PE may exacerbate the situation by potentially inducing hepatic damage [13].

Examining AST activity in PE and normotensive pregnancies, both with and without HIV infection, may provide clarity of the complex relationships among hypertensive diseases of

pregnancy, viral infection, and hepatic function. Determining whether HIV infection and ART exacerbate or mitigate AST changes in PE could yield essential insights into maternal disease progression and neonatal consequences. Therefore, the aim of this study is to assess AST levels in PE and normotensive pregnancies, both with and without HIV infection, to determine the effects of PE and HIV on maternal liver function.

2. Materials and Methods

2.1. Study population and design

This study was conducted as a cross-sectional study, with samples collected at a single time point, as previously reported by other scholars [14]. It adhered to the principles of the Declaration of Helsinki [15], and informed consent was obtained from participants prior to participation. Institutional ethical approval was obtained from the University of South Africa-College of Agriculture & Environmental Sciences Health Research Ethics Committee (2025/CAES_HREC/7327), approved on 08/05/2025, and the gatekeeper's permission was granted to make use of the samples. The hospital regulatory permission was also obtained from Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), South Africa. After written consent was obtained, Normotensive (N) and pre-eclamptic (PE) HIV-negative and HIV-positive pregnant women were recruited at CMJAH. Normotensive (n = 48, age range: 18 ± 43 years) and PE (n = 24, age range: 18 ± 40 years) patients were recruited. Pre-eclampsia is characterised by new-onset hypertension of ≥140 mmHg systolic or ≥90 mmHg diastolic, measured on two separate occasions four hours apart, with or without proteinuria. Normotensive pregnant participants were defined as individuals exhibiting a blood pressure of ≤120/80 mmHg [16]. Demographic data for all research participants were obtained from their maternity case records. HIV testing was done after counselling using a rapid point-of-care test kit initially, as is the standard of care in South Africa. Maternal weight was categorised as normal weight (BMI: 18.5 - 24.9 kg/m²), overweight (BMI: 25.0 – 29.9 kg/m²), and obese (BMI: >30 kg/m²). To ensure anthropometric consistency, all women participating in the study reported being non-smokers and abstaining from alcohol and recreational drugs, while all HIV-positive participants were receiving highly active antiretroviral therapy (HAART: tenofovir, emtricitabine, and efavirenz) in accordance with South African National HIV guidelines [17]. The administration of HAART during pregnancy is crucial for minimising mother-to-child transmission through mechanisms such as reducing maternal antepartum viral load and providing preexposure and postexposure prophylaxis for the infant [18]. Women with additional chronic medical issues were excluded from the study.

2.2. Sample Collection

This study utilised 72 archived maternal plasma samples, comprising 48 from normotensive individuals and 24 from those with PE, collected at the Charlotte Maxeke Johannesburg Academic Hospital. A competent nurse (Ms PN Zulu) utilised a 21-gauge needle for venipuncture. Approximately 6 mL of blood samples were obtained from each research participant in EDTA tubes (July-October 2023). Following sample collection, the blood was processed within one hour to preserve plasma integrity. The EDTA tubes were centrifuged at 1500 × g for 10–15 minutes at 4°C to isolate plasma from cellular components. The isolated plasma was subsequently preserved in a freezer at -80°C for future utilisation.

2.3. Human AST (aspartate Aminotransferase) ELISA

AST measurement was conducted utilising the Human Aspartate Aminotransferase (AST) ELISA Kit (Cat. No. ELK1966, Lot. 33405676). This assay utilises a sandwich enzyme-linked immunosorbent assay (ELISA) methodology, with microplates that are pre-coated with a capture antibody specific to human AST. Samples and standards were introduced into the wells and bound to the immobilised antibody. A biotinylated anti-AST antibody was subsequently added, followed by streptavidin linked to horseradish peroxidase (HRP). Following the addition of the substrate (TMB), a colorimetric reaction ensued, which was then halted with a stop solution. Optical density (OD) was quantified at 450 nm utilising a microplate reader.

All samples were diluted to a ratio of 1:4 in accordance with the assay specifications. 100 µL of standards, blanks, and plasma samples were added to specified wells and incubated for 80 minutes at 37 °C. Following three washes with 1× wash buffer, where the contents were decanted and 200 µL of wash buffer added to each well, 100 µL of biotinylated antibody working solution was dispensed into each well and incubated for 50 minutes at 37 °C. Subsequent to an additional wash step, 100 µL of streptavidin-HRP working solution was added and incubated for 50 minutes at 37 °C. Subsequent to five washes, 90 µL of TMB substrate was dispensed into each well and incubated in the dark for 20 minutes at 37 °C. The reaction was halted using 50 µL of stop solution, and absorbance was measured immediately at 450 nm.

A standard curve was constructed with AST concentrations represented on the x-axis and optical density (OD) values on the y-axis. AST concentrations in plasma samples were derived using this curve. Samples exhibiting OD values over the detection range (0.32–20 U/L) were reanalysed using suitable dilutions.

2.4. Statistical Analysis

Microsoft Excel 365 was used to construct the standard curve and determine AST concentrations using the calculated regression line. GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California, USA) was used to analyse the data. The Shapiro–Wilk test was used to assess the normality of the data distribution. When the distribution was parametric, descriptive statistics for continuous data were presented as mean ± standard deviation, whilst nonparametrically distributed data were presented as median and interquartile range (IQR). Group comparisons were conducted using one-way analysis of variance (ANOVA) for parametric data, followed by a Tukey post hoc test. The Kruskal–Wallis test was utilised for non-parametric data. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinical Characteristics of Participants

Table 1 summarises the clinical and demographic features of the study population. As expected, systolic and diastolic blood pressure (BP) differed between the normotensive and PE groups ($p \leq 0.0001$). Similarly, maternal age ($p = 0.0425$) and BMI ($p = 0.0373$) were significantly different between the normotensive and PE groups. There were no significant differences in maternal weight ($p = 0.1062$), maternal height ($p = 0.9917$), and gestational age ($p = 0.1515$) between normotensive and PE groups.

Table 1: Patient demographic features of the study groups (normotensive = 48, pre-eclampsia = 24)

Variables	**Groups**	**Median**	**Q1 – Q3**	**Mean ± SD**	***p*-value**
Maternal age (years)	N	32	21 – 37.5	31.68 ± 6.56	0.0425
	PE	36	34 – 38	35.96 ± 2.38	
Maternal Weight (kg)	N	73	68 – 90	78.24 ± 14.73	0.1062
	PE	87	71.75 – 100.5	88.6 ± 18.26	
Maternal Height (m)	N	159.5	155 – 167	158.9 ± 4.79	0.9917
	PE	158	157.3 162.5	159.8 ± 5.48	
BMI (kg/m^2)	N	28.87	27.17 – 37.02	31.39 ± 5.48	0.0373
	PE	39.39	34.08 – 47.67	39.02 ± 8.64	
Systolic blood pressure (mmHg)	N	106	96 – 116.3	110.1 ± 6.53	*** <0.0001
	PE	149	104 – 154.8	15 ± 19.41	
	N	66.5	60.25 – 72	66.83 ± 7.67	*** <0.0001

Diastolic blood pressure (mmHg)	PE	96.5	91.25 – 106	99.3 ± 14.78	
Gestational age (weeks)	N	23	16 – 30	22.44 ± 7.76	0.1515
	PE	19	13 – 26	19.48 ± 8.47	

N: Normotensive; PE; Pre-eclampsia

3.2. Plasma concentration levels of AST

3.2.1. Across all groups

There was no significant difference in AST levels across all study groups (Kruskal-Wallis = 0.3475, p = 0.9509) as shown in Figure.1

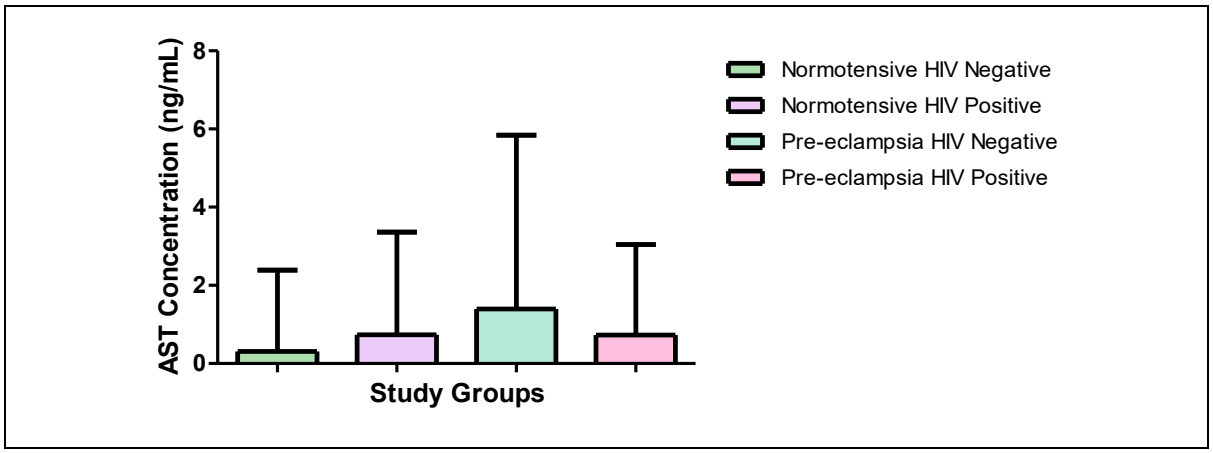


Figure 1: Plasma concentration levels of AST (U/L): Across all groups: Normotensive HIV negative (N-); Normotensive HIV positive (N+); Pre-eclamptic HIV negative (PE-); Pre-eclamptic HIV positive (PE+).

3.2.2. Pregnancy Type

i) Normotensive vs. pre-eclamptic:

AST levels were increased in the pre-eclamptic group (median = -0.4063 U/L; 95% CI: 2.788 -0.4439) compared with the normotensive group (median = -0.5667 U/L; 95% CI: 1.107 - 0.2042); however, this did not reach statistical significance (Mann-Whitney U = 554.0; p = 0.7973). Figure 2 (A).

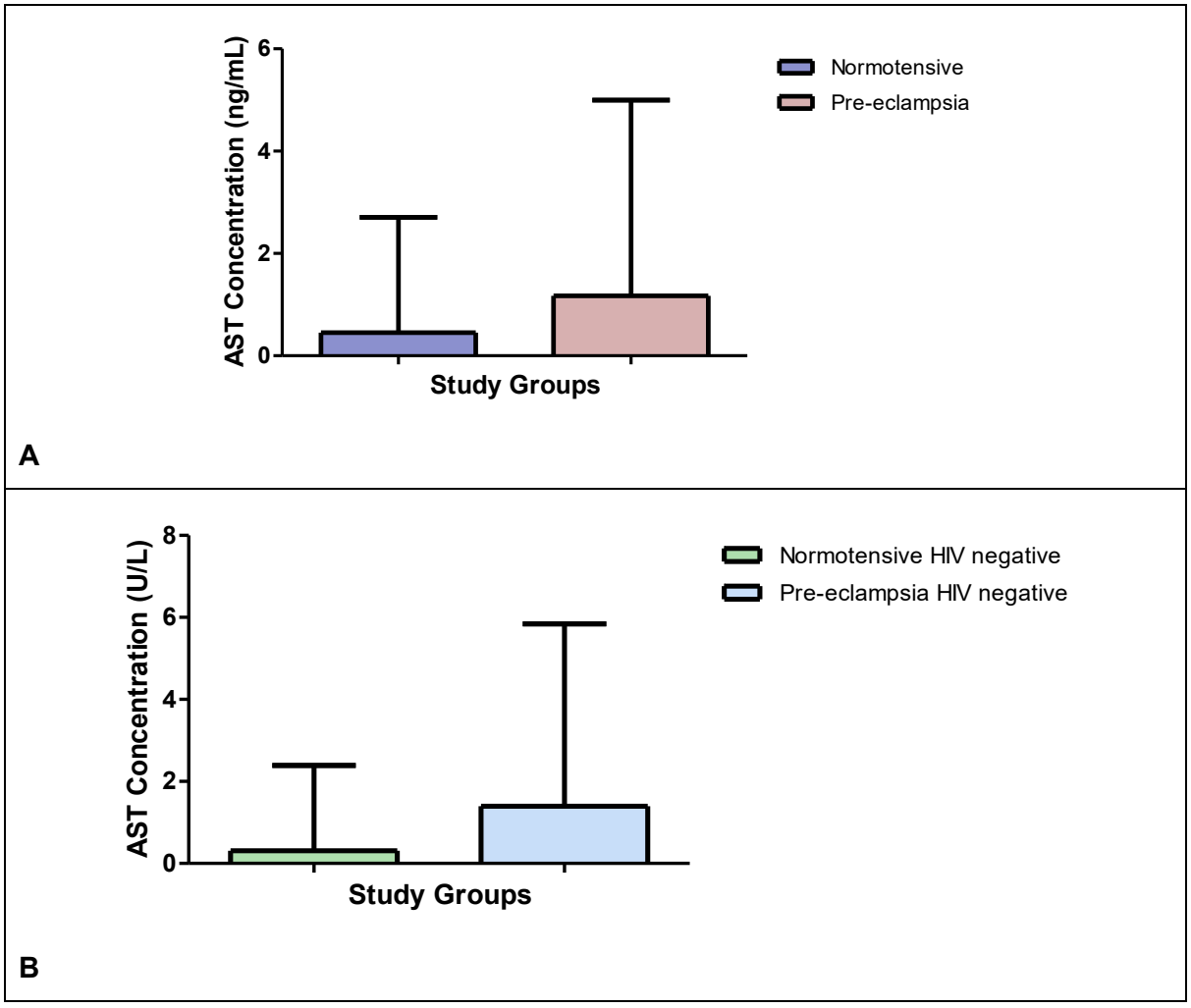
ii) HIV negative Normotensive vs. pre-eclamptic:

AST levels were increased in the pre-eclamptic group (median = -0.5923 U/L; 95% CI: 3.765 - 0.9758) compared with the normotensive group (median = -0.4318 U/L; 95% CI: 1.059 -

0.4400) however this did not reach statistical significance (Mann-Whitney U = 250.0; p = 0.9043). Figure 2 (B).

iii) HIV Positive Normotensive vs. pre-eclamptic:

There was no significant difference in AST levels in the normotensive group (median = -0.6652 U/L; 95% CI: 2.137 -0.6660) compared with the pre-eclamptic group (median = -0.1929 U/L; 95% CI: 2.666 -1.212) however this did not reach statistical significance (Mann-Whitney U = 55.00; p = 0.6027). Figure 2 (C).



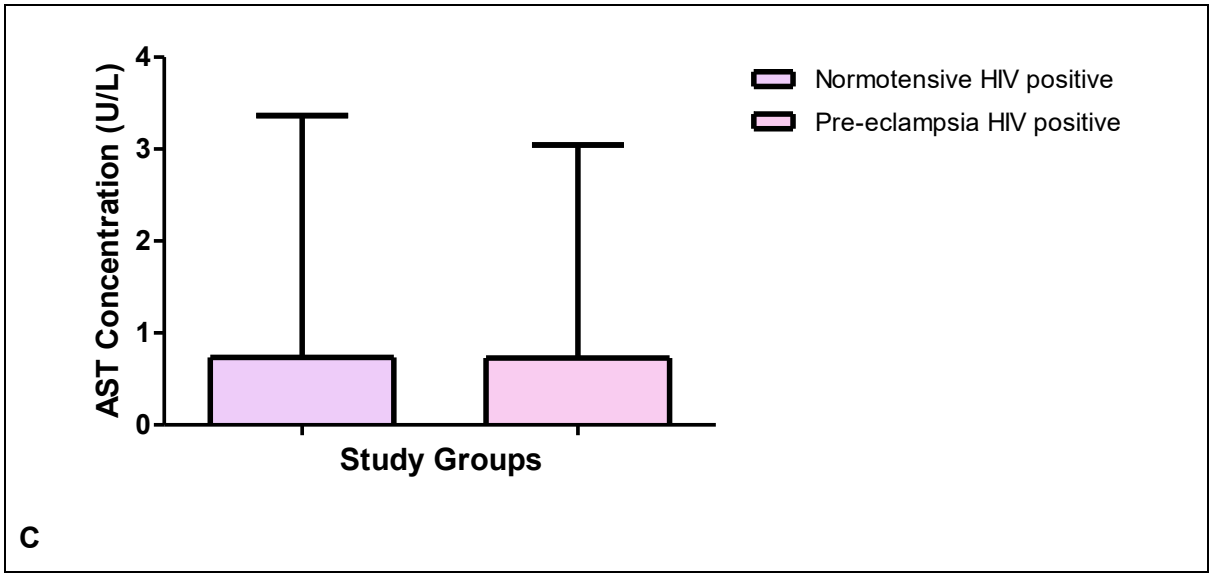


Figure 2: Plasma concentration levels of ARST (U/L) +) in Pregnancy type: **(A)** N vs. PE; **(B)** N- vs PE-; **(C)** N+ vs. PE+.

3.2.3. HIV status

i) HIV negative normotensive vs. HIV positive normotensive:

AST levels were increased in the HIV positive normotensive group (median = -0.6652 U/L; 95% CI: 2.137 -0.6660) compared with the HIV negative normotensive group (median = -0.4318 U/L; 95% CI: 1.059 -0.4400) however this did not reach statistical significance (Mann-Whitney U = 252.5; p = 0.9477). Figure 3 (A).

ii) HIV negative pre-eclamptic vs. HIV positive pre-eclamptic:

AST levels were reduced in the HIV positive pre-eclamptic group (median = -0.1929 U/L; 95% CI: 2.666 -1.212) compared with the HIV negative pre-eclamptic group (median = -0.5923 U/L; 95% CI: 3.765 -0.9758) however no statistical significance was established (Mann-Whitney U = 56.00; p = 0.6460). Figure 3 (B).

iii) All HIV negative groups vs. All HIV positive groups:

There was no significant difference in AST levels in all HIV negative groups (median = -0.4500 U/L; 95% CI: 1.563 -0.2206) compared with all HIV positive groups (median = -0.5230 U/L; 95% CI: 1.780 -0.3143) however no statistical significance was established (Mann-Whitney U = 560.5; p = 0.8578). Figure 3 (C).

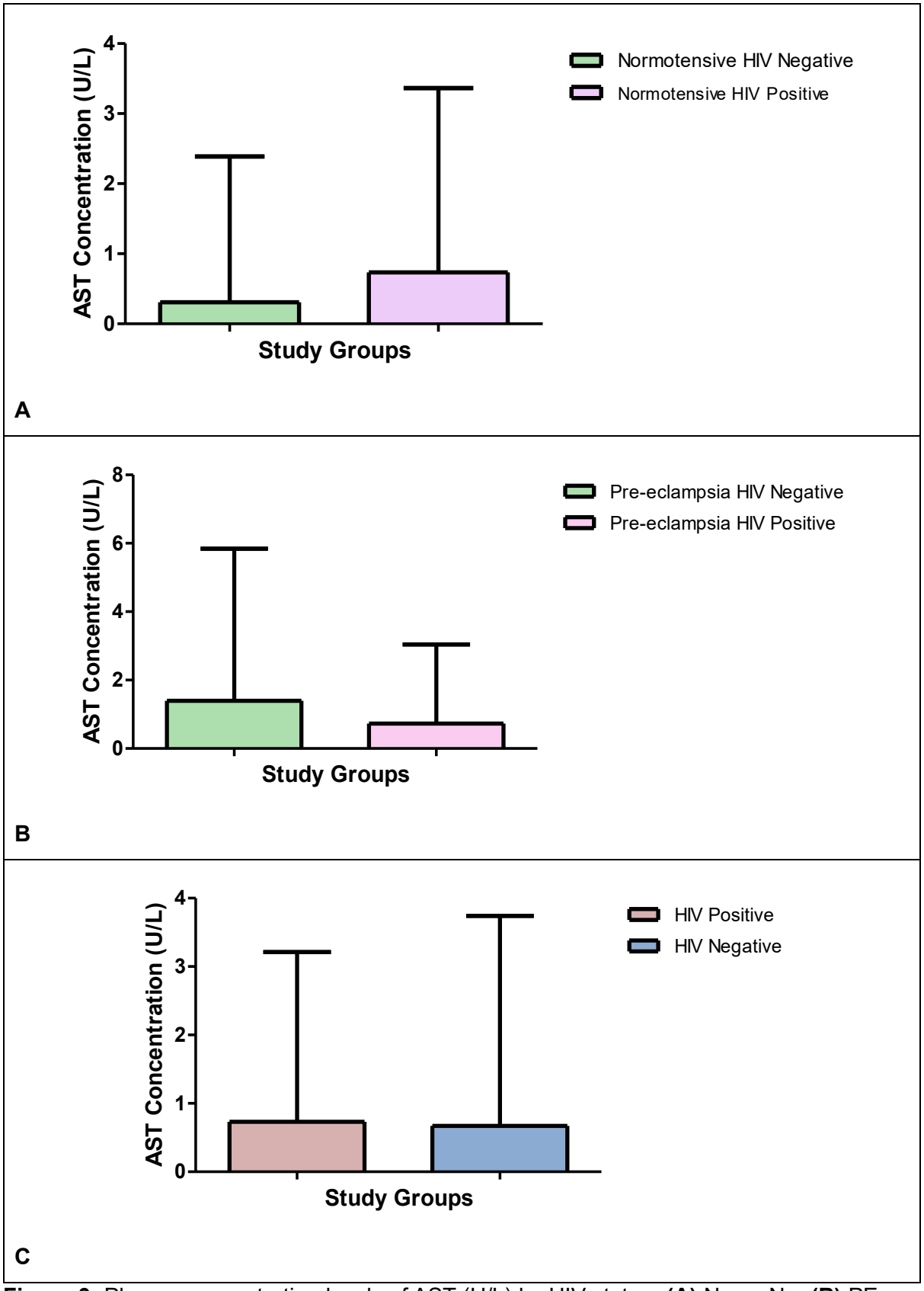


Figure 3: Plasma concentration levels of AST (U/L) by HIV status**: (A)** N- vs. N+; **(B)** PE- vs. PE+; and **(C)** HIV- vs. HIV+. HIV- vs. HIV+.

3.2.4. HIV status - Negative

i) HIV negative normotensive vs. all pre-eclamptic:

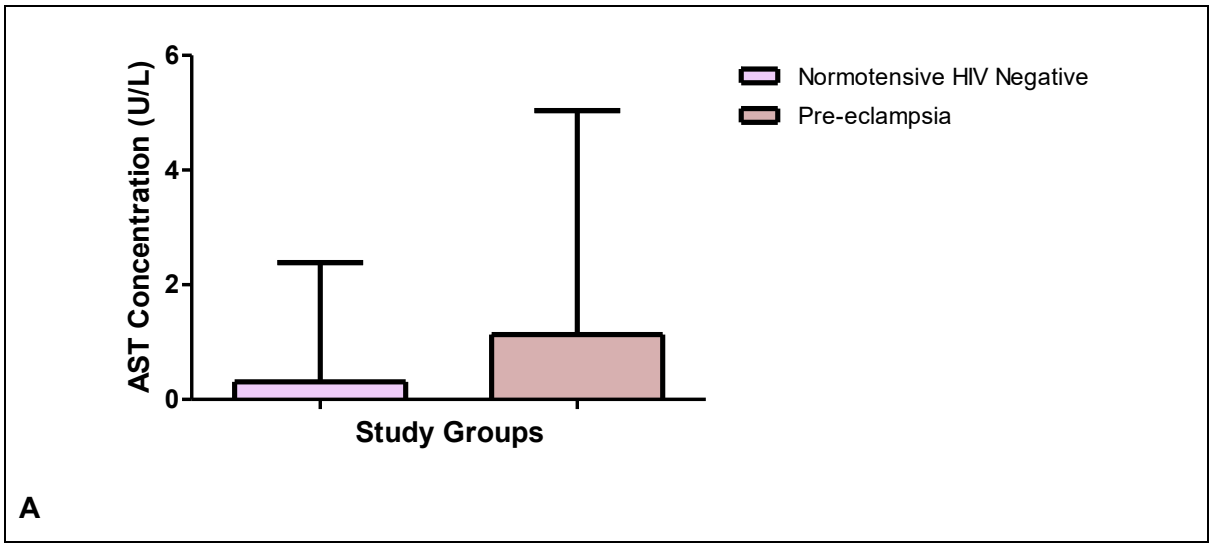
AST levels were reduced in the HIV negative normotensive group (median = -0.4318 U/L; 95% CI: 1.059 -0.440) compared with all pre-eclamptic groups (median = -0.4756 U/L; 95% CI: 2.821 -0.5581) however no statistical significance was established (Mann-Whitney U = 366.0; p = 0.9796). Figure 4 (A)

ii) HIV negative normotensive vs. pre-eclamptic HIV negative vs. pre-eclamptic HIV positive:

AST levels higher in the pre-eclamptic HIV negative group (median = -0.1929 U/L; 95% CI: 3.765 – 0.9758), lower in the pre-eclamptic HIV positive group (mean = 0.7270 ± 2.319 U/L; 95% CI: 2.666 -1.212), compared to the lowest in the normotensive HIV negative group (median = -0.4318 U/L; 95% CI: 1.059 -0.4400), however there was no statistical significance establish amongst the groups (Kruskal Wallis = 0.3042; p = 0.8589). Figure 4 (B)

iii) HIV negative normotensive vs. pre-eclamptic HIV positive:

AST levels were lower in the HIV negative normotensive group (median = -0.4318 U/L; 95% CI: 1.059 -0.4400), compared to the pre-eclamptic HIV positive group (mean = 0.7270 ± 2.319 U/L; 95% CI: 2.666 -1.212), however no statistical significance was established (Mann-Whitney U = 113.0; p = 0.6239). Figure 4 (C)



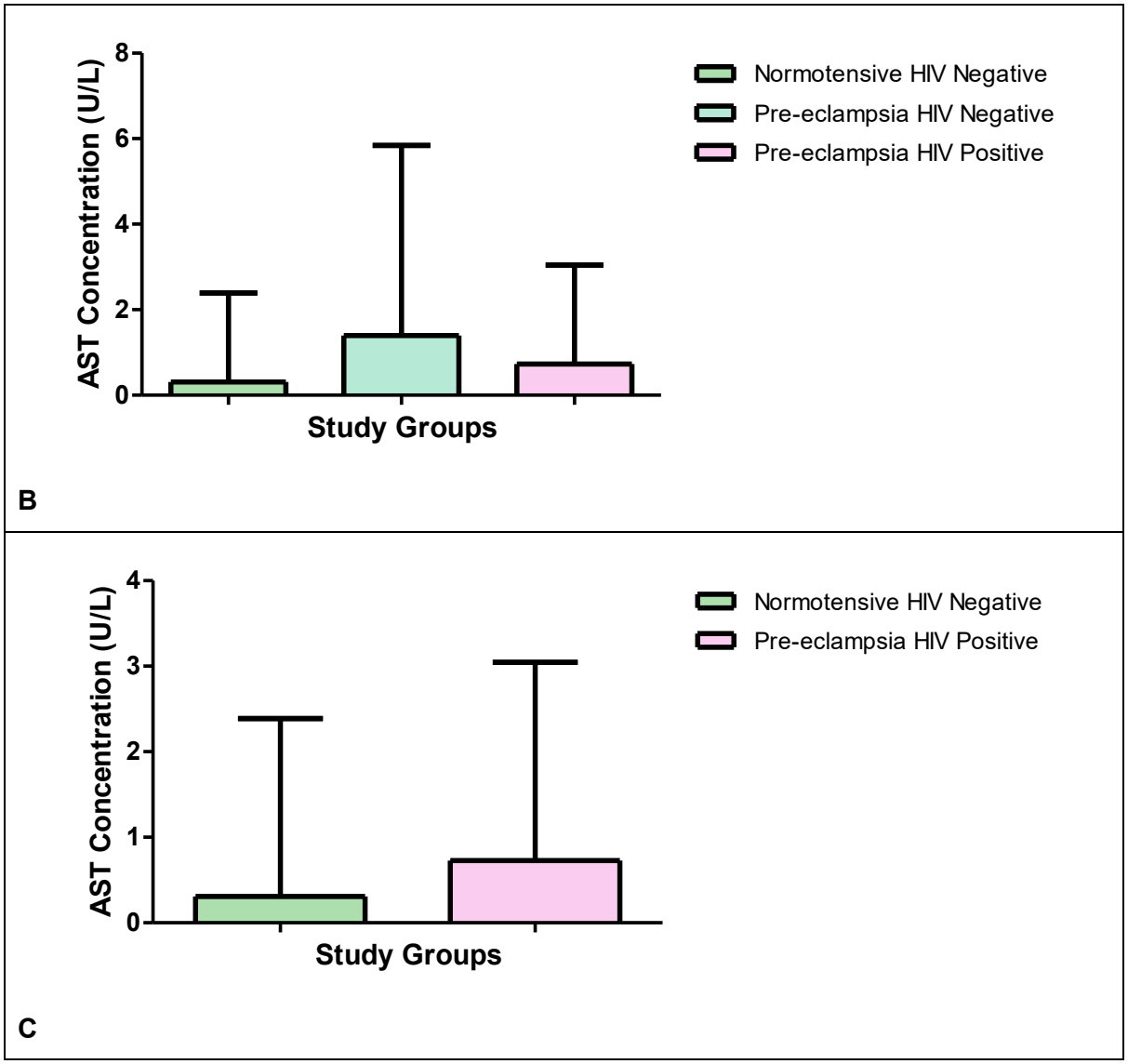


Figure 4: Plasma concentration levels of AST (U/L) by HIV status – Negative; **(A)** HIV- vs. all PE; **(B)** HIV- vs. PE- vs. PE+; **(C)** HIV- vs. PE+.

3.2.5. HIV status - Positive

i) HIV positive normotensive vs. all pre-eclamptic:

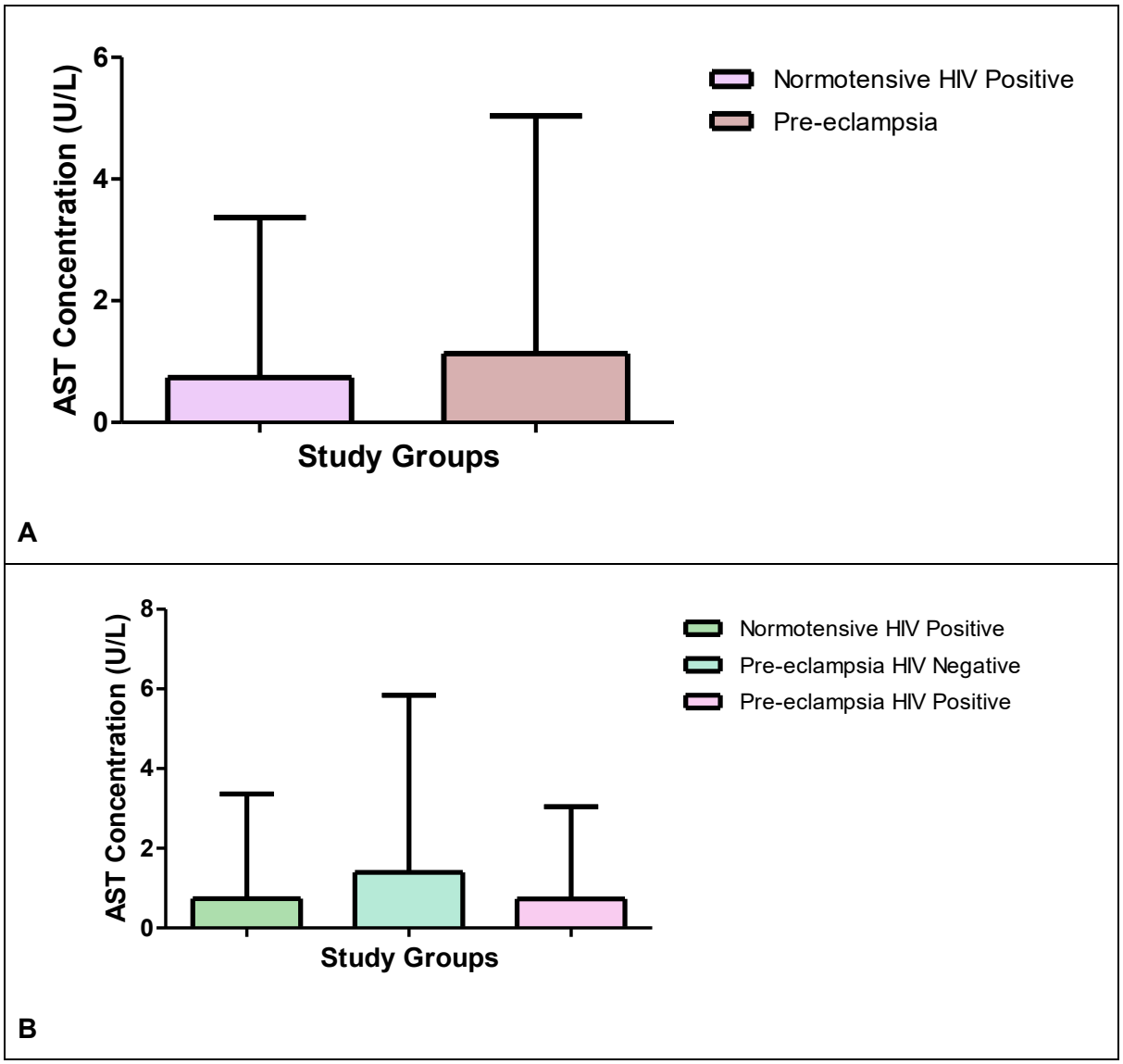
AST levels were higher in the pre-eclamptic group (median = -0.4756 U/L; 95% CI: 2.821 - 0.5581), compared to the lower levels of AST in the HIV positive normotensive group (median = -0.6652 U/L; 95% CI: 2.137 -0.6660), however no statistical significance was established (Mann-Whitney U = 176.0; p = 0.8304). Figure 5 (A)

ii) HIV positive normotensive vs. pre-eclamptic HIV negative vs. pre-eclamptic HIV positive:

AST levels higher in the pre-eclamptic HIV negative group (median = -0.5923 U/L; 95% CI: 3.765 -0.9758), lower in the pre-eclamptic HIV positive group (mean = 0.7270 ± 2.319 U/L; 95% CI: 2.666 -1.212), compared to the lowest in the normotensive HIV negative group (median = -0.6652 U/L; 95% CI: 2.137 -0.6660), however there was no statistical significance establish amongst the groups (Kruskal Wallis = 0.3489; *p* = 0.8399). Figure 5 (B)

iii) HIV positive normotensive vs pre-eclamptic HIV negative:

AST levels were lower in the HIV positive normotensive group (median = -0.6652 U/L; 95% CI: 2.137 -0.6660), compared to the pre-eclamptic HIV negative group (mean = -0.5923 U/L; 95% CI: 3.765 -0.9758), however no statistical significance was established (Mann-Whitney U = 124.0; *p* = 0.8951). Figure 5 (C)



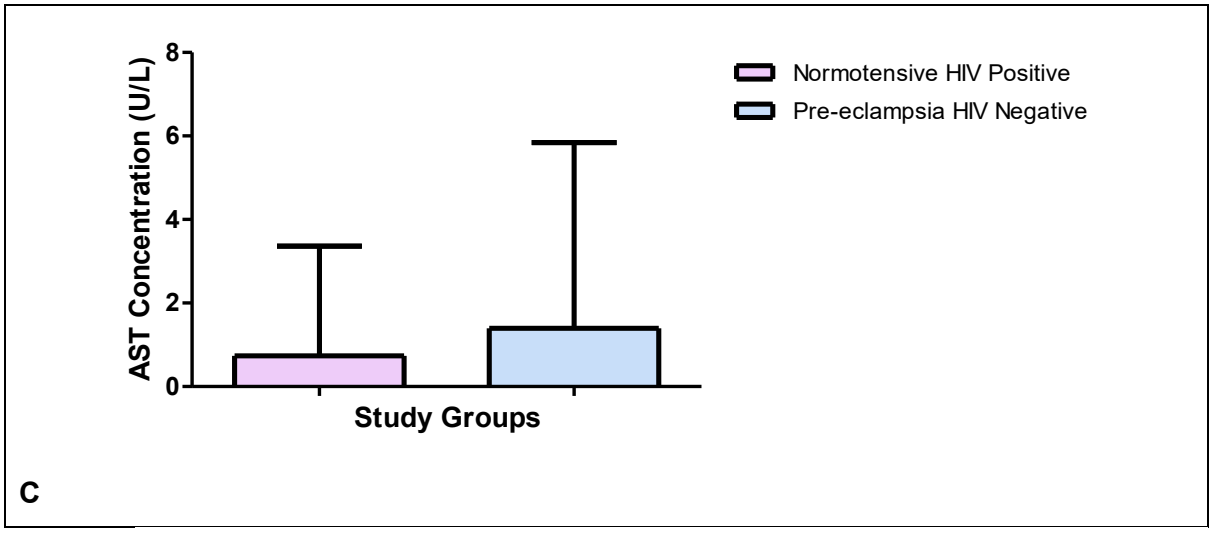


Figure 5: Plasma concentration levels of AST (U/L) by HIV status – Positive; **(A)** HIV+ vs. all PE; **(B)** HIV+ vs. PE- vs. PE+; **(C)** HIV+ vs. PE-.

4. Discussion

This study examined plasma AST levels in PE and normotensive women, both with and without HIV infection, to assess the cumulative effect of hypertensive disorder of pregnancy and HIV on maternal liver function. The results showed higher AST levels in women with PE than in normotensive groups, but the difference was not statistically significant. PE is usually associated with elevated liver enzyme levels, which are caused by endothelial dysfunction, microangiopathic haemolysis, and liver cell damage [19]. Contrary to our findings, another study by Dajac et al. (2016) reported that women with PE had significantly higher AST levels than healthy pregnant women [20]. As well as Munazza et al., (2011), reported a significant increase in AST levels in women with PE (41.34 ± 10.764 U/L) compared to normotensive women (8.24 ± 2.544 U/L) [21]. The lack of statistically significant differences in AST levels among the groups can be attributed to the sample size and statistical power, as the overall cohort consisted of 72 participants, with the PE cohort being smaller. Another potential explanation is that AST is not exclusively liver-specific. It is also present in cardiac and skeletal muscle, as well as in erythrocytes [22]. Consequently, any minor muscular injury or haemolytic event may elevate circulating AST levels, thereby obscuring subtle hepatic variations between normotensive and PE patients.

AST, or Serum Glutamic-Oxaloacetic Transaminase (SGOT), is an enzyme that plays a role in amino acid metabolism. It facilitates the reversible transfer of an amino group between aspartate and α-ketoglutarate, forming oxaloacetate and glutamate [23]. AST is synthesised in multiple tissues across the body, with the highest concentrations seen in the liver, heart,

skeletal muscles, kidneys, and red blood cells [22]. The widespread distribution of AST means that elevated levels in the blood may indicate tissue injury in any of these organs. AST levels were generally elevated in HIV-positive normotensive women relative to their HIV-negative counterparts, although this difference lacked statistical significance. The increased AST values in HIV-positive normotensive women relative to HIV-negative controls may indicate the cumulative impact of persistent systemic inflammation and potential hepatotoxicity from highly active antiretroviral therapy (HAART) [24]. Chronic immune system activation, viral replication, and ART-induced hepatotoxicity may raise AST levels over time, even in the absence of visible liver disease [25], [26]. Interestingly, in this study, HIV-positive women with PE demonstrated reduced AST levels in comparison to HIV-negative women with PE. Various reasons may explain this paradox. This unexpected outcome may be due to ART-related hepatic normalisation [27], reduced endothelial & hepatic inflammation leading to less hepatic injury [28], variations in illness severity, or potential protective benefits of HAART on systemic inflammation in specific situations [29]. Another consideration is that AST, expressed in various organs including muscle and red blood cells, is less liver-specific than other liver function markers [22]. The PE group had significantly elevated maternal BMI and age, both recognised risk factors for PE and potential influencers of altered hepatic function [30]. These factors may obscure the correlation between illness condition and AST levels.

Between HIV-negative normotensive women and all PE groups, AST levels were observed to be lower in the normotensive group; however, this difference again lacked statistical significance. Comparing HIV-negative normotensive, PE HIV-negative, and PE HIV-positive groups showed that PE HIV-negative women had slightly higher AST levels, while pre-eclamptic HIV-positive women had values that were about the same. This may be due to the mitigating factors of ART exposure in the HIV-positive group and may indicate that hepatic enzyme increase is likely to PE exposure [31]. Analysis involving HIV-positive women, PE participants typically demonstrated elevated AST levels compared to their normotensive peers, indicative of hepatic stress or endothelial dysfunction associated with PE [32]. When linking groups based on HIV status and hypertension (HIV-positive normotensive versus PE HIV-negative and PE HIV-positive), there was a similar but not significant trend. The PE groups were more likely to have high AST levels. This may indicate modest hepatic involvement that did not meet clinical criteria or could stem from variations in disease severity, ART regimen, or individual immunological response. The lack of substantial differences in AST among the groups likely reflects both biological and methodological influences, such as limited subgroup sizes and clinical severity diversity. Nevertheless, the identified patterns highlight the intricacy of hepatic responses during gestation, especially in individuals impacted by both PE and HIV.

Ongoing monitoring of liver enzymes in these patients is essential, since even slight increases may indicate early hepatic distress, particularly in individuals on prolonged ART.

5. Conclusion

The results of this study found no significant difference in AST between pregnant women with and without PE. Furthermore, there was no difference in AST between those with or without HIV. This suggests pregnant HIV women with or without PE might not be at high risk of liver dysfunction. However, due to the limited sample size, the results must be interpreted with caution. These data indicate that AST alone may inadequately represent hepatic impairment in the context of PE and HIV. It is advisable to conduct larger studies that include supplementary biomarkers to elucidate the synergistic impacts of PE and HIV on maternal liver health.

6. Limitations

The overall sample size (n = 72) and the subgroup sizes (PE + HIV^+, PE + HIV^-) were comparatively small due to limited willingness among pregnant women to participate. Small sample sizes limit the study's ability to detect differences and increase the risk of Type II error. Only AST was assessed as a hepatic function indicator. AST is not exclusive to the liver and may also be elevated owing to muscular injury or haemolysis.

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Chapter 3.2: Manuscript 2

Alanine Aminotransferase Levels in HIV-Positive and HIV-Negative Pregnant Women with and Without Pre-Eclampsia

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Abstract

Pre-eclampsia (PE) is a hypertensive condition in pregnancy characterised by hypertension and proteinuria after 20 weeks of gestation, which leads to systemic endothelial dysfunction and multi-organ impairment, including the liver. Hepatic changes during pregnancy respond to metabolic demands and hormonal fluctuations, often reflected in elevated alanine aminotransferase (ALT) levels resulting from hepatocellular injury. The study aims to compare serum ALT levels in PE women with and without HIV and to determine the impact of HIV infection and hypertensive disorder on AST as a marker of liver function. A prospective case-control study was conducted at the Charlotte Maxeke Johannesburg Academic Hospital in South Africa, including 18 PE and 22 normotensive women. Participants were categorised based on HIV status and type of pregnancy. ALT activity was assessed via a colourimetric enzymatic test, and statistical analyses were conducted using Statisty. This study found a significant increase in ALT in HIV-positive pregnant women with PE compared to HIV-negative pregnant women with PE. No significant difference in ALT level in normotensive pregnant women with or without HIV ($p > 0.05$). Among HIV-positive pregnant women with or without PE, there was no difference in ALT. In contrast, the normotensive HIV-negative women exhibited higher ALT levels than the PE women. The results showed that HIV-positive women with PE have higher ALT activity than HIV-negative women with PE, suggesting that HIV infection and ART may exacerbate the liver dysfunction in PE by increasing hepatic ALT. These findings clarify the complex regulation of hepatic enzymes during pregnancy.

Keywords: Pre-eclampsia; ALT; HIV; Pregnancy; Hepatic function; Antiretroviral therapy

1. Introduction

Pre-eclampsia (PE) is a hypertensive condition occurring during pregnancy, defined by the emergence of hypertension and proteinuria after 20 weeks of gestation, frequently associated with systemic endothelial dysfunction and multi-organ involvement, including the liver [1]. Pregnancy impairs biological and physiological processes that facilitate foetal development and maternal well-being [2]. Hepatic function experiences significant alteration to meet heightened metabolic requirements and hormonal effects [3]. Liver involvement in PE may lead to increased liver enzyme levels, particularly alanine aminotransferase (ALT), due to hepatocellular necrosis or ischaemia resulting from diminished hepatic perfusion [4]. ALT, a crucial hepatic enzyme involved in amino acid metabolism, functions as a sensitive biomarker for hepatocellular damage [5]. Increased ALT levels often indicate hepatic stress or injury and are commonly employed to evaluate liver function in clinical and research settings [6]. ALT, previously known as serum glutamic-pyruvic transaminase (SGPT), is a critical liver enzyme commonly employed to assess hepatic function [7]. It facilitates the reversible transamination reaction between alanine and α-ketoglutarate, resulting in pyruvate and glutamate, essential cofactors in amino acid metabolism and gluconeogenesis [8]. Due to ALT's predominant presence in the cytoplasm of hepatocytes and its release into circulation after cellular damage, its blood levels act as a highly specific marker of hepatocellular integrity [7]. Increased ALT levels indicate hepatocellular damage resulting from several sources, including viral infections, ischaemia, toxins, or drug-induced injury [9]. An increase in ALT has been linked to liver adversity, thus making it a possible predictor of liver impairment in PE women. Previous quantitative study showed that HIV infection and ART administration are associated with elevated liver function test enzymes, suggesting that each may contribute to liver dysfunction among PLWH [10]. However, there have been conflicting findings with recent evidence showing lower ALT in PE than in normotensives [11]. These conflicting findings make it difficult to understand the contribution of PE to liver dysfunction

In communities with a significant prevalence of HIV, such as sub-Saharan Africa, the coexistence of PE with HIV infection complicates maternal liver function during gestation [12]. HIV infection, when combined with antiretroviral therapy (ART), can affect liver enzyme activity through direct viral effects, immune-mediated damage, or drug-induced hepatotoxicity [13,14]. Thus, differentiating whether hepatic enzyme alteration arises from HIV infection, ART use, PE, or their interplay presents a clinical and research challenge. Therefore, examining the variance in ALT activity among normotensive and PE pregnant women with and without HIV could shed light on the contribution of these factors in clinical settings, especially in areas of the HIV pandemic, like SA. As a result, this study aims to investigate the level of serum ALT in pre-eclamptic and normotensive pregnant women living with and without HIV to better

understand the combined and independent effects of HIV infection and hypertensive pregnancy disorders on hepatic function.

2. Materials and Methods

2.1. Study Design, Population

This prospective case-control study was conducted at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), South Africa. Following the acquisition of written consent, normotensive (N) and pre-eclamptic (PE) HIV-negative and HIV-positive pregnant women were enlisted at CMJAH. Forty-eight normotensive women (aged 18 to 43 years) and 24 women with PE (aged 18 to 40 years) were recruited.

2.2. Ethics Approval and Participant's Consent

The project received ethics approval from the University of South Africa-College of Agriculture and Environmental Sciences Health research ethics committee (2022/CAES_HREC/005), was subsequently approved by CAES HREC (2025/CAES_HREC/7327), and received authorisation from the gatekeeper to utilise the samples. Regulatory approval was obtained from Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), South Africa. All patients provided written informed consent at the time of recruitment, indicating that their data would be used for future publication, provided their confidentiality is maintained.

2.3. Participant's Characteristics and Measurement

Demographic information for all participants was obtained from their maternity case records. HIV testing was conducted post-counselling using a rapid point-of-care test kit, in accordance with South African standards of care. Weight (kg) and height (m) were measured according to standard protocols, with shoes removed and participants wearing light clothing. Body mass index (BMI) was calculated using the participant's weight (kg) and height (m) according to the formula: BMI = weight (kg)/height (m^2). The BMI was classified as normal weight (18-25 kg/m^2), overweight (25-30 kg/m^2), and obesity (>30 kg/m^2). PE is defined by the emergence of hypertension (SBP/DBP ≥140/90 mmHg) recorded on two distinct occasions four hours apart, with or without accompanying proteinuria. Normotensive pregnant participants were characterised as those with an SBP/DBP of ≤120/80 mmHg [15]. Systolic and diastolic blood pressure were measured using an automated blood pressure monitor (OMRON Healthcare, Kyoto, Japan) according to the manufacturer's protocol.

2.4. Inclusion and exclusion criteria

To maintain anthropometric uniformity, all participants in the study identified as non-smokers and refrained from alcohol and recreational drug use, while all HIV-positive individuals were undergoing highly active antiretroviral therapy (HAART: tenofovir, emtricitabine, and efavirenz) in accordance with the South African National HIV guidelines (Department of Health, Republic of South Africa, 2015). The implementation of HAART during pregnancy is essential for reducing mother-to-child transmission via several mechanisms, including lowering maternal antepartum viral load and providing preexposure and postexposure prophylaxis to the infant [16]. Women with concomitant chronic medical conditions were excluded from the trial.

2.5. Sample Collection, Preparation, and Storage

Of the 40 stored maternal plasma samples, 18 were from PE patients and 22 from normotensive patients. The sample was collected by a professional nurse at Charlotte Maxeke Johannesburg Academic Hospital using a 21-gauge venipuncture needle. Approximately 6 mL of blood was collected from each participant in EDTA tubes (July-October 2023). After the sample collection, the blood was processed within one hour to maintain plasma integrity. The EDTA tubes were centrifuged at 1500 x g for 10-15 minutes at 4°C to separate plasma from cellular components. The separated plasma was subsequently stored in a freezer at -80 °C for future use.

2.6. Alanine Aminotransferase (ALT) Activity Assay

ALT concentrations were quantified using the Alanine Aminotransferase (ALT/GLT) Activity Assay Kit (Elabscience, Cat No. E-BC-K235M), which is based on the principle that ALT catalyses the transamination of alanine with α-ketoglutarate, yielding pyruvate and glutamate. Pyruvate subsequently interacts with phenylhydrazine to produce a reddish-brown phenylhydrazone in alkaline circumstances. The colour intensity, assessed at 510 nm, correlates with ALT enzymatic activity in the sample.

In compliance with the kit instructions, 20 µL of preheated substrate solution and 5 µL of the serum sample were added to each well allocated for sample measurement. Control wells received only substrate solution, whereas standard wells received varying amounts of sodium pyruvate to establish the calibration curve. After mixing, the plate was incubated at 37 °C for 30 minutes. Subsequently, 20 µL of chromogenic agent was added to each well, followed by a 20-minute incubation at 37 °C. Subsequently, 200 µL of freshly prepared alkali working solution was added, thoroughly mixed, and the plate was left at room temperature for 15 minutes. Absorbance was quantified at 510 nm using a microplate reader.

The ALT activity in serum samples was determined by subtracting the optical density (OD) of control wells from that of sample wells (ΔA510). A standard curve ($y = ax^2 + bx + c$) was created by graphing absolute optical density values of standards against their respective activities. ALT activity was determined in international units (IU/L) using the manufacturer's conversion factor (1 Carmen unit = 0.482 IU/L).

2.7. Statistical Analysis

Microsoft Excel 365 was used to generate the standard curve and determine AST concentrations using the computed regression line. The data were analysed using Statisty, an online statistical analysis tool (https://statisty.app/online-statistics-software; accessed 25 November 2025). The Shapiro–Wilk test was used to assess the distribution of data. For parametric distributions, descriptive statistics for continuous data are reported as mean ± standard deviation; for nonparametric distributions, the median and 95% confidence intervals (CIs) are reported. Due to the non-distributional nature of the data, the Mann-Whitney U test was used. Two-way ANOVA was used to assess the main effect of HIV and PE statuses on CRP. The statistical significance was $p < 0.05$.

3. Results

3.1. Clinical Characteristics of Participants

Table 1 summarizes the clinical and demographic features of the study population. PE women had higher SBP and DBP than normotensives ($p < 0.0001$). Additionally, maternal weight ($p = 0.0345$) and BMI ($p = 0.0394$) were statistically different between the normotensive pregnant and PE groups. There were no significant differences in maternal age ($p = 0.3043$), height (p = 0.4838), or gestational age ($p = 0.1131$) between PE and normotensive women.

Table 1. Patient demographic features of the study groups.

Variables	Pre-eclampsia	Normotensive	*P*-value
Sample size	18	22	
Maternal age (years)	35.5 (21.0–40.0)	37.0 (21–40.2)	0.3043
Maternal weight (kg)	88.0 (80.25–101.5)	72.5 (63–87.75)	**0.0345**
Maternal height (m)	158 (157.3–162.5)	161 (156.8–164)	0.4838
BMI (kg/m^2)	39.39 (34.08–33.45)	27.78 (26.91–33.54)	**0.0394**
Gestational age (weeks)	17.5 (13.0–26.5)	23.5 (16.75–29.25)	0.1131
SBP (mmHg)	150.5 (147–156.5)	109 (105.8–111.5)	**<0.0001**
DBP (mmHg))	97.5 (90–105.3)	65.5 (54.75–69.0)	**<0.0001**

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, kg: kilogram, Data reported as median and 95%CI, the bolded present statistically significant results

3.2 The level of ALT in pre-eclamptic pregnant women with and without HIV

In pre-eclamptic pregnant women, ALT levels differed significantly between HIV-negative and HIV-positive women, Mann-Whitney U = 15, p = 0.027 (Figure 1). HIV-positive women had increased ALT, Median = -53.75 IU/L, 95%CI (-57.71, 48.54) compared to HIV-negative women, Median = -63.33 IU/L, 95%CI (-68.33, -56.88). Furthermore, the effect size was large (r = 0.52), showing a substantial influence of HIV status on ALT concentrations.

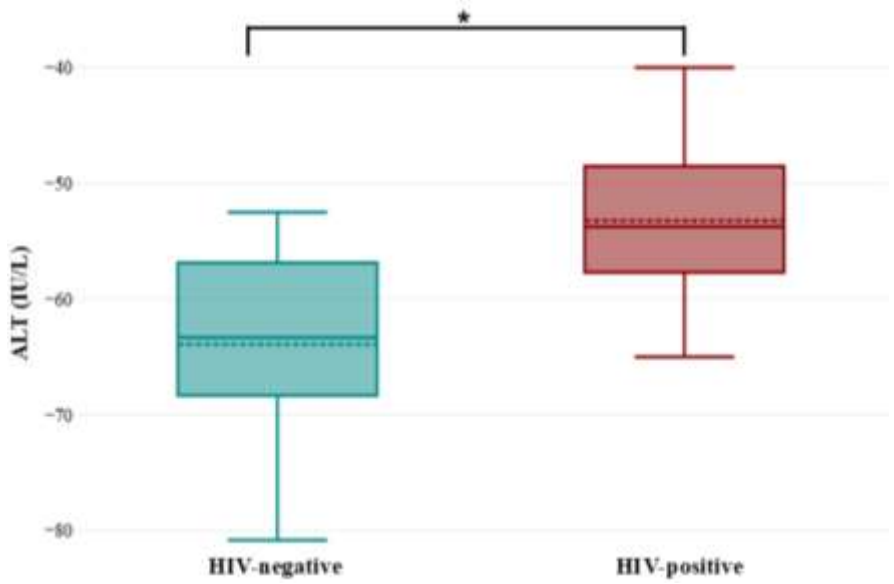

Figure 1. ALT levels in pre-eclamptic women with and without HIV. *Shows significant differences.

3.3 The level of ALT in normotensive pregnant women with and without HIV

The descriptive statistics show that the HIV-negative group had higher levels of ALT, Median = -51.25 IU/L, 95%CI (-64.38, 128.96) than the HIV-positive women, Median = -57.5 IU/L, 95%CI (-66.67, -50.83). However, the Mann-Whitney U-Test showed that these differences between HIV-negative and HIV-positive women were not statistically significant, U = 40, p = 0.203. Moreover, the observed effect size was small (r = 0.28).

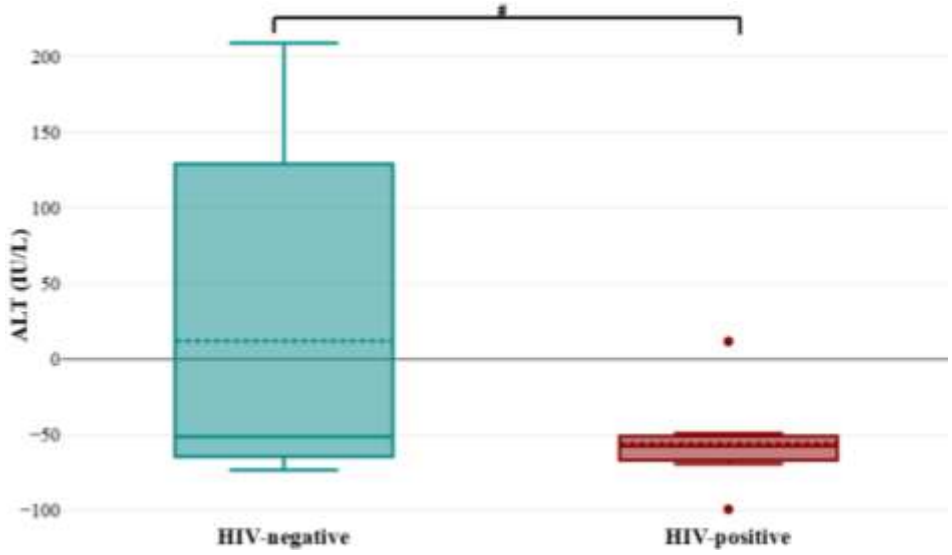

Figure 2. ALT in normotensive pregnant women with and without HIV. # shows no significant effect.

A two-way ANOVA investigated the effect of HIV and PE status on ALT (Figure 3). The PE results revealed a significant main effect, F (1,36) = 4.26, p = 0.046, η^2p = 0.11. In contrast, HIV status did not have a significant effect, F (1, 36) = 2.69, p = 0.109, η^2p = 0.07. The HIV and PE interaction approached significance, F (1,36) = 4.05, p = 0.052, η^2p = 0.10, suggesting a differential pattern between the groups.

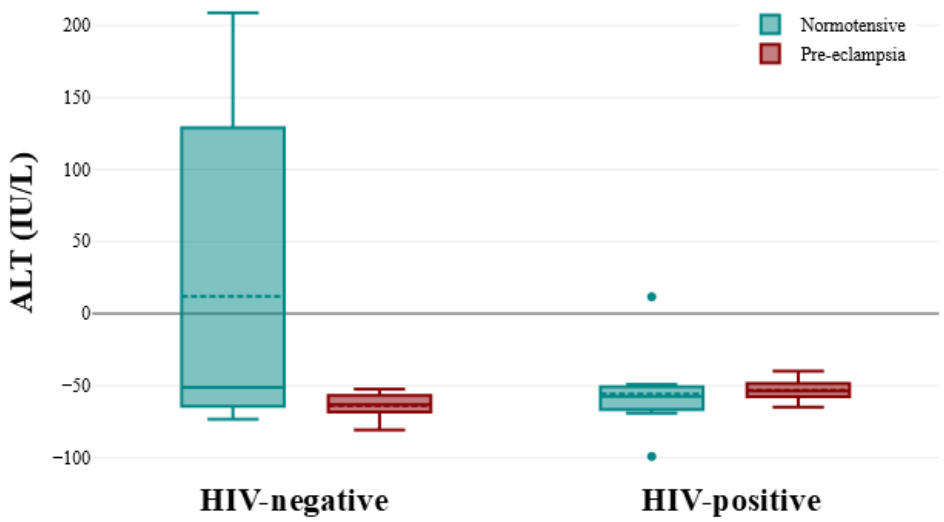

Figure 3. Effect of PE and HIV status on CRP.

4. Discussion

This study examined the combined and independent effects of HIV infection and PE on maternal liver function, using ALT as a biomarker. Results revealed that HIV-positive women with PE exhibit significantly elevated ALT levels in comparison to their HIV-negative counterparts. This indicates that HIV infection, especially when coupled with ART, exacerbates hepatic vulnerability during hypertensive disorder of pregnancy. HIV infection leads to persistent immunological activation, modified metabolic pathways, and perhaps direct viral interaction with hepatocytes [17]. When combined with PE-induced ischaemia and endothelial dysfunction, this results in increased hepatocyte membrane damage and enzyme leakage, as evidenced by elevated ALT levels [18]. The differences in ALT activity patterns between HIV-positive and HIV-negative women may indicate the intricate interplay among HIV infection, ART, and the hepatic implications of PE. HIV infection affects hepatic function through many mechanisms: direct viral infiltration of hepatocytes, immune-mediated inflammation, and drug-induced hepatotoxicity due to ART [13]. Prolonged exposure to ART has been linked to hepatocellular adaptation, resulting in a diminished propensity for transaminase leakage despite persistent hepatic stress [19]. In normotensive women, HIV status alone did not significantly affect ALT concentrations. This trend contradicts known research, such as a study by Kushner et al. (2022) on pregnant women in the USA, which found that 13.2% exhibited elevated ALT levels, defined as exceeding 25 IU/L. Most of these women did not have ALT testing during routine prenatal care [20], indicating that hepatic involvement is characteristic of PE and presents as endothelial dysfunction, ischaemia, and hepatocellular necrosis [21]. PE-associated hepatic impairment results from vasospasm and microangiopathy within hepatic sinusoids, compromising perfusion and causing hepatocyte

damage, leading to enzyme leakage into the bloodstream [22]. However, PE has a diverse clinical spectrum, with moderate to severe variants causing varying degrees of hepatic enzyme increase [23]. Even non-significant elevations in ALT may suggest subclinical hepatic stress, particularly in early or medium PE cases where overt liver impairment has yet to emerge. The lower ALT levels observed in this study can be attributed to several factors, including the mild nature of PE, effects of HIV and ART on enzyme expression, differences in sample timing, and physiological responses. Interestingly, normotensive HIV-negative women exhibited higher ALT levels than PE women, indicating physiological enzyme variability, body composition variations, or subclinical metabolic stress unrelated to hypertension disease. The two-way ANOVA indicated that PE exerted a significant main effect on ALT, and the interaction between HIV and PE approached statistical significance. This suggests that HIV alters the hepatic response to PE and that the impact of PE on ALT varies depending on HIV status. Collectively, these findings underscore a physiological synergism whereby HIV infection amplifies the hepatic effects of PE, despite HIV alone not substantially affecting ALT levels under normotensive conditions.

The findings highlight the importance of interpreting liver enzyme changes in pregnant women in relation to their HIV status and ART regimen. While ALT is still a reliable indicator of hepatocellular damage, its diagnostic sensitivity may be lowered in HIV-positive people due to ART-induced biochemical modulation. This has significant clinical implications for tracking hepatic function in HIV-positive pregnancies complicated by hypertension problems. Several factors must be considered when interpreting the results of this investigation. First, the sample size was relatively small, especially after stratifying participants based on HIV and PE status. This reduced statistical power to detect significant differences between groups, potentially leading to a lack of statistical significance in some comparisons despite discernible trends. Second, while all HIV-positive patients were on HAART, the study did not consider treatment duration, adherence, or specific drug-related hepatotoxic effects, which could have altered ALT levels independent of HIV infection or PE.

5. Conclusion

The findings drawn from this study showed that HIV-positive pregnant women with PE had significantly higher ALT levels when compared to HIV-negative pregnant women with PE. However, there was no significant difference in ALT level in normotensive pregnant women with or without HIV. Among HIV-positive pregnant women with or without PE, there was no difference in ALT. On the other hand, in contrast, the normotensive HIV-negative women exhibited higher ALT levels than the PE women. These findings support the significant main effect of PE, borderline HIV and PE interaction, indicating that the effect of PE on ALT differed

by HIV status. As the result showed that HIV-positive women with PE have higher ALT activity than HIV-negative women with PE, this suggests that HIV infection and ART may exacerbate the liver dysfunction in PE by increasing hepatic ALT. These findings clarify the complex regulation of hepatic enzymes during pregnancy.

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Chapter 3.3: Manuscript 3

Placental Alkaline Phosphatase (PLAP) Levels in HIV-Positive and HIV-Negative Pregnant Women with and Without Pre-Eclampsia

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Abstract

Background: Placental Alkaline Phosphatase (PLAP) is a glycoprotein enzyme synthesised by the placenta and released into maternal circulation, serving as an indicator of placental function and development. Changes in PLAP activity are associated with pregnancy complications like intrauterine growth restriction and pre-eclampsia (PE), which lead to maternal and perinatal morbidity. PE involves hypertension and proteinuria due to abnormal placentation. HIV infection complicates pregnancy by affecting immune responses and placental function, while antiretroviral therapy can influence PLAP function.

Objective: This study aims to analyse PLAP levels in HIV-positive and HIV-negative pregnant women, with and without PE, to enhance understanding of liver function and improve care strategies.

Methods: A prospective case-control study using stored plasma samples from 72 pregnant women (48 normotensive and 24 with PE), collected at Charlotte Maxeke Johannesburg Academic Hospital in South Africa. PLAP concentrations were measured using a sandwich ELISA. Statistical analyses utilised the Kruskal–Wallis and Mann–Whitney U tests, with significance established at $p < 0.05$.

Results: PLAP levels did not differ substantially among the study groups (Kruskal-Wallis = 0.8595; $p = 0.8352$). PE women had slightly higher mean PLAP levels than normotensive women (6.778 ± 3.336 ng/mL vs. 6.490 ng/mL; $p = 0.8112$). HIV-positive normotensive women had lower PLAP levels than HIV-negative normotensive women (4.905 ng/mL versus 6.585 ng/mL; $p = 0.5047$). There were no significant differences between the HIV-positive and HIV-negative pre-eclamptic groups ($p > 0.05$).

Conclusion: Despite slight variations in PLAP concentrations between groups, no significant changes were found. A slight rise in PLAP in PE may indicate placental hypoxia and endothelial dysfunction. In contrast, low PLAP in HIV-positive women may be due to ART-induced mitochondrial stress and altered trophoblastic activity. Collectively, these findings suggest that PLAP, while not dramatically altered, may nonetheless serve as a biochemical marker of mild placental and hepatic stress in complicated pregnancies.

Keywords: Placental Alkaline Phosphatase (PLAP), Pre-eclampsia, HIV, Antiretroviral therapy, Pregnancy, Liver function

1. Introduction

Placental Alkaline Phosphatase (PLAP) is a glycoprotein enzyme synthesised in the placenta and secreted into the maternal bloodstream during gestation [1]. It functions as a biochemical indicator of placental performance and development [2]. Altered PLAP activity, whether increased or decreased, has been associated with pregnancy complications, such as intrauterine growth restriction (IUGR), premature delivery, and hypertensive diseases [3]. Pregnancy is a multifaceted physiological state marked by substantial metabolic, immunological, and circulatory changes that promote maternal health and facilitate optimal foetal development [4]. Among pregnancy-related complications, pre-eclampsia (PE) is a significant contributor to maternal and perinatal morbidity and mortality globally [5]. PE is characterised by the emergence of hypertension and proteinuria after 20 weeks of gestation, principally linked to abnormal placentation, endothelial dysfunction, and a dysregulation of pro- and anti-angiogenic factors [6], [7]. The placenta is pivotal in the pathogenesis of PE, as deficient trophoblastic invasion and insufficient remodelling of the spiral arteries result in placental ischaemia and oxidative stress, ultimately causing systemic maternal inflammation [8], [9]. Altered PLAP expression may indicate placental dysfunction and help elucidate the disease's pathophysiology [10].

Human Immunodeficiency Virus (HIV) infection adds difficulty to pregnancy by altering immune responses and affecting placental development and function [11]. The extensive use of antiretroviral therapy (ART) has improved maternal and foetal outcomes in HIV-positive women. Yet, ART exposure and HIV-associated immunological modifications may continue to influence placental enzyme function and vascular integrity [12]. Liver involvement in PE indicates the systemic endothelial damage characteristic of the condition [13]. The hepatic endothelium is particularly susceptible to microangiopathy and vasospasm, which are induced by anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) [14]. These factors disrupt vascular homeostasis and impede hepatic perfusion [15].

This study aims to explore PLAP levels in HIV-positive and HIV-negative pregnant women with and without PE. By investigating differences in PLAP activity among different groups, the study hopes to gain insight into liver function under the influence of HIV infection and hypertensive disorders of pregnancy.

2. Materials and Methods

2.1. Study Population and Design

This study was executed as a cross-sectional study. Ethical approval for the study was secured from the University of South Africa-College of Agriculture & Environmental Sciences Health Research Ethics Committee (2025/CAES_HREC/7327), approved on 08/05/2025, and authorisation from the gatekeeper was obtained to utilise the samples. Regulatory approval from the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa was also secured. Following the acquisition of written consent, normotensive (N) and pre-eclamptic (PE) HIV-negative and HIV-positive pregnant women were enlisted at CMJAH. Normotensive patients (n = 48, age range: 18 to 43 years) and patients with preeclampsia (PE) (n = 24, age range: 18 to 40 years) were recruited. Pre-eclampsia is defined by the emergence of hypertension of ≥140/90 mmHg recorded on two distinct occasions four hours apart, with or without accompanying proteinuria. Normotensive pregnant participants were characterised as those with a blood pressure of ≤120/80 mmHg [16]. Demographic information for all research participants was obtained from their maternity case records. HIV testing was conducted following counselling using a rapid point-of-care test kit, in accordance with South African standards of care. Maternal weight was classified as normal weight (BMI: 18-25 kg/m²), overweight (BMI: 25-30 kg/m²), and obesity (BMI: >30 kg/m²). To maintain anthropometric uniformity, all female participants in the study confirmed their status as non-smokers and their abstention from alcohol and recreational drugs, while all HIV-positive individuals were undergoing highly active antiretroviral therapy (HAART: tenofovir, emtricitabine, and efavirenz) in compliance with the South African National HIV guidelines (Department of Health, Republic of South Africa, 2015). The implementation of HAART during pregnancy is essential for reducing mother-to-child transmission by lowering maternal antepartum viral load and offering preexposure and postexposure prophylaxis for the infant [17]. Women with concomitant chronic medical conditions were excluded from the trial.

2.2. Sample Collection

This study will employ 72 archived maternal plasma samples, comprising 48 from normotensive subjects and 24 from individuals with preeclampsia, obtained from the Charlotte Maxeke Johannesburg Academic Hospital. A qualified nurse collected blood samples via venipuncture using a 21-gauge needle. Approximately 6 ml of blood samples were collected from each research participant in EDTA tubes (July-October 2023). After sample collection, the blood was processed within 1 hour to maintain plasma integrity. The EDTA tubes were

centrifuged at 1500 × g for 10-15 minutes at 4°C to separate plasma from cellular components. The separated plasma was subsequently stored in a freezer at -80° for future use.

2.3. Human PLAP (Placental Alkaline Phosphatase) ELISA

ALP concentrations were quantified using the Human Placental Alkaline Phosphatase (PLAP) ELISA kit (Elabscience, Cat No: E-EL-H1976), which employs a sandwich enzyme-linked immunosorbent assay (ELISA) methodology. The 96-well microplates, pre-coated with an antibody specific to human PLAP, collected the analyte from samples or standards. A biotinylated detection antibody was subsequently inserted, followed by avidin coupled to horseradish peroxidase (HRP). Upon introduction of the chromogenic substrate (TMB), a blue colour appeared, which turned yellow upon addition of the stop solution. The optical density (OD) was measured at 450 ± 2 nm, with OD directly proportional to PLAP concentration.

All reagents and standards were prepared according to the manufacturer's instructions. Samples were diluted 1:5 by adding 160 µL of diluent to 40 µL of sample to obtain 200 µL of diluted samples. The lyophilised PLAP standard was reconstituted to yield a working solution of 10 ng/mL. Serial dilutions were prepared to generate a standard curve at concentrations of 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, and 0 ng/mL. The wash buffer was created by diluting the 25× concentrate with deionised water. The biotinylated detection antibody and HRP conjugate were diluted 1:100 with their respective diluents just before application.

100 µL of standards, blanks, and serum samples (diluted as required) were added to the designated wells and incubated for 90 minutes at 37 °C. Without prior washing, 100 µL of biotinylated detection antibody was introduced, followed by a 60-minute incubation at 37 °C. The wells were washed three times, then 100 µL of HRP conjugate was added and incubated for 30 minutes at 37 °C. After five washes, 90 µL of TMB substrate solution was introduced and incubated in the dark for 15 minutes at 37 °C. The reaction was terminated with 50 µL of stop solution, and absorbance was promptly measured at 450 nm using a microplate reader.

A standard curve was created with PLAP concentrations represented on the x-axis and OD values on the y-axis. PLAP concentrations in serum samples were determined using this curve. Samples exhibiting OD values beyond the detection range (0.39 – 25 ng/mL) were reanalysed with suitable dilutions.

2.4. Statistical Analysis

Microsoft Excel 365 was used to create the standard curve and determine AST concentrations using the computed regression line. Data analysis was conducted using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California, USA). The Shapiro–Wilk test was utilised to assess the normality of data distribution. For parametric distributions, descriptive statistics for continuous data are reported as mean ± standard deviation (SD), whereas for nonparametric distributions, they are reported as median and interquartile range (IQR). Group comparisons were performed via one-way analysis of variance (ANOVA) for parametric data, followed by Tukey's post hoc test. The Kruskal–Wallis test was employed for non-parametric data. The statistical significance was $p < 0.05$.

3. Results

3.1. Clinical Characteristics of Participants

Table 1 summarises the clinical and demographic characteristics of the study population. Systolic and diastolic blood pressures (BP) significantly differed between the normotensive and PE groups ($p < 0.0001$). Maternal age ($p = 0.0425$) and BMI ($p = 0.0373$) exhibited statistically significant differences between the normotensive pregnant group and the PE group. No significant differences were observed in maternal weight ($p = 0.1062$), maternal height ($p = 0.9917$), or gestational age ($p = 0.1515$) between the normotensive and PE groups.

Table 1. Patient demographic features of the study groups (normotensive = 48 women, pre-eclampsia = 24 women).

Variables	**Groups**	**Median**	**Q1 – Q3**	**Mean ± SD**	***p*-value**
Maternal age (years)	N	32	21 – 37.5	31.68 ± 6.56	0.0425
	PE	36	34 – 38	35.96 ± 2.38	
Maternal Weight (kg)	N	73	68 – 90	78.24 ± 14.73	0.1062
	PE	87	71.75 – 100.5	88.6 ± 18.26	
Maternal Height (m)	N	159.5	155 – 167	158.9 ± 4.79	0.9917
	PE	158	157.3 162.5	159.8 ± 5.48	
BMI (kg/m^2)	N	28.87	27.17 – 37.02	31.39 ± 5.48	0.0373

	PE	39.39	34.08 – 47.67	39.02 ± 8.64	
Systolic blood pressure (mmHg)	N	106	96 – 116.3	110.1 ± 6.53	<0.0001
	PE	149	104 – 154.8	15 ± 19.41	
Diastolic blood pressure (mmHg)	N	66.5	60.25 – 72	66.83 ± 7.67	<0.0001
	PE	96.5	91.25 – 106	99.3 ± 14.78	
Gestational age (weeks)	N	23	16 – 30	22.44 ± 7.76	0.1515
	PE	19	13 – 26	19.48 ± 8.47	

N: Normotensive; PE; Pre-eclampsia

3.2. Plasma concentration levels of PLAP

3.2.1. Across all groups

There was no significant difference in PLAP levels among the study groups (Kruskal-Wallis = 0.8595, p = 0.8352), as shown in Figure 1.

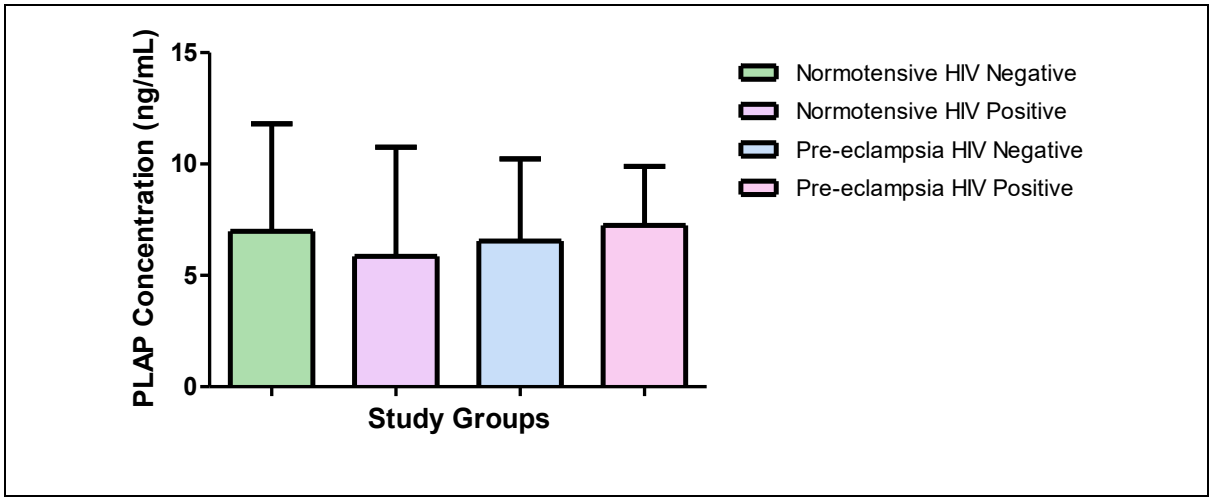


Figure 1: Plasma concentration levels of PLAP (ng/ml=l): Across all groups: Normotensive HIV negative (N-); Normotensive HIV positive (N+); Pre-eclamptic HIV negative (PE-); Pre-eclamptic HIV positive (PE+).

3.2.2. Pregnancy Type

i) Normotensive vs. pre-eclamptic:

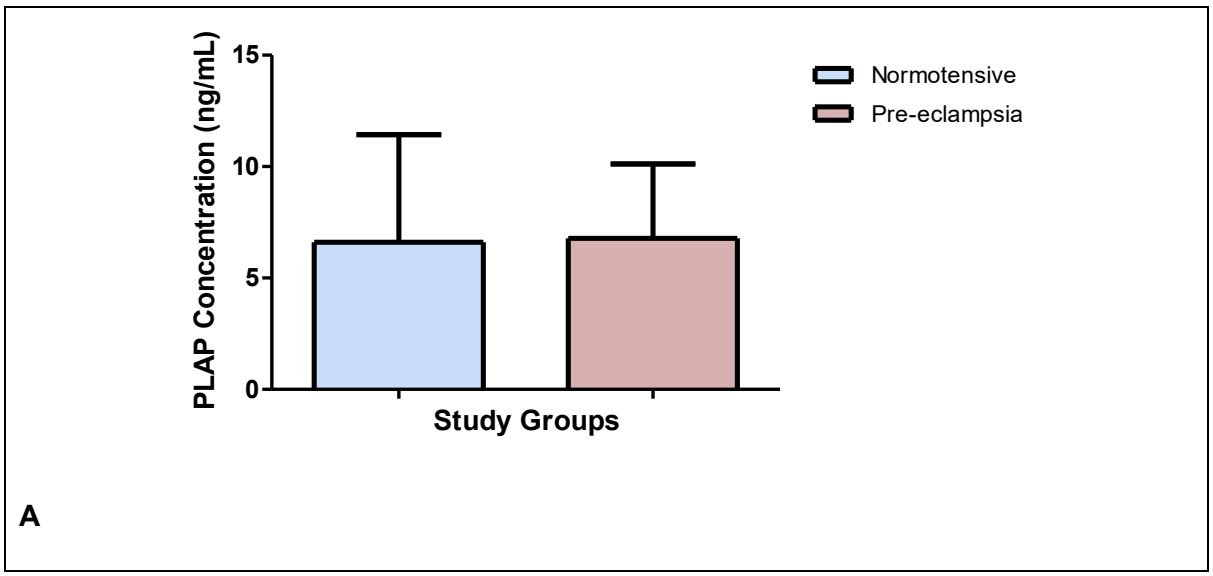
PLAP levels were increased in the pre-eclamptic group (mean = 6.778 ± 3.336 ng/mL; 95% CI: 8.186; 5.369) compared with the normotensive group (median = 6.490 ng/mL; 95% CI: 8.008; 5.203); however, this did not reach statistical significance (Mann-Whitney U = 555.5; *p* = 0.8112). Figure 2 (A).

ii) HIV negative Normotensive vs. pre-eclamptic:

PLAP levels were increased in the pre-eclamptic group (mean = 6.544 ± 3.692 ng/mL; 95% CI: 8.511; 4.577) compared with the normotensive group (median = 6.585 ng/mL; 95% CI: 8.722; 5.241); however, this did not reach statistical significance (Mann-Whitney U = 243.0; *p* = 0.7846). Figure 2 (B).

iii) HIV Positive Normotensive vs. pre-eclamptic:

There was no significant difference in PLAP levels in the normotensive group (mean = 5.853 ± 4.901 ng/mL; 95% CI: 8.465; 3.241) compared with the pre-eclamptic group (mean = 7.245 ± 2.644 ng/mL; 95% CI: 9.455; 5.034) however this did not reach statistically significant level (Mann-Whitney U = 54.00; *p* = 0.5607 Figure 2 (C).



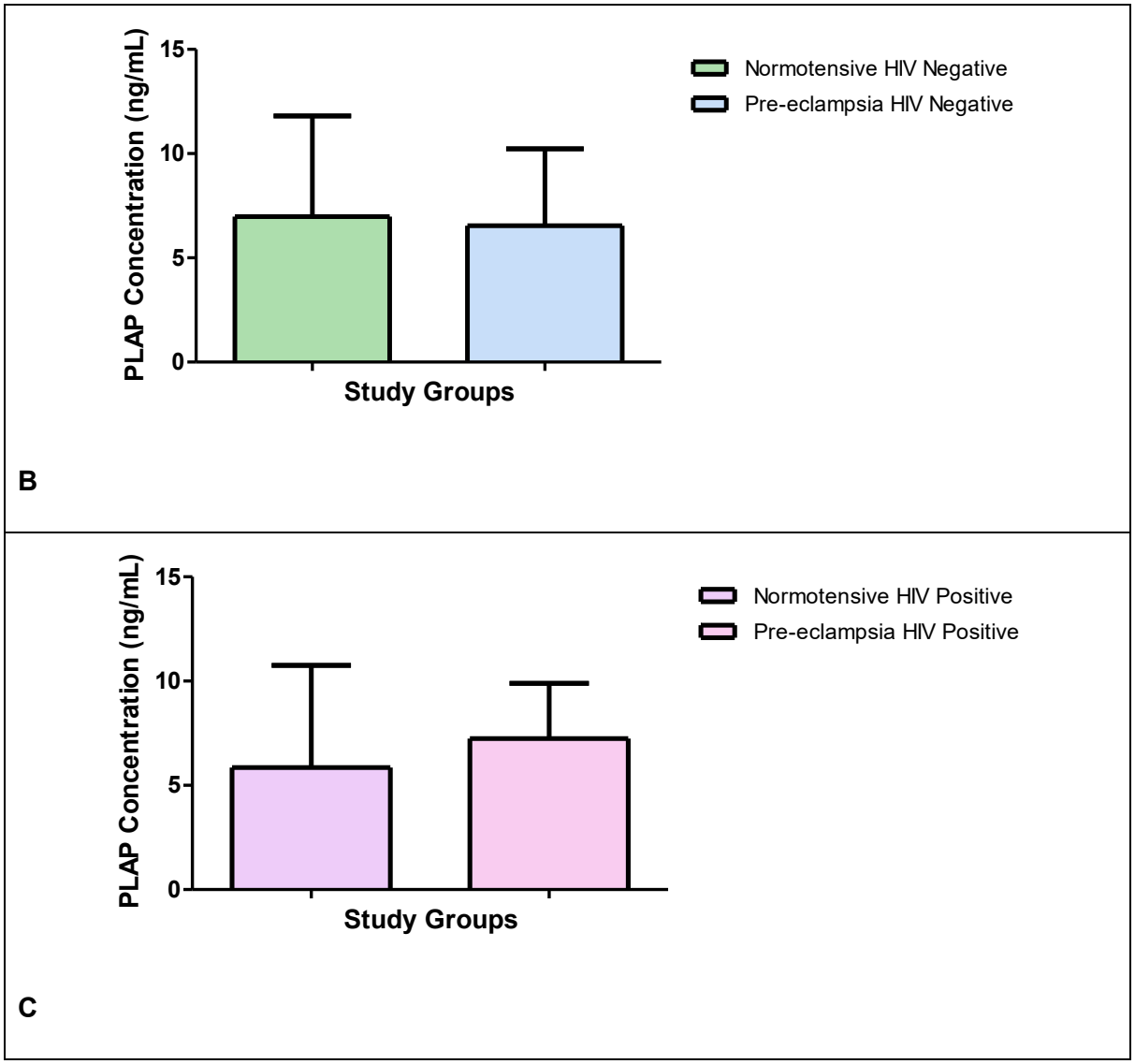


Figure 2: Plasma concentration levels of PLAP (ng/mL) +) in Pregnancy type: **(A)** N vs. PE; **(B)** N- vs PE-; **(C)** N+ vs. PE+.

3.2.3. HIV status

i) HIV negative normotensive vs. HIV positive normotensive:

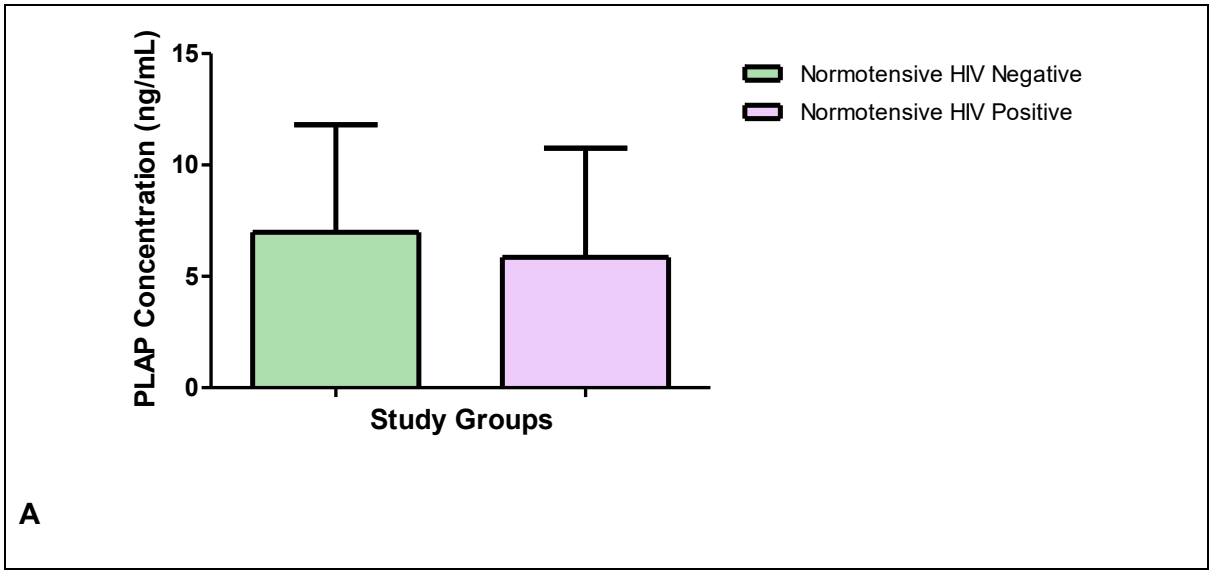
PLAP levels were lower in the HIV positive normotensive group (median = 4.905 ng/mL; 95% CI: 4.905; 3.241) compared with the HIV negative normotensive group (median = 6.585 ng/mL; 95% CI: 8.722; 5.241); however, this did not reach statistical significance (Mann-Whitney U = 225.0; p = 0.5047). Figure 3 (A).

ii) HIV negative pre-eclamptic vs. HIV positive pre-eclamptic:

PLAP levels were reduced in the HIV positive pre-eclamptic group (median = 7.708 ng/mL; 95% CI: 9.455; 5.034) compared with the HIV negative pre-eclamptic group (median = 6.264 ng/mL; 95% CI: 8.511; 4.577); however, no statistical significance was established (Mann-Whitney U = 50.00; p = 0.4084). Figure 3 (B).

iii) All HIV negative groups vs. All HIV positive groups:

There was no significant difference in PLAP levels in all HIV negative groups (median = 6.552 ng/mL; 95% CI: 8.126; 5.545) compared with all HIV positive groups (median = 7.490 ng/mL; 95% CI: 8.121; 4.513) (Mann-Whitney U = 549.5; p = 0.7561). Figure 3 (C).



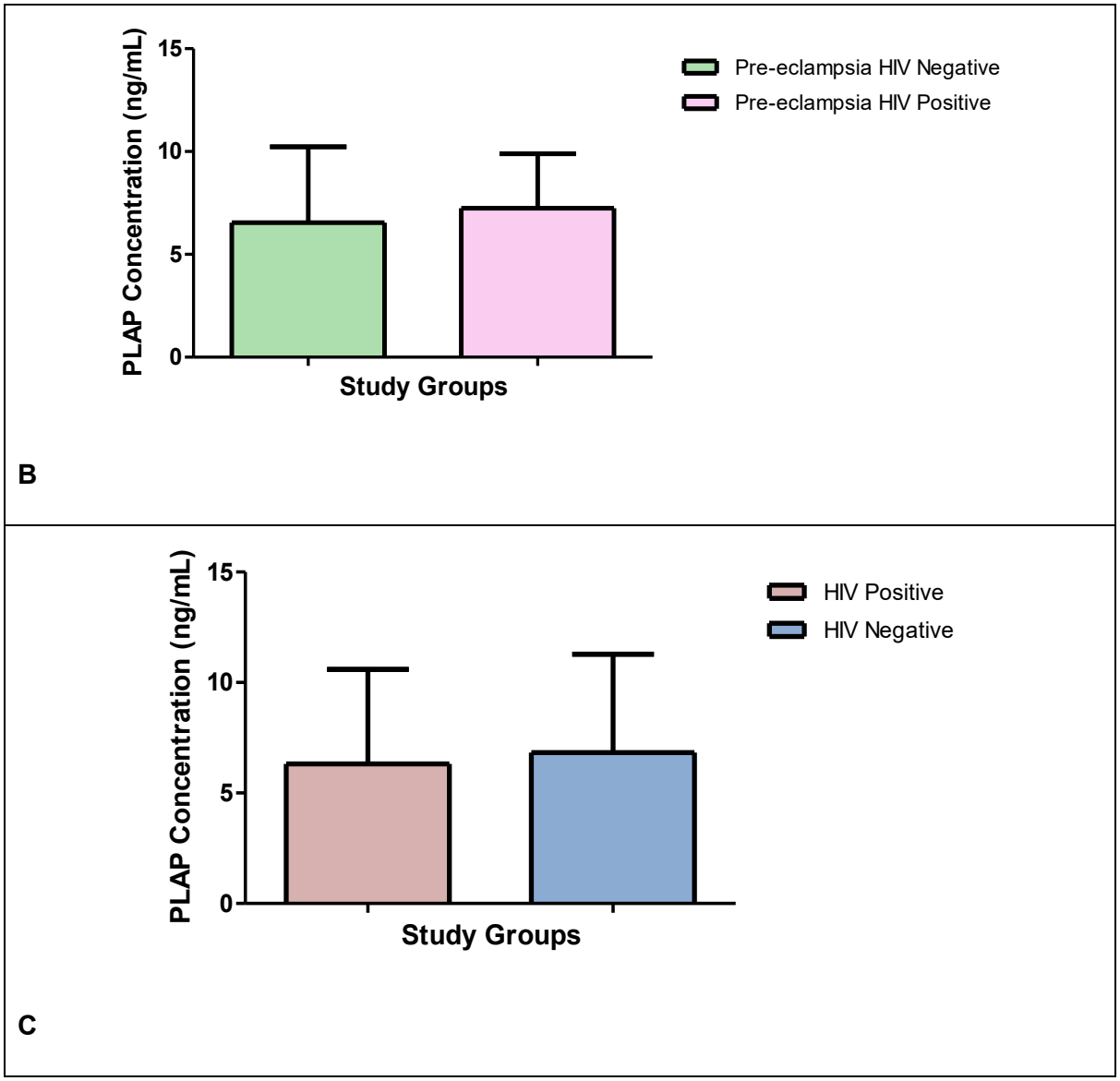


Figure 3: Plasma concentration levels of PLAP (ng/ml=l) by HIV status**: (A)** N- vs. N+; **(B)** PE- vs. PE+; and **(C)** HIV- vs. HIV+. HIV- vs. HIV+.

3.2.4. HIV status - Negative

i) HIV negative normotensive vs. all pre-eclamptic:

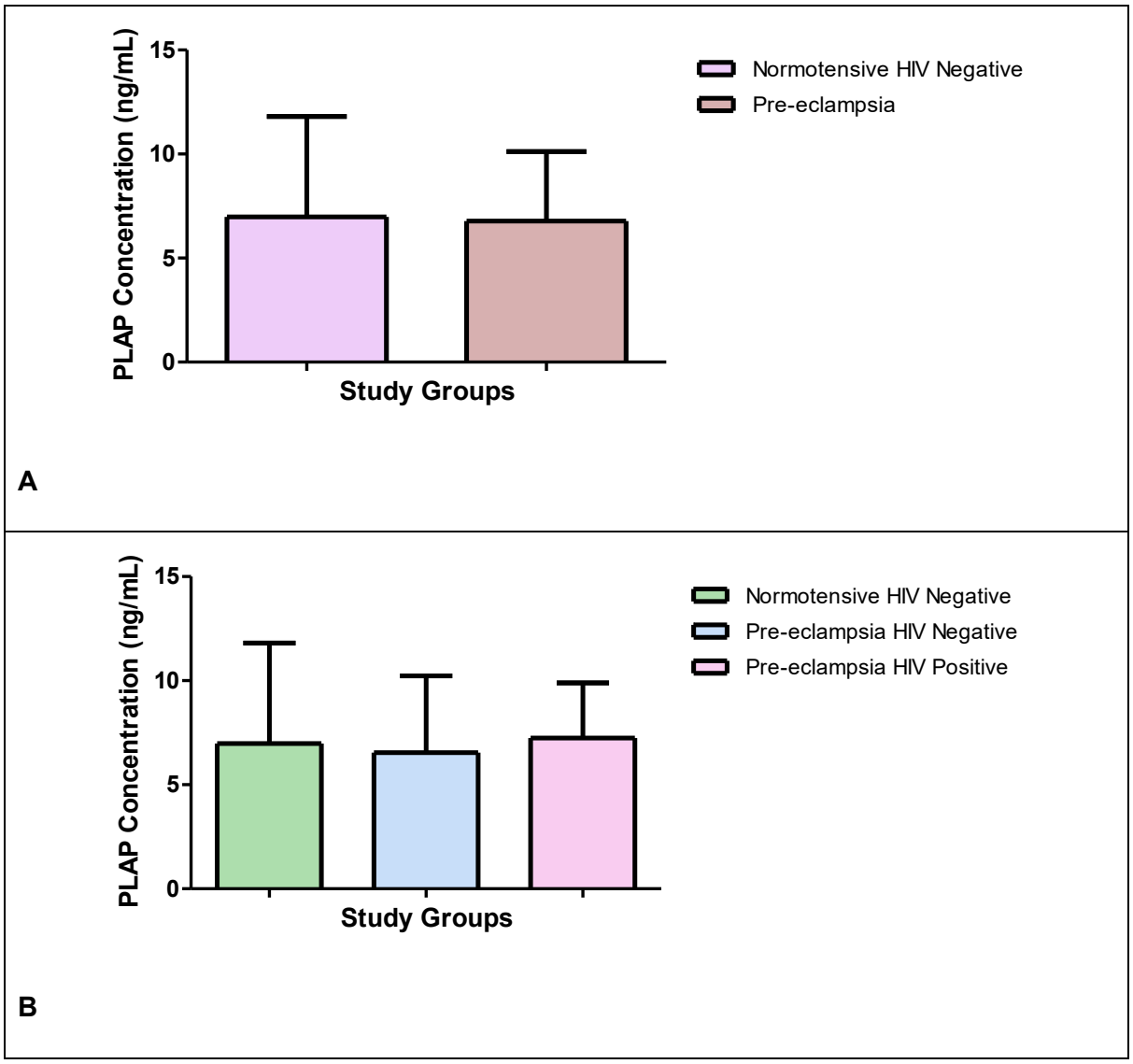
PLAP levels were increased in the HIV negative normotensive group (median = 6.585 ng/mL; 95% CI: 8.722; 5.241) compared with all pre-eclamptic groups (mean = 6778 ± 3.336 ng/mL; 95% CI: 8.186; 5.369); however, no statistical significance was established (Mann-Whitney U = 378.0; p = 0.9274). Figure 4 (A)

ii) HIV negative normotensive vs. pre-eclamptic HIV negative vs. pre-eclamptic HIV positive:

PLAP levels were higher in the pre-eclamptic HIV positive group (mean = 7.245 ± 2.644 ng/mL; 95% CI: 9.455 5.034), lower in the pre-eclamptic HIV negative group (mean = 6.544 ± 3.692 ng/mL; 95% CI: 8.511; 4.577), compared to the lowest in the HIV negative normotensive group (median = 6.585 ng/mL; 95% CI: 8.722 5.241), however there was no statistical significance establish amongst the groups (Kruskal Wallis = 0.3828; *p* = 0.8258). Figure 4 (B)

iii) HIV negative normotensive vs. pre-eclamptic HIV positive:

PLAP levels were lower in the normotensive HIV-negative group (median = 6.585 ng/mL; 95% CI: 8.722; 5.241) than in the pre-eclamptic HIV-positive group (mean = 7.245 ± 2.644 ng/mL; 95% CI: 9.455; 5.034). No statistical significance was established (Mann-Whitney U = 121.0; *p* = 0.8260). Figure 4 (C)



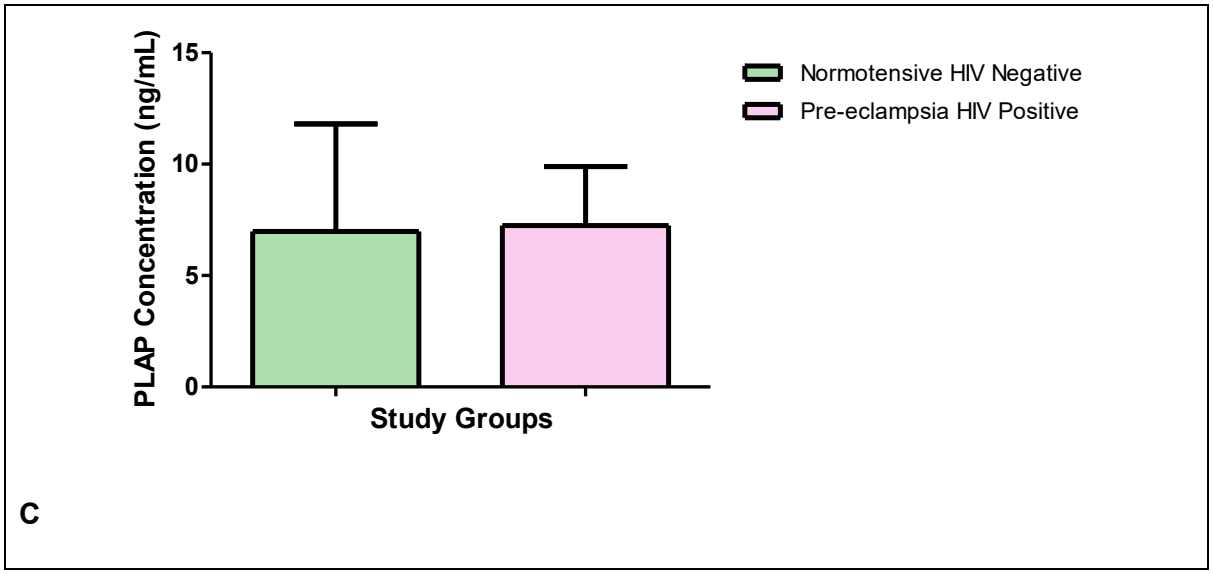


Figure 4: Plasma concentration levels of PLAP (ng/ml=l) by HIV status – Negative; **(A)** HIV- vs. all PE; **(B)** HIV- vs. PE- vs. PE+; **(C)** HIV- vs. PE+.

3.2.5. HIV status - Positive

i) HIV positive normotensive vs. all pre-eclamptic:

PLAP levels were lower in the HIV positive normotensive group (mean = 5.583 ± 4.901 ng/mL; 95% CI: 8.465; 3.241) compared to the lower levels of AST in the pre-eclamptic group (mean = 6.788 ± 3.336 ng/mL; 95% CI: 8.186; 5.369), however no statistical significance was established (Mann-Whitney U = 165.5; *p* = 0.4729). Figure 5 (A)

ii) HIV positive normotensive vs. pre-eclamptic HIV negative vs. pre-eclamptic HIV positive:

PLAP levels higher in the pre-eclamptic HIV positive group (mean = 7.245 ± 2.644 ng/mL; 95% CI: 9.455; 5.034), lower in the pre-eclamptic HIV negative group (mean = 6.544 ± 3.692 ng/mL; 95% CI: 8.511; 4.577), compared to the lowest in the normotensive HIV negative group (mean = 5.853 ± 4.901 mg/mL; 95% CI: 8.465 3.241), however there was no statistical significance establish amongst the groups (Kruskal Wallis = 0.8509; *p* = 0.6535). Figure 5 (B)

iii) HIV positive normotensive vs pre-eclamptic HIV negative:

PLAP levels were higher in the pre-eclamptic HIV negative group (mean = 6.544 ± 3.692 ng/mL; 95% CI: 8.511; 4.577), compared to the normotensive HIV positive group (mean = 5.853 ± 4.901 ng/mL; 95% CI: 8.465; 3.241), however no statistical significance was established (Mann-Whitney U = 111.5; *p* = 0.5464). Figure 5 (C)

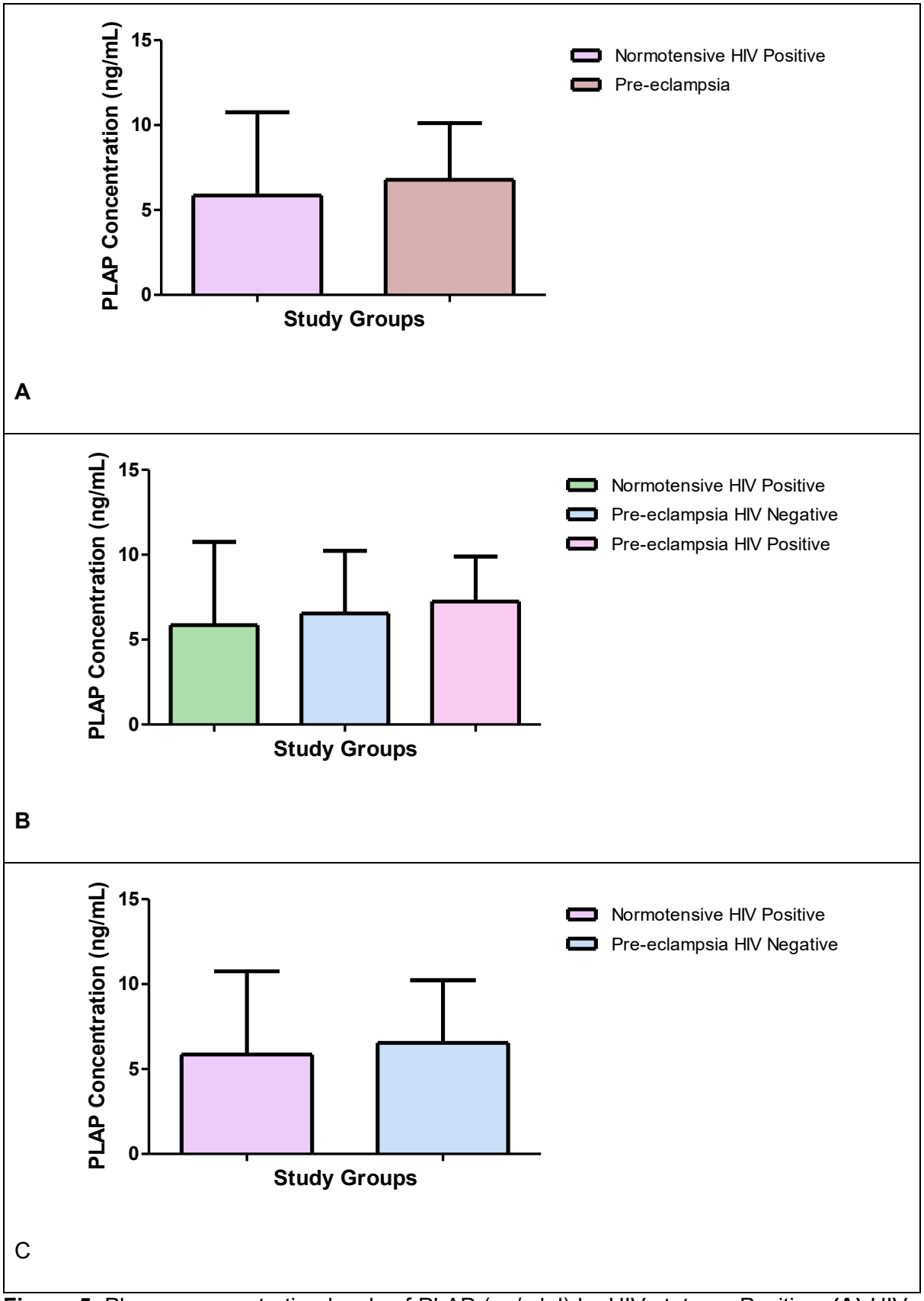


Figure 5: Plasma concentration levels of PLAP (ng/ml=l) by HIV status – Positive; **(A)** HIV+ vs. all PE; **(B)** HIV+ vs. PE- vs. PE+; **(C)** HIV+ vs. PE-.

4. Discussion

The study examined PLAP levels in normotensive and pre-eclamptic (PE) pregnancies, stratified by HIV infection on placental function and potential hepatic involvement. The PE group showed elevated PLAP levels compared with the normotensive group; however, this difference did not reach statistical significance. This trend corresponds with earlier research by Rajagambeeram et al. (2014), which identified elevated PLAP activity in PE pregnancies [18], indicating enhanced trophoblastic shedding and syncytiotrophoblast membrane turnover due to placental hypoxia [19]. Hypoxic stress, a characteristic of PE, may induce the production and secretion of placental enzymes, such as PLAP, as a compensatory mechanism for reduced placental perfusion [20]. A more recent retrospective study by Chaparro et al., (2021) found that 4.3% of pregnancies progressed from normotensive to PE, with GCF-PLAP concentrations being three to six times greater than those in plasma samples [21]. Several factors may explain variation in the significance of the observed data, including sample size, gestational age at sampling, assay sensitivity, and disease severity across study populations. Although PE patients had significantly higher blood pressure and BMI than normotensive women, the lack of statistical significance in PLAP variation may be due to the small cohort size or heterogeneity in PE severity.

When analysed according to HIV status, PLAP levels were marginally lower in HIV-positive normotensive women than in their HIV-negative counterparts, although the difference lacked statistical significance. This observation may be related to antiretroviral therapy (ART), as certain ART agents have been associated with mitochondrial dysfunction in trophoblastic cells, which may alter placental enzyme activity [22]. Additionally, the development of trophoblasts, crucial for sustaining PLAP levels, is hindered by inflammation and villous immaturity linked to HIV [23]. HIV-positive women with PE demonstrated slightly elevated PLAP levels compared to HIV-negative women with PE. This variance among groups indicates that PE, HIV infection, and ART all may influence placental enzyme expression [24]. Furthermore, the persistent immune activation associated with HIV infection may interfere with placental endocrine signalling and diminish enzyme secretion into maternal circulation [25], while ART exposure may also affect PLAP expression; research indicates that tenofovir and efavirenz can induce mitochondrial and endoplasmic reticulum stress in trophoblastic cells, potentially modifying enzymatic pathways [26], [27]. The slight elevation in PLAP levels in HIV-positive women with PE may indicate cumulative placental stress resulting from both HIV-induced inflammation and ischaemia associated with PE [28]. PLAP shares structural similarities with other alkaline phosphatase isoenzymes, including hepatic alkaline phosphatase [29]. In PE, endothelial dysfunction and reduced hepatic perfusion may contribute to hepatocellular stress and the release of liver-associated enzymes into the circulation [30]. Although PLAP is primarily

placental in origin, elevated circulating levels may reflect broader systemic stress involving both placental and hepatic pathways. Impaired hepatic clearance under hypoxic or inflammatory conditions may further contribute to increased circulating PLAP concentrations [31]. Consequently, alterations in PLAP levels may reflect integrated placental and systemic physiological disturbances in pregnancies complicated by PE and HIV infection

5. Conclusion

Overall, PLAP concentrations did not differ significantly between the HIV-positive pregnant women with PE and those without PE. Although the difference was not significant, the mean value differed, suggesting the study was underpowered.

6. Limitations

The primary limitations of the study include its small sample size, the cross-sectional design of data collection, and the lack of gestational-age-matched controls for each group. Furthermore, variations in ART regimens and the duration of HIV infection were not investigated, which could affect placental enzyme expression. Subsequent research using histological analysis of placental tissue may clarify the correlation between PLAP expression, trophoblast shape, and vascular alterations in pregnancies affected by HIV and hypertension.

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Chapter 3.4. Manuscript 4

The Interaction Between HIV Infection and Pre-Eclampsia on C-Reactive Protein Levels in Pregnant Women

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Student contributions: Conceptualisation, methodology, software, formal analysis, investigation, data curation, original draft preparation, reviewing and editing, and visualisation.

Abstract

Background: Pre-eclampsia (PE) is a pregnancy-specific hypertension disorder defined by systemic inflammation and endothelial dysfunction. In sub-Saharan Africa, the high prevalence of Human Immunodeficiency Virus (HIV) infection among women of reproductive age, along with the coexistence of PE, poses a distinct immunological and clinical health problem. This study aimed to examine the relationships among PE, HIV, and maternal systemic inflammation, using C-reactive protein (CRP) as a clinical biomarker.

Method: A retrospective cross-sectional study was performed with 72 pregnant women (48 normotensive and 24 pre-eclamptic), categorised by HIV status. Plasma CRP levels were assessed with a sandwich enzyme-linked immunosorbent test (ELISA), and statistical analyses were conducted using Statisty online version.

Results: CRP levels showed no significant difference between women with pre-eclampsia (PE) and normotensive expectant women, nor between HIV-positive and HIV-negative participants ($p > 0.05$). Similarly, CRP levels did differ significantly between moderate and severe PE. Two-way ANOVA revealed no significant main effect of PE status, HIV status, or their interaction on CRP levels. Multiple regression analysis confirmed that neither HIV nor PE status is a predictor of CRP. However, the analysis of clinical criteria at 5 ng/mL revealed that women with preeclampsia (PE) had slightly higher CRP levels compared to normotensive women when CRP was below this threshold. Conversely, when CRP exceeded 5 ng/mL, the PE group showed lower levels than the normotensive group ($p < 0.001$). However, CRP did not reliably distinguish between inflammatory status, PE severity, or HIV infection.

Conclusion: The study found that neither HIV nor PE status predicted significant differences in CRP levels; additionally, CRP levels did differ between mild and severe PE. For a better understanding of this complex interaction between HIV and hypertensive disorders, large-scale prospective studies measuring various inflammatory markers are warranted.

Keywords: Pre-eclampsia; HIV; Antiretroviral Therapy; Systemic Inflammation; C-reactive Protein; Pregnancy

1. Introduction

Pregnancy entails significant physiological, immunological, and metabolic changes that promote foetal development and preserve maternal health [1]. Maternal health during gestation relies on a fragile balance between immunological tolerance towards the foetus and maintaining sufficient immune activity to defend against infections [2]. Disruption of these physiological processes may result in gestational problems, including pre-eclampsia (PE), a hypertensive disorder associated with maternal and perinatal morbidity and mortality globally [3]. PE is characterised by elevated systolic (SBP) and diastolic (DBP) blood pressure, along with proteinuria and impaired maternal organ function after 20 weeks of gestation [4,5]. Accompanied by systemic endothelial dysfunction, heightened inflammatory responses, and compromised placental development [6]. The precise pathophysiological processes of PE are complex and multifactorial; nonetheless, evidence suggests that systemic inflammation, endothelial dysfunction, and dysregulated immune activation play a major role in its onset and progression [7]. Although PE is traditionally associated with heightened inflammation, some recent studies have reported lower inflammatory indices, including neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), systemic inflammatory response index (SIRI), and aggregate index of systemic inflammation (AISI) [8]. This contradicts the results of other studies, which showed no differences in SIRI, SII, NLR, platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR) [9,10]. Another well-recognised marker of inflammation, C-reactive protein (CRP), is an acute-phase reactant whose levels increase in inflammatory states such as infection or tissue damage [11,12]. It is predominantly synthesised by the liver in response to pro-inflammatory cytokines, including interleukin 6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor-alpha (TNF-α) [12]. An elevated CRP is associated with an increased risk of cardiovascular complications, metabolic dysregulation, adverse pregnancy outcomes, and neonatal complications. Elevated maternal CRP levels were also associated with an increased risk of small-for-gestational-age offspring [13–16]. In PE, elevated CRP levels may promote maternal inflammation, which contributes to endothelial dysfunction [17]. Evidence from a previous meta-analysis showed no difference in CRP and IL-6 between PE and normotensive [18]. Conversely, another meta-analysis reported higher levels of CRP, TNF-α, and IL-6 in PE compared with normal pregnancy [19]. These contradictory findings make it challenging to understand the role of CRP in maternal inflammation, especially in pregnancy-related disorders. Given the burden of hypertensive disorders of pregnancy in Africa, where the prevalence of superimposed PE, PE, and severe PE is 44.0%, 22.1%, and 14.7%, respectively [20], it is therefore important to examine CRP levels in this population.

In regions like sub-Saharan Africa, where Human Immunodeficiency Virus (HIV) infection is prevalent among women of reproductive age, the coexistence of HIV and PE poses a distinct clinical and immunological challenge [21]. HIV infection triggers persistent immunological activation and systemic inflammation, both of which may affect pathways associated with PE, potentially exacerbating the inflammatory processes underlying PE [1,22]. The extensive use of antiretroviral therapy (ART) has altered this link, thus independently affecting inflammatory responses and maternal outcomes [23]. For instance, ART has been shown to ameliorate inflammation, potentially reducing the incidence of PE in women infected with HIV [24]. Nevertheless, the exact mechanisms by which ART influences the onset of PE in this demographic remain poorly understood and warrant further investigation [25,26]. Previous evidence has shown an increased high-sensitivity C-reactive protein (hs-CRP) in people living with HIV on ART when compared to those not on ART or HIV-negative individuals, suggesting ART may be an exacerbating factor for inflammation [27]. Hence, it is important to assess CRP levels in patients with PE. This can be used to quantify maternal inflammatory status and provide insight into the pathophysiological mechanisms driving disease severity. The findings may help identify inflammatory patterns associated with disease progression, serve as a predictive biomarker, and further help tailor treatment targeting inflammatory pathways in PE. Therefore, examining CRP levels in pregnant women with and without PE provides clarity on the effects of hypertensive disorder on maternal inflammation.

This study aimed to investigate the relationship between PE and maternal systemic inflammation, using CRP levels as a clinical biomarker.

2. Methods and Materials

2.1. Study population and design

This research was a retrospective cross-sectional study, with samples collected at a single time point, as previously reported by other scholars [28] It adhered to the principles of the Declaration of Helsinki [29], and informed consent was obtained from participants prior to participation, as samples were collected from participants at a single time point and stored at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa.

2.2. Ethics approval and patient Consents

Ethical approval was obtained from the University of South Africa-College of Agriculture and Environmental Sciences Health Research Ethics Committee (2025/CAES_HREC/7327),

approved on 08/05/2025, and authorisation from the gatekeeper was obtained to utilise the samples. Regulatory approval was obtained from CMJAH in South Africa. All patients provided written informed consent at the time of data collection, indicating that the data would be used for future publication. Following the acquisition of written consent, normotensive and PE HIV-negative and HIV-positive pregnant women were enrolled at CMJAH.

2.3. Participant's Characteristics and Measurement

A total of 72 participants were recruited, comprising 48 normotensive patients (aged 18 to 43 years) and 24 patients with PE (aged 18 to 40 years). Systolic and diastolic blood pressure were measured using an automated blood pressure monitor (OMRON Healthcare, Kyoto, Japan) according to the manufacturer's protocol. PE was defined as the emergence of hypertension ≥140/90 mmHg, recorded on two distinct occasions 4 hours apart, with or without accompanying proteinuria. Normotensive pregnancy was defined as a blood pressure ≤120/80 mmHg [46]. Demographic information for all research participants was obtained from their maternity case records. HIV testing was conducted following counselling using a rapid point-of-care test kit, in accordance with the standard of care in South Africa [47]. Maternal weight was classified as normal weight (BMI: 18.5 – 24.9 kg/m²), overweight (BMI: 25.0 – 29.9 kg/m²), and obesity (BMI: >30 kg/m²). To maintain anthropometric uniformity, all participants in the study confirmed their status as non-smokers and their abstention from alcohol and recreational drugs, while all HIV-positive individuals were undergoing highly active antiretroviral therapy (HAART: tenofovir, emtricitabine, and efavirenz) in compliance with the South African National HIV guidelines (Department of Health, Republic of South Africa, 2015). The implementation of HAART during pregnancy is essential for reducing mother-to-child transmission via multiple mechanisms, including lowering maternal antepartum viral load and offering preexposure and postexposure prophylaxis for the infant [48]. Women having concurrent chronic medical conditions were excluded from the study.

2.4. Sample Collection

This investigation used 72 archived maternal plasma samples, comprising 48 from normotensive individuals and 24 from individuals with preeclampsia, collected at the Charlotte Maxeke Johannesburg Academic Hospital. A competent nurse used a 21-gauge needle for venipuncture. Approximately 6 mL of blood was collected from each research participant in EDTA tubes. After sample collection, the blood was processed within 1 hour to maintain plasma integrity. The EDTA tubes were centrifuged at 1500 × g for 10-15 minutes at 4°C to separate plasma from cellular components. The separated plasma was subsequently stored in a freezer at −80 °C for future use.

2.5. Quantification of Human CRP (C-Reactive Protein) through an ELISA kit

CRP concentrations were measured with the Human C-Reactive Protein (CRP) ELISA Kit (Elabscience Biotechnology Co., Ltd., Houston, TX, USA, Cat No: E-EL-H0043), which operates on the basis of a sandwich enzyme-linked immunosorbent assay (ELISA). This has a sensitivity of 0.23 ng/mL and a detection range of 0.39–25 ng/mL, which was used for inflammation measurement according to the manufacturer's instructions. The 96-well plates were pre-treated with a monoclonal antibody targeting human CRP. Samples or standards were introduced and permitted to adhere to the immobilised antibody. A biotinylated anti-CRP detection antibody was subsequently added, followed by an avidin-horseradish peroxidase (HRP) combination. Following the addition of the substrate, a colorimetric reaction was initiated and then terminated using a stop solution. The absorbance was quantified at 450 ± 2 nm utilising a microplate reader.

Samples were diluted 1:1000 in a three-step dilution method. Firstly, 180 µL diluent was added to 20 µL of the undiluted sample to obtain a 1:10 dilution, followed by 100 µL diluent added to 20 µL of the diluted sample to obtain a 1:100 dilution, and lastly, 190 µL diluent was added to 10 µL of the diluted sample to obtain a 1:1000 dilution. 100 µL of standards, blanks, and plasma samples (diluted as required) were added in duplicate to the designated wells and incubated for 90 minutes at 37 °C. Without prior washing, 100 µL of biotinylated detection antibody was introduced, followed by a 60-minute incubation at 37 °C. The wells were washed 3 times, then 100 µL of HRP-conjugate was added and incubated for 30 minutes at 37 °C. After the five washes, 90 µL of TMB substrate solution was added, and the mixture was incubated in the dark at 37 °C for 15 minutes. The reaction was halted by adding 50 µL of stop solution, and the absorbance was promptly assessed at 450 nm using a microplate reader.

A four-parameter logistic curve was fitted to CRP concentrations on the x-axis and optical density (OD) on the y-axis using Boster Bio's online ELISA data analysis tool. Serum sample CRP concentrations were obtained utilising this curve. Samples with OD values beyond the detection range (0.39 – 25 ng/mL) were reanalysed with appropriate dilutions.

2.6. Statistical Analysis

Boster ELISA calculator was used to create a four-parameter (4PL) logistic curve and determine unknown CRP concentrations. The data was analysed using Statisty App (https://statisty.app/ accessed on the 20th of November 2025). We used the Shapiro-Wilk test to assess the normality of the data. The CRP data were first log-transformed to meet the assumption of normality. Descriptive data were reported as the mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical data were reported as numbers and percentages. Comparisons between PE and HIV status were performed using a two-way

analysis of variance (ANOVA) for parametric data. A Bonferroni post hoc test was conducted to ascertain statistical significance among all groups. The statistical significance was $p < 0.05$.

3. Results

3.1. Clinical Characteristics of Participants

Table 1 summarizes the clinical and demographic characteristics of the study population. SBP was significantly higher in PE than in normotensives ($p < 0.0001$). Additionally, DBP was significantly higher in PE than in normotensives ($p < 0.0001$). However, no significant differences in maternal age, BMI, weight, height, gestational age, or Hb were observed between the PE and normotensive groups ($p > 0.05$). Although CRP showed a decrease in PE compared with normotensive subjects, the difference was not statistically significant ($p = 0.697$). There were almost three times as many mild PE cases (75%) compared to severe PE cases (25%).

Table 1. Patient demographic features of the study groups.

Variables	Pre-eclampsia	Normotensive	P value
Sample size (n)	24	48	
HIV+, n (%)	8 (33)	16 (33)	
HIV-, n (%)	16 (67)	32 (67)	
Mild PE	18 (75)	-	
Severe PE	6 (25)	-	
Maternal age (years)	36 (34-38)	34.5 (27-38)	0.159
Maternal weight (kg)	87 (73.75-97.75)	73 (68-90)	0.124
Maternal height (cm)	158 (157.75-161.5)	159 (155-163)	0.958
BMI (kg/m²)	33.2 (28.46-39.81)	31.2 (28.5-37.58)	0.697
SBP (mmHg)	149 (146.75-154.25)	110 (106-114.75)	<0.0001
DBP (mmHg)	98.5 (91.75-106)	66.5 (60.75-72)	<0.0001
Gestational age (weeks)	19 (14-25)	23 (16-30)	0.153
Hb (g/dL)	11.5 (10.2-11.8)	8.5 (10.6-12.13)	0.258
CRP (ng/mL)	1.39 ± 0.95	1.49 ± 1.13	0.697

Data reported as mean and standard deviation, median and interquartile range (IQR) or number (%), PE: pre-eclampsia, N: normotensive, SBP: systolic blood pressure, DBP: diastolic blood pressure, Hb: haemoglobin, HIV+: Human Immune Deficiency Virus positive, HIV-: Human Immune Deficiency Virus negative, CRP: C-reactive protein. CRP level log transformed.

3.2. The CRP PE and normotensives, and based on the severity of PE

The CRP level was slightly lower in PE than in normotensive women; however, this difference was not statistically significant (Table 1). Although women with mild PE had higher CRP levels than those with severe PE, this difference was not statistically significant (Figure 1). Mild PE presented with SBP, 147 (145-149.79), compared to severe PE, 161 (154-168.75), $p < 0.001$. Moreover, the DBP was 93.5 (90.75-99.5) in mild PE compared with 110 (104-116.75) in severe PE ($p < 0.001$).

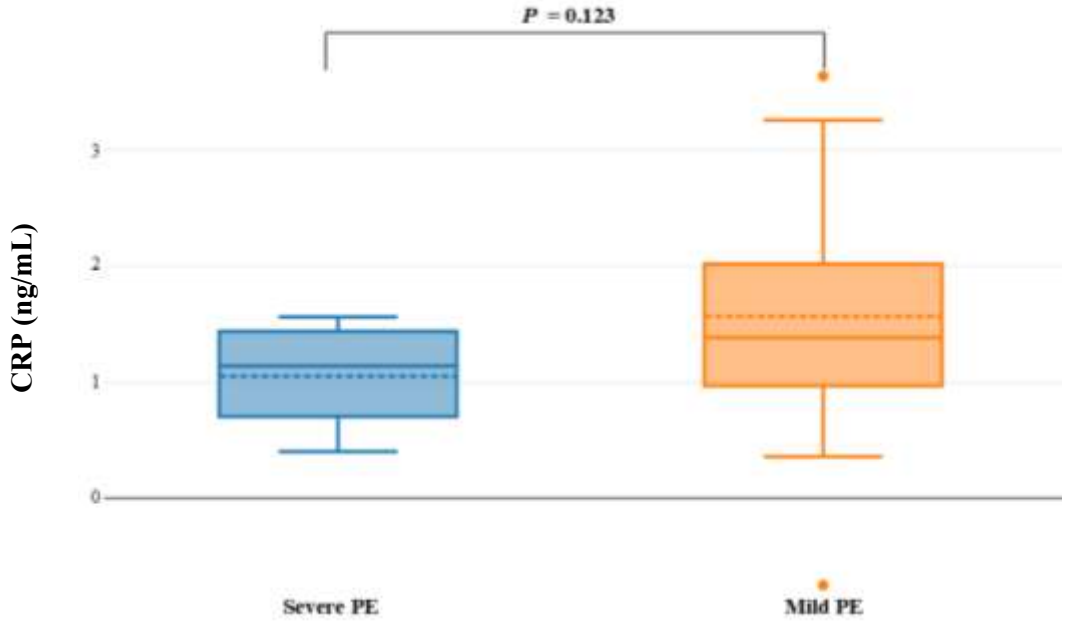


Figure 1: CRP levels in women with severe compared to those with mild PE. Log-transformed data reported as mean and SD. Severe PE (SBP ≥ 160 mmHg and DBP ≥ 110 mmHg), mild PE (SBP range of 140-159 mmHg and DBP ranging between 90-109 mmHg)

3.3. Clinical classification of CRP in PE and normotensives

CRP levels were classified using a clinical cutoff in both groups. Of the 24 women with PE, 17 (71%) had CRP less than 5 ng/mL, compared with the group with CRP greater than 5 ng/mL (Table 2). In contrast, 60.4% of the normotensive group had CRP greater than 5 ng/mL. The PE group that was clinically classified as having a CRP level < 5 ng/mL had a median CRP level of 2.63 ng/mL, significantly higher than that of normotensive patients (2.57 ng/mL) (Figure 2A and B). However, the PE group with greater than 5 ng/mL had significantly lower CRP levels (9.57 ng/mL) than the normotensive (11.52 ng/mL).

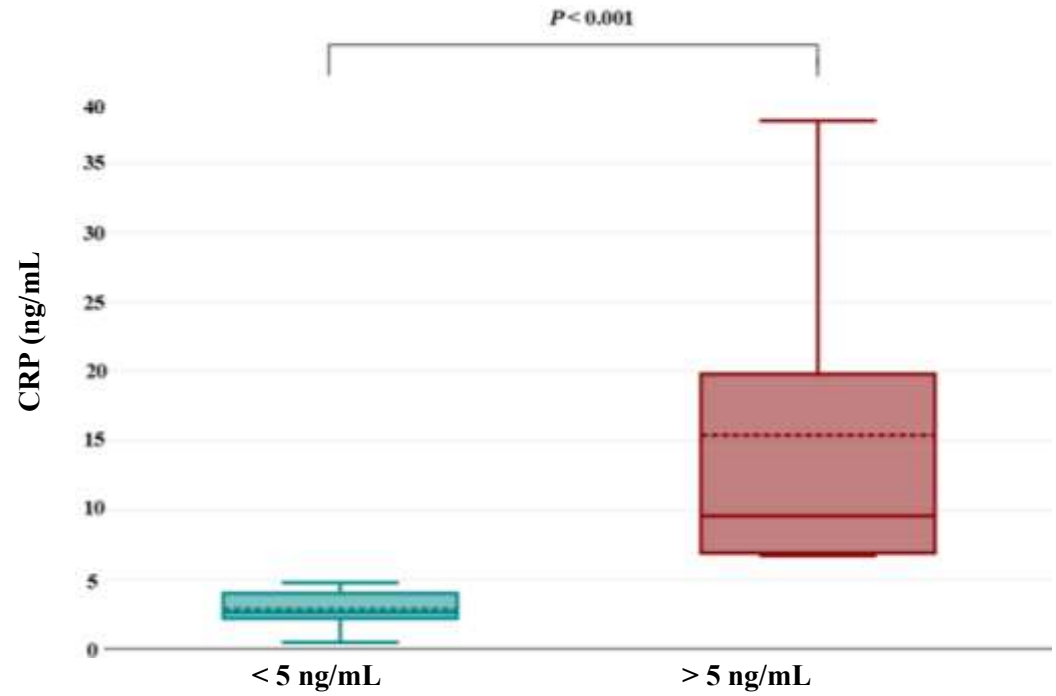
Table 2: The level of CRP based on clinical cut-off ranges in PE and normotensive

CRP category	N, %	Pre-eclampsia	N, %	Normotensive
< 5 ng/mL	17 (71.0)	2.63 (2.19-4.00)	19 (39.6)	2.57 (1.43-3.8)

> 5 ng/mL	7 (29.0)	9.57 (6.92-19.79)	29 (60.4)	11.52 (6.61-23.77)
P-value		< 0.001		< 0.001

Data reported as median and interquartile range (IQR) or number and percentages, N: number of participants in each group, CRP: C-reactive protein. CRP is reported as raw data to meet the clinical classification.

A



B

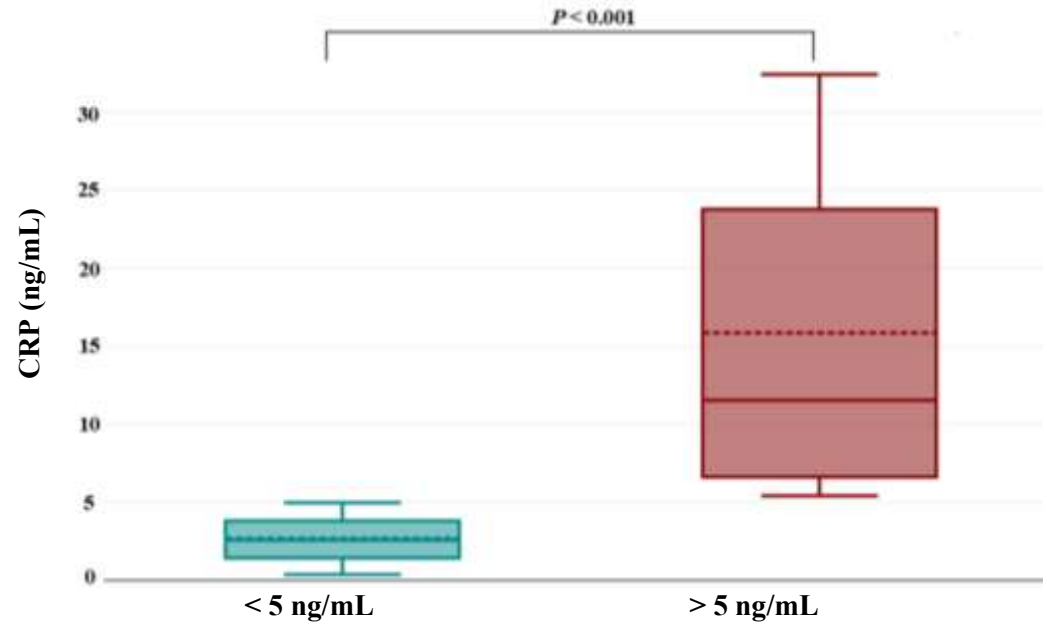


Figure 2: The clinical cut-off of the difference in CRP in the PE and normotensive group. A: pre-eclamptic women, B: normotensive women. Data reported as Median and interquartile ranges (IQR). *A p* < 0.001 signifies statistical significance.

3.4. Plasma concentration levels of CRP based on HIV and PE status and the effect of interactions

The result of a two-way ANOVA showed no significant main effect of PE status on CRP, F (1, 68) = 0.13, *p* = 0.716, η²p = 0. This indicates that the levels of CRP did not differ between pregnant women who are HIV-positive when compared to those who were HIV-negative based on HIV status (Figure 3). Additionally, the results of a two-way ANOVA showed that the main effect of HIV was not significant, F (1, 68) = 0.56, *p* = 0.457, η²p = 0.01. Indicating no difference in CRP level between pregnant women with and without PE (Figure 3). Moreover, the interaction between HIV status and PE was not significant, F (1, 68) = 0.08, p = 0.78, η²p = 0.00, indicating that HIV status did not affect CRP levels by PE status (Figure 3). These results suggest that neither PE nor HIV status influenced the CRP levels.

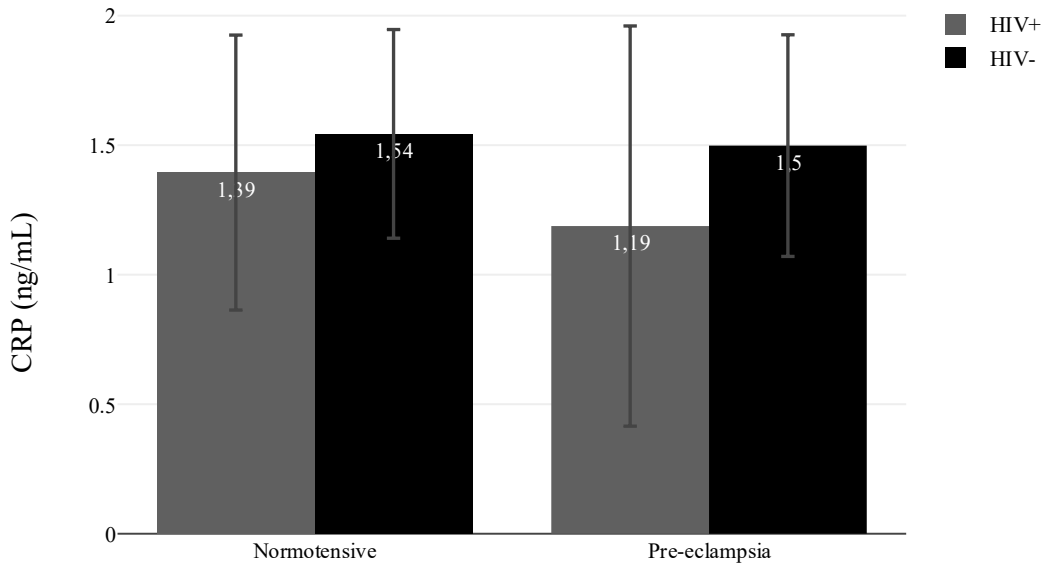


Figure 3. Plasma concentration levels of CRP (ng/mL) in PE and normotensive women with and without HIV. Data log transformed, reported as mean and IQR.

3.5. Association between PE, HIV status and CRP levels

A multiple regression analysis was conducted to determine whether HIV status and PE status predicted the CRP levels, as presented in Table 2. The overall model showed that neither HIV status nor PE status made a significant contribution to the prediction. HIV status was not a significant predictor (B = 0.2, *p* = 0.452, 95% CI [−0.19; 0.28]). Similarly, PE status was not a significant predictor (B = -0.1, *p* = 0.714, 95% CI [−0.15; 0.32]). These results indicate that neither HIV nor PE status meaningfully influences the CRP levels.

Table 3. Association of HIV, PE, and CRP.

	Unstandardized Coefficients	**Standardized Coefficients**			**95% CI for B**	
Model	**B**	**Beta**	**SE**	**p**	**LB**	**UB**
Constant	1.36		0.14	<0.001	0.33	0.87
HIV Status: HIV-negative	0.2	0.09	0.27	0.452	-0.33	0.74
PE Status: normotensive	-0.1	-0.04	0.27	0.714	-0.64	0.44

PE: pre-eclampsia, SE: standard error, HIV: human immunodeficiency virus, SE: standard error, CI: confidence intervals, LB: lower bound, UB: upper bound.

3.6. The levels of CRP in HIV+, HIV -pregnant women with and without PE

The results showed no statistically significant difference in CRP levels between the groups. The HIV+ group with PE was 1.19 ± 1.11, and normotensive, 1.39 ± 1.08 (*p* = 0.667; Figure 4A). In the HIV- group with PE, CRP was 1.5 ± 0.87, whereas in the normotensive group, it was 1.54 ± 1.16 (*p* = 0.891; Figure 4B).

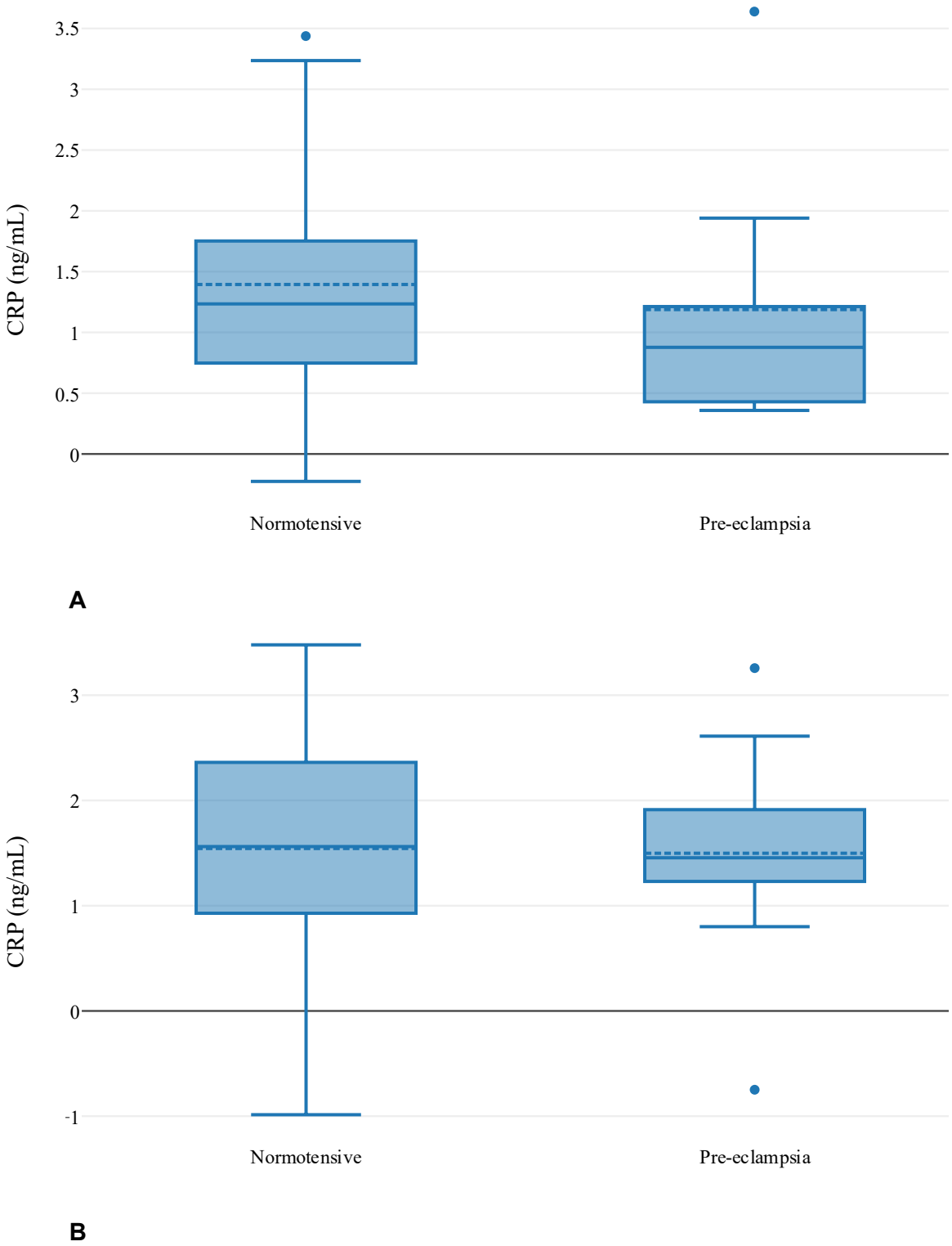


Figure 4. A: CRP levels in HIV-positive individuals with or without PE. **B:** CRP levels in HIV-negative individuals with and without PE.

4. Discussion

This study investigated the interaction of HIV infection and PE on maternal CRP as a biomarker of inflammation. Our findings revealed no significant difference in CRP levels between pregnant women with or without HIV. Additionally, there was no significant difference in CRP between pregnant women with PE and normotensive. Furthermore, we found no difference in CRP between women with mild or severe PE. There was a clinical cutoff difference in CRP between PE and normotensives. For instance, the PE group with CRP below 5 ng/mL had higher CRP levels than the normotensive group; however, the PE group with CRP above 5 ng/mL had lower CRP levels than the normotensive group. These findings are supported by previous studies that showed no significant differences in CRP levels between PE and normotensives [30–32]. Moreover, other studies have used hs-CRP and still reported no significant difference between PE and normotensive [33,34]. In contrast, other reports have shown increased CRP levels in women with PE compared to those with normotensive pregnancies, indicating heightened maternal systemic inflammation and hepatic acute-phase responses [35–37]. Similarly, a report by Begum et al. found that CRP levels were significantly higher in the PE group (10.52 ± 10.24 µg/mL) than in the normotensive group (5.10 ± 6.20 µg/mL) [38]. This study included over 200 pregnant women with at least 100 PE; the PE women were relatively young, with a gestational age of 10 weeks, compared to our current study, which included 24 PE women and relatively older reproductive-age women (36 years). These factors may explain this discrepancy despite studies employing the same methodology. Sample size and statistical power are critical; however, with only 72 samples, the study may lack sufficient power to detect small or moderate group differences, leading to a type II error. Variability in coexisting metabolic variables can influence CRP levels. For example, maternal BMI was significantly increased in the PE group, and obesity independently increased baseline CRP levels [39].

CRP is a principal acute-phase reactant synthesized mainly by hepatocytes in response to pro-inflammatory cytokines, including IL-6, I-1β, and TNF-α [12]. It functions as a dependable marker of systemic inflammation and tissue damage [11]. However, during pregnancy, CRP serves a dual function: its moderate increase may indicate low-grade inflammation associated with placental development [13]; pathologically, elevated CRP levels have been associated with adverse pregnancy outcomes, including PE and intrauterine growth restriction (IUGR) [40]. Therefore, assessing CRP levels provides significant insight into the systemic inflammatory condition of pregnant women, particularly in situations marked by increased immune activation, such as HIV infection and PE.

HIV infection is often associated with persistent immunological activation and systemic inflammation, as indicated by elevated CRP levels [41]. In this study, neither PE status nor

HIV infection was a significant predictor of CRP levels. While HIV treated patients have been reported to exhibit reduced inflammation [42]. This was not the case in our study. Other studies show persistent inflammation, as evidenced by elevated CRP levels even after ART initiation [43]. This suggests that ART may exacerbate inflammation in HIV infection. On the other hand, Wilson and colleagues reported that HIV patients: ART can inhibit viral replication, diminish immunological activation, and progressively restore immune homeostasis, thereby lowering systemic inflammatory markers [44]. ART, specifically regimens incorporating tenofovir, emtricitabine, and efavirenz, has demonstrated efficacy in suppressing viral replication and diminishing immunological activation, thereby normalizing inflammatory marker levels [28,45]. In pregnancy, ART-induced immunological reconstitution may reduce the inflammatory load, thereby mitigating PE-associated elevations in CRP. Although these contradictory data, it's important to note that our findings are yet supported by other previous findings, which reported no difference in CRP between HIV positive individuals and HIV negative individuals [46,47]. The research demonstrates no substantial interaction between HIV status and PE regarding CRP levels, indicating that HIV does not meaningfully modify the inflammatory profile in PE. Therefore, our findings show that CRP cannot be used as a potential biomarker for PE in HIV pregnant women with HIV. Therefore, it will provide more insight into various markers of inflammation, including IL-6, IL-1β, IL-10, IL-4, transforming growth factor beta (TGF-β), and TNF-α, to give a clear picture of the inflammatory status. This study had multiple limitations. The small sample size reduced the statistical power to detect small differences among groups. Retrospective design and reliance on archived data limited control over potential confounding factors. Moreover, CRP levels may be affected by non-pregnancy-related factors such as latent infections, obesity, or dietary status, which were not thoroughly monitored.

5. Conclusion

This study found that CRP levels did not differ significantly between HIV-positive or negative women with PE and normotensive women, nor between mild or severe PE. These findings suggest that CRP does not consistently reflect the inflammatory differences attributable to HIV infection or PE. However, after applying the clinical cutoff of 5 ng/mL, there was a notable difference: women with PE showed higher CRP than normotensives in the <5 ng/mL category, whereas those in the >5 ng/mL category showed lower CRP in PE than in normotensives. These trends suggest that CRP may not be a specific biomarker of inflammatory status in PE.

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CHAPTER 4: SYNTHESIS OF FINDINGS

Overview

This research aimed to examine liver function (AST, ALT and PLAP) and inflammatory marker CRP in HIV-positive and HIV-negative pregnant women with and without pre-eclampsia (PE). The analysis combined data from meta-analysis and cross-sectional cohort studies to clarify the relationship between hepatic, placental, and inflammatory responses associated with PE and HIV infection during pregnancy. Collectively, these findings emphasise that PE is a complex syndrome involving hepatic, placental, and inflammatory processes, which may be further exacerbated by HIV infection.

1. Overall Findings

The result from meta-analysis showed an association between liver dysfunction and use of antiretroviral therapy in those with PE when compared to those without PE. Additionally, another meta-analysis of observational studies showed that PE is significantly associated with elevated maternal hepatic enzyme levels, indicating hepatocellular damage and impaired liver function. Furthermore, we found that through another quantitative analysis, HIV infection and ART administration are associated with elevated liver function test enzymes, suggesting that each may contribute to liver dysfunction among PWLWHIV. However, the results from our cross-sectional studies showed no significant difference in AST, ALT, PLAP, and CRP in HIV pregnant women with or without PE.

1.1. Hepatic Enzymes (AST & ALT)

Chapter 2 provided evidence, through systematic review and meta-analysis, indicating that both PE and HIV infection are independently linked to significant increases in liver enzymes, including AST and ALT. The meta-analysis of PE-induced maternal liver dysfunction revealed significant elevations in AST and ALT levels in women with PE compared with normotensive controls, reinforcing the notion that PE is characterised by hepatic injury resulting from endothelial dysfunction, hypoxia, and inflammation [1,2]. The meta-analysis of HIV infection and ART exposure revealed that PLWHIV, regardless of ART status, demonstrate increased AST and ALT levels compared to HIV-negative persons, underscoring the combined effects of viral infection and pharmaceutical exposure on hepatic stress.

The experimental results in Chapter 3 validated these observations at a cohort level. AST and ALT levels were consistently elevated in PE women compared to normotensive pregnant women, with the most significant increases noted in those with concurrent HIV infection as well as PE. The results demonstrated that AST levels were elevated in women with PE relative

to normotensive groups, however, this difference did not achieve statistical significance (p = 0.7973). A two-way ANOVA examining the impact of HIV and PE on ALT indicated a borderline significant influence of both HIV and PE on ALT levels, $p = 0.052$. Stratified studies indicated that HIV status influenced the extent of liver enzyme increase, implying a synergistic or additive interaction among HIV-related immunological activation, ART-induced hepatotoxicity, and PE-related endothelial dysfunction.

1.2. Placental Alkaline Phosphatase (PLAP)

PLAP has emerged as a significant biomarker connecting placental failure to maternal hepatic stress. Chapter 2 emphasised the significance of placental pathology and angiogenic imbalance in PE, whereas Chapter 3 presented new findings indicating that PLAP levels were altered in PE, especially among HIV-positive women. Increased PLAP levels in PE pregnancies indicate placental damage, compromised trophoblast function, and altered placental perfusion, all of which are characteristic features of PE.

The integration of PLAP findings with hepatic enzyme data substantiates the idea that placental failure indirectly induces maternal liver injury by exacerbating systemic inflammation, oxidative stress, and endothelial activation. In HIV-positive women, ART-induced placental oxidative stress may intensify these effects, establishing PLAP as a potential intermediary biomarker connecting placental pathology to maternal hepatic outcomes.

1.3. C-Reactive Protein (CRP)

The CRP data offered an essential understanding of the inflammatory environment contributing to liver impairment in PE and HIV. Chapter 2 systematically evaluated evidence indicating that inflammation is a primary mechanism responsible for endothelial damage and multi-organ involvement in PE. Chapter 3 supported this evidence by illustrating markedly higher CRP levels in women with PE compared to normotensive controls, with CRP concentrations escalating further with the severity of preeclampsia.

HIV-positive women demonstrated elevated baseline CRP levels, though not statistically significant, $p = 0.667$, even without PE, indicating chronic immunological activation linked to HIV infection. The simultaneous presence of HIV and PE led to elevated CRP values, reinforcing the notion of an exacerbated inflammatory burden. These findings emphasise CRP as a sensitive indicator of disease severity and underscore inflammation as a common mechanism connecting HIV, ART exposure, PE, and hepatic dysfunction.

2. Interpretation of Findings

2.1. Hepatic Enzyme Findings (AST & ALT)

AST and ALT are important transaminases that catalyse amino acid metabolism and act as sensitive measures of hepatocellular health [3]. Elevated enzyme activity in PE women is associated with hepatic stress and endothelial dysfunction, both of which are frequent features of PE [4]. In HIV-negative individuals, increased enzyme activity in the PE group compared to normotensive pregnancies suggests hepatic involvement as PE advances. However, a more recent study conducted by Edebiri et al., [5], concluded that there is a significant ($p < 0.001$) decrease in AST and ALT in PE women compared to normotensive women, which contradicts previous findings [5]. This inconsistent result suggests that more research is required to completely understand the association between liver enzymes and PE. Edebiri et al., [5], discovered that PE women have lower AST and ALT levels, which may indicate a different mechanism of hepatic involvement in PE than previously considered. These findings emphasise the complexities of PE and the necessity for additional research into its pathogenesis.

HIV-positive women exhibited a lower increase in AST and ALT levels. This may arise from the immunomodulatory effects of HIV infection and ART, potentially affecting hepatic enzyme expression or obscuring normal patterns associated with PE. Certain ART regimens are associated with mild hepatotoxicity; however, prolonged exposure may induce adaptive hepatic responses that regulate enzyme release [6]. These data indicate a complex hepatic response influenced by the interplay of PE and HIV pathogenesis.

2.2. Placental Marker Integration (PLAP)

PLAP is a glycoprotein enzyme produced in the syncytiotrophoblast layer of the placenta, indicating placental development and functional capability [7]. The study demonstrated modified PLAP levels in PE pregnancies, signifying placental stress and potential hypoxia-induced regulation of enzyme production. Increased PLAP levels in PE may have result from increased shedding of syncytiotrophoblast into the maternal circulation, a phenomenon associated with placental ischaemia and oxidative stress.

In HIV-positive pregnancies, PLAP levels exhibited fluctuating trends, likely affected by HIV-associated placental pathology, such as villous immaturity and inflammatory lesions [8]. HIV infection can compromise trophoblastic function, potentially leading to either suppression or dysregulation of PLAP release. The synergistic effects of PE and HIV create compounded difficulties for placental function, affecting foetal growth and nutrient transport.

2.3. Inflammatory Marker Interpretation (CRP)

CRP, an acute-phase reactant synthesised by the liver in response to inflammatory cytokines including interleukin-6 (IL-6), offers insight into systemic inflammatory activity [9]. Elevated CRP levels in women with PE underscore the perception of PE as a chronic inflammatory disorder marked by endothelial activation and oxidative stress. The extent of the CRP rise corresponded to illness severity, thereby reaffirming its diagnostic and prognostic value in PE.

In HIV-positive individuals, CRP levels were somewhat increased even in normotensive pregnancies, indicating a baseline immunological activation linked to HIV infection. The differentiation between PE and normotensive conditions was less evident in this sample. This pattern may indicate the concurrent inflammatory responses triggered by both HIV and PE, a confusing interpretation while highlighting common pathophysiological mechanisms.

2.4. Interaction between HIV, ART and PE

This study significantly demonstrated that HIV status alters the biochemical expression of liver disease associated with PE. Although PE alone can cause considerable hepatic enzyme alterations, HIV infection and ART exposure seem to exacerbate these consequences. This interaction may elucidate the discrepancies noted in previous research about the hepatotoxic effects of ART during pregnancy, as many did not consider hypertensive diseases such as PE.

The data indicate that ART functions within a complex biological environment influenced by pregnancy-associated vascular and inflammatory alterations. In PE, where endothelial integrity is already compromised, ART-related hepatic and placental stress may have more significant clinical consequences.

3. Clinical and Biological Implications

The combined measurement of AST, ALT, PLAP, and CRP provided a more complete biochemical profile of PE in the HIV setting. Understanding how these indicators interact improves diagnostic accuracy and may influence risk stratification and therapeutic management. For example, concomitant elevations in hepatic enzymes and CRP may indicate impending hepatic dysfunction in PE and warrant additional monitoring. Similarly, altered PLAP activity may suggest placental insufficiency, guiding obstetric treatments.

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CHAPTER 5: LIMITATIONS, CONCLUSION, AND RECOMMENDATION

1. Limitations

The relatively small sample size reduced statistical power for subgroup analyses, especially among HIV-positive women. The cross-sectional design prevented causal inference and limited the ability to examine biomarker progression. Variations in antiretroviral therapy (ART) regimens may have contributed to biochemical heterogeneity among participants. The absence of histopathology or cytokine data limited mechanistic interpretation.

2. Conclusion

The evidence presented in this study indicates that maternal liver impairment during pregnancy cannot be attributed to a singular mechanism, especially in cases of HIV and PE. It may arise from the placental failure, systemic inflammation, endothelial damage, viral infection, or ART exposure. PE was consistently associated with substantial increases in hepatic enzymes. The HIV infection exacerbated these abnormalities by chronic immunological activation and inflammatory stress, while ART exposure added another dimension of hepatocellular susceptibility.

The coexistence of HIV and PE was significantly linked to the most pronounced abnormalities in liver enzymes and inflammatory markers, indicating that these diseases operate synergistically rather than independently. Increased AST and ALT levels indicated hepatocellular damage, altered PLAP concentrations emphasised placental-hepatic interactions, and elevated CRP levels highlighted the significance of systemic inflammation. Collectively, these biomarkers offered convergent evidence that liver impairment in pregnant women with HIV is exacerbated by PE and may be influenced by common inflammatory and endothelial pathways.

3. Recommendations

Future investigations should employ longitudinal cohort methodologies to evaluate liver biomarkers during pregnancy and the postpartum phase, facilitating the distinction between pre-existing liver dysfunction, pregnancy-associated alterations, and the impacts of HIV and pre-eclampsia. Substantial sample numbers are crucial for strong statistical power in subgroup analyses. It is advisable to stratify by ART regimen and adherence to appropriately assess hepatotoxic consequences. Future research should encompass a diverse array of biomarkers and, placental histopathology or liver imaging to further the understanding of placental-hepatic interactions.

CHAPTER 6: APPENDICES

Appendix 1: Ethics Approval

UNISA university of south africa

College of Agriculture and Environmental Sciences_Health REC

NHREC Registration # : REC-170616-051
Ref #: 2025/CAES_HREC/7327
Name: Miss Kay-Lee Strauss
Student #: 68188242

Date: 08/05/2025

Dear: Miss Kay-Lee Strauss

Decision: Ethics Approval from 08/05/2025 to 30/04/2028

Researcher: Miss Kay-Lee Strauss
68188242@mylife.unisa.ac.za 0724715749

Supervisor: Mr Kabelo Mokgalaboni mokgak@unisa.ac.za

Co-Supervisor: Dr Wendy Phoswa phoswwn@unisa.ac.za

Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa

Qualification: MSc Life Science

Thank you for the application for research ethics clearance by the College of Agriculture and Environmental Sciences_Health REC for the above mentioned research study. Ethics approval is granted for three years, **subject to submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report is submitted.**

Due date for progress report: 30 April 2026

The **low risk application** was **reviewed** by College of Agriculture and Environmental Sciences_Health REC on 08 May 2025 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the College of Agriculture and Environmental Sciences_Health REC.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.

Page 1 of 2

6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data requires additional ethics clearance.

7. No field work activities may continue after the expiry date (30 April 2026). Submission of a completed research ethics progress report will constitute an application for renewal, for Ethics Research Committee approval.

Additional Conditions

1. Disclosure of data to third parties is prohibited without explicit consent from Unisa.
2. De-identified data must be safely stored on password protected PCs.
3. Care should be taken by the researcher when publishing the results to protect the confidentiality and privacy of the university.
4. Adherence to the National Statement on Ethical Research and Publication practices, principle 7 referring to Social awareness, must be ensured: "Researchers and institutions must be sensitive to the potential impact of their research on society, marginal groups or individuals, and must consider these when weighing the benefits of the research against any harmful effects, with a view to minimising or avoiding the latter where possible." Unisa will not be liable for any failure to comply with this principle.
5. Kindly note that the College of Agriculture and Environmental Sciences_Health REC requires the submission of regular progress reports to be submitted annually in line with section 7.2 of the Unisa Policy on Research Ethics (2024).

Note

The reference number 2025/CAES_HREC/7327 should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.

Kind regards,

Dr MD Matlala
Chair of College of Agriculture and Environmental Sciences_Health REC
E-mail: matlamd1@unisa.ac.za

Prof NO Maphoti
Executive Dean / By delegation from the Executive Dean of College of Agriculture and Environmental Sciences_Health REC
E-mail: maphon@unisa.ac.za

Appendix 2: Letter of Permission

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES
DEPARTMENT OF INTERNAL MEDICINE
7 York Road, Parktown, 2193
Johannesburg, South Africa

10 March 2025

The Ethics Committee
University of South Africa
Preller St
Muckleneuk
Pretoria
0002

To the Chair

Re: The study, "Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa"

This letter serves to grant permission for Kay-Lee Elrechia Strauss to process and stores samples in the Department of Internal Research Laboratory.

All samples will be stored safely in ultra-low temperature freezers that are under a networked temperature monitoring system linked to a generator backup. The freezers are located in an access- controlled laboratory.

Please do not hesitate if you wish to have any additional information.

Thanking you.

Yours sincerely,

Duarte R. (PhD)
Associate Professor
Head: Internal Medicine: Translational Research
Email: raquel.duarte@wits.ac.za
Cell: 0725315317

Appendix 3: Research proposal outcome

Department of Life and Consumer Sciences
School of Agriculture and Life Sciences
College of agriculture and Environmental Sciences
Private Bag X6
Florida
1710

To: KE Strauss (Student no: **681-882-42**)

Subject: Outcome of your research proposal

It gives me great pleasure to inform you that your MSc research proposal titled: **"Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa"** has been approved.

Comments and suggested improvements were provided by the review committee. These comments will be communicated to you by your supervisor.

Good luck with the rest of your studies.

Best regards

Masebe TM ..... Date 21/02/2025

Dr. TM Masebe
CoD: Department of Life and Consumer Sciences

Appendix 4: Conference Name: UNISA Research & Innovation Postgraduate Student Showcase

Appendix 5: Conference Name: UNISA North Eastern Region 14th Annual Postgraduate Student Conference

Presenters Certificate

This is to certify that the Research Paper Entitled

"Comparative Impact of Antiretroviral Therapy on Liver Function among HIV infected pregnant women with and those without Pre-eclampsia in South Africa"

Was part of the 14th Annual North Eastern Regional Postgraduate Students' Conference, 01 to 03 October 2025.

Presented by

Kay-Lee Strauss

UNISA university of south africa
North Eastern Region

Prof T Makgakga
Chairperson: North Eastern Region Research Forum

01 October 2025
Date

Appendix 6 - Pre-eclampsia-Induced Maternal Liver Dysfunction: Meta-Analysis of observation Studies

1. **Table S1**: Subgroup analysis showing effect of different factors on liver function

		Effect Size	Std. Error	Z	*P*	95% Confidence Interval		
						Lower	Upper	I^2
	Design							
AST	Case control	1.199	0.2666	4.496	<0.001	0.676	1.721	93.8
	Cohort	0.971	0.2994	3.243	0.001	0.384	1.558	99.7
	Cross Sectional	2.462	0.3433	7.171	<0.001	1.789	3.135	99.9
	Overall	1.810	0.1512	11.974	<0.001	1.514	2.107	99.1
	Quality							
	High	1.266	0.1910	6.630	<0.001	0.892	1.641	99.4
	Moderate	2.466	0.3683	6.696	<0.001	1.744	3.187	98.0
	Overall	1.810	0.1512	11.974	<0.001	1.514	2.107	99.1
	Continents							
	Africa	1.738	0.4398	3.952	<0.001	0.876	2.600	96.8
	Asia	1.960	0.1799	10.898	<0.001	1.608	2.313	99.3
	Europe	0.730	0.3524	2.070	0.038	0.039	1.420	92.2
	Overall	1.810	0.1512	11.974	<0.001	1.514	2.107	99.1
	Maternal age (years)							
	< 30 years	1.843	0.3098	5.949	<0.001	1.236	2.450	95.7
	> 30 years	1.836	0.1954	9.396	<0.001	1.453	2.219	99.4
	NR	1.719	0.7420	2.316	0.021	0.264	3.173	97.8
	Overall	1.810	0.1512	11.974	<0.001	1.514	2.107	99.1
	BMI							
	Normal	0.926	0.2203	4.202	<0.001	0.494	1.357	97.2
	NR	1.981	0.2486	7.971	0.001	1.494	2.469	98.2

	Obese	1.773	0.9540	1.859	0.063	-0.096	3.643	95.7

	Overweight	2.090	0.3389	6.166	<0.001	1.425	2.754	97.1
	Gestation duration at diagnosis (weeks)							
	<20	1.790	0.7594	2.357	0.018	0.302	3.278	97.6
	>20	2.008	0.2385	8.421	<0.001	1.541	2.475	99.3
	>30	1.705	0.7985	2.135	0.033	0.139	3.270	98.0
	NR	1.012	0.2769	3.656	<0.001	0.470	1.555	91.9
	Overall	1.810	0.1512	11.974	<0.001	1.514	2.107	99.1
ALT	Case control	1.384	0.3427	4.038	<0.001	0.712	2.055	96,4
	Cohort	0.851	0.3946	2.156	0.031	0.077	1.624	99.9
	Cross Sectional	2.256	0.3210	7.027	<0.001	1.627	2.885	97.9
	Overall	1.729	0.1739	9.944	<0.001	1.388	2.070	99.3
	< 30 years	1.607	0.2759	5.826	<0.001	1.067	2.148	95.9
	> 30 years	1.887	0.2764	6.827	<0.001	1.345	2.429	99.7
	NR	1.731	0.4808	3.601	<0.001	0.789	2.674	97.2
	Overall	1.729	0.1739	9.944	<0.001	1.388	2.070	99.3
	Africa	1.359	0.5069	2.681	0.007	0.365	2.352	97.9
	Asia	2.030	0.2063	9.840	<0.001	1.626	2.434	99.5
	Europe	0.096	0.4025	0.240	0.811	-0.692	0.885	94.4
	Overall	1.729	0.1739	9.944	<0.001	1.388	2.070	99.3
	<20	5.700	2.6694	2.135	0.033	0.468	10.931	97.2
	>20	1.779	0.2235	7.961	<0.001	1.341	2.217	99.5
	>30	0.949	0.4439	2.137	0.033	0.079	1.819	95.8
	NR	1.561	0.4495	3.472	<0.001	0.680	2.442	97.9
	Overall	1.729	0.1739	9.944	<.0001	1.388	2.070	99.3

	High	1.381	0.2345	5.889	<0.001	0.921	1.841	99.6

	Moderate	2.183	0.3285	6.646	<0.001	1.539	2.827	97.7
	Overall	1.729	0.1739	9.944	<0.001	1.388	2.070	99.3
	Normal	0.667	0.3350	1.991	0.046	0.010	1.324	99.7
	NR	2.092	0.3101	6.747	<0.001	1.484	2.700	98.1
	Obese	1.892	1.9729	0.959	0.337	-1.974	5.759	98.7
	Overweight	1.846	0.3610	5.113	<0.001	1.138	2.553	97.4
	Overall	1.729	0.1739	9.944	<0.001	1.388	2.070	99.3
Bilirubin	Case control	0.623	0.2421	2.574	0.010	0.149	1.098	84.0
	Cohort	-0.169	0.0291	-5.805	<0.001	-0.226	-0.0112	0.0
	Cross Sectional	0.831	0.1769	4.698	<0.001	0.484	1.178	89.8
	Overall	0.621	0.1337	4.644	<0.001	0.359	0.883	93.7
	< 30 years	1.080	0.3104	3.479	<0.001	0.472	1.688	94.6
	> 30 years	0.322	0.1330	2.421	0.015	0.061	0.582	88.8
	NR	0.636	0.2543	2.502	0.012	0.138	1.135	73.4
	Overall	0.621	0.1337	4.644	<0.001	0.359	0.883	93.7
	Africa	0.306	0.2403	1.271	0.204	-0.165	0.776	80.6
	Asia	0.695	0.1538	4.518	<0.001	0.393	0.996	94.5
	Overall	0.621	0.1337	4.644	<0.001	0.359	0.883	93.7
	<20	0.519	0.4536	1.145	0.252	-0.370	1.408	84.7
	>20	0.583	0.1602	3.638	<0.001	0.269	0.897	94.2
	>30	0.772	0.1553	4.970	<0.001	0.467	1.076	51.1
	NR	0.778	0.9225	0.843	0.399	-1.030	2.586	96.3
	Overall	0.621	0.1337	4.644	<0.001	0.359	0.883	93.7
	High	0.146	0.1124	1.301	0.193	-0.074	0.367	82.6
	Moderate	1.006	0.1941	5.185	<0.001	0.626	1.387	90.3
	Overall	0.621	0.1337	4.644	<0.001	0.359	0.883	93.7
	Normal	0.674	0.2650	2.542	0.011	0.154	1.193	73.2

	NR	0.900	0.2291	3.929	<0.001	0.451	1.349	93.0

	Obese	0.438	0.2263	1.936	0.053	-0.005	0.882	.
	Overweight	0.280	0.1664	1.682	0.093	-0.046	0.606	89.9
	Overall	0.621	0.1337	4.644	<0.001	0.359	0.883	93.7
	Design							
ALP	Case control	1.689	0.4937	3.420	<0.001	0.721	2.656	96.3
	Cohort	0.972	0.4295	2.263	0.024	0.130	1.814	99.3
	Cross Sectional	1.413	0.4781	2.956	0.003	0.476	2.350	97.3
	Overall	1.423	0.2326	6.118	<0.001	0.967	1.879	98.3
	Continent							
	Africa	-1.646	1.2677	-1.299	0.194	-4.131	0.838	98.2
	Asia	1.800	0.2422	7.432	<0.001	1.325	2.275	98.4
	Overall	1.423	0.2326	6.118	<0.001	0.967	1.879	98.3
	Maternal age							
	<20 years	2.308	0.6125	3.769	<0.001	1.108	3.509	99.5
	>20 years	1.279	0.3725	3.433	<0.001	0.549	2.009	97.8
	>30 years	1.303	0.3849	3.385	<0.001	0.548	2.057	87.7
	NR	0.549	0.8959	0.613	0.540	-1.207	2.305	96.3
	Overall	1.423	0.2326	6.118	<.001	0.967	1.879	98.3
	Quality							
	High	.751	.03296	2.277	0.023	0.105	1.397	99.0
	Moderate	2.208	0.3722	5.933	<0.001	1.479	2.938	95.8
	Overall	1.423	0.2326	6.118	<0.001	0.967	1.879	98.3
	BMI							
	Normal	1.114	0.8366	1.331	0.183	-0.526	2.753	99.8
	NR	1.550	0.3567	4.344	<0.001	0.850	2.249	96.9

	Obese	1.042	0.2383	4.374	<0.001	0.575	1.509	.

	Overweight	1.241	0.4329	2.868	0.004	0.393	2.090	96.3
	Overall	1.423	0.2326	6.118	<0.001	0.967	1.879	98.3

Table S2: Quality assessment of Cohort Studies

	Selection				**Comparability**	**Outcome**			**Score**
	Representativeness of exposed cohort (1)	Selection of non-exposed cohort (1)	Ascertainment of exposure (1)	Demonstration that outcome of interest was not present at the start of study (1)	Comparability of cohorts on the basis of the design or analysis controlled for confounders (2)	Assessment of outcome (2)	Was follow-up long enough for outcomes to occur (1)	Adequacy of follow-up cohorts (1)	10
Cho et al.. 2022	★	★	★	★	★★	★★	★	★	10/10
Fang et al.. 2024	★	★	★	★	★★	★★	★	★	10/10
Haggai et al.. 2022	★	★	★	★	★★	★★	★	★	10/10
Nie et al.. 2025	★	★	★	★	★★	★★	★	★	10/10
Zhang et al.. 2022	★	★	★	★	★★	★★	★	★	10/10
Shahid et al.. 2019	★	★	★	★	★	★★	★	☆	8/10
Zhang et al.. 2025	★	★	★	★	★★	★★	★	★	10/10

Table S3: Quality assessment of Cross-sectional studies

	Selection				**Comparability**	**Outcome**			**Score**
	Representativeness of the sample (1)	Sample size (1)	Non-included subjects (1)	Ascertainment of exposure (1)	Based on design and analysis (2)	Assessment of outcome (2)	Statistical test (1)	Reporting outcomes and precision (1)	10
Atiba et al.. 2016	★	☆	☆	★	★	★★	★	★	7/10
Hamed et al.. 2023	★	☆	☆	★	★	★★	★	★	7/10
Hassen et al.. 2022	★	★	☆	★	★★	★★	★	★	9/10
Makuyana et al.. 2002	★	★	☆	★	★	★★	★	★	8/10
Saha. 2022	★	★	☆	★	★	★★	★	★	8/10
Salman. 2016	★	☆	☆	★	★	★★	★	★	7/10
Hazari et al.. 2014	★	☆	☆	★	★	★★	★	★	7/10
Afroz et al.. 2020	★	☆	☆	★	★	★★	★	★	7/10
Al-Sultan et al.. 2025	★	☆	☆	★	★	★★	★	★	7/10
Nainani and Bhargava. 2019	★	☆	☆	★	★	★★	★	★	7/10
Edebiri et al.. 2025	★	★	☆	★	★	★★	★	★	8/10

Singh et al.. 2017	★	☆	☆	★	★	★★	★	★	7/10
Al-Jameil et al.. 2015	★	☆	☆	★	★	★★	★	★	7/10
Khan et al.. 2023	★	☆	☆	★	★	★★	★	★	7/10
Roy and Lodhi. 2019	★	☆	☆	★	★	★★	★	★	7/10
Mondal et al.. 2016	★	☆	☆	★	★	★★	★	★	7/10
Munazza et al.. 2013	★	☆	☆	★	★	★★	★	★	7/10
Taimoor et al.. 2017	★	☆	☆	★	★	★★	★	★	7/10
Walle et al.. 2022	★	☆	☆	★	★	★★	★	★	7/10
Sakr et al.. 2019	★	☆	☆	★	★	★★	★	★	7/10
Zhestkova et al.. 2023	★	☆	☆	★	★	★★	★	★	7/10
Ohotu et al.. 2023	★	★	☆	★	★	★★	★	★	8/10
Hendawy et al.. 2020	★	☆	☆	★	★	★★	★	★	7/10
Das et al.. 2013	★	☆	☆	★	★	★★	★	★	7/10
Ekun et al.. 2018	☆	☆	☆	★	★	★★	★	☆	5/10

Ghazali et al.. 2014	★	☆	☆	★	★	★★	★	★	7/10

Table S4: Quality assessment of case-control studies

	Selection				**Comparability**	**Exposure**			**Score**
	Is the case definition adequate?	Representativeness of the case	Selection of controls	Definition of controls	Comparability of cases and controls on the bases of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	10
Hassanpour and Karami. 2018	★	★	★	★	☆	★★	★	★	8/10
Mishra et al.. 2021	★	★	★	★	★	★★	★	★	9/10
Qassim and Ameen. 2021	★	★	★	★	★	★★	★	★	9/10
Asha and Varghese. 2017	★	☆	★	★	☆	★	★	★	6/10
Singh and Rachna. 2025	★	★	★	★	☆	★★	★	★	8/10
Sultana et al.. 2021	★	★	★	★	★	★★	★	★	9/10

Uckan and Sahin. 2018	★	★	★	★	★	★★	★	★	9/10
Udenze et al.. 2014	★	★	★	★	★	★★	★	★	9/10
Ipek et al.. 2024	★	★	★	★	★	★★	★	★	9/10
Chen et al.. 2022	★	★	★	★	★★	★★	★	★	9/10
Lu et al.. 2025	★	★	★	★	★	★★	★★	★	10/10
Albayrak and Arslan. 2025	★	★	★	★	★★	★	★	★	9/10

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Appendix 7 – HIV Infection and Antiretroviral Therapy Impair Liver Function in People Living with HIV: Systematic Review and Meta-Analysis

Table S1. General overview of characteristics of included studies

Author, year and reference	Country	Study design	Population Size	Age	Treatments and duration	Baseline CD4 of the ART/ART-naïve group Mean ± SD	Male In the ART group n (%)	Effect on liver enzymes
Abdulmumin et al., 2024 [1]	Nigeria	Cross-sectional	100 people living with HIV (PLWH) on HAART 100 HIV-negative individuals	18–50	Tenofovir, Lamivudine, and Efavirenz; (TDF+3TC+EFV) (70%) and 30% were on Zidovudine, Lamivudine, and Efavirenz for 4–12 years	NR	25 (25)	HAART significantly increased ALT and AST levels compared to HIV-negative individuals, with no effect on ALP.
Abriba et al., 2024 [2]	Nigeria	Case-control	17 PLWH on ART 15 PLWH not on ART 12 HIV-negative	18–65	NR	NR	23 (45)	Significant increase in ALT, ALP, and AST in ART compared to HIV negative. However, no changes in ALP despite an increased AST and ALT in ART-naïve compared to HIV-negative.
Deshmukh et al., 2024 [3]	India	Case-control	150 PLWH on ART 50 HIV-negative	16-50	NR	≤ 200 20–500 ≥ 500 cells/µl	NR	Significant increase in AST and ALT in PLWH on ART compared to the HIV-negative.

Odegbemi et al., 2024 [4]	Nigeria	Cross-sectional	120 PLWH on ART 50 HIV-negative	18–65	Tenofovir/Lamivudine, Dolutegravir (TLD)	NR	52 (43.3)	Significant decrease in AST without change in ALT in ART compared to HIV-negative.
Tamuno–Boma et al., 2023 [5]	Nigeria	Cross-sectional	83 pregnant women living with HIV on ART 82 non-pregnant women living with HIV on ART 84 pregnant HIV- negative 81 non-pregnant HIV negative	15–60	HAART for over 6 months	NR	0 (0)	Significant increase in ALP, ALT, and AST levels in both groups compared to the control group.

Author, year and reference	Country	Study design	Population Size	Age	Treatments and duration	Baseline CD4 of the ART/ART-naïve group Mean ± SD	Male In the ART group n (%)	Effect on liver enzymes
Gbolohan et al., 2023 [6]	Nigeria	Cross-sectional	59 PLWH on HAART 34 PLWH who are pre-HAART. 50 HIV-negative	NR	HAART	NR	41 (44)	Compared to healthy individuals, there was a significant increase in AST and ALT levels among PLWH on HAART and pre-HAART.
Gospel et al., 2023 [7]	Nigeria	Cross-sectional Comparative	100 PLWH on ART 100 HIV without ART 100 HIV-negative	20 –70	HAART for 7 months	NR	NR	Significant increases in ALT and ALP levels and decreased AST in ART and ART-naïve compared to HIV-negative individuals.
Mutuma et al., 2023 [8]	Kenya	Cross-sectional	47 PLWH who are HAART adherent 23 HAART-naïve PLWH 51 HIV-negative	18–60	HAART (Tenofovir Disoproxil Fumarate, Lamivudine, and Efavirenz) for more than 6 months	≥ 500 CD4+ cells/ µl 350 – 499 cells/ µl 200 – 349 cells/ µl < 200 cells/ µl	32 (68)	Significant increase in AST and ALP in ART and ART naïve compared to HIV negative. ALT increased in ART without a change in ATR-naïve.
Younis et al., 2022 [9]	Lybia	Case-control	101 PLWH on ART 21 ART-naïve 70 HIV-negative	20–45	ARV triple combination (FTC + TDF + EFV; FTC+ TDF + LPV/r, FTC+ TAF + EVG/c or RAL) more than 24 weeks	648.3 ± 400.5 884.5 ± 369.8	NR	Significant increase in ALT and AST in ART and ART-naïve compared to HIV - negative.

Ezeugwunne et al., 2021 [10]	Nigeria	Case-control	45 PLWH on ART 26 PLWH not on ART 26 HIV-negative	18–60	Lamivudine, Stavudine, and Nevirapine	616.16±359.22 390.27±117.15	31 (68.9)	Significant increase in ALT without change in AST and ALP when ART and ART naïve are compared to HIV-negative.
Ambad et al., 2021 [11]	India	Cross-sectional comparative	20 PLWH-naïve who further receive ART after 6 months. 20 HIV-negative	15–60	On HAART for 6 months	NR	0 (0)	Significant increase in ALP, AST, and ALT levels after 6 months of ART treatment in PLWH compared to HIV-negative individuals.
Ikekpeazu et al., 2019 [12]	Nigeria	Comparative cross-sectional	30 pregnant women living with HIV on HAART	20–40	Nevirapine-based HAART	NR	0 (0)	Significant increase in ALP and ALT activities in PLWH compared

Author, year and reference	Country	Study design	Population Size	Age	Treatments and duration	Baseline CD4 of the ART/ART-naïve group Mean ± SD	Male In the ART group n (%)	Effect on liver enzymes
			30 pregnant women living with HIV not on HAART 30 HIV-negative					to HIV-negative individuals in all trimesters.
Quaye et al., 2019 [13]	Ghana	Observational cross-sectional	105 PLWH on ART 77 ART-naïve PLHIV 60 HIV-negative	36.05 – 48.86	Lamivudine (3TC), Zidovudine (AZT), Efavirenz (EFV), Nevirapine (NVP), Atazanavir (ATV), and Lopinavir (LPV for 3.7 years	< 200 200–350 > 500	45 (43)	PLWH showed significantly elevated ALP and AST without changes in ALT compared to HIV– negative.
Emokpae et al., 2018 [14]	Nigeria	Comparative cross-sectional	50 PLWH on HAART 50 PLWH no ART 50 HIV-negative	33.53 – 40.60	HAART for at least 4 months	537.64±49.38 352.10±42.86 939.20±23.67	6 (16)	Significant increase in AST, ALT, and ALP in ART, ART-naïve compared to HIV-negative
Olisekodiaka et al., 2018 [15]	Nigeria	Comparative cross-sectional	40 PLWH on ART 40 ART-naive 40 HIV-negative	18–55	HAART for at least 6 months	467.3±19.4 188.5 ± 21.7 886.6± 12.6	20 (50)	Significant increase in AST and ALT in ART and ART-naïve compared to HIV-negative
Ashakarin et al., 2018 [16]	India	Cross-sectional case-control	90 PLWH 90 HIV-negative	20–60	NR	NR	37 (41)	Significant increase in AST, ALT, and ALP levels in PLWH on ART compared to HIV-negative individuals.

Agbecha & Ikyernum 2018 [17]	Nigeria	Case-control	20 PLWH on ART 20 ART-naïve PLWH 20 HIV-negative	18–60	NR	451.20±229.18	NR	Significant increase in ALT without changes in AST and ALP in ART compared to HIV–negative. Significant increase in AST, ALT, and ALP in ART-naïve compared to HIV-negative.
Aniagolu et al., 2017 [18]	Nigeria	Cohort	43 PLWH who are HAART naïve (given ART after 8 months) 20 HIV-negative	NR	Combivir N used as HAART	247± 71 319±139	NR	Significant increase in ALT and AST levels following 8 months of treatment.

Author, year and reference	Country	Study design	Population Size	Age	Treatments and duration	Baseline CD4 of the ART/ART-naïve group Mean ± SD	Male In the ART group n (%)	Effect on liver enzymes
					(Zidovudine, Lamivudine and Nevirapine) for 4 and then 8 months			
Ebot et al., 2015 [19]	Cameroon	Cross-sectional	100 PLWH on ART 100 ART-naïve individuals 100 HIV-negative	25.86 – 49.94	On the first line ART regimen for six months	435.5 ± 45.2 352.1 ± 28.7	96 (32)	Significant increase in AST and ALT without change in ALP activities compared to ART and ART-naïve, are compared to HIV-negative.
Prathinia et al., 2015 [20]	India	Comparative cross-sectional	200 PLWH no ART 200 HIV-negative	19.01 – 61.30	None	NR	118 (59)	Significant increase in serum AST and ALT in PLHIV compared to HIV-negative
Nwosu et al., 2015 [21]	Nigeria	Comparative cross-sectional	20 PLWH on ART 20 HIV–negative	53	ART for 1–10 months	NR	9 (45)	Significant decrease in AST and ALT and increased ALP in PLWH on ART compared to HIV-negative individuals.

Ayelagbe et al., 2014 [22]	Nigeria	Comparative cross-sectional	45 PLWH on HAART 40 HAART-naïve 40 HIV-negative.	18.27 – 55.65	HAART Didanosine + Emtricitabine or Lamivudine + Nevirapine or Efavirenz Zidovudine + Lamivudine + Nevirapine or Efavirenz Stavudine + Lamivudine + Nevirapine or Efavirenz for 6 months.	NR	NR	Significant increase in AST, ALT, and ALP in HAART compared to HIV-negative. Significant increase in AST and ALT without changes in ALP in HAART-naïve compared to HIV-negative.
Abubakar et al., 2014 [23]	Nigeria	Cross-sectional comparative	25 PLWH on ART 25 PLWH without ART 25 HIV-negative individuals	20–39	2 NRTIs + NNRTIs, 2 NRTIs + A boosted protease inhibitor (Indinavir or Ritonavir) for 1 year or less	516.28±37.51	16 (64)	ART significantly reduced ALP compared to the non-ART group. No differences in AST and ALT in ART and no-–ART groups.

Author, year and reference	Country	Study design	Population Size	Age	Treatments and duration	Baseline CD4 of the ART/ART-naïve group Mean ± SD	Male In the ART group n (%)	Effect on liver enzymes
Ibeh et al., 2013 [24]	Nigeria	Prospective Cohort	50 PLWH on winniecure ART 100 HIV-negative	17 and above	Winniecure ART for 12 weeks	50–200 200–350 cells/μL	24 (48)	A significant increase in ALP, ALT, and AST levels following the administration of ART was observed when compared to HIV-negative individuals.
Analike et al., 2008 [25]	Nigeria	Cross-sectional	100 PLWH on ART 50 PWLH not on ART 100 HIV-negative	18-55	Combination of Zidovudine, Lamivudine, and Nevirapine for six months	NR	NR	A significant increase in AST, ALT, and ALP in ART compared to HIV- negative patients.
Betuine et al., 2007 [26]	Brazil	Prospective cohort	45 pregnant women living with HIV on ART 12 HIV-negative females	16–43	Zidovudine, and Triple T (ZDV + 3TC + NFV)	NR	0 (0)	No significant difference in AST and ALT levels in pregnant women living with HIV on ART compared to HIV-negative women.

PLWH: Pepple living with HIV, TDF: Tenofovir Disoproxil Fumarate, EFV: Efavirenz, NR: Not Reported, HAART: Highly Active Antiretroviral Therapy, ALT: Alanine Aminotransferase, AST: Aspartate Transaminase, ALP: Alkaline Phosphatase, HIV: Humam Immunodeficiency Virus, ART: Antiretroviral Therapy, TLD: Tenofovir/Lamivudine, Dolutegravir, ARV: Antiretroviral, NRTI: Nucleoside reverse transcriptase inhibitor, NNRTI: Non-nucleoside reverse transcriptase inhibitors.

Quality Assessment of included by Newcastle-Oflawa Scale (NOS) Table S2: Quality assessment of cohort studies

Table S2: Quality assessment of cohort studies

	Selection				**Comparability**	**Outcome**			**Score**	**Quality**	**ROB**
	Representativeness of exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at the start of study	Comparability of cohorts on the basis of the design or analysis controlled for confounders	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow-up cohorts			
Angiagolu et al., 2017	□	□	□	☆	□	☆	□	□	6	Moderate	Moderate
Betuine et al., 2007	□	□	□	□	☆	☆	□	□	6	Moderate	Moderate
Ibeh et al., 2013	□	□	□	☆	□	☆	□	□	6	Moderate	Moderate

ROB: Risk of bias

Table S3: Quality assessment of cross-sectional studies

	Selection				Comparability	Outcome		Score	Quality	ROB
	Representativeness of the sample	**Sample size**	**Non-included subjects**	**Ascertainment of exposure**	**Based on design and analysis**	**Assessment of outcome**	**Statistical test**			
Abdulmumin et al., 2024	□	☆	□	□	□□	□	□	7	High	Low
Adubakar et al., 2014	□	☆	□	□	□□	☆	□	6	Moderate	Moderate
Ambad et al., 2021	□	☆	□	□	□	☆	□	5	Moderate	Moderate
Ashakarin et al., 2018	□	☆	□	□	□	☆	□	5	Moderate	Moderate
Ebot et al., 2025	□	☆	□	□	□	□	□	7	High	Low
Gbolohan et al., 2023	□	□	□	□	□□	□	☆	7	High	Low
Gospel et al., 2023	□	□	□	□	□□	☆	□	7	High	Low
Mutuma et al., 2023	□	☆	□	□	□□	□	□	7	High	Low
Odegbemi et al., 2025	□	□	□	□	□□	□	☆	7	High	Low
Quaye et al., 2019	□	☆	☆	□	□□	☆	□	6	Moderate	Moderate
Tamuno-Boma et al., 2023	□	□	□	□	□□	☆	□	7	High	Low
Ikekpeazu et al., 2019	□	☆	□	☆	□□	☆	□	5	Moderate	Moderate
Emokpae et al., 2018	□	☆	□	□	□□	□	□	7	High	Low
Olisekodiaka et al., 2018	□	☆	□	□	□□	□	□	7	High	Low
Prathima et al., 2015	□	☆	□	□	□	□	□	6	Moderate	Moderate

Nwosu et al., 2015	□	☆	□	□	□□	□	□	7	High	Low
Ayelagbe et al., 2014	□	☆	□	□	□□	□	☆	6	Moderate	Moderate
Analike et al., 2008	□	☆	□	□	□	□	□	6	Moderate	Moderate

ROB: Risk of bias

Table S4: Quality assessment of case control studies

	Selection				**Comparability**	**Exposure**			**Score**	**Quality**	**ROB**
	Is the case definition adequate?	**Representativeness of the case**	**Selection of controls**	**Definition of controls**	**Comparability of cases and controls on the bases of the design or analysis**	**Ascertainment of exposure**	**Same method of ascertainment for cases and controls**	**Non-Response rate**			
Agbecha & Ikyernum 2018	□	□	□	□	□□	□	□	☆	8	High	Low
Ezeugwunne et al., 2021	□	□	□	□	□□	□	☆	□	8	High	Low
Abriba et al., 2024	□	□	□	□	□□	□	□	☆	8	High	Low
Deshmikh et al, 2024	□	□	□	□	□	□	□	☆	7	High	Low
Younis et al., 2022	□	□	□	□	□□	□	□	☆	8	High	Low

ROB: Risk of bias

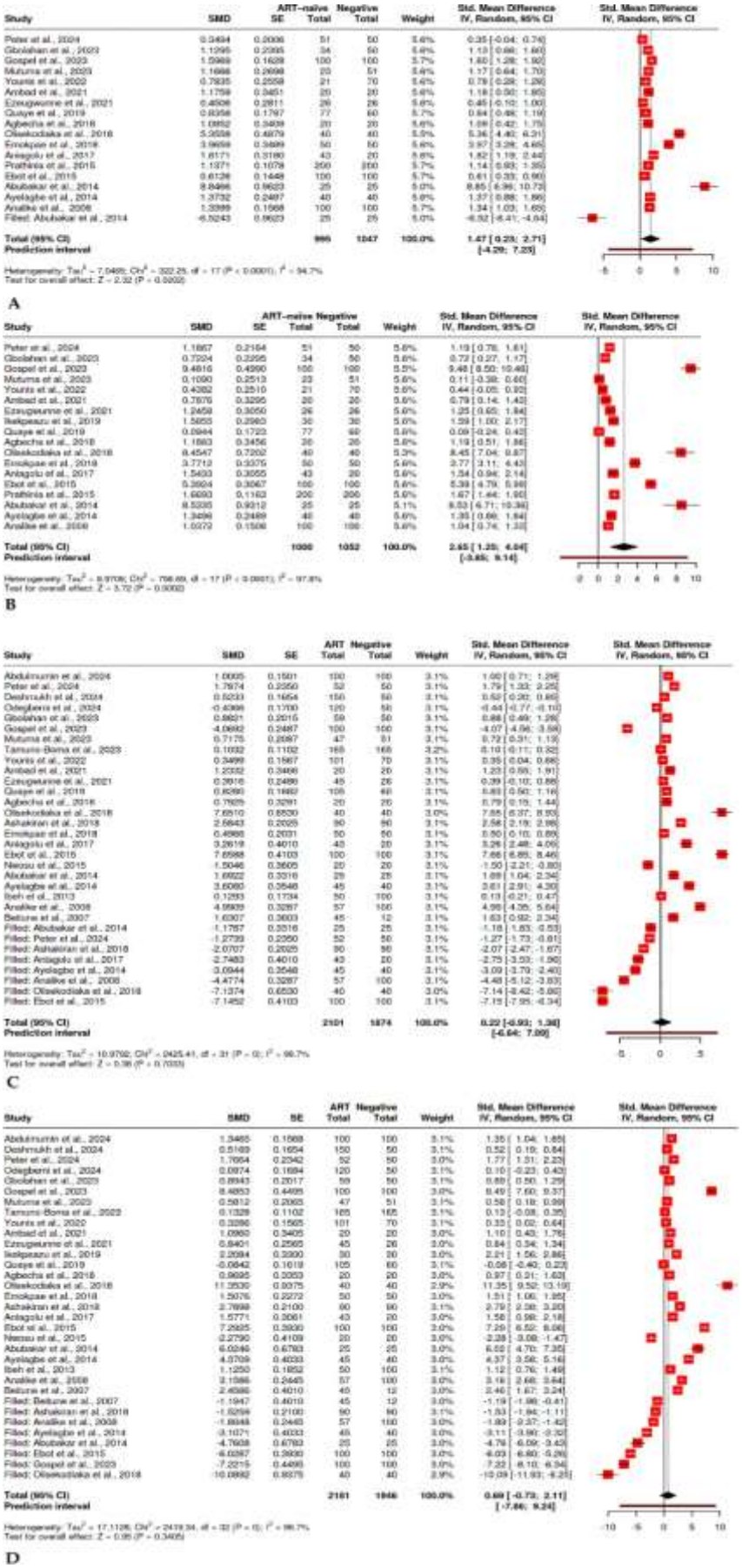


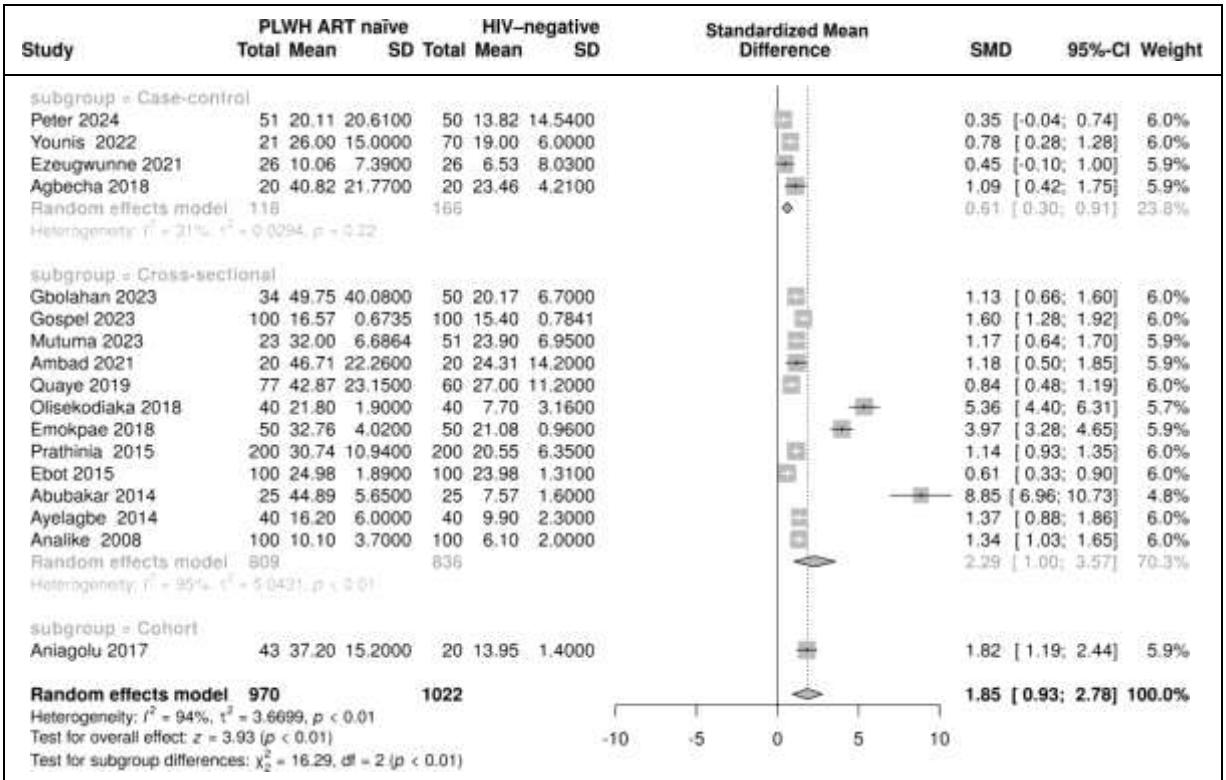
Figure S1: Result of the sensitivity analysis based on the trim and fill test. A: AST in ART-naïve compared with HIV negative. B: ALT in ART-naïve compared with HIV negative. C: AST in ART compared with HIV negative. D: ALT in ART compared with HIV negative**.**

Table S5: Sensitivity analysis using one study exclusion at a time on AST in PLWH compared to HIV negative

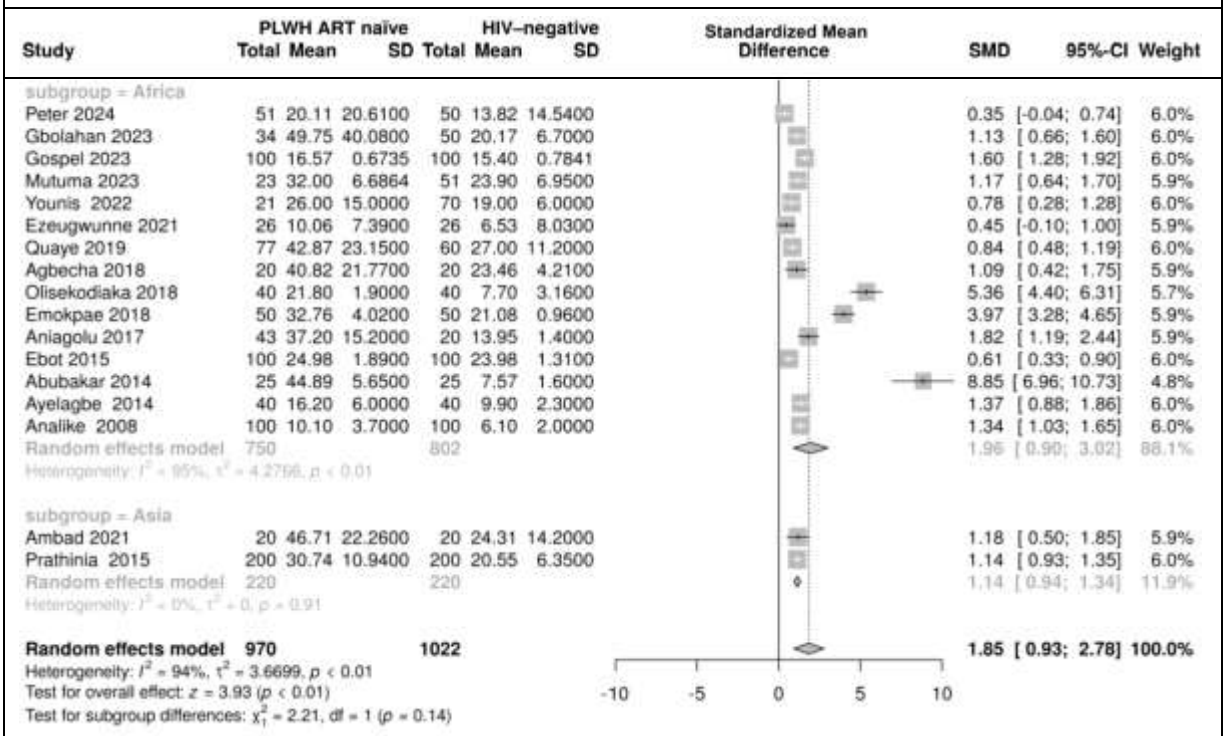
Studies	Effect size (SMD)	Lower Confidence intervals	Upper Confidence intervals	P
Abdulmumin	1.51	0.46	2.56	<0.05
Peter	1.48	0.42	2.53	<0.05
Deshmukh	1.53	0.48	2.59	<0.05
Odegbemi	1.58	0.53	2.62	<0.05
Gbolahan	1.52	0.46	2.57	<0.05
Gospel	1.73	0.80	2.66	<0.05
Mutuma	1.53	0.47	2.58	<0.05
Tamuno-Boma	1.55	0.50	2.60	<0.05
Younis	1.54	0.49	2.59	<0.05
Ambad	1.50	0.45	2.56	<0.05
Ezeugwunne	1.54	0.49	2.59	<0.05
Quaye	1.52	0.47	2.58	<0.05
Agbecha	1.52	0.47	2.58	<0.05
Olisekodiaka	1.23	0.32	2.15	<0.05
Ashakiran	1.44	0.39	2.50	<0.05
Emokpae	1.54	0.48	2.59	<0.05
Aniagolu	1.42	0.37	2.46	<0.05
Ebot	1.22	0.32	2.12	<0.05
Nwosu	1.62	0.60	2.64	<0.05
Abubakar	1.48	0.43	2.54	<0.05
Ayelagbe	1.40	0.36	2.44	<0.05
Ibeh	1.55	0.50	2.50	<0.05
Analike	1.34	0.33	2.35	<0.05
Beitune	1.49	0.43	2.54	<0.05

Table S6. Sensitivity analysis using one study exclusion at a time on ALT in PLWH compared to HIV negative

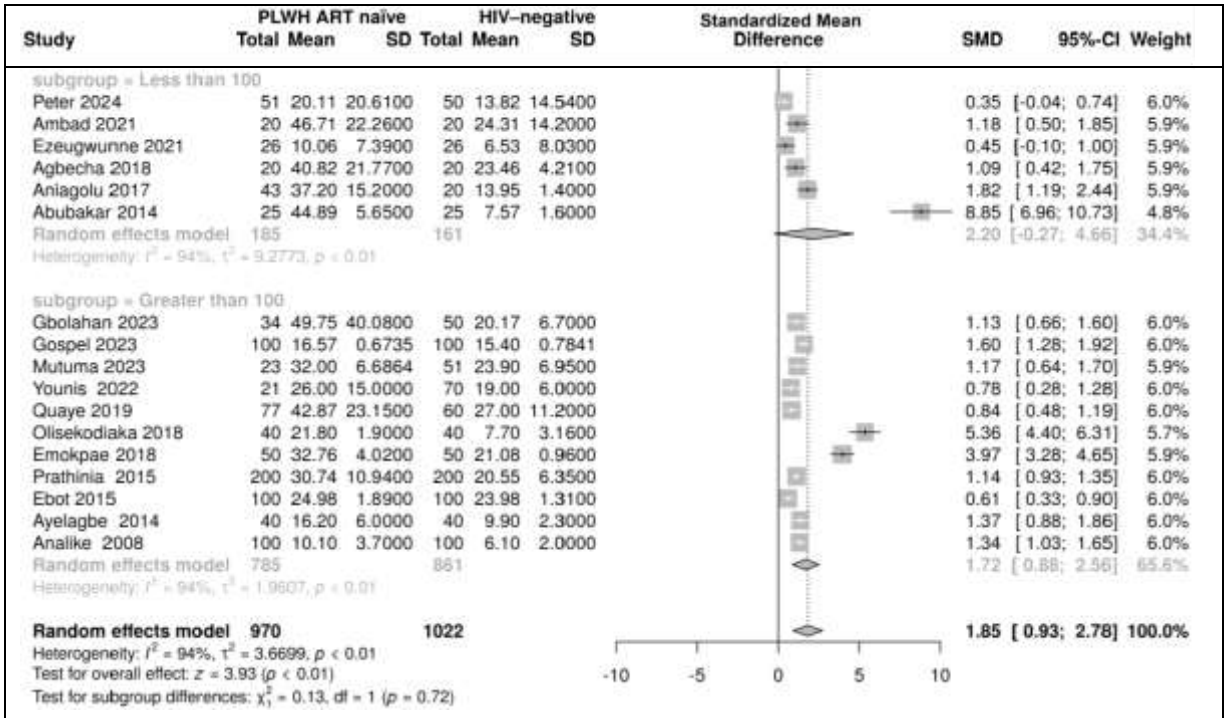
Studies	Effect size (SMD)	Lower CI	Upper CI	P
Abdulmumin	2.34	1.14	3.54	<0.05
Peter	2.37	1.18	3.57	<0.05
Deshmukh	2.32	1.12	3.52	<0.05
Odegbemi	2.39	1.20	3.58	<0.05
Gbolahan	2.36	1.16	3.56	<0.05
Gospel	2.03	0.96	3.11	<0.05
Mutuma	2.37	1.18	3.57	<0.05
Tamuno-Boma	2.39	1.20	3.58	<0.05
Younis	2.38	1.19	3.57	<0.05
Ambad	2.35	1.15	3.55	<0.05
Ezeugwunne	2.36	1.16	3.56	<0.05
Ikekpeazu	2.30	1.10	3.51	<0.05
Quaye	2.40	1.21	3.58	<0.05
Agbecha	2.35	1.16	3.55	<0.05
Olisekodiaka	1.94	0.98	2.91	<0.05
Emokpae	2.33	1.13	3.53	<0.05
Ashakiran	2.28	1.08	3.48	<0.05
Aniagolu	2.33	1.13	3.53	<0.05
Ebot	2.09	0.97	3.20	<0.05
Nwosu	2.48	1.35	3.62	<0.05
Abubakar	2.15	0.99	3.31	<0.05
Ayelagbe	2.21	1.02	3.40	<0.05
Ibeh	2.35	1.15	3.55	<0.05
Analike	2.26	1.06	3.46	<0.05
Beitune	2.29	1.09	3.50	<0.05


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Case-control
Peter 2024 51 20.11 20.6100 50 13.82 14.5400 0.35 [-0.04; 0.74] 6.0%
Younis 2022 21 26.00 15.0000 70 19.00 6.0000 0.78 [0.28; 1.28] 6.0%
Ezeugwunne 2021 26 10.06 7.3900 26 6.53 8.0300 0.45 [-0.10; 1.00] 5.9%
Agbecha 2018 20 40.82 21.7700 20 23.46 4.2100 1.09 [0.42; 1.75] 5.9%
Random effects model 118 166 0.61 [0.30; 0.91] 23.8%
Heterogeneity: I^2 = 21%, τ^2 = 0.0294, p = 0.22
subgroup = Cross-sectional
Gbolahan 2023 34 49.75 40.0800 50 20.17 6.7000 1.13 [0.66; 1.60] 6.0%
Gospel 2023 100 16.57 0.6735 100 15.40 0.7841 1.60 [1.28; 1.92] 6.0%
Mutuma 2023 23 32.00 6.6864 51 23.90 6.9500 1.17 [0.64; 1.70] 5.9%
Ambad 2021 20 46.71 22.2600 20 24.31 14.2000 1.18 [0.50; 1.85] 5.9%
Quaye 2019 77 42.87 23.1500 60 27.00 11.2000 0.84 [0.48; 1.19] 6.0%
Olisekodiaka 2018 40 21.80 1.9000 40 7.70 3.1600 5.36 [4.40; 6.31] 5.7%
Emokpae 2018 50 32.76 4.0200 50 21.08 0.9600 3.97 [3.28; 4.65] 5.9%
Prathinia 2015 200 30.74 10.9400 200 20.55 6.3500 1.14 [0.93; 1.35] 6.0%
Ebot 2015 100 24.98 1.8900 100 23.98 1.3100 0.61 [0.33; 0.90] 6.0%
Abubakar 2014 25 44.89 5.6500 25 7.57 1.6000 8.85 [6.96; 10.73] 4.8%
Ayelagbe 2014 40 16.20 6.0000 40 9.90 2.3000 1.37 [0.88; 1.86] 6.0%
Analike 2008 100 10.10 3.7000 100 6.10 2.0000 1.34 [1.03; 1.65] 6.0%
Random effects model 809 836 2.29 [1.00; 3.57] 70.3%
Heterogeneity: I^2 = 95%, τ^2 = 5.0431, p < 0.01
subgroup = Cohort
Aniagolu 2017 43 37.20 15.2000 20 13.95 1.4000 1.82 [1.19; 2.44] 5.9%
Random effects model 970 1022 1.85 [0.93; 2.78] 100.0%
Heterogeneity: I^2 = 94%, τ^2 = 3.6699, p < 0.01
Test for overall effect: z = 3.93 (p < 0.01)
Test for subgroup differences: χ^2_2 = 16.29, df = 2 (p < 0.01)
-10 -5 0 5 10

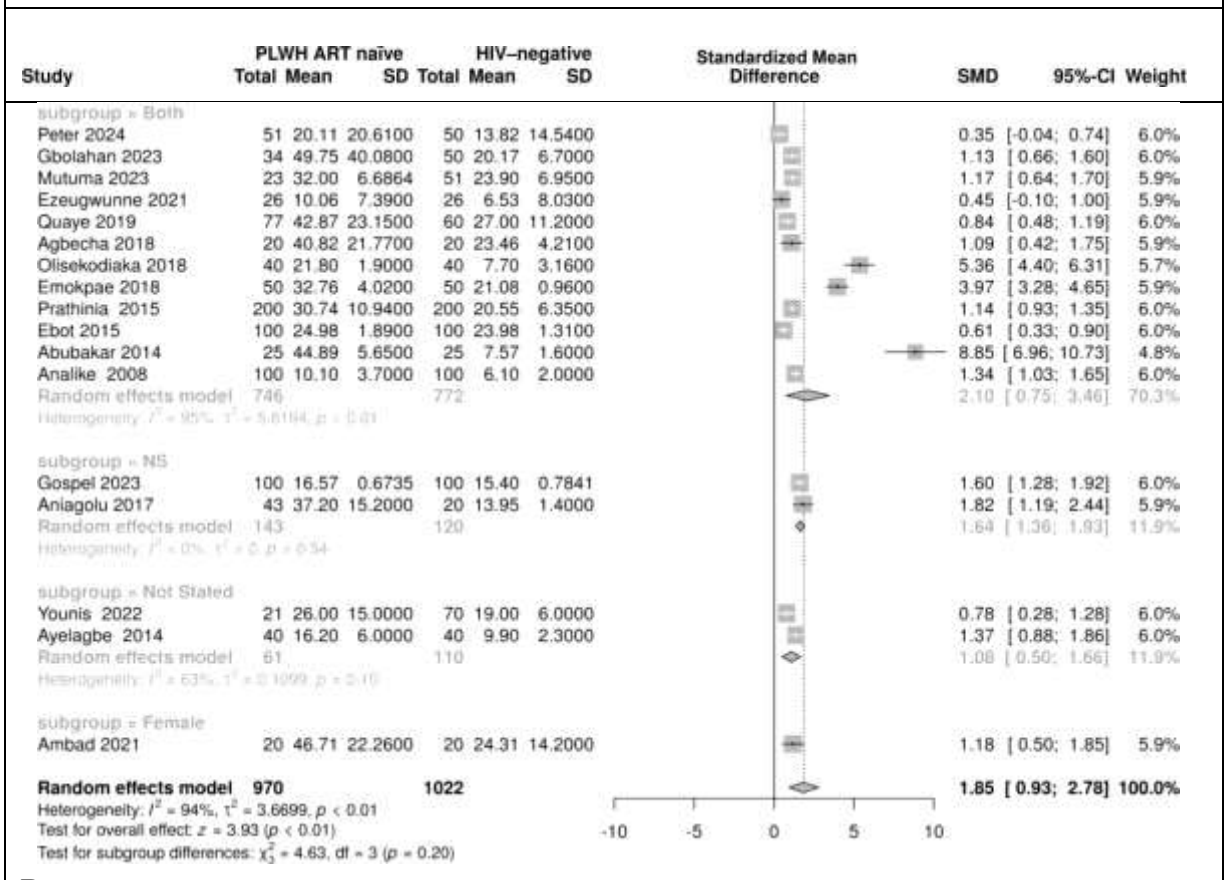
A


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Africa
Peter 2024 51 20.11 20.6100 50 13.82 14.5400 0.35 [-0.04; 0.74] 6.0%
Gbolahan 2023 34 49.75 40.0800 50 20.17 6.7000 1.13 [0.66; 1.60] 6.0%
Gospel 2023 100 16.57 0.6735 100 15.40 0.7841 1.60 [1.28; 1.92] 6.0%
Mutuma 2023 23 32.00 6.6864 51 23.90 6.9500 1.17 [0.64; 1.70] 5.9%
Younis 2022 21 26.00 15.0000 70 19.00 6.0000 0.78 [0.28; 1.28] 6.0%
Ezeugwunne 2021 26 10.06 7.3900 26 6.53 8.0300 0.45 [-0.10; 1.00] 5.9%
Quaye 2019 77 42.87 23.1500 60 27.00 11.2000 0.84 [0.48; 1.19] 6.0%
Agbecha 2018 20 40.82 21.7700 20 23.46 4.2100 1.09 [0.42; 1.75] 5.9%
Olisekodiaka 2018 40 21.80 1.9000 40 7.70 3.1600 5.36 [4.40; 6.31] 5.7%
Emokpae 2018 50 32.76 4.0200 50 21.08 0.9600 3.97 [3.28; 4.65] 5.9%
Aniagolu 2017 43 37.20 15.2000 20 13.95 1.4000 1.82 [1.19; 2.44] 5.9%
Ebot 2015 100 24.98 1.8900 100 23.98 1.3100 0.61 [0.33; 0.90] 6.0%
Abubakar 2014 25 44.89 5.6500 25 7.57 1.6000 8.85 [6.96; 10.73] 4.8%
Ayelagbe 2014 40 16.20 6.0000 40 9.90 2.3000 1.37 [0.88; 1.86] 6.0%
Analike 2008 100 10.10 3.7000 100 6.10 2.0000 1.34 [1.03; 1.65] 6.0%
Random effects model 750 802 1.96 [0.90; 3.02] 88.1%
Heterogeneity: I^2 = 95%, τ^2 = 4.2766, p < 0.01
subgroup = Asia
Ambad 2021 20 46.71 22.2600 20 24.31 14.2000 1.18 [0.50; 1.85] 5.9%
Prathinia 2015 200 30.74 10.9400 200 20.55 6.3500 1.14 [0.93; 1.35] 6.0%
Random effects model 220 220 1.14 [0.94; 1.34] 11.9%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.91
Random effects model 970 1022 1.85 [0.93; 2.78] 100.0%
Heterogeneity: I^2 = 94%, τ^2 = 3.6699, p < 0.01
Test for overall effect: z = 3.93 (p < 0.01)
Test for subgroup differences: χ^2_1 = 2.21, df = 1 (p = 0.14)
-10 -5 0 5 10

B


Study
PLWH ART naive
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Less than 100
Peter 2024 51 20.11 20.6100 50 13.82 14.5400 0.35 [-0.04; 0.74] 6.0%
Ambad 2021 20 46.71 22.2600 20 24.31 14.2000 1.18 [0.50; 1.85] 5.9%
Ezeugwunne 2021 26 10.06 7.3900 26 6.53 8.0300 0.45 [-0.10; 1.00] 5.9%
Agbecha 2018 20 40.82 21.7700 20 23.46 4.2100 1.09 [0.42; 1.75] 5.9%
Aniagolu 2017 43 37.20 15.2000 20 13.95 1.4000 1.82 [1.19; 2.44] 5.9%
Abubakar 2014 25 44.89 5.6500 25 7.57 1.6000 8.85 [6.96; 10.73] 4.8%
Random effects model 185 161 2.20 [-0.27; 4.66] 34.4%
Heterogeneity: I^2 = 94%, τ^2 = 9.2773, p < 0.01
subgroup = Greater than 100
Gbolahan 2023 34 49.75 40.0800 50 20.17 6.7000 1.13 [0.66; 1.60] 6.0%
Gospel 2023 100 16.57 0.6735 100 15.40 0.7841 1.60 [1.28; 1.92] 6.0%
Mutuma 2023 23 32.00 6.6864 51 23.90 6.9500 1.17 [0.64; 1.70] 5.9%
Younis 2022 21 26.00 15.0000 70 19.00 6.0000 0.78 [0.28; 1.28] 6.0%
Quaye 2019 77 42.87 23.1500 60 27.00 11.2000 0.84 [0.48; 1.19] 6.0%
Olisekodiaka 2018 40 21.80 1.9000 40 7.70 3.1600 5.36 [4.40; 6.31] 5.7%
Emokpae 2018 50 32.76 4.0200 50 21.08 0.9600 3.97 [3.28; 4.65] 5.9%
Prathinia 2015 200 30.74 10.9400 200 20.55 6.3500 1.14 [0.93; 1.35] 6.0%
Ebot 2015 100 24.98 1.8900 100 23.98 1.3100 0.61 [0.33; 0.90] 6.0%
Ayelagbe 2014 40 16.20 6.0000 40 9.90 2.3000 1.37 [0.88; 1.86] 6.0%
Analike 2008 100 10.10 3.7000 100 6.10 2.0000 1.34 [1.03; 1.65] 6.0%
Random effects model 785 861 1.72 [0.88; 2.56] 65.6%
Heterogeneity: I^2 = 94%, τ^2 = 1.9607, p < 0.01
Random effects model 970 1022 1.85 [0.93; 2.78] 100.0%
Heterogeneity: I^2 = 94%, τ^2 = 3.6699, p < 0.01
Test for overall effect: z = 3.93 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.13, df = 1 (p = 0.72)
-10 -5 0 5 10

C


Study
PLWH ART naive
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Both
Peter 2024 51 20.11 20.6100 50 13.82 14.5400 0.35 [-0.04; 0.74] 6.0%
Gbolahan 2023 34 49.75 40.0800 50 20.17 6.7000 1.13 [0.66; 1.60] 6.0%
Mutuma 2023 23 32.00 6.6864 51 23.90 6.9500 1.17 [0.64; 1.70] 5.9%
Ezeugwunne 2021 26 10.06 7.3900 26 6.53 8.0300 0.45 [-0.10; 1.00] 5.9%
Quaye 2019 77 42.87 23.1500 60 27.00 11.2000 0.84 [0.48; 1.19] 6.0%
Agbecha 2018 20 40.82 21.7700 20 23.46 4.2100 1.09 [0.42; 1.75] 5.9%
Olisekodiaka 2018 40 21.80 1.9000 40 7.70 3.1600 5.36 [4.40; 6.31] 5.7%
Emokpae 2018 50 32.76 4.0200 50 21.08 0.9600 3.97 [3.28; 4.65] 5.9%
Prathinia 2015 200 30.74 10.9400 200 20.55 6.3500 1.14 [0.93; 1.35] 6.0%
Ebot 2015 100 24.98 1.8900 100 23.98 1.3100 0.61 [0.33; 0.90] 6.0%
Abubakar 2014 25 44.89 5.6500 25 7.57 1.6000 8.85 [6.96; 10.73] 4.8%
Analike 2008 100 10.10 3.7000 100 6.10 2.0000 1.34 [1.03; 1.65] 6.0%
Random effects model 746 772 2.10 [0.75; 3.46] 70.3%
Heterogeneity: I^2 = 95%, τ^2 = 5.6184, p < 0.01
subgroup = NS
Gospel 2023 100 16.57 0.6735 100 15.40 0.7841 1.60 [1.28; 1.92] 6.0%
Aniagolu 2017 43 37.20 15.2000 20 13.95 1.4000 1.82 [1.19; 2.44] 5.9%
Random effects model 143 120 1.64 [1.36; 1.93] 11.9%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.54
subgroup = Not Stated
Younis 2022 21 26.00 15.0000 70 19.00 6.0000 0.78 [0.28; 1.28] 6.0%
Ayelagbe 2014 40 16.20 6.0000 40 9.90 2.3000 1.37 [0.88; 1.86] 6.0%
Random effects model 61 110 1.08 [0.50; 1.66] 11.9%
Heterogeneity: I^2 = 63%, τ^2 = 0.1099, p = 0.10
subgroup = Female
Ambad 2021 20 46.71 22.2600 20 24.31 14.2000 1.18 [0.50; 1.85] 5.9%
Random effects model 970 1022 1.85 [0.93; 2.78] 100.0%
Heterogeneity: I^2 = 94%, τ^2 = 3.6699, p < 0.01
Test for overall effect: z = 3.93 (p < 0.01)
Test for subgroup differences: χ^2_3 = 4.63, df = 3 (p = 0.20)
-10 -5 0 5 10

D

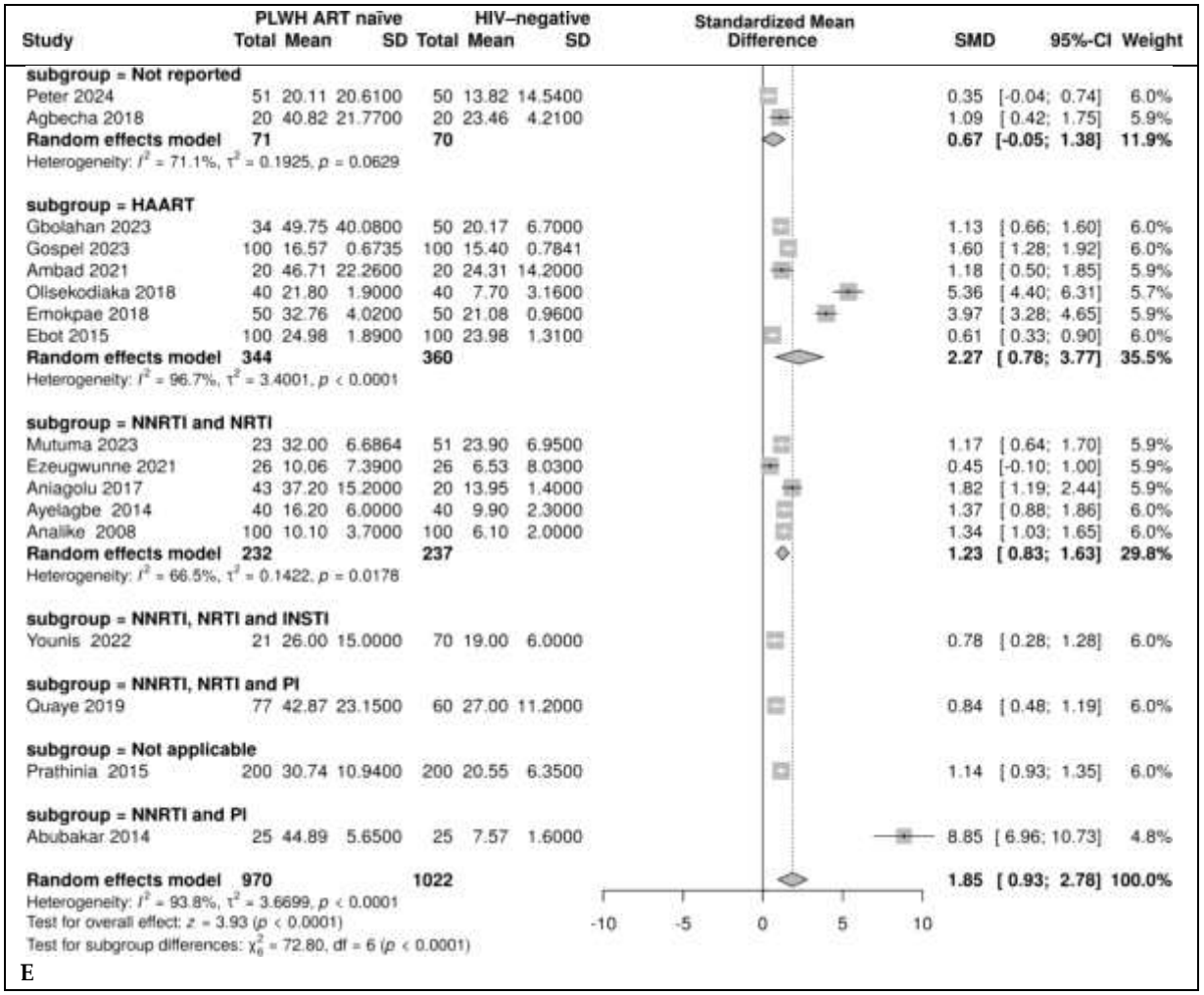
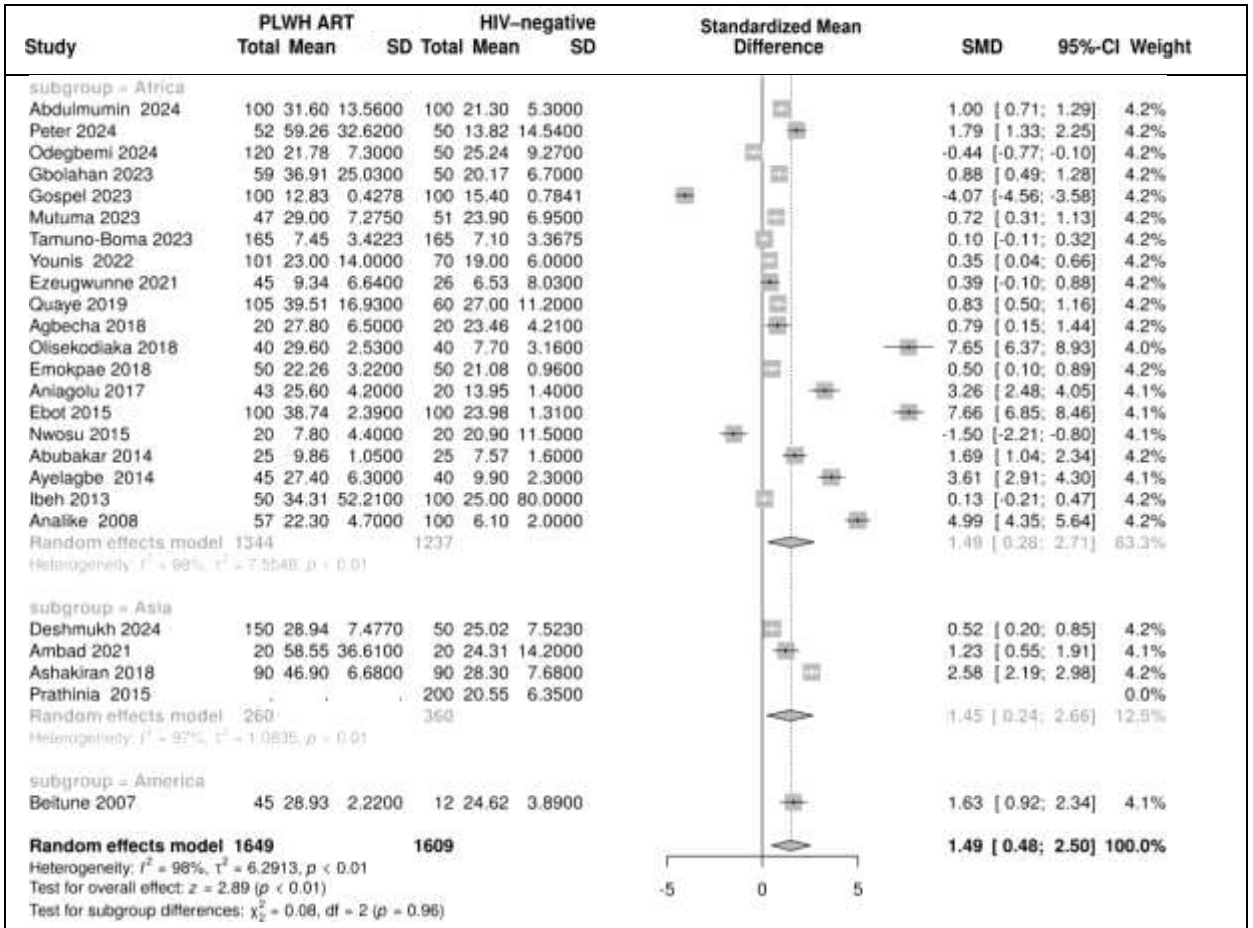
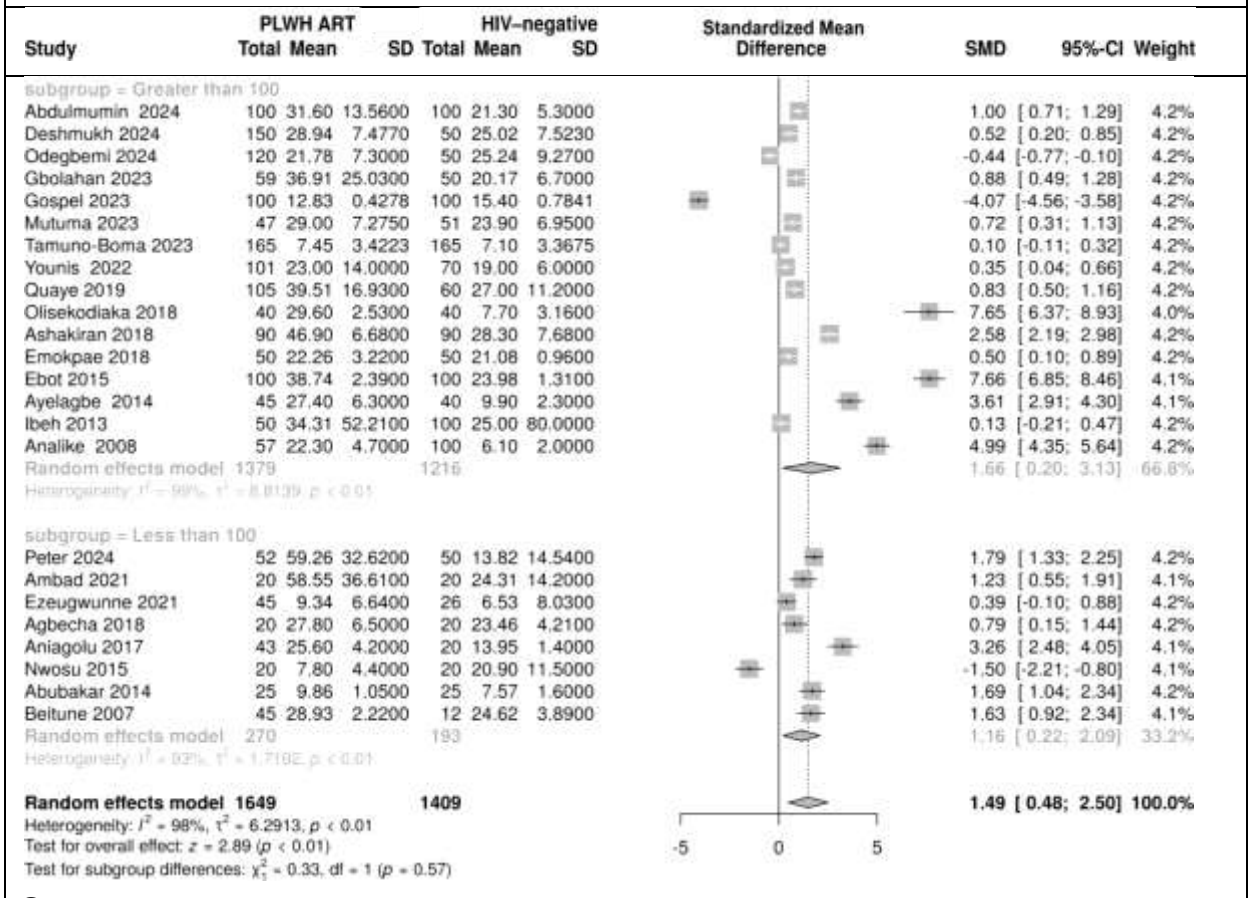


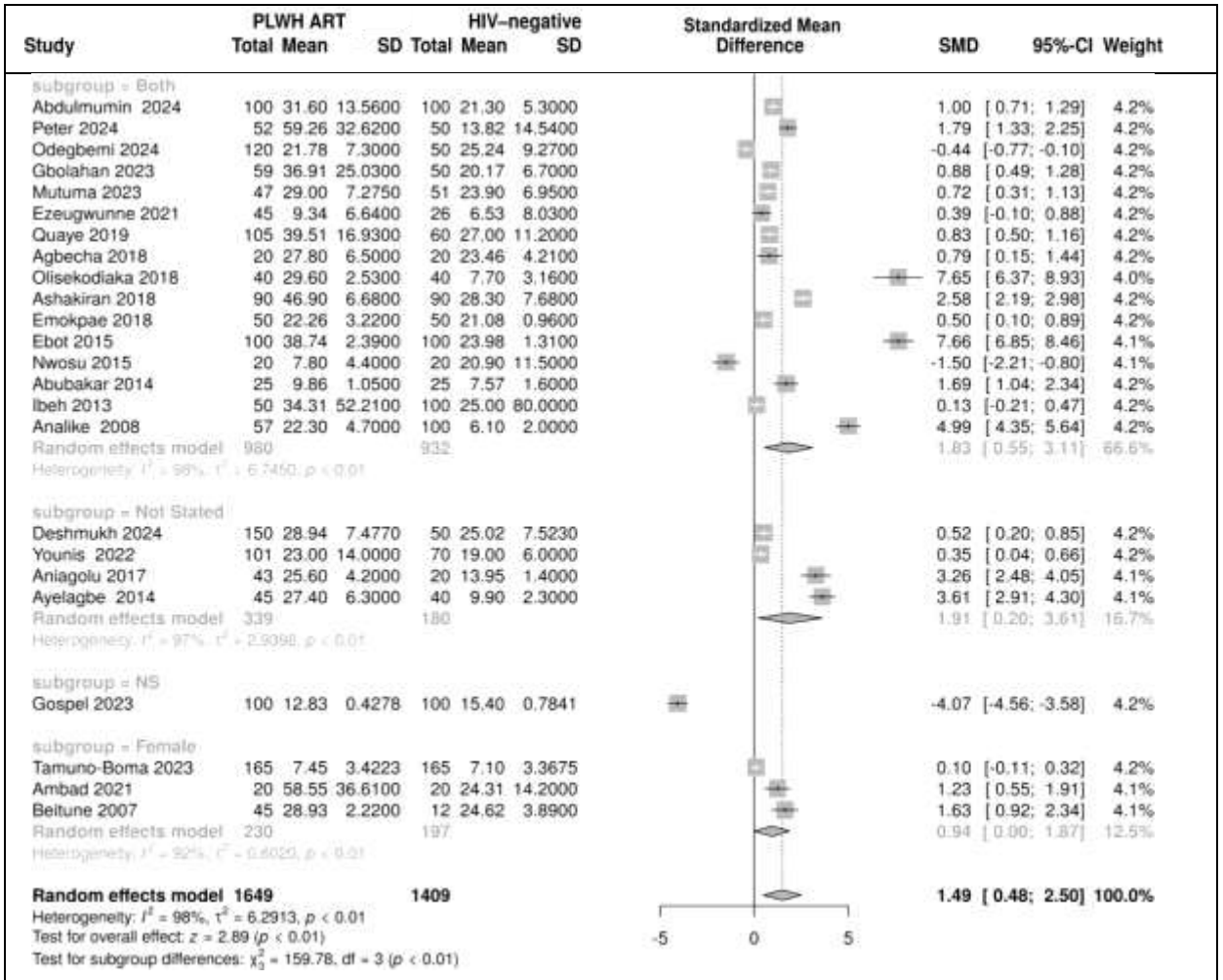
Figure S2: Subgroup analysis on AST in ART-naïve compared to HIV-negative individuals. A: The level of AST in PLWH who were ART-naïve versus HIV negative, based on study design. B: ART-naïve versus HIV negative on AST based on continent. C: AST levels in PLWH who are ART-naïve versus HIV negative, based on sample size. D: AST levels in ART-naïve versus HIV negative, based on gender distribution. E: AST levels in ART-naïve versus HIV negative, based on class of ART. The solid line shows the line of no effect, the dashed line shows the effect size, the gray block shows the weight of the study, the horizontal line crossing the gray block shows the confidence intervals, diamond plot shows the combined effect size. NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, NtRTI: nucleotide reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase-nucleoside strand transfer inhibitors


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Africa
Abdulmumin 2024 100 31.60 13.5600 100 21.30 5.3000 1.00 [0.71; 1.29] 4.2%
Peter 2024 52 59.26 32.6200 50 13.82 14.5400 1.79 [1.33; 2.25] 4.2%
Odegbemi 2024 120 21.78 7.3000 50 25.24 9.2700 -0.44 [-0.77; -0.10] 4.2%
Gbolahan 2023 59 36.91 25.0300 50 20.17 6.7000 0.88 [0.49; 1.28] 4.2%
Gospel 2023 100 12.83 0.4278 100 15.40 0.7841 -4.07 [-4.56; -3.58] 4.2%
Mutuma 2023 47 29.00 7.2750 51 23.90 6.9500 0.72 [0.31; 1.13] 4.2%
Tamuno-Boma 2023 165 7.45 3.4223 165 7.10 3.3675 0.10 [-0.11; 0.32] 4.2%
Younis 2022 101 23.00 14.0000 70 19.00 6.0000 0.35 [0.04; 0.66] 4.2%
Ezeugwunne 2021 45 9.34 6.6400 26 6.53 8.0300 0.39 [-0.10; 0.88] 4.2%
Quaye 2019 105 39.51 16.9300 60 27.00 11.2000 0.83 [0.50; 1.16] 4.2%
Agbecha 2018 20 27.80 6.5000 20 23.46 4.2100 0.79 [0.15; 1.44] 4.2%
Olisekodiaka 2018 40 29.60 2.5300 40 7.70 3.1600 7.65 [6.37; 8.93] 4.0%
Emokpae 2018 50 22.26 3.2200 50 21.08 0.9600 0.50 [0.10; 0.89] 4.2%
Aniagolu 2017 43 25.60 4.2000 20 13.95 1.4000 3.26 [2.48; 4.05] 4.1%
Ebot 2015 100 38.74 2.3900 100 23.98 1.3100 7.66 [6.85; 8.46] 4.1%
Nwosu 2015 20 7.80 4.4000 20 20.90 11.5000 -1.50 [-2.21; -0.80] 4.1%
Abubakar 2014 25 9.86 1.0500 25 7.57 1.6000 1.69 [1.04; 2.34] 4.2%
Ayelagbe 2014 45 27.40 6.3000 40 9.90 2.3000 3.61 [2.91; 4.30] 4.1%
Ibeh 2013 50 34.31 52.2100 100 25.00 80.0000 0.13 [-0.21; 0.47] 4.2%
Analike 2008 57 22.30 4.7000 100 6.10 2.0000 4.99 [4.35; 5.64] 4.2%
Random effects model 1344 1237 1.49 [0.28; 2.71] 63.3%
Heterogeneity: I^2 = 98%, τ^2 = 7.5548, p < 0.01
subgroup = Asia
Deshmukh 2024 150 28.94 7.4770 50 25.02 7.5230 0.52 [0.20; 0.85] 4.2%
Ambad 2021 20 58.55 36.6100 20 24.31 14.2000 1.23 [0.55; 1.91] 4.1%
Ashakiran 2018 90 46.90 6.6800 90 28.30 7.6800 2.58 [2.19; 2.98] 4.2%
Prathinia 2015 200 20.55 6.3500 0.0%
Random effects model 260 360 1.45 [0.24; 2.66] 12.5%
Heterogeneity: I^2 = 97%, τ^2 = 1.0835, p < 0.01
subgroup = America
Beitune 2007 45 28.93 2.2200 12 24.62 3.8900 1.63 [0.92; 2.34] 4.1%
Random effects model 1649 1609 1.49 [0.48; 2.50] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 6.2913, p < 0.01
Test for overall effect: z = 2.89 (p < 0.01)
Test for subgroup differences: χ^2_2 = 0.08, df = 2 (p = 0.96)
-5
0
5

B


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Greater than 100
Abdulmumin 2024 100 31.60 13.5600 100 21.30 5.3000 1.00 [0.71; 1.29] 4.2%
Deshmukh 2024 150 28.94 7.4770 50 25.02 7.5230 0.52 [0.20; 0.85] 4.2%
Odegbemi 2024 120 21.78 7.3000 50 25.24 9.2700 -0.44 [-0.77; -0.10] 4.2%
Gbolahan 2023 59 36.91 25.0300 50 20.17 6.7000 0.88 [0.49; 1.28] 4.2%
Gospel 2023 100 12.83 0.4278 100 15.40 0.7841 -4.07 [-4.56; -3.58] 4.2%
Mutuma 2023 47 29.00 7.2750 51 23.90 6.9500 0.72 [0.31; 1.13] 4.2%
Tamuno-Boma 2023 165 7.45 3.4223 165 7.10 3.3675 0.10 [-0.11; 0.32] 4.2%
Younis 2022 101 23.00 14.0000 70 19.00 6.0000 0.35 [0.04; 0.66] 4.2%
Quaye 2019 105 39.51 16.9300 60 27.00 11.2000 0.83 [0.50; 1.16] 4.2%
Olisekodiaka 2018 40 29.60 2.5300 40 7.70 3.1600 7.65 [6.37; 8.93] 4.0%
Ashakiran 2018 90 46.90 6.6800 90 28.30 7.6800 2.58 [2.19; 2.98] 4.2%
Emokpae 2018 50 22.26 3.2200 50 21.08 0.9600 0.50 [0.10; 0.89] 4.2%
Ebot 2015 100 38.74 2.3900 100 23.98 1.3100 7.66 [6.85; 8.46] 4.1%
Ayelagbe 2014 45 27.40 6.3000 40 9.90 2.3000 3.61 [2.91; 4.30] 4.1%
Ibeh 2013 50 34.31 52.2100 100 25.00 80.0000 0.13 [-0.21; 0.47] 4.2%
Analike 2008 57 22.30 4.7000 100 6.10 2.0000 4.99 [4.35; 5.64] 4.2%
Random effects model 1379 1216 1.66 [0.20; 3.13] 66.8%
Heterogeneity: I^2 = 98%, τ^2 = 8.8139, p < 0.01
subgroup = Less than 100
Peter 2024 52 59.26 32.6200 50 13.82 14.5400 1.79 [1.33; 2.25] 4.2%
Ambad 2021 20 58.55 36.6100 20 24.31 14.2000 1.23 [0.55; 1.91] 4.1%
Ezeugwunne 2021 45 9.34 6.6400 26 6.53 8.0300 0.39 [-0.10; 0.88] 4.2%
Agbecha 2018 20 27.80 6.5000 20 23.46 4.2100 0.79 [0.15; 1.44] 4.2%
Aniagolu 2017 43 25.60 4.2000 20 13.95 1.4000 3.26 [2.48; 4.05] 4.1%
Nwosu 2015 20 7.80 4.4000 20 20.90 11.5000 -1.50 [-2.21; -0.80] 4.1%
Abubakar 2014 25 9.86 1.0500 25 7.57 1.6000 1.69 [1.04; 2.34] 4.2%
Beitune 2007 45 28.93 2.2200 12 24.62 3.8900 1.63 [0.92; 2.34] 4.1%
Random effects model 270 193 1.16 [0.22; 2.09] 33.2%
Heterogeneity: I^2 = 93%, τ^2 = 1.7102, p < 0.01
Random effects model 1649 1409 1.49 [0.48; 2.50] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 6.2913, p < 0.01
Test for overall effect: z = 2.89 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.33, df = 1 (p = 0.57)
-5
0
5

C


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Both
Abdulmumin 2024 100 31.60 13.5600 100 21.30 5.3000 1.00 [0.71; 1.29] 4.2%
Peter 2024 52 59.26 32.6200 50 13.82 14.5400 1.79 [1.33; 2.25] 4.2%
Odegbemi 2024 120 21.78 7.3000 50 25.24 9.2700 -0.44 [-0.77; -0.10] 4.2%
Gbolahan 2023 59 36.91 25.0300 50 20.17 6.7000 0.88 [0.49; 1.28] 4.2%
Mutuma 2023 47 29.00 7.2750 51 23.90 6.9500 0.72 [0.31; 1.13] 4.2%
Ezeugwunne 2021 45 9.34 6.6400 26 6.53 8.0300 0.39 [-0.10; 0.88] 4.2%
Quaye 2019 105 39.51 16.9300 60 27.00 11.2000 0.83 [0.50; 1.16] 4.2%
Agbecha 2018 20 27.80 6.5000 20 23.46 4.2100 0.79 [0.15; 1.44] 4.2%
Olisekodiaka 2018 40 29.60 2.5300 40 7.70 3.1600 7.65 [6.37; 8.93] 4.0%
Ashakiran 2018 90 46.90 6.6800 90 28.30 7.6800 2.58 [2.19; 2.98] 4.2%
Emokpae 2018 50 22.26 3.2200 50 21.08 0.9600 0.50 [0.10; 0.89] 4.2%
Ebot 2015 100 38.74 2.3900 100 23.98 1.3100 7.66 [6.85; 8.46] 4.1%
Nwosu 2015 20 7.80 4.4000 20 20.90 11.5000 -1.50 [-2.21; -0.80] 4.1%
Abubakar 2014 25 9.86 1.0500 25 7.57 1.6000 1.69 [1.04; 2.34] 4.2%
Ibeh 2013 50 34.31 52.2100 100 25.00 80.0000 0.13 [-0.21; 0.47] 4.2%
Analike 2008 57 22.30 4.7000 100 6.10 2.0000 4.99 [4.35; 5.64] 4.2%
Random effects model 980 932 1.83 [0.55; 3.11] 66.6%
Heterogeneity: I^2 = 98%, τ^2 = 6.7450, p < 0.01
subgroup = Not Stated
Deshmukh 2024 150 28.94 7.4770 50 25.02 7.5230 0.52 [0.20; 0.85] 4.2%
Younis 2022 101 23.00 14.0000 70 19.00 6.0000 0.35 [0.04; 0.66] 4.2%
Aniagolu 2017 43 25.60 4.2000 20 13.95 1.4000 3.26 [2.48; 4.05] 4.1%
Ayelagbe 2014 45 27.40 6.3000 40 9.90 2.3000 3.61 [2.91; 4.30] 4.1%
Random effects model 339 180 1.91 [0.20; 3.61] 16.7%
Heterogeneity: I^2 = 97%, τ^2 = 2.9098, p < 0.01
subgroup = NS
Gospel 2023 100 12.83 0.4278 100 15.40 0.7841 -4.07 [-4.56; -3.58] 4.2%
subgroup = Female
Tamuno-Boma 2023 165 7.45 3.4223 165 7.10 3.3675 0.10 [-0.11; 0.32] 4.2%
Ambad 2021 20 58.55 36.6100 20 24.31 14.2000 1.23 [0.55; 1.91] 4.1%
Beitune 2007 45 28.93 2.2200 12 24.62 3.8900 1.63 [0.92; 2.34] 4.1%
Random effects model 230 197 0.94 [0.00; 1.87] 12.5%
Heterogeneity: I^2 = 92%, τ^2 = 0.6020, p < 0.01
Random effects model 1649 1409 1.49 [0.48; 2.50] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 6.2913, p < 0.01
Test for overall effect: z = 2.89 (p < 0.01)
Test for subgroup differences: χ^2_3 = 159.78, df = 3 (p < 0.01)
-5
0
5

D

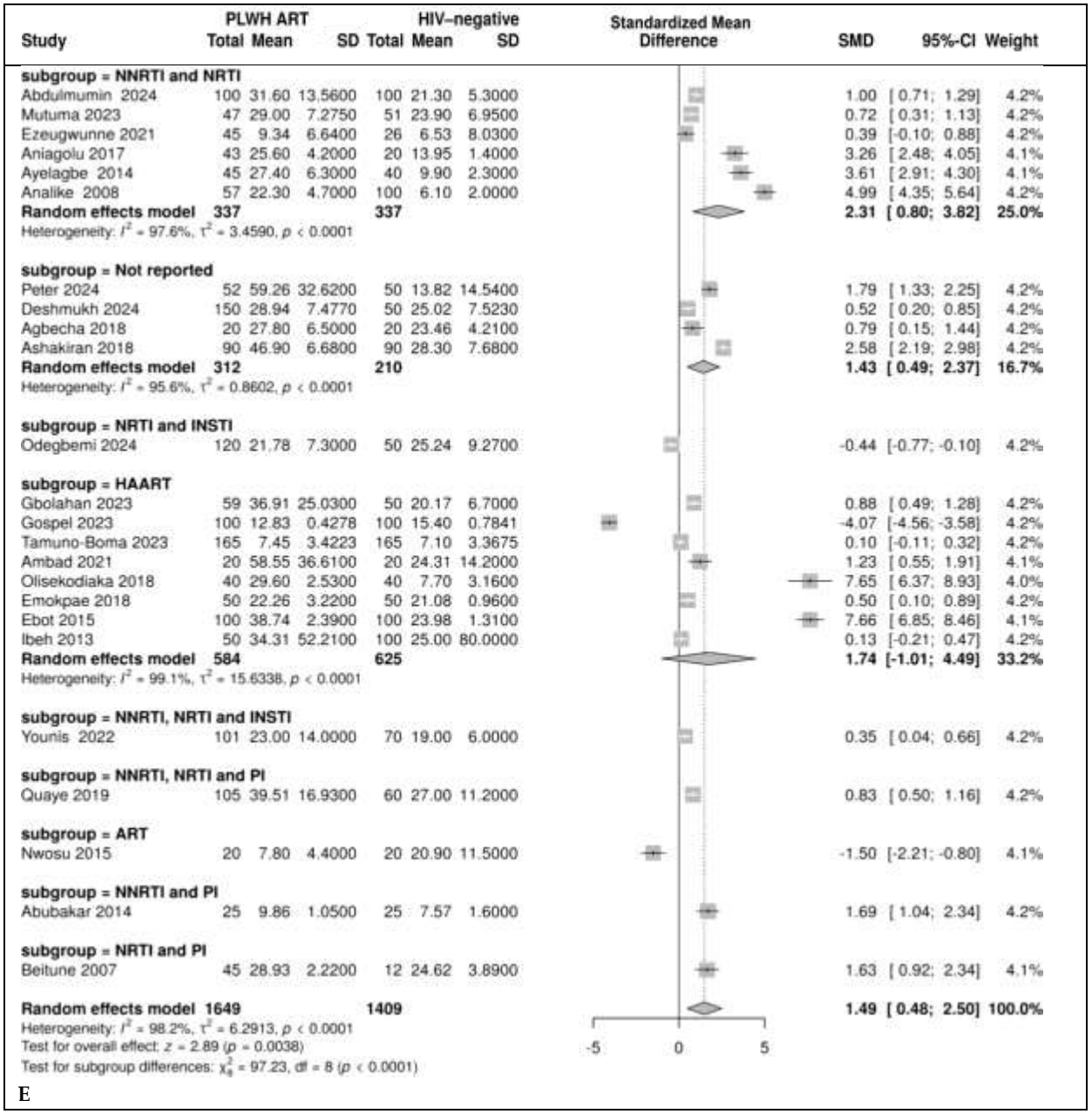
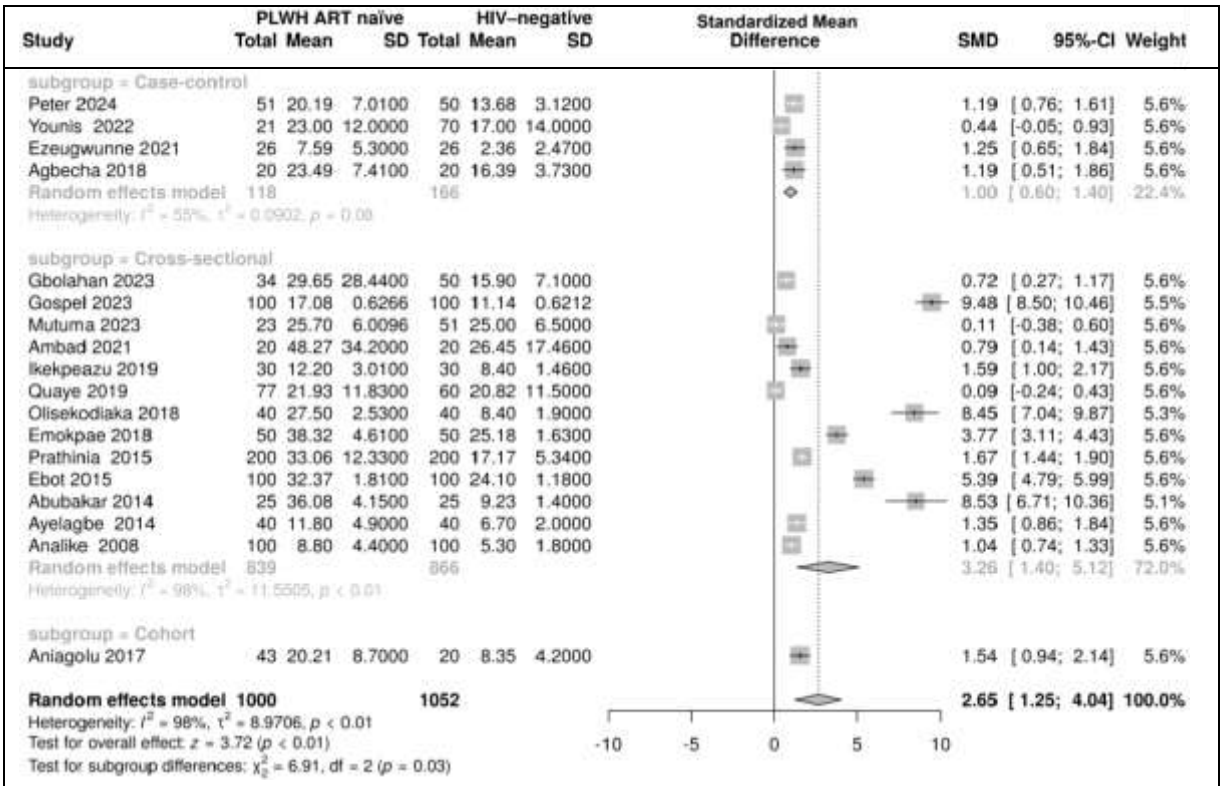
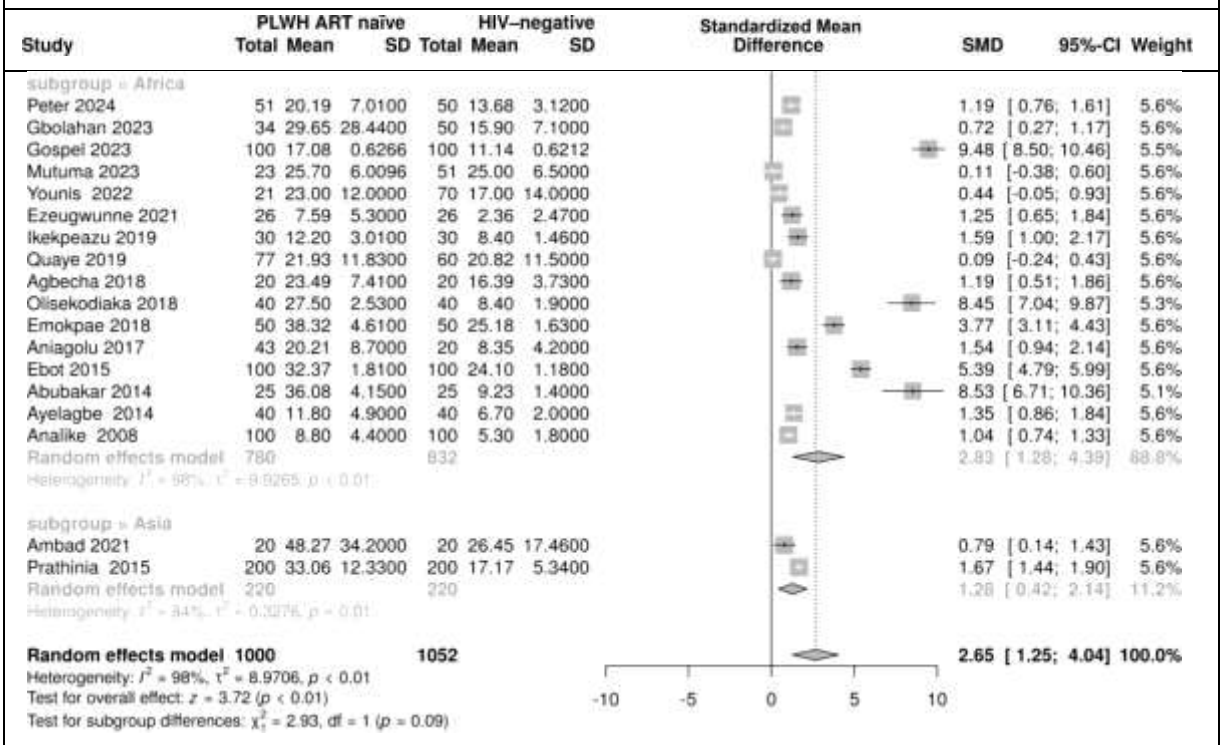


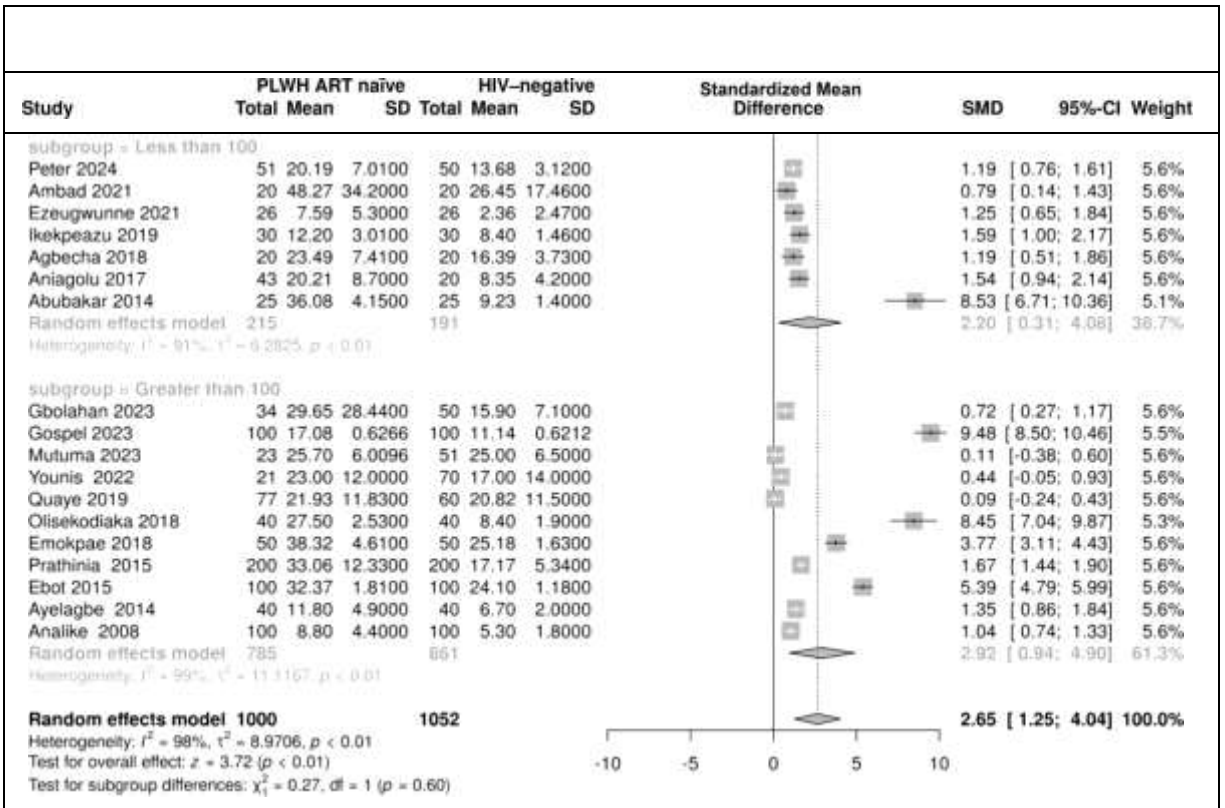
Figure S3: Subgroup analysis on AST in PLWH exposed to ART compared with HIV-negative individuals. A: AST in PLWH exposed to ART compared with HIV-negative individuals based on study design. B: AST in PLWH exposed to ART compared with HIV-negative individuals based on the continent of publication. C: AST in PLWH exposed to ART compared with HIV- negative individuals based on sample size. D: AST in PLWH exposed to ART compared with HIV-negative individuals based on gender distribution. E: AST in PLWH exposed to ART compared with HIV-negative individuals based on the class of ART. The solid line shows the line of no effect, the dashed line shows the effect size, the gray block shows the weight of the study, the horizontal line crossing the gray block shows the confidence intervals, diamond plot shows the combined effect size. NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, NtRTI: nucleotide reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase-nucleoside strand transfer inhibitors.


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Case-control
Peter 2024 51 20.19 7.0100 50 13.68 3.1200 1.19 [0.76; 1.61] 5.6%
Younis 2022 21 23.00 12.0000 70 17.00 14.0000 0.44 [-0.05; 0.93] 5.6%
Ezeugwunne 2021 26 7.59 5.3000 26 2.36 2.4700 1.25 [0.65; 1.84] 5.6%
Agbecha 2018 20 23.49 7.4100 20 16.39 3.7300 1.19 [0.51; 1.86] 5.6%
Random effects model 118 166 1.00 [0.60; 1.40] 22.4%
Heterogeneity: I^2 = 55%, τ^2 = 0.0902, p = 0.08
subgroup = Cross-sectional
Gbolahan 2023 34 29.65 28.4400 50 15.90 7.1000 0.72 [0.27; 1.17] 5.6%
Gospel 2023 100 17.08 0.6266 100 11.14 0.6212 9.48 [8.50; 10.46] 5.5%
Mutuma 2023 23 25.70 6.0096 51 25.00 6.5000 0.11 [-0.38; 0.60] 5.6%
Ambad 2021 20 48.27 34.2000 20 26.45 17.4600 0.79 [0.14; 1.43] 5.6%
Ikekpeazu 2019 30 12.20 3.0100 30 8.40 1.4600 1.59 [1.00; 2.17] 5.6%
Quaye 2019 77 21.93 11.8300 60 20.82 11.5000 0.09 [-0.24; 0.43] 5.6%
Olisekodiaka 2018 40 27.50 2.5300 40 8.40 1.9000 8.45 [7.04; 9.87] 5.3%
Emokpae 2018 50 38.32 4.6100 50 25.18 1.6300 3.77 [3.11; 4.43] 5.6%
Prathinia 2015 200 33.06 12.3300 200 17.17 5.3400 1.67 [1.44; 1.90] 5.6%
Ebot 2015 100 32.37 1.8100 100 24.10 1.1800 5.39 [4.79; 5.99] 5.6%
Abubakar 2014 25 36.08 4.1500 25 9.23 1.4000 8.53 [6.71; 10.36] 5.1%
Ayelagbe 2014 40 11.80 4.9000 40 6.70 2.0000 1.35 [0.86; 1.84] 5.6%
Analike 2008 100 8.80 4.4000 100 5.30 1.8000 1.04 [0.74; 1.33] 5.6%
Random effects model 839 866 3.26 [1.40; 5.12] 72.0%
Heterogeneity: I^2 = 98%, τ^2 = 11.5505, p < 0.01
subgroup = Cohort
Aniagolu 2017 43 20.21 8.7000 20 8.35 4.2000 1.54 [0.94; 2.14] 5.6%
Random effects model 1000 1052 2.65 [1.25; 4.04] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.9706, p < 0.01
Test for overall effect: z = 3.72 (p < 0.01)
Test for subgroup differences: χ^2_2 = 6.91, df = 2 (p = 0.03)
-10 -5 0 5 10

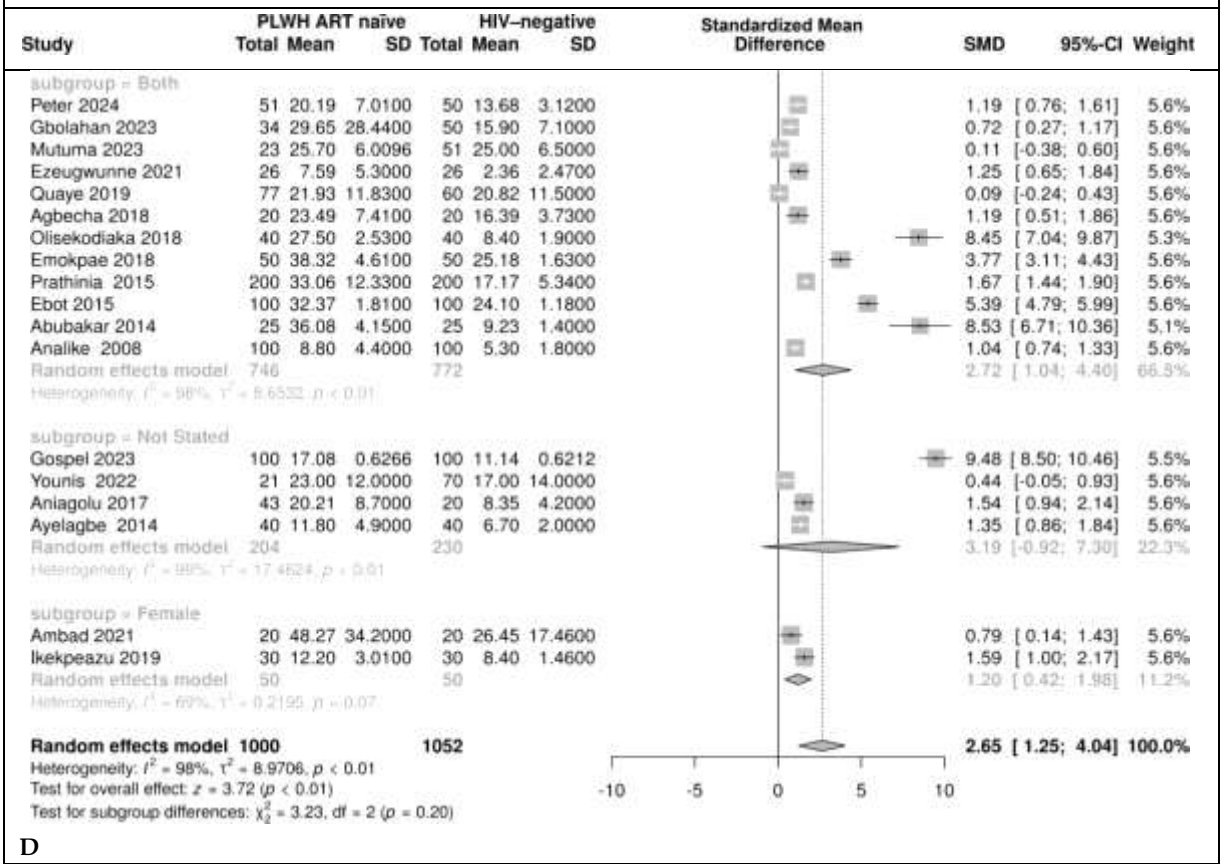
A


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Africa
Peter 2024 51 20.19 7.0100 50 13.68 3.1200 1.19 [0.76; 1.61] 5.6%
Gbolahan 2023 34 29.65 28.4400 50 15.90 7.1000 0.72 [0.27; 1.17] 5.6%
Gospel 2023 100 17.08 0.6266 100 11.14 0.6212 9.48 [8.50; 10.46] 5.5%
Mutuma 2023 23 25.70 6.0096 51 25.00 6.5000 0.11 [-0.38; 0.60] 5.6%
Younis 2022 21 23.00 12.0000 70 17.00 14.0000 0.44 [-0.05; 0.93] 5.6%
Ezeugwunne 2021 26 7.59 5.3000 26 2.36 2.4700 1.25 [0.65; 1.84] 5.6%
Ikekpeazu 2019 30 12.20 3.0100 30 8.40 1.4600 1.59 [1.00; 2.17] 5.6%
Quaye 2019 77 21.93 11.8300 60 20.82 11.5000 0.09 [-0.24; 0.43] 5.6%
Agbecha 2018 20 23.49 7.4100 20 16.39 3.7300 1.19 [0.51; 1.86] 5.6%
Olisekodiaka 2018 40 27.50 2.5300 40 8.40 1.9000 8.45 [7.04; 9.87] 5.3%
Emokpae 2018 50 38.32 4.6100 50 25.18 1.6300 3.77 [3.11; 4.43] 5.6%
Aniagolu 2017 43 20.21 8.7000 20 8.35 4.2000 1.54 [0.94; 2.14] 5.6%
Ebot 2015 100 32.37 1.8100 100 24.10 1.1800 5.39 [4.79; 5.99] 5.6%
Abubakar 2014 25 36.08 4.1500 25 9.23 1.4000 8.53 [6.71; 10.36] 5.1%
Ayelagbe 2014 40 11.80 4.9000 40 6.70 2.0000 1.35 [0.86; 1.84] 5.6%
Analike 2008 100 8.80 4.4000 100 5.30 1.8000 1.04 [0.74; 1.33] 5.6%
Random effects model 780 832 2.83 [1.28; 4.39] 88.8%
Heterogeneity: I^2 = 98%, τ^2 = 9.0265, p < 0.01
subgroup = Asia
Ambad 2021 20 48.27 34.2000 20 26.45 17.4600 0.79 [0.14; 1.43] 5.6%
Prathinia 2015 200 33.06 12.3300 200 17.17 5.3400 1.67 [1.44; 1.90] 5.6%
Random effects model 220 220 1.28 [0.42; 2.14] 11.2%
Heterogeneity: I^2 = 84%, τ^2 = 0.3276, p = 0.01
Random effects model 1000 1052 2.65 [1.25; 4.04] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.9706, p < 0.01
Test for overall effect: z = 3.72 (p < 0.01)
Test for subgroup differences: χ^2_1 = 2.93, df = 1 (p = 0.09)
-10 -5 0 5 10

B


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Less than 100
Peter 2024 51 20.19 7.0100 50 13.68 3.1200 1.19 [0.76; 1.61] 5.6%
Ambad 2021 20 48.27 34.2000 20 26.45 17.4600 0.79 [0.14; 1.43] 5.6%
Ezeugwunne 2021 26 7.59 5.3000 26 2.36 2.4700 1.25 [0.65; 1.84] 5.6%
Ikekpeazu 2019 30 12.20 3.0100 30 8.40 1.4600 1.59 [1.00; 2.17] 5.6%
Agbecha 2018 20 23.49 7.4100 20 16.39 3.7300 1.19 [0.51; 1.86] 5.6%
Aniagolu 2017 43 20.21 8.7000 20 8.35 4.2000 1.54 [0.94; 2.14] 5.6%
Abubakar 2014 25 36.08 4.1500 25 9.23 1.4000 8.53 [6.71; 10.36] 5.1%
Random effects model 215 191 2.20 [0.31; 4.08] 38.7%
Heterogeneity: I^2 = 91%, τ^2 = 6.2825, p < 0.01
subgroup = Greater than 100
Gbolahan 2023 34 29.65 28.4400 50 15.90 7.1000 0.72 [0.27; 1.17] 5.6%
Gospel 2023 100 17.08 0.6266 100 11.14 0.6212 9.48 [8.50; 10.46] 5.5%
Mutuma 2023 23 25.70 6.0096 51 25.00 6.5000 0.11 [-0.38; 0.60] 5.6%
Younis 2022 21 23.00 12.0000 70 17.00 14.0000 0.44 [-0.05; 0.93] 5.6%
Quaye 2019 77 21.93 11.8300 60 20.82 11.5000 0.09 [-0.24; 0.43] 5.6%
Olisekodiaka 2018 40 27.50 2.5300 40 8.40 1.9000 8.45 [7.04; 9.87] 5.3%
Emokpae 2018 50 38.32 4.6100 50 25.18 1.6300 3.77 [3.11; 4.43] 5.6%
Prathinia 2015 200 33.06 12.3300 200 17.17 5.3400 1.67 [1.44; 1.90] 5.6%
Ebot 2015 100 32.37 1.8100 100 24.10 1.1800 5.39 [4.79; 5.99] 5.6%
Ayelagbe 2014 40 11.80 4.9000 40 6.70 2.0000 1.35 [0.86; 1.84] 5.6%
Analike 2008 100 8.80 4.4000 100 5.30 1.8000 1.04 [0.74; 1.33] 5.6%
Random effects model 785 661 2.92 [0.94; 4.90] 61.3%
Heterogeneity: I^2 = 99%, τ^2 = 11.1167, p < 0.01
Random effects model 1000 1052 2.65 [1.25; 4.04] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.9706, p < 0.01
Test for overall effect: z = 3.72 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.27, df = 1 (p = 0.60)
-10 -5 0 5 10

C


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Both
Peter 2024 51 20.19 7.0100 50 13.68 3.1200 1.19 [0.76; 1.61] 5.6%
Gbolahan 2023 34 29.65 28.4400 50 15.90 7.1000 0.72 [0.27; 1.17] 5.6%
Mutuma 2023 23 25.70 6.0096 51 25.00 6.5000 0.11 [-0.38; 0.60] 5.6%
Ezeugwunne 2021 26 7.59 5.3000 26 2.36 2.4700 1.25 [0.65; 1.84] 5.6%
Quaye 2019 77 21.93 11.8300 60 20.82 11.5000 0.09 [-0.24; 0.43] 5.6%
Agbecha 2018 20 23.49 7.4100 20 16.39 3.7300 1.19 [0.51; 1.86] 5.6%
Olisekodiaka 2018 40 27.50 2.5300 40 8.40 1.9000 8.45 [7.04; 9.87] 5.3%
Emokpae 2018 50 38.32 4.6100 50 25.18 1.6300 3.77 [3.11; 4.43] 5.6%
Prathinia 2015 200 33.06 12.3300 200 17.17 5.3400 1.67 [1.44; 1.90] 5.6%
Ebot 2015 100 32.37 1.8100 100 24.10 1.1800 5.39 [4.79; 5.99] 5.6%
Abubakar 2014 25 36.08 4.1500 25 9.23 1.4000 8.53 [6.71; 10.36] 5.1%
Analike 2008 100 8.80 4.4000 100 5.30 1.8000 1.04 [0.74; 1.33] 5.6%
Random effects model 746 772 2.72 [1.04; 4.40] 66.8%
Heterogeneity: I^2 = 98%, τ^2 = 8.6532, p < 0.01
subgroup = Not Stated
Gospel 2023 100 17.08 0.6266 100 11.14 0.6212 9.48 [8.50; 10.46] 5.5%
Younis 2022 21 23.00 12.0000 70 17.00 14.0000 0.44 [-0.05; 0.93] 5.6%
Aniagolu 2017 43 20.21 8.7000 20 8.35 4.2000 1.54 [0.94; 2.14] 5.6%
Ayelagbe 2014 40 11.80 4.9000 40 6.70 2.0000 1.35 [0.86; 1.84] 5.6%
Random effects model 204 230 3.19 [-0.92; 7.30] 22.3%
Heterogeneity: I^2 = 98%, τ^2 = 17.4624, p < 0.01
subgroup = Female
Ambad 2021 20 48.27 34.2000 20 26.45 17.4600 0.79 [0.14; 1.43] 5.6%
Ikekpeazu 2019 30 12.20 3.0100 30 8.40 1.4600 1.59 [1.00; 2.17] 5.6%
Random effects model 50 50 1.20 [0.42; 1.98] 11.2%
Heterogeneity: I^2 = 69%, τ^2 = 0.2195, p = 0.07
Random effects model 1000 1052 2.65 [1.25; 4.04] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.9706, p < 0.01
Test for overall effect: z = 3.72 (p < 0.01)
Test for subgroup differences: χ^2_2 = 3.23, df = 2 (p = 0.20)
-10 -5 0 5 10

D

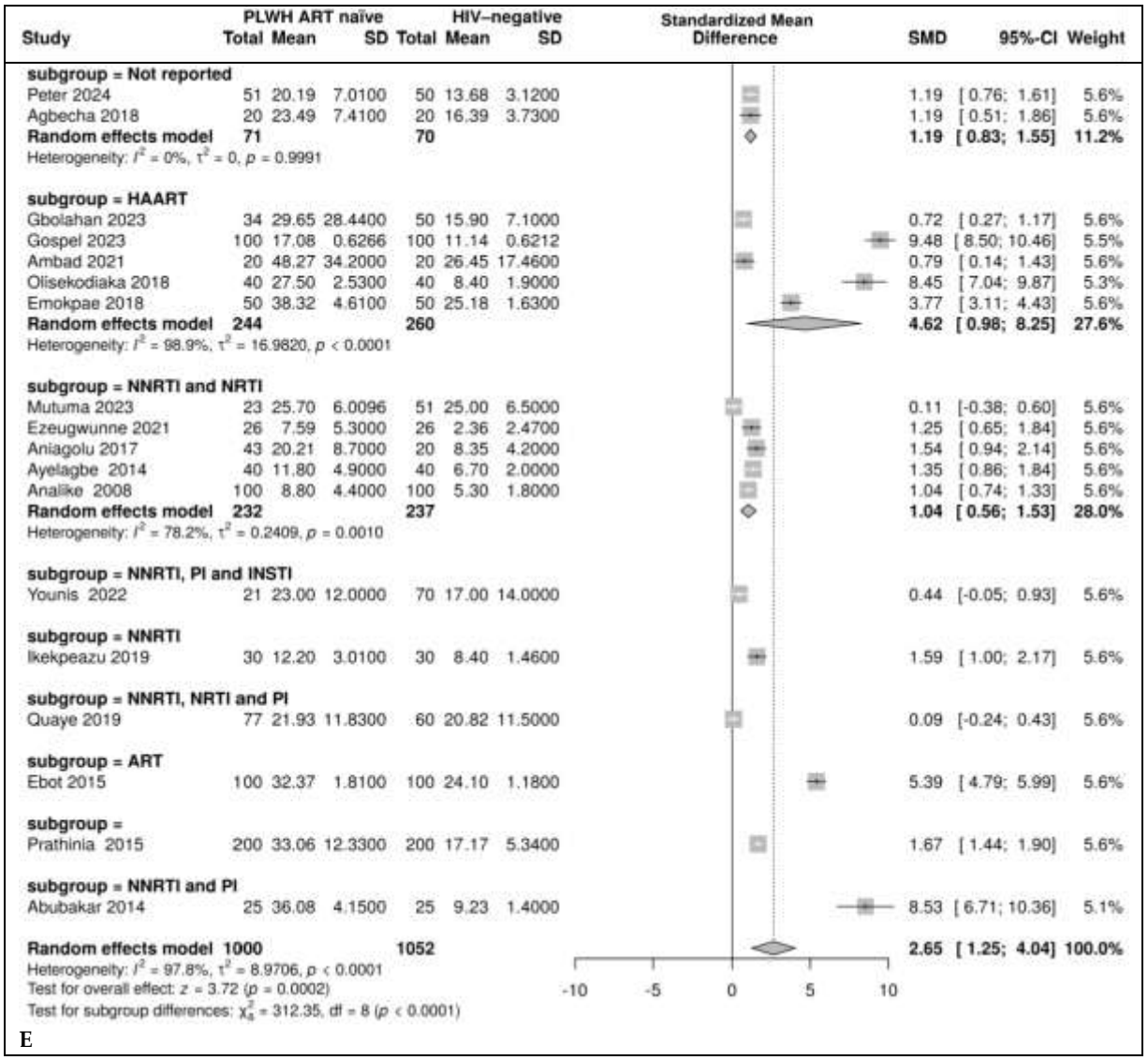
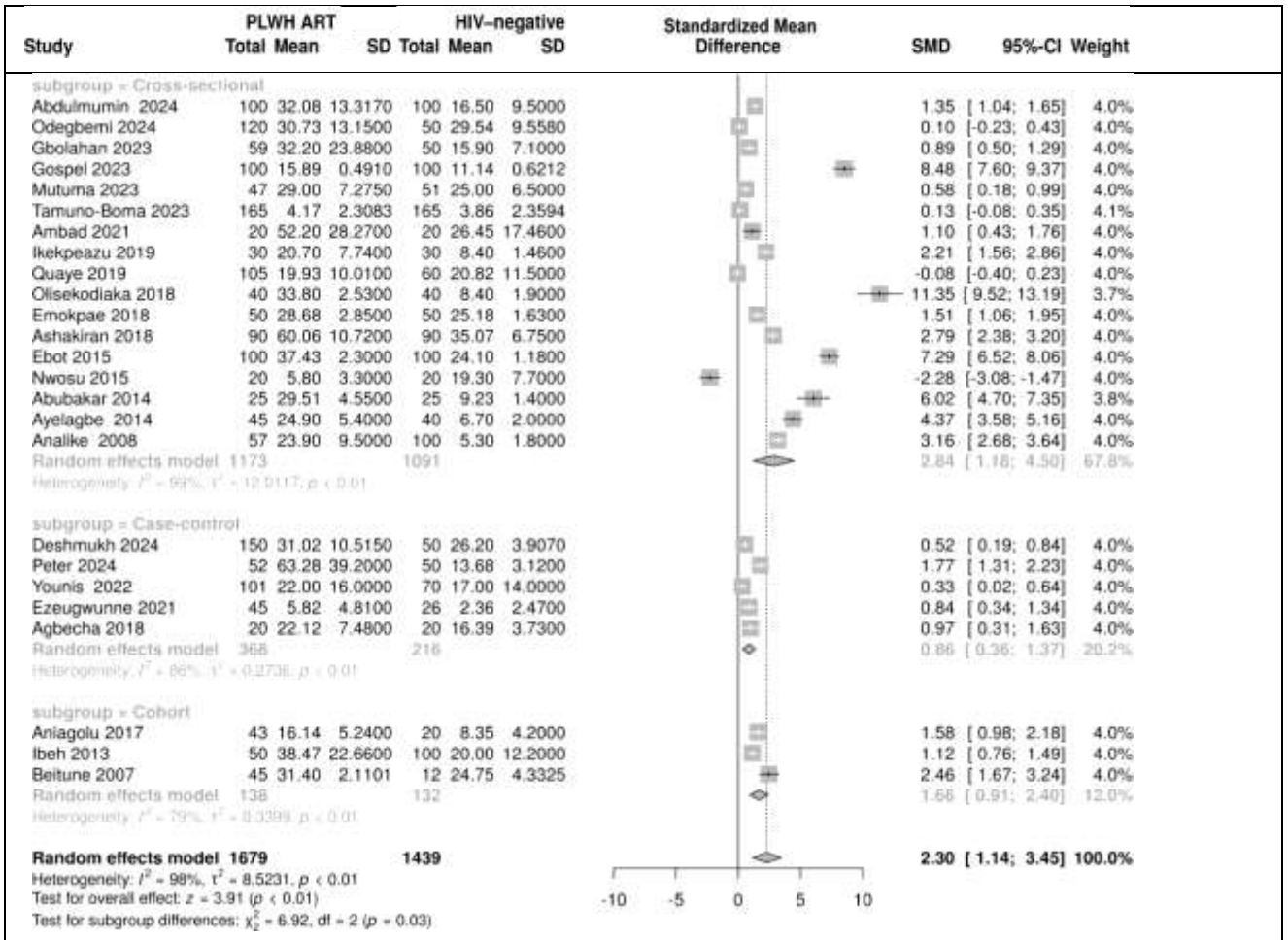
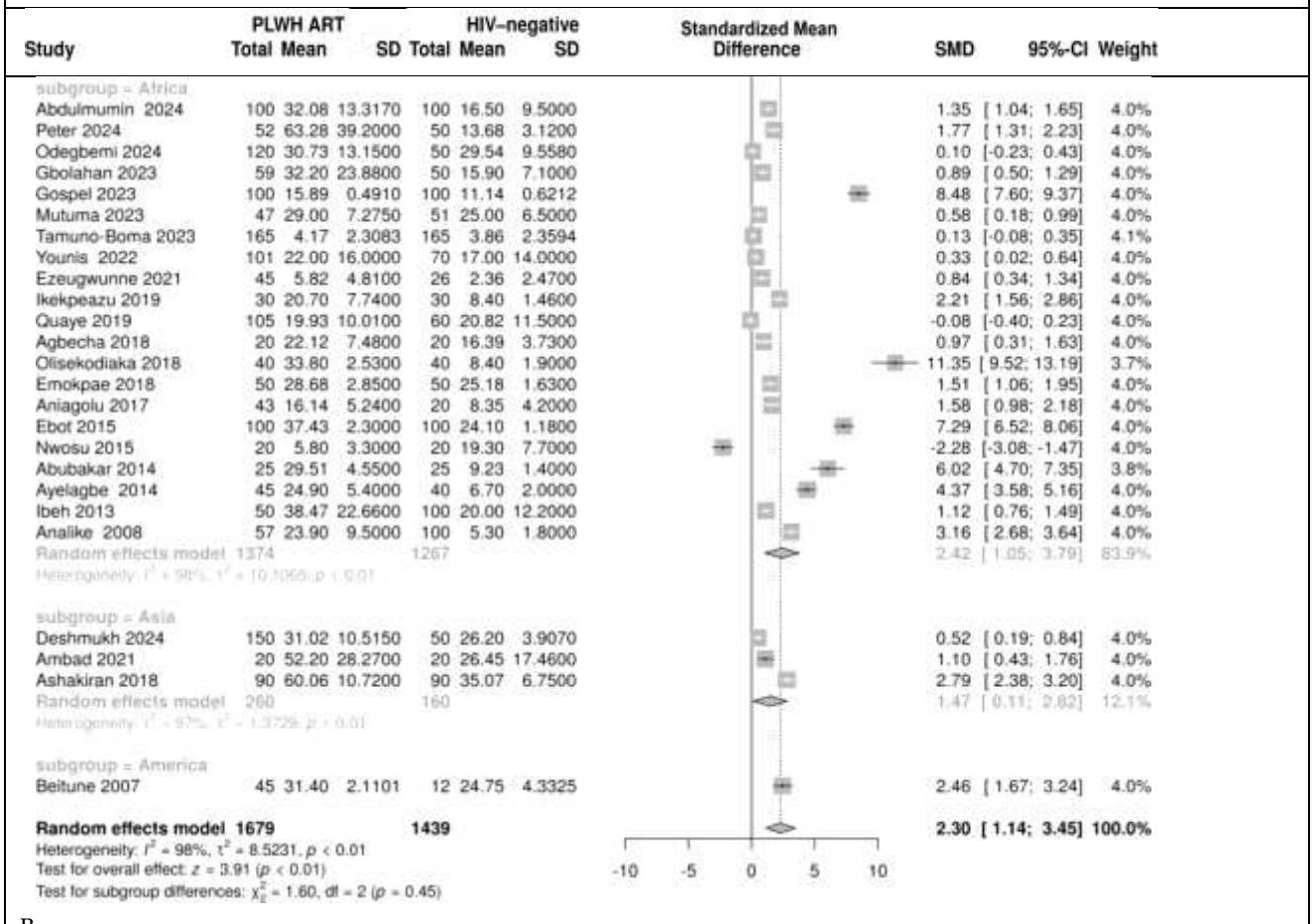


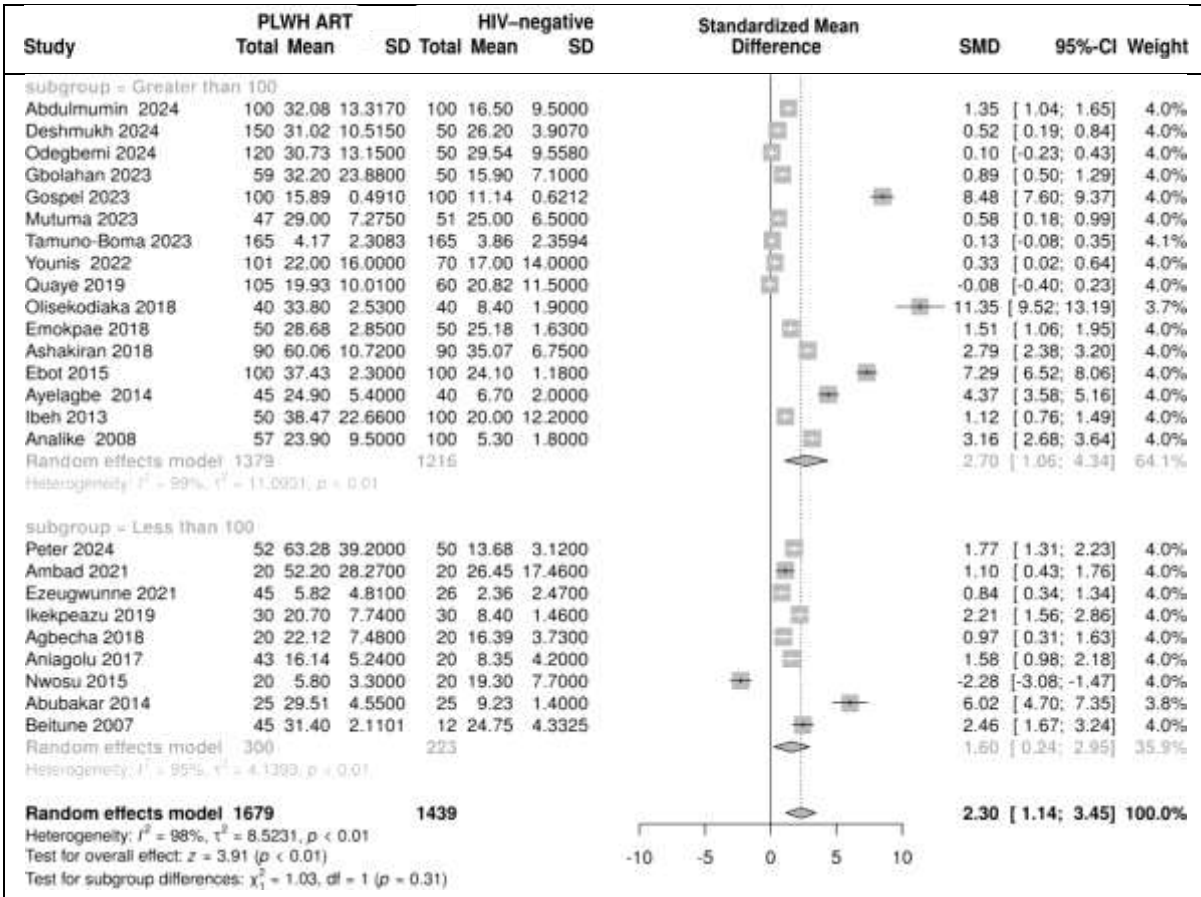
Figure S4: Subgroup analysis on ALT in ART-naïve compared with HIV-negative individuals. A: ALT level in PLWH on ART- naïve group compared with HIV negative, based on study design. B: Levels of ALT in ART-naïve PLWH compared with HIV negative, based on the continent of publication. C: ALT level in PLWH on ART-naïve compared with HIV negative, based on sample size. D: ALT levels in PLWH on ART-naïve compared with HIV negative, based on gender distribution. E: ALT levels in PLWH on ART-naïve group compared with HIV negative based on class of ART regimens. The solid line shows the line of no effect, the dashed line shows the effect size, the gray block shows the weight of the study, the horizontal line crossing the gray block shows the confidence intervals, diamond plot shows the combined effect size. NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, NtRTI: nucleotide reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase-nucleoside strand transfer inhibitors.


Study
PLWH ART Total Mean SD
HIV−negative Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Cross-sectional
Abdulmumin 2024 100 32.08 13.3170 100 16.50 9.5000 1.35 [1.04; 1.65] 4.0%
Odegbemi 2024 120 30.73 13.1500 50 29.54 9.5580 0.10 [-0.23; 0.43] 4.0%
Gbolahan 2023 59 32.20 23.8800 50 15.90 7.1000 0.89 [0.50; 1.29] 4.0%
Gospel 2023 100 15.89 0.4910 100 11.14 0.6212 8.48 [7.60; 9.37] 4.0%
Muturna 2023 47 29.00 7.2750 51 25.00 6.5000 0.58 [0.18; 0.99] 4.0%
Tamuno-Boma 2023 165 4.17 2.3083 165 3.86 2.3594 0.13 [-0.08; 0.35] 4.1%
Ambad 2021 20 52.20 28.2700 20 26.45 17.4600 1.10 [0.43; 1.76] 4.0%
Ikekpeazu 2019 30 20.70 7.7400 30 8.40 1.4600 2.21 [1.56; 2.86] 4.0%
Quaye 2019 105 19.93 10.0100 60 20.82 11.5000 -0.08 [-0.40; 0.23] 4.0%
Olisekodiaka 2018 40 33.80 2.5300 40 8.40 1.9000 11.35 [9.52; 13.19] 3.7%
Emokpae 2018 50 28.68 2.8500 50 25.18 1.6300 1.51 [1.06; 1.95] 4.0%
Ashakiran 2018 90 60.06 10.7200 90 35.07 6.7500 2.79 [2.38; 3.20] 4.0%
Ebot 2015 100 37.43 2.3000 100 24.10 1.1800 7.29 [6.52; 8.06] 4.0%
Nwosu 2015 20 5.80 3.3000 20 19.30 7.7000 -2.28 [-3.08; -1.47] 4.0%
Abubakar 2014 25 29.51 4.5500 25 9.23 1.4000 6.02 [4.70; 7.35] 3.8%
Ayelagbe 2014 45 24.90 5.4000 40 6.70 2.0000 4.37 [3.58; 5.16] 4.0%
Analike 2008 57 23.90 9.5000 100 5.30 1.8000 3.16 [2.68; 3.64] 4.0%
Random effects model 1173 1091 2.84 [1.18; 4.50] 67.8%
Heterogeneity: I^2 = 99%, τ^2 = 12.9117, p < 0.01
subgroup = Case-control
Deshmukh 2024 150 31.02 10.5150 50 26.20 3.9070 0.52 [0.19; 0.84] 4.0%
Peter 2024 52 63.28 39.2000 50 13.68 3.1200 1.77 [1.31; 2.23] 4.0%
Younis 2022 101 22.00 16.0000 70 17.00 14.0000 0.33 [0.02; 0.64] 4.0%
Ezeugwunne 2021 45 5.82 4.8100 26 2.36 2.4700 0.84 [0.34; 1.34] 4.0%
Agbecha 2018 20 22.12 7.4800 20 16.39 3.7300 0.97 [0.31; 1.63] 4.0%
Random effects model 368 216 0.86 [0.36; 1.37] 20.2%
Heterogeneity: I^2 = 86%, τ^2 = 0.2736, p < 0.01
subgroup = Cohort
Aniagolu 2017 43 16.14 5.2400 20 8.35 4.2000 1.58 [0.98; 2.18] 4.0%
Ibeh 2013 50 38.47 22.6600 100 20.00 12.2000 1.12 [0.76; 1.49] 4.0%
Beitune 2007 45 31.40 2.1101 12 24.75 4.3325 2.46 [1.67; 3.24] 4.0%
Random effects model 138 132 1.66 [0.91; 2.40] 12.0%
Heterogeneity: I^2 = 79%, τ^2 = 0.3299, p < 0.01
Random effects model 1679 1439 2.30 [1.14; 3.45] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.5231, p < 0.01
Test for overall effect: z = 3.91 (p < 0.01)
Test for subgroup differences: χ^2_2 = 6.92, df = 2 (p = 0.03)
-10 -5 0 5 10

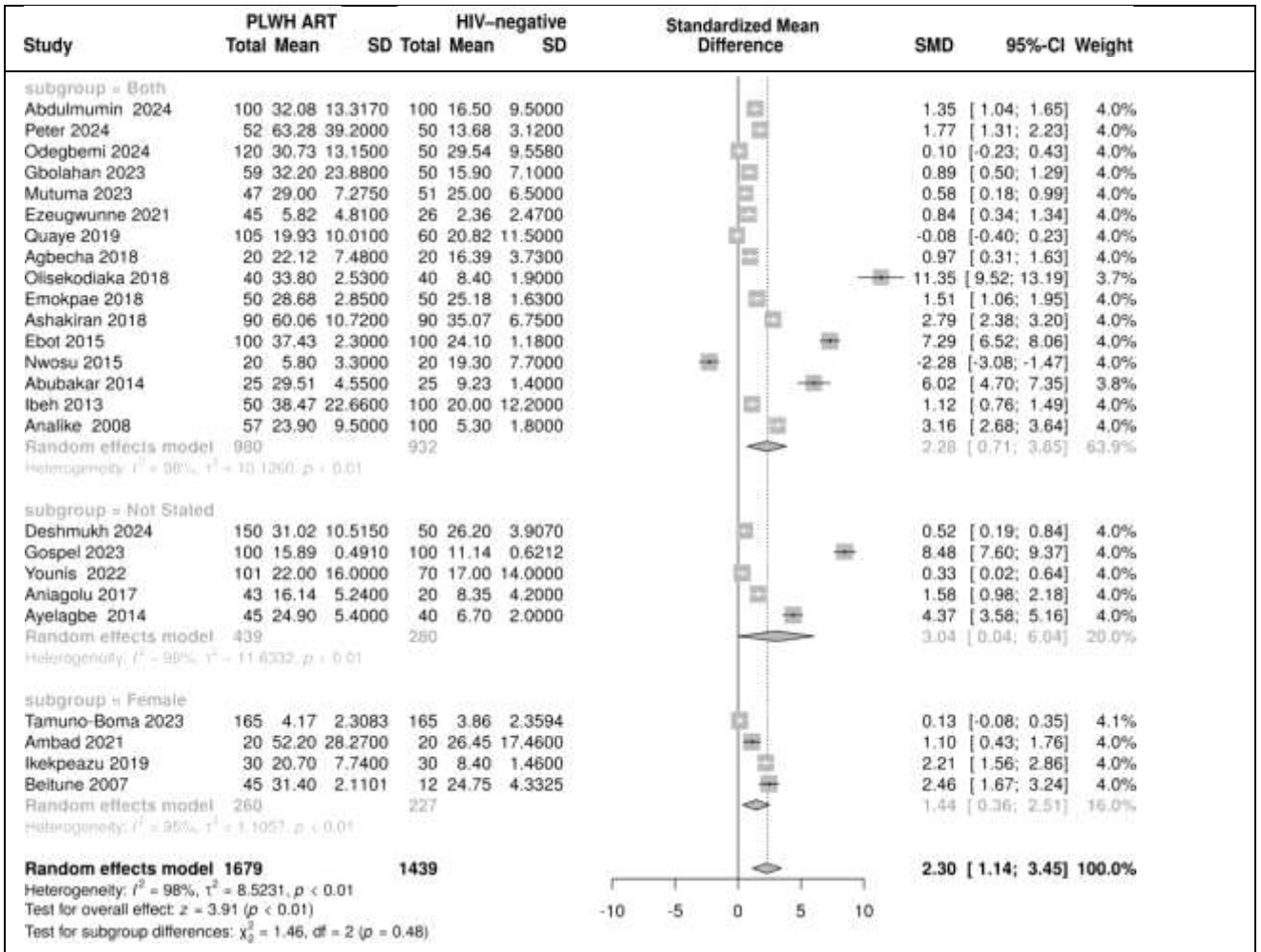
A


Study
PLWH ART Total Mean SD
HIV−negative Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Africa
Abdulmumin 2024 100 32.08 13.3170 100 16.50 9.5000 1.35 [1.04; 1.65] 4.0%
Peter 2024 52 63.28 39.2000 50 13.68 3.1200 1.77 [1.31; 2.23] 4.0%
Odegbemi 2024 120 30.73 13.1500 50 29.54 9.5580 0.10 [-0.23; 0.43] 4.0%
Gbolahan 2023 59 32.20 23.8800 50 15.90 7.1000 0.89 [0.50; 1.29] 4.0%
Gospel 2023 100 15.89 0.4910 100 11.14 0.6212 8.48 [7.60; 9.37] 4.0%
Muturna 2023 47 29.00 7.2750 51 25.00 6.5000 0.58 [0.18; 0.99] 4.0%
Tamuno-Boma 2023 165 4.17 2.3083 165 3.86 2.3594 0.13 [-0.08; 0.35] 4.1%
Younis 2022 101 22.00 16.0000 70 17.00 14.0000 0.33 [0.02; 0.64] 4.0%
Ezeugwunne 2021 45 5.82 4.8100 26 2.36 2.4700 0.84 [0.34; 1.34] 4.0%
Ikekpeazu 2019 30 20.70 7.7400 30 8.40 1.4600 2.21 [1.56; 2.86] 4.0%
Quaye 2019 105 19.93 10.0100 60 20.82 11.5000 -0.08 [-0.40; 0.23] 4.0%
Agbecha 2018 20 22.12 7.4800 20 16.39 3.7300 0.97 [0.31; 1.63] 4.0%
Olisekodiaka 2018 40 33.80 2.5300 40 8.40 1.9000 11.35 [9.52; 13.19] 3.7%
Emokpae 2018 50 28.68 2.8500 50 25.18 1.6300 1.51 [1.06; 1.95] 4.0%
Aniagolu 2017 43 16.14 5.2400 20 8.35 4.2000 1.58 [0.98; 2.18] 4.0%
Ebot 2015 100 37.43 2.3000 100 24.10 1.1800 7.29 [6.52; 8.06] 4.0%
Nwosu 2015 20 5.80 3.3000 20 19.30 7.7000 -2.28 [-3.08; -1.47] 4.0%
Abubakar 2014 25 29.51 4.5500 25 9.23 1.4000 6.02 [4.70; 7.35] 3.8%
Ayelagbe 2014 45 24.90 5.4000 40 6.70 2.0000 4.37 [3.58; 5.16] 4.0%
Ibeh 2013 50 38.47 22.6600 100 20.00 12.2000 1.12 [0.76; 1.49] 4.0%
Analike 2008 57 23.90 9.5000 100 5.30 1.8000 3.16 [2.68; 3.64] 4.0%
Random effects model 1374 1267 2.42 [1.05; 3.79] 83.9%
Heterogeneity: I^2 = 98%, τ^2 = 10.1065, p < 0.01
subgroup = Asia
Deshmukh 2024 150 31.02 10.5150 50 26.20 3.9070 0.52 [0.19; 0.84] 4.0%
Ambad 2021 20 52.20 28.2700 20 26.45 17.4600 1.10 [0.43; 1.76] 4.0%
Ashakiran 2018 90 60.06 10.7200 90 35.07 6.7500 2.79 [2.38; 3.20] 4.0%
Random effects model 260 160 1.47 [0.11; 2.82] 12.1%
Heterogeneity: I^2 = 97%, τ^2 = 1.3729, p < 0.01
subgroup = America
Beitune 2007 45 31.40 2.1101 12 24.75 4.3325 2.46 [1.67; 3.24] 4.0%
Random effects model 1679 1439 2.30 [1.14; 3.45] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.5231, p < 0.01
Test for overall effect: z = 3.91 (p < 0.01)
Test for subgroup differences: χ^2_2 = 1.60, df = 2 (p = 0.45)
-10 -5 0 5 10

B


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Greater than 100
Abdulmumin 2024 100 32.08 13.3170 100 16.50 9.5000 1.35 [1.04; 1.65] 4.0%
Deshmukh 2024 150 31.02 10.5150 50 26.20 3.9070 0.52 [0.19; 0.84] 4.0%
Odegbemi 2024 120 30.73 13.1500 50 29.54 9.5580 0.10 [-0.23; 0.43] 4.0%
Gbolahan 2023 59 32.20 23.8800 50 15.90 7.1000 0.89 [0.50; 1.29] 4.0%
Gospel 2023 100 15.89 0.4910 100 11.14 0.6212 8.48 [7.60; 9.37] 4.0%
Mutuma 2023 47 29.00 7.2750 51 25.00 6.5000 0.58 [0.18; 0.99] 4.0%
Tamuno-Boma 2023 165 4.17 2.3083 165 3.86 2.3594 0.13 [-0.08; 0.35] 4.1%
Younis 2022 101 22.00 16.0000 70 17.00 14.0000 0.33 [0.02; 0.64] 4.0%
Quaye 2019 105 19.93 10.0100 60 20.82 11.5000 -0.08 [-0.40; 0.23] 4.0%
Olisekodiaka 2018 40 33.80 2.5300 40 8.40 1.9000 11.35 [9.52; 13.19] 3.7%
Emokpae 2018 50 28.68 2.8500 50 25.18 1.6300 1.51 [1.06; 1.95] 4.0%
Ashakiran 2018 90 60.06 10.7200 90 35.07 6.7500 2.79 [2.38; 3.20] 4.0%
Ebot 2015 100 37.43 2.3000 100 24.10 1.1800 7.29 [6.52; 8.06] 4.0%
Ayelagbe 2014 45 24.90 5.4000 40 6.70 2.0000 4.37 [3.58; 5.16] 4.0%
Ibeh 2013 50 38.47 22.6600 100 20.00 12.2000 1.12 [0.76; 1.49] 4.0%
Analike 2008 57 23.90 9.5000 100 5.30 1.8000 3.16 [2.68; 3.64] 4.0%
Random effects model 1379 1216 2.70 [1.06; 4.34] 64.1%
Heterogeneity: I^2 = 99%, τ^2 = 11.0931, p < 0.01
subgroup = Less than 100
Peter 2024 52 63.28 39.2000 50 13.68 3.1200 1.77 [1.31; 2.23] 4.0%
Ambad 2021 20 52.20 28.2700 20 26.45 17.4600 1.10 [0.43; 1.76] 4.0%
Ezeugwunne 2021 45 5.82 4.8100 26 2.36 2.4700 0.84 [0.34; 1.34] 4.0%
Ikekpeazu 2019 30 20.70 7.7400 30 8.40 1.4600 2.21 [1.56; 2.86] 4.0%
Agbecha 2018 20 22.12 7.4800 20 16.39 3.7300 0.97 [0.31; 1.63] 4.0%
Aniagolu 2017 43 16.14 5.2400 20 8.35 4.2000 1.58 [0.98; 2.18] 4.0%
Nwosu 2015 20 5.80 3.3000 20 19.30 7.7000 -2.28 [-3.08; -1.47] 4.0%
Abubakar 2014 25 29.51 4.5500 25 9.23 1.4000 6.02 [4.70; 7.35] 3.8%
Beitune 2007 45 31.40 2.1101 12 24.75 4.3325 2.46 [1.67; 3.24] 4.0%
Random effects model 300 223 1.60 [0.24; 2.95] 35.9%
Heterogeneity: I^2 = 95%, τ^2 = 4.1393, p < 0.01
Random effects model 1679 1439 2.30 [1.14; 3.45] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.5231, p < 0.01
Test for overall effect: z = 3.91 (p < 0.01)
Test for subgroup differences: χ^2_1 = 1.03, df = 1 (p = 0.31)
-10
-5
0
5
10

C


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Both
Abdulmumin 2024 100 32.08 13.3170 100 16.50 9.5000 1.35 [1.04; 1.65] 4.0%
Peter 2024 52 63.28 39.2000 50 13.68 3.1200 1.77 [1.31; 2.23] 4.0%
Odegbemi 2024 120 30.73 13.1500 50 29.54 9.5580 0.10 [-0.23; 0.43] 4.0%
Gbolahan 2023 59 32.20 23.8800 50 15.90 7.1000 0.89 [0.50; 1.29] 4.0%
Mutuma 2023 47 29.00 7.2750 51 25.00 6.5000 0.58 [0.18; 0.99] 4.0%
Ezeugwunne 2021 45 5.82 4.8100 26 2.36 2.4700 0.84 [0.34; 1.34] 4.0%
Quaye 2019 105 19.93 10.0100 60 20.82 11.5000 -0.08 [-0.40; 0.23] 4.0%
Agbecha 2018 20 22.12 7.4800 20 16.39 3.7300 0.97 [0.31; 1.63] 4.0%
Olisekodiaka 2018 40 33.80 2.5300 40 8.40 1.9000 11.35 [9.52; 13.19] 3.7%
Emokpae 2018 50 28.68 2.8500 50 25.18 1.6300 1.51 [1.06; 1.95] 4.0%
Ashakiran 2018 90 60.06 10.7200 90 35.07 6.7500 2.79 [2.38; 3.20] 4.0%
Ebot 2015 100 37.43 2.3000 100 24.10 1.1800 7.29 [6.52; 8.06] 4.0%
Nwosu 2015 20 5.80 3.3000 20 19.30 7.7000 -2.28 [-3.08; -1.47] 4.0%
Abubakar 2014 25 29.51 4.5500 25 9.23 1.4000 6.02 [4.70; 7.35] 3.8%
Ibeh 2013 50 38.47 22.6600 100 20.00 12.2000 1.12 [0.76; 1.49] 4.0%
Analike 2008 57 23.90 9.5000 100 5.30 1.8000 3.16 [2.68; 3.64] 4.0%
Random effects model 980 932 2.28 [0.71; 3.85] 63.9%
Heterogeneity: I^2 = 98%, τ^2 = 10.1260, p < 0.01
subgroup = Not Stated
Deshmukh 2024 150 31.02 10.5150 50 26.20 3.9070 0.52 [0.19; 0.84] 4.0%
Gospel 2023 100 15.89 0.4910 100 11.14 0.6212 8.48 [7.60; 9.37] 4.0%
Younis 2022 101 22.00 16.0000 70 17.00 14.0000 0.33 [0.02; 0.64] 4.0%
Aniagolu 2017 43 16.14 5.2400 20 8.35 4.2000 1.58 [0.98; 2.18] 4.0%
Ayelagbe 2014 45 24.90 5.4000 40 6.70 2.0000 4.37 [3.58; 5.16] 4.0%
Random effects model 439 260 3.04 [0.04; 6.04] 20.0%
Heterogeneity: I^2 = 96%, τ^2 = 11.6332, p < 0.01
subgroup = Female
Tamuno-Boma 2023 165 4.17 2.3083 165 3.86 2.3594 0.13 [-0.08; 0.35] 4.1%
Ambad 2021 20 52.20 28.2700 20 26.45 17.4600 1.10 [0.43; 1.76] 4.0%
Ikekpeazu 2019 30 20.70 7.7400 30 8.40 1.4600 2.21 [1.56; 2.86] 4.0%
Beitune 2007 45 31.40 2.1101 12 24.75 4.3325 2.46 [1.67; 3.24] 4.0%
Random effects model 260 227 1.44 [0.36; 2.51] 16.0%
Heterogeneity: I^2 = 95%, τ^2 = 1.1057, p < 0.01
Random effects model 1679 1439 2.30 [1.14; 3.45] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 8.5231, p < 0.01
Test for overall effect: z = 3.91 (p < 0.01)
Test for subgroup differences: χ^2_2 = 1.46, df = 2 (p = 0.48)
-10
-5
0
5
10

D

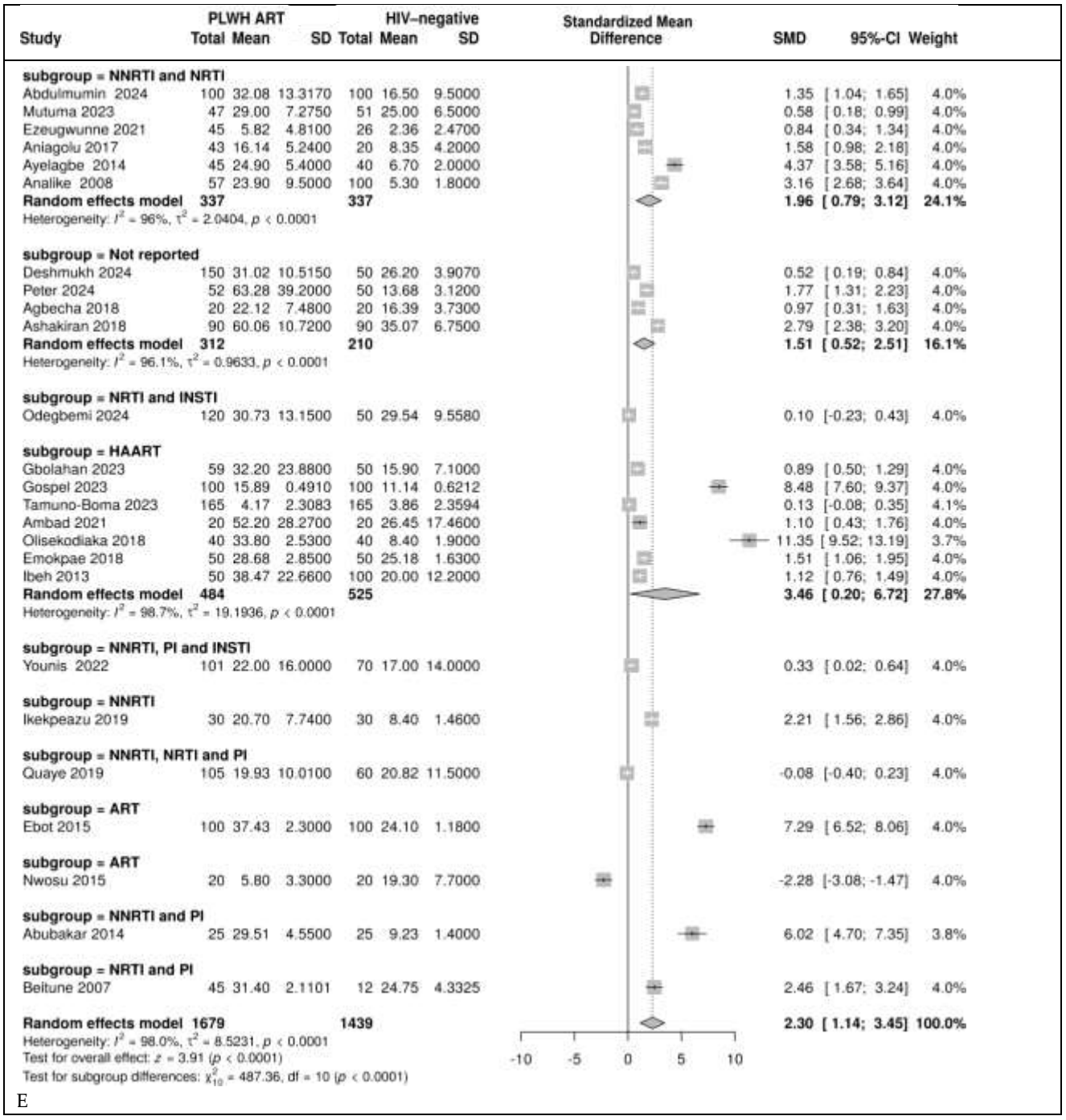
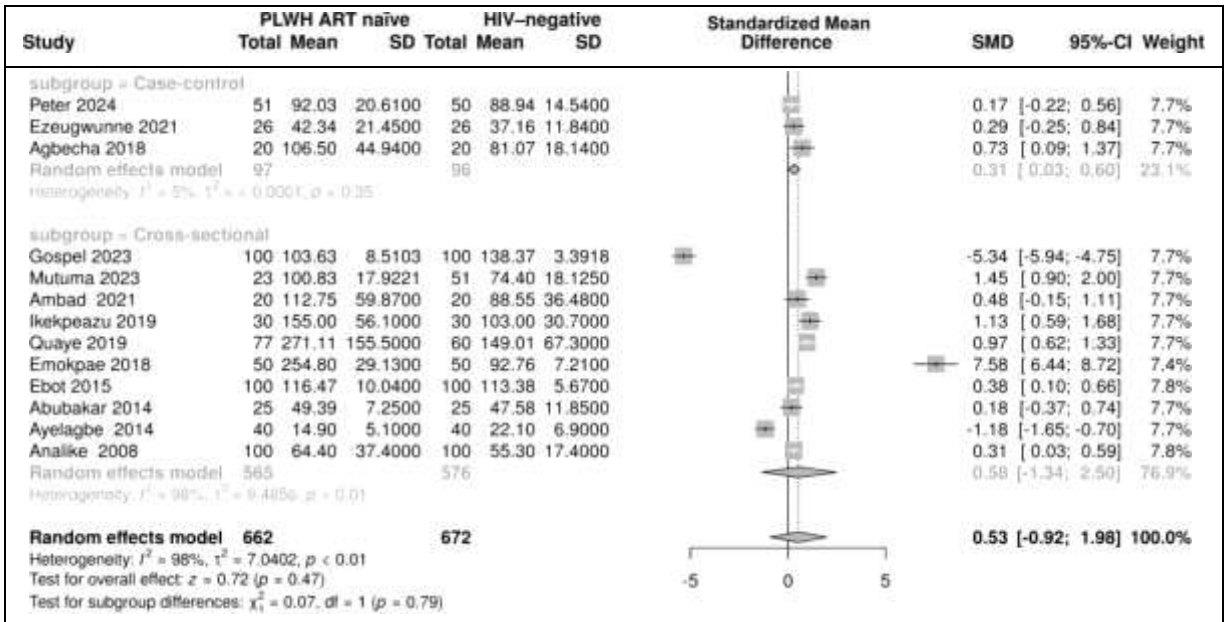
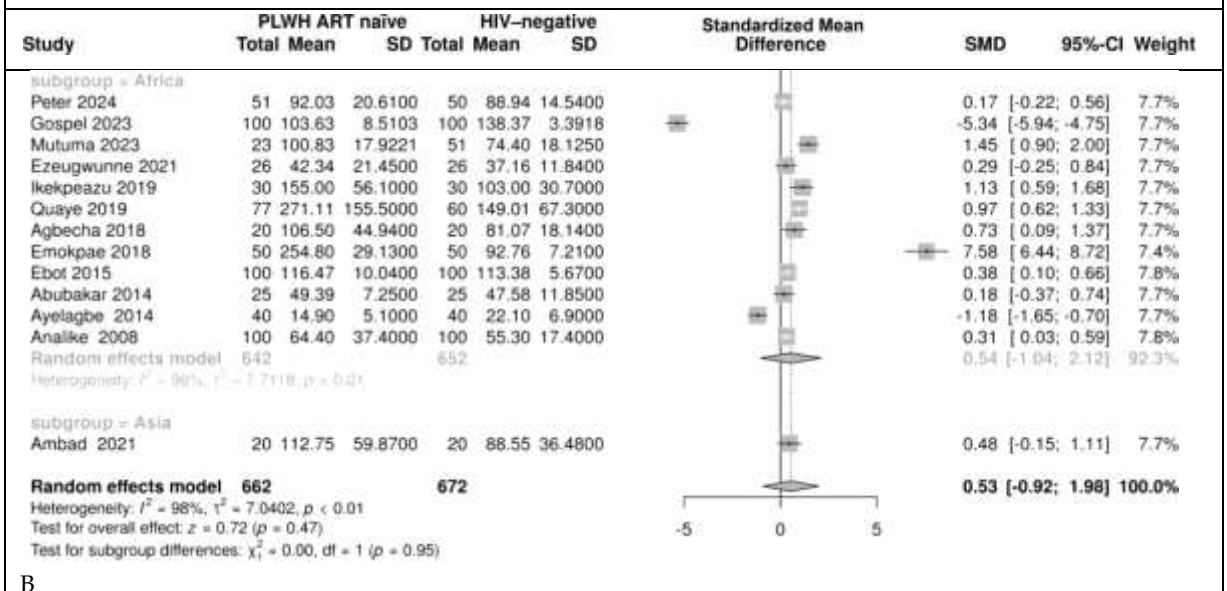


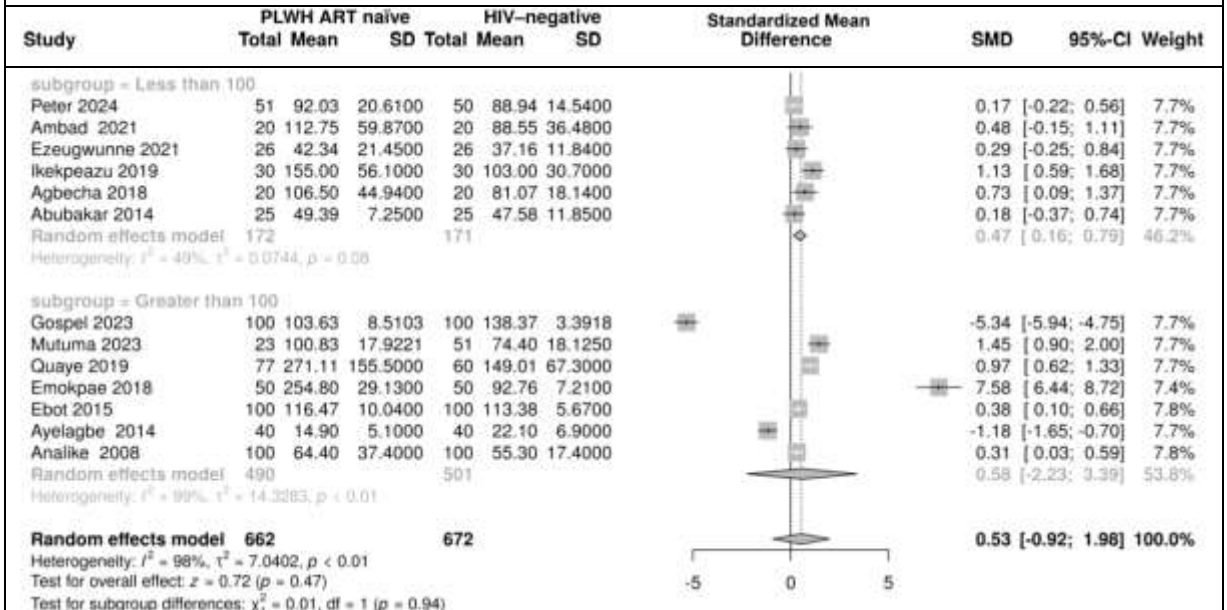
Figure S5: Subgroup analysis on ALT among PLWH on ART compared with HIV-negative individuals. A: ALT among PLWH on ART compared with HIV-negative individuals based on study design. B: ALT among PLWH on ART compared with HIV negative individuals based on continent. C: ALT among PLWH on ART compared with HIV-negative individuals based on sample size. D: ALT among PLWH on ART compared with HIV-negative individuals based on gender distribution. E: ALT among PLWH on ART compared with HIV-negative individuals based on the class of ART regimens. The solid line shows the line of no effect, the dashed line shows the effect size, the gray block shows the weight of the study, the horizontal line crossing the gray block shows the confidence intervals, diamond plot shows the combined effect size. NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, NtRTI: nucleotide reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase-nucleoside strand transfer inhibitors.


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Case-control
Peter 2024 51 92.03 20.6100 50 88.94 14.5400 0.17 [-0.22; 0.56] 7.7%
Ezeugwunne 2021 26 42.34 21.4500 26 37.16 11.8400 0.29 [-0.25; 0.84] 7.7%
Agbecha 2018 20 106.50 44.9400 20 81.07 18.1400 0.73 [0.09; 1.37] 7.7%
Random effects model 97 96 0.31 [0.03; 0.60] 23.1%
Heterogeneity: I^2 = 5%, τ^2 = < 0.0001, p = 0.35
subgroup = Cross-sectional
Gospel 2023 100 103.63 8.5103 100 138.37 3.3918 -5.34 [-5.94; -4.75] 7.7%
Mutuma 2023 23 100.83 17.9221 51 74.40 18.1250 1.45 [0.90; 2.00] 7.7%
Ambad 2021 20 112.75 59.8700 20 88.55 36.4800 0.48 [-0.15; 1.11] 7.7%
Ikekpeazu 2019 30 155.00 56.1000 30 103.00 30.7000 1.13 [0.59; 1.68] 7.7%
Quaye 2019 77 271.11 155.5000 60 149.01 67.3000 0.97 [0.62; 1.33] 7.7%
Emokpae 2018 50 254.80 29.1300 50 92.76 7.2100 7.58 [6.44; 8.72] 7.4%
Ebot 2015 100 116.47 10.0400 100 113.38 5.6700 0.38 [0.10; 0.66] 7.8%
Abubakar 2014 25 49.39 7.2500 25 47.58 11.8500 0.18 [-0.37; 0.74] 7.7%
Ayelagbe 2014 40 14.90 5.1000 40 22.10 6.9000 -1.18 [-1.65; -0.70] 7.7%
Analike 2008 100 64.40 37.4000 100 55.30 17.4000 0.31 [0.03; 0.59] 7.8%
Random effects model 565 576 0.58 [-1.34; 2.50] 76.9%
Heterogeneity: I^2 = 98%, τ^2 = 9.4856, p < 0.01
Random effects model 662 672 0.53 [-0.92; 1.98] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 7.0402, p < 0.01
Test for overall effect: z = 0.72 (p = 0.47)
Test for subgroup differences: χ^2_1 = 0.07, df = 1 (p = 0.79)
-5
0
5

A


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Africa
Peter 2024 51 92.03 20.6100 50 88.94 14.5400 0.17 [-0.22; 0.56] 7.7%
Gospel 2023 100 103.63 8.5103 100 138.37 3.3918 -5.34 [-5.94; -4.75] 7.7%
Mutuma 2023 23 100.83 17.9221 51 74.40 18.1250 1.45 [0.90; 2.00] 7.7%
Ezeugwunne 2021 26 42.34 21.4500 26 37.16 11.8400 0.29 [-0.25; 0.84] 7.7%
Ikekpeazu 2019 30 155.00 56.1000 30 103.00 30.7000 1.13 [0.59; 1.68] 7.7%
Quaye 2019 77 271.11 155.5000 60 149.01 67.3000 0.97 [0.62; 1.33] 7.7%
Agbecha 2018 20 106.50 44.9400 20 81.07 18.1400 0.73 [0.09; 1.37] 7.7%
Emokpae 2018 50 254.80 29.1300 50 92.76 7.2100 7.58 [6.44; 8.72] 7.4%
Ebot 2015 100 116.47 10.0400 100 113.38 5.6700 0.38 [0.10; 0.66] 7.8%
Abubakar 2014 25 49.39 7.2500 25 47.58 11.8500 0.18 [-0.37; 0.74] 7.7%
Ayelagbe 2014 40 14.90 5.1000 40 22.10 6.9000 -1.18 [-1.65; -0.70] 7.7%
Analike 2008 100 64.40 37.4000 100 55.30 17.4000 0.31 [0.03; 0.59] 7.8%
Random effects model 642 652 0.54 [-1.04; 2.12] 92.3%
Heterogeneity: I^2 = 98%, τ^2 = 7.7118, p < 0.01
subgroup = Asia
Ambad 2021 20 112.75 59.8700 20 88.55 36.4800 0.48 [-0.15; 1.11] 7.7%
Random effects model 662 672 0.53 [-0.92; 1.98] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 7.0402, p < 0.01
Test for overall effect: z = 0.72 (p = 0.47)
Test for subgroup differences: χ^2_1 = 0.00, df = 1 (p = 0.95)
-5
0
5

B


Study
PLWH ART naïve
Total Mean SD
HIV–negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Less than 100
Peter 2024 51 92.03 20.6100 50 88.94 14.5400 0.17 [-0.22; 0.56] 7.7%
Ambad 2021 20 112.75 59.8700 20 88.55 36.4800 0.48 [-0.15; 1.11] 7.7%
Ezeugwunne 2021 26 42.34 21.4500 26 37.16 11.8400 0.29 [-0.25; 0.84] 7.7%
Ikekpeazu 2019 30 155.00 56.1000 30 103.00 30.7000 1.13 [0.59; 1.68] 7.7%
Agbecha 2018 20 106.50 44.9400 20 81.07 18.1400 0.73 [0.09; 1.37] 7.7%
Abubakar 2014 25 49.39 7.2500 25 47.58 11.8500 0.18 [-0.37; 0.74] 7.7%
Random effects model 172 171 0.47 [0.16; 0.79] 46.2%
Heterogeneity: I^2 = 49%, τ^2 = 0.0744, p = 0.08
subgroup = Greater than 100
Gospel 2023 100 103.63 8.5103 100 138.37 3.3918 -5.34 [-5.94; -4.75] 7.7%
Mutuma 2023 23 100.83 17.9221 51 74.40 18.1250 1.45 [0.90; 2.00] 7.7%
Quaye 2019 77 271.11 155.5000 60 149.01 67.3000 0.97 [0.62; 1.33] 7.7%
Emokpae 2018 50 254.80 29.1300 50 92.76 7.2100 7.58 [6.44; 8.72] 7.4%
Ebot 2015 100 116.47 10.0400 100 113.38 5.6700 0.38 [0.10; 0.66] 7.8%
Ayelagbe 2014 40 14.90 5.1000 40 22.10 6.9000 -1.18 [-1.65; -0.70] 7.7%
Analike 2008 100 64.40 37.4000 100 55.30 17.4000 0.31 [0.03; 0.59] 7.8%
Random effects model 490 501 0.58 [-2.23; 3.39] 53.8%
Heterogeneity: I^2 = 99%, τ^2 = 14.3283, p < 0.01
Random effects model 662 672 0.53 [-0.92; 1.98] 100.0%
Heterogeneity: I^2 = 98%, τ^2 = 7.0402, p < 0.01
Test for overall effect: z = 0.72 (p = 0.47)
Test for subgroup differences: χ^2_1 = 0.01, df = 1 (p = 0.94)
-5
0
5

C

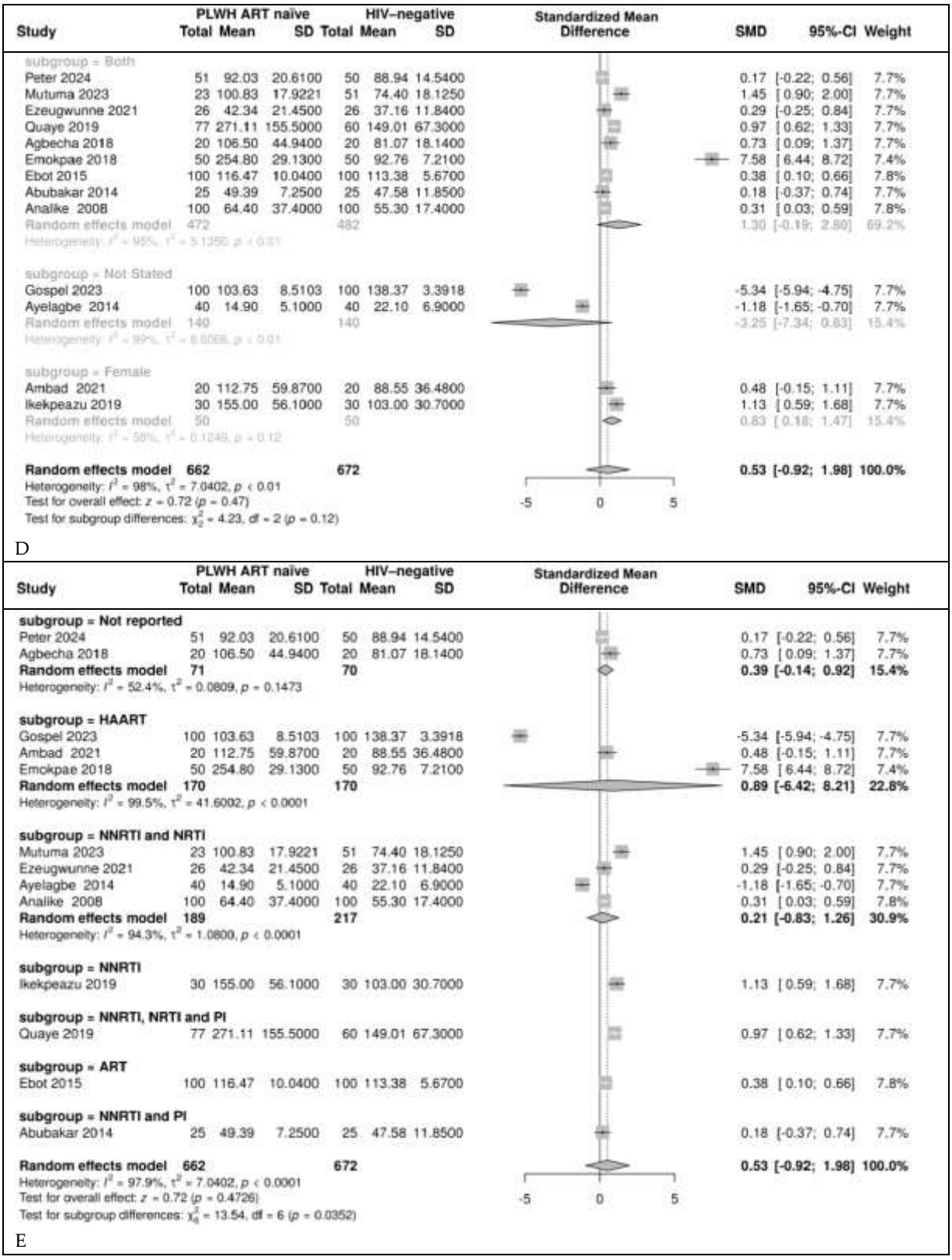
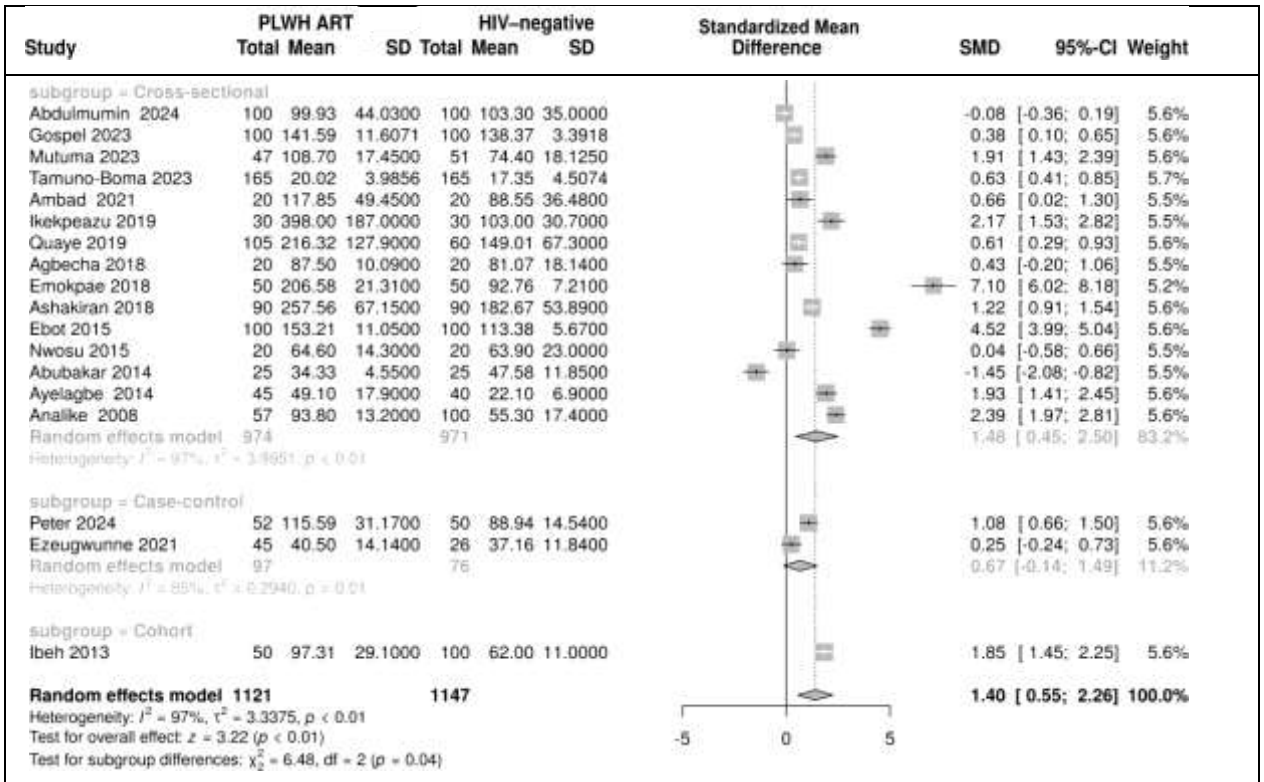
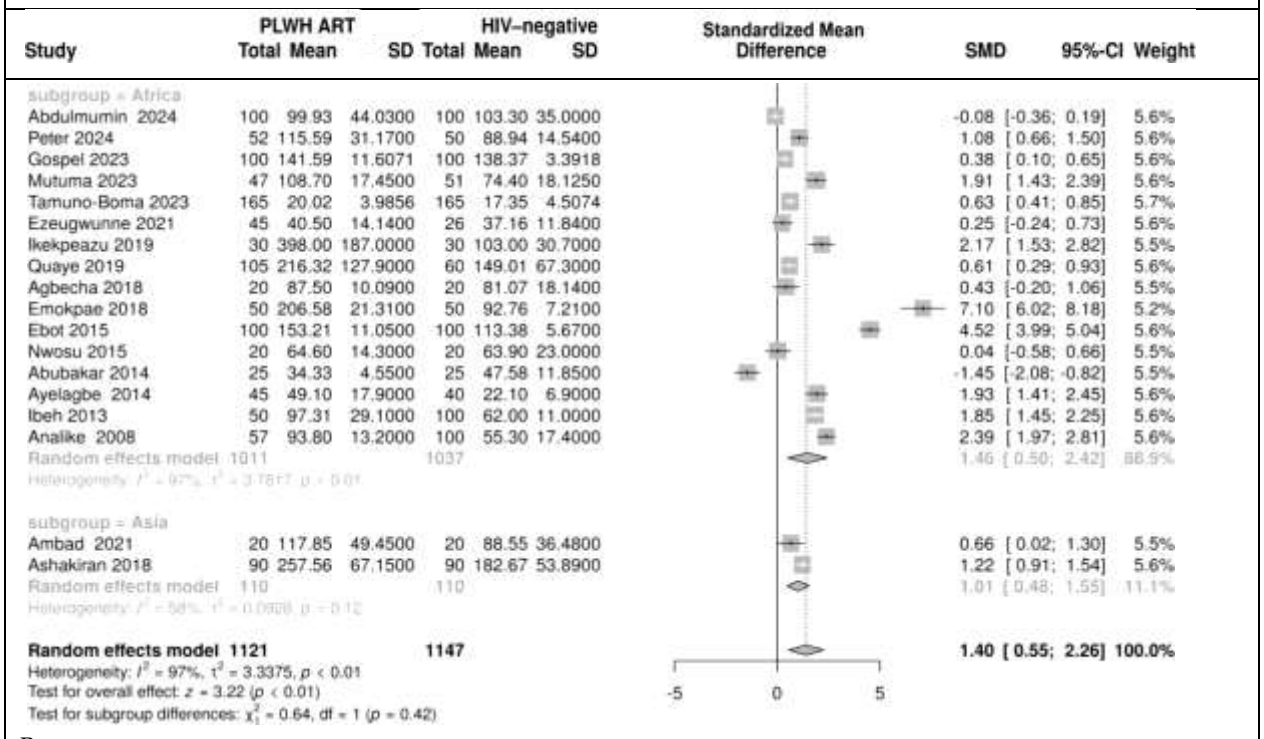


Figure S6: Subgroup analysis showing the effect of different factors on ALP in PLWH who are ART-naïve compared with HIV negative. A: ALP levels in PLWH who are ART-naïve compared with HIV-negative individuals, based on study design. B: ALP level in PLWH who are ART-naïve vs. HIV negative, based on the continent of publication. C: ALP levels in PLWH who are ART-naïve compared with HIV negative, based on sample size. D: ALP levels in PLWH who are ART-naïve compared with HIV negative, based on gender. E: ALP levels in PLWH who are ART-naïve compared with HIV negative, based on the class of ART regimens. The solid line shows the line of no effect, the dashed line shows

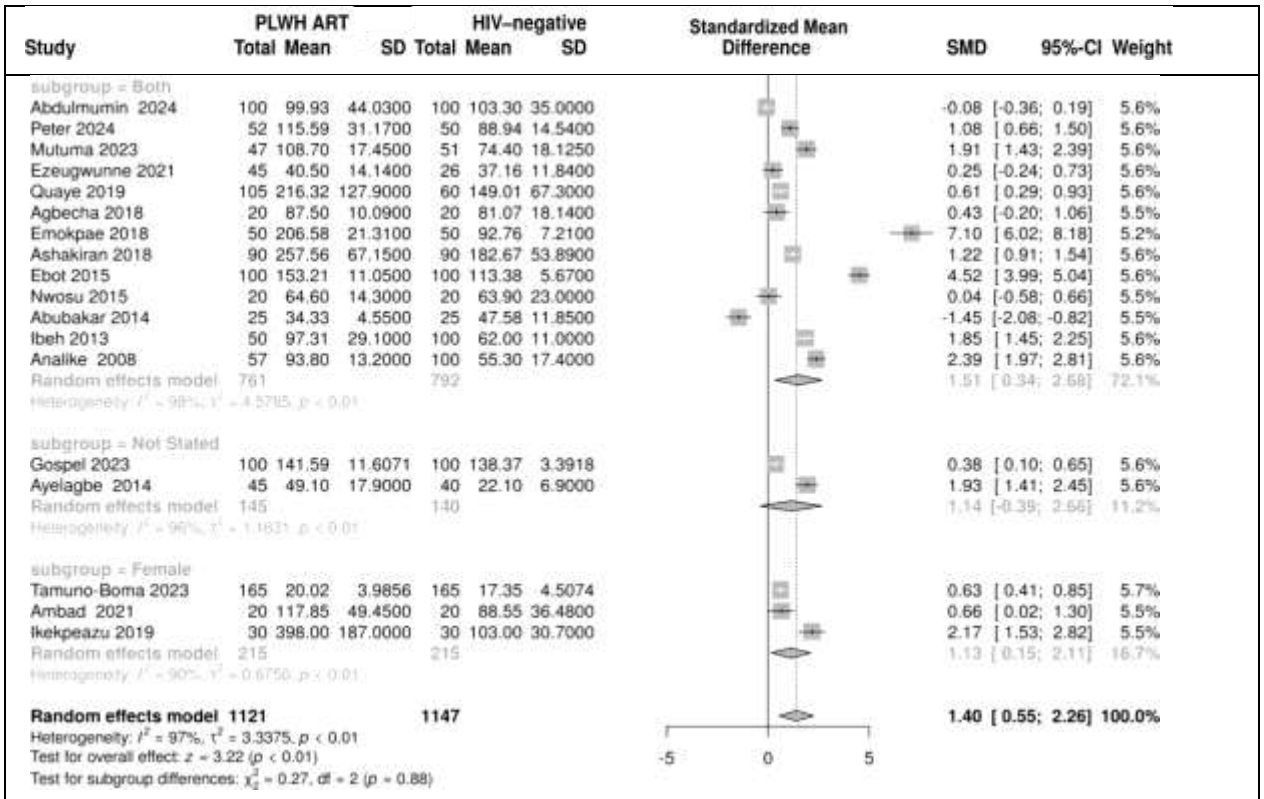
the effect size, the gray block shows the weight of the study, the horizontal line crossing the gray block shows the confidence intervals, diamond plot shows the combined effect size. NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, NtRTI: nucleotide reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase-nucleoside strand transfer inhibitors.


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Cross-sectional
Abdulmumin 2024 100 99.93 44.0300 100 103.30 35.0000 -0.08 [-0.36; 0.19] 5.6%
Gospel 2023 100 141.59 11.6071 100 138.37 3.3918 0.38 [0.10; 0.65] 5.6%
Mutuma 2023 47 108.70 17.4500 51 74.40 18.1250 1.91 [1.43; 2.39] 5.6%
Tamuno-Boma 2023 165 20.02 3.9856 165 17.35 4.5074 0.63 [0.41; 0.85] 5.7%
Ambad 2021 20 117.85 49.4500 20 88.55 36.4800 0.66 [0.02; 1.30] 5.5%
Ikekpeazu 2019 30 398.00 187.0000 30 103.00 30.7000 2.17 [1.53; 2.82] 5.5%
Quaye 2019 105 216.32 127.9000 60 149.01 67.3000 0.61 [0.29; 0.93] 5.6%
Agbecha 2018 20 87.50 10.0900 20 81.07 18.1400 0.43 [-0.20; 1.06] 5.5%
Emokpae 2018 50 206.58 21.3100 50 92.76 7.2100 7.10 [6.02; 8.18] 5.2%
Ashakiran 2018 90 257.56 67.1500 90 182.67 53.8900 1.22 [0.91; 1.54] 5.6%
Ebot 2015 100 153.21 11.0500 100 113.38 5.6700 4.52 [3.99; 5.04] 5.6%
Nwosu 2015 20 64.60 14.3000 20 63.90 23.0000 0.04 [-0.58; 0.66] 5.5%
Abubakar 2014 25 34.33 4.5500 25 47.58 11.8500 -1.45 [-2.08; -0.82] 5.5%
Ayelagbe 2014 45 49.10 17.9000 40 22.10 6.9000 1.93 [1.41; 2.45] 5.6%
Analike 2008 57 93.80 13.2000 100 55.30 17.4000 2.39 [1.97; 2.81] 5.6%
Random effects model 974 971 1.48 [0.45; 2.50] 83.2%
Heterogeneity: I^2 = 97%, τ^2 = 3.9651, p < 0.01
subgroup = Case-control
Peter 2024 52 115.59 31.1700 50 88.94 14.5400 1.08 [0.66; 1.50] 5.6%
Ezeugwunne 2021 45 40.50 14.1400 26 37.16 11.8400 0.25 [-0.24; 0.73] 5.6%
Random effects model 97 76 0.67 [-0.14; 1.49] 11.2%
Heterogeneity: I^2 = 85%, τ^2 = 0.2940, p = 0.01
subgroup = Cohort
Ibeh 2013 50 97.31 29.1000 100 62.00 11.0000 1.85 [1.45; 2.25] 5.6%
Random effects model 1121 1147 1.40 [0.55; 2.26] 100.0%
Heterogeneity: I^2 = 97%, τ^2 = 3.3375, p < 0.01
Test for overall effect: z = 3.22 (p < 0.01)
Test for subgroup differences: χ^2_2 = 6.48, df = 2 (p = 0.04)
-5
0
5

A


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Africa
Abdulmumin 2024 100 99.93 44.0300 100 103.30 35.0000 -0.08 [-0.36; 0.19] 5.6%
Peter 2024 52 115.59 31.1700 50 88.94 14.5400 1.08 [0.66; 1.50] 5.6%
Gospel 2023 100 141.59 11.6071 100 138.37 3.3918 0.38 [0.10; 0.65] 5.6%
Mutuma 2023 47 108.70 17.4500 51 74.40 18.1250 1.91 [1.43; 2.39] 5.6%
Tamuno-Boma 2023 165 20.02 3.9856 165 17.35 4.5074 0.63 [0.41; 0.85] 5.7%
Ezeugwunne 2021 45 40.50 14.1400 26 37.16 11.8400 0.25 [-0.24; 0.73] 5.6%
Ikekpeazu 2019 30 398.00 187.0000 30 103.00 30.7000 2.17 [1.53; 2.82] 5.5%
Quaye 2019 105 216.32 127.9000 60 149.01 67.3000 0.61 [0.29; 0.93] 5.6%
Agbecha 2018 20 87.50 10.0900 20 81.07 18.1400 0.43 [-0.20; 1.06] 5.5%
Emokpae 2018 50 206.58 21.3100 50 92.76 7.2100 7.10 [6.02; 8.18] 5.2%
Ebot 2015 100 153.21 11.0500 100 113.38 5.6700 4.52 [3.99; 5.04] 5.6%
Nwosu 2015 20 64.60 14.3000 20 63.90 23.0000 0.04 [-0.58; 0.66] 5.5%
Abubakar 2014 25 34.33 4.5500 25 47.58 11.8500 -1.45 [-2.08; -0.82] 5.5%
Ayelagbe 2014 45 49.10 17.9000 40 22.10 6.9000 1.93 [1.41; 2.45] 5.6%
Ibeh 2013 50 97.31 29.1000 100 62.00 11.0000 1.85 [1.45; 2.25] 5.6%
Analike 2008 57 93.80 13.2000 100 55.30 17.4000 2.39 [1.97; 2.81] 5.6%
Random effects model 1011 1037 1.46 [0.50; 2.42] 88.9%
Heterogeneity: I^2 = 97%, τ^2 = 3.7617, p < 0.01
subgroup = Asia
Ambad 2021 20 117.85 49.4500 20 88.55 36.4800 0.66 [0.02; 1.30] 5.5%
Ashakiran 2018 90 257.56 67.1500 90 182.67 53.8900 1.22 [0.91; 1.54] 5.6%
Random effects model 110 110 1.01 [0.48; 1.55] 11.1%
Heterogeneity: I^2 = 58%, τ^2 = 0.0928, p = 0.12
Random effects model 1121 1147 1.40 [0.55; 2.26] 100.0%
Heterogeneity: I^2 = 97%, τ^2 = 3.3375, p < 0.01
Test for overall effect: z = 3.22 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.64, df = 1 (p = 0.42)
-5
0
5

B


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Both
Abdulmumin 2024 100 99.93 44.0300 100 103.30 35.0000 -0.08 [-0.36; 0.19] 5.6%
Peter 2024 52 115.59 31.1700 50 88.94 14.5400 1.08 [0.66; 1.50] 5.6%
Mutuma 2023 47 108.70 17.4500 51 74.40 18.1250 1.91 [1.43; 2.39] 5.6%
Ezeugwunne 2021 45 40.50 14.1400 26 37.16 11.8400 0.25 [-0.24; 0.73] 5.6%
Quaye 2019 105 216.32 127.9000 60 149.01 67.3000 0.61 [0.29; 0.93] 5.6%
Agbecha 2018 20 87.50 10.0900 20 81.07 18.1400 0.43 [-0.20; 1.06] 5.5%
Emokpae 2018 50 206.58 21.3100 50 92.76 7.2100 7.10 [6.02; 8.18] 5.2%
Ashakiran 2018 90 257.56 67.1500 90 182.67 53.8900 1.22 [0.91; 1.54] 5.6%
Ebot 2015 100 153.21 11.0500 100 113.38 5.6700 4.52 [3.99; 5.04] 5.6%
Nwosu 2015 20 64.60 14.3000 20 63.90 23.0000 0.04 [-0.58; 0.66] 5.5%
Abubakar 2014 25 34.33 4.5500 25 47.58 11.8500 -1.45 [-2.08; -0.82] 5.5%
Ibeh 2013 50 97.31 29.1000 100 62.00 11.0000 1.85 [1.45; 2.25] 5.6%
Analike 2008 57 93.80 13.2000 100 55.30 17.4000 2.39 [1.97; 2.81] 5.6%
Random effects model 761 792 1.51 [0.34; 2.68] 72.1%
Heterogeneity: I^2 = 98%, τ^2 = 4.5785, p < 0.01
subgroup = Not Stated
Gospel 2023 100 141.59 11.6071 100 138.37 3.3918 0.38 [0.10; 0.65] 5.6%
Ayelagbe 2014 45 49.10 17.9000 40 22.10 6.9000 1.93 [1.41; 2.45] 5.6%
Random effects model 145 140 1.14 [-0.39; 2.66] 11.2%
Heterogeneity: I^2 = 96%, τ^2 = 1.1631, p < 0.01
subgroup = Female
Tamuno-Boma 2023 165 20.02 3.9856 165 17.35 4.5074 0.63 [0.41; 0.85] 5.7%
Ambad 2021 20 117.85 49.4500 20 88.55 36.4800 0.66 [0.02; 1.30] 5.5%
Ikekpeazu 2019 30 398.00 187.0000 30 103.00 30.7000 2.17 [1.53; 2.82] 5.5%
Random effects model 215 215 1.13 [0.15; 2.11] 16.7%
Heterogeneity: I^2 = 90%, τ^2 = 0.6750, p < 0.01
Random effects model 1121 1147 1.40 [0.55; 2.26] 100.0%
Heterogeneity: I^2 = 97%, τ^2 = 3.3375, p < 0.01
Test for overall effect: z = 3.22 (p < 0.01)
Test for subgroup differences: χ^2_2 = 0.27, df = 2 (p = 0.88)
-5
0
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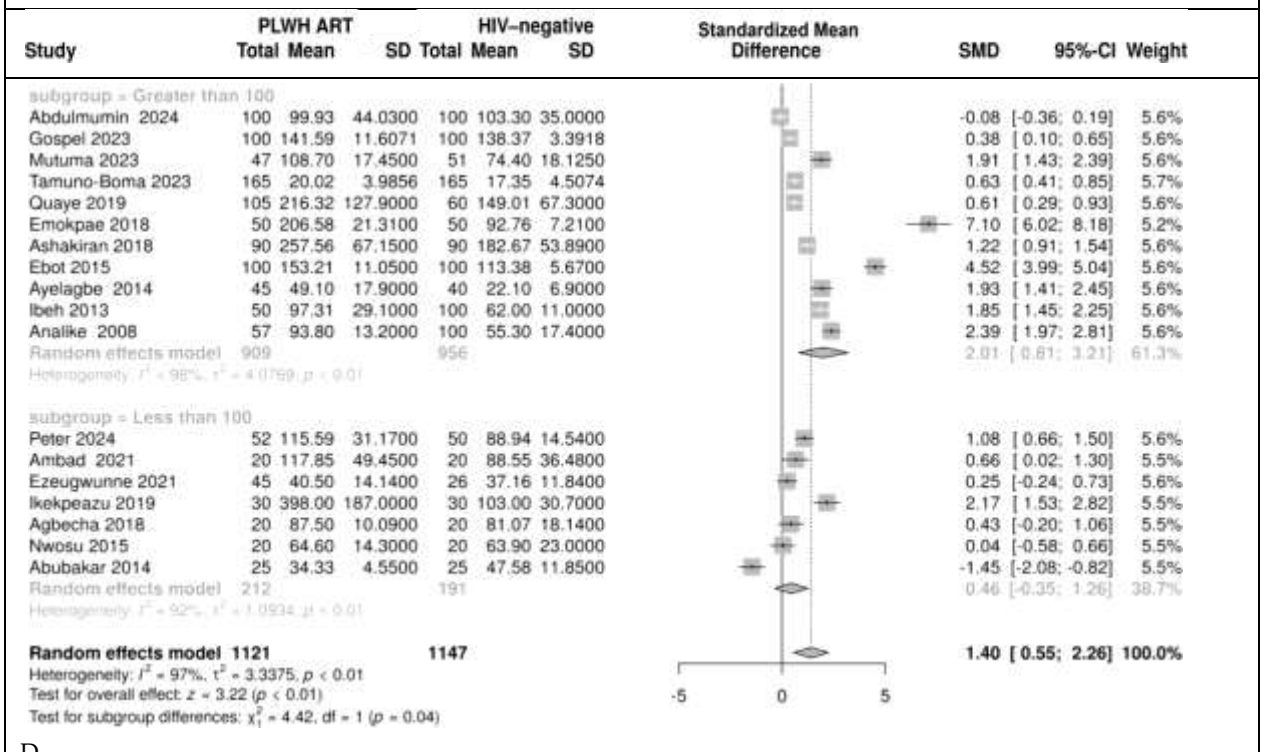
C


Study
PLWH ART
Total Mean SD
HIV−negative
Total Mean SD
Standardized Mean Difference
SMD 95%-CI Weight
subgroup = Greater than 100
Abdulmumin 2024 100 99.93 44.0300 100 103.30 35.0000 -0.08 [-0.36; 0.19] 5.6%
Gospel 2023 100 141.59 11.6071 100 138.37 3.3918 0.38 [0.10; 0.65] 5.6%
Mutuma 2023 47 108.70 17.4500 51 74.40 18.1250 1.91 [1.43; 2.39] 5.6%
Tamuno-Boma 2023 165 20.02 3.9856 165 17.35 4.5074 0.63 [0.41; 0.85] 5.7%
Quaye 2019 105 216.32 127.9000 60 149.01 67.3000 0.61 [0.29; 0.93] 5.6%
Emokpae 2018 50 206.58 21.3100 50 92.76 7.2100 7.10 [6.02; 8.18] 5.2%
Ashakiran 2018 90 257.56 67.1500 90 182.67 53.8900 1.22 [0.91; 1.54] 5.6%
Ebot 2015 100 153.21 11.0500 100 113.38 5.6700 4.52 [3.99; 5.04] 5.6%
Ayelagbe 2014 45 49.10 17.9000 40 22.10 6.9000 1.93 [1.41; 2.45] 5.6%
Ibeh 2013 50 97.31 29.1000 100 62.00 11.0000 1.85 [1.45; 2.25] 5.6%
Analike 2008 57 93.80 13.2000 100 55.30 17.4000 2.39 [1.97; 2.81] 5.6%
Random effects model 909 956 2.01 [0.81; 3.21] 61.3%
Heterogeneity: I^2 = 98%, τ^2 = 4.0769, p < 0.01
subgroup = Less than 100
Peter 2024 52 115.59 31.1700 50 88.94 14.5400 1.08 [0.66; 1.50] 5.6%
Ambad 2021 20 117.85 49.4500 20 88.55 36.4800 0.66 [0.02; 1.30] 5.5%
Ezeugwunne 2021 45 40.50 14.1400 26 37.16 11.8400 0.25 [-0.24; 0.73] 5.6%
Ikekpeazu 2019 30 398.00 187.0000 30 103.00 30.7000 2.17 [1.53; 2.82] 5.5%
Agbecha 2018 20 87.50 10.0900 20 81.07 18.1400 0.43 [-0.20; 1.06] 5.5%
Nwosu 2015 20 64.60 14.3000 20 63.90 23.0000 0.04 [-0.58; 0.66] 5.5%
Abubakar 2014 25 34.33 4.5500 25 47.58 11.8500 -1.45 [-2.08; -0.82] 5.5%
Random effects model 212 191 0.46 [-0.35; 1.26] 38.7%
Heterogeneity: I^2 = 92%, τ^2 = 1.0934, p < 0.01
Random effects model 1121 1147 1.40 [0.55; 2.26] 100.0%
Heterogeneity: I^2 = 97%, τ^2 = 3.3375, p < 0.01
Test for overall effect: z = 3.22 (p < 0.01)
Test for subgroup differences: χ^2_1 = 4.42, df = 1 (p = 0.04)
-5
0
5

D

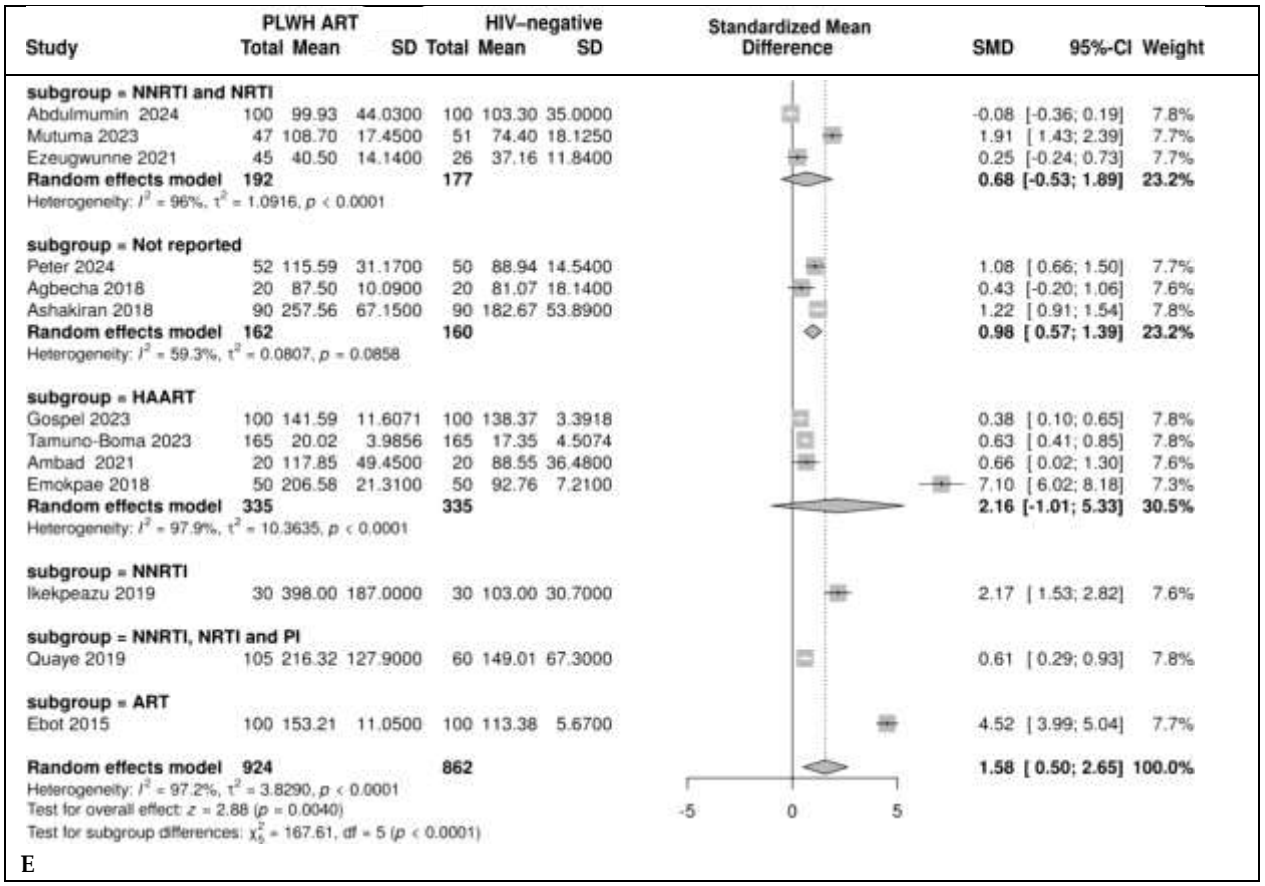


Figure S7: Subgroup analysis showing the effect of different factors on ALP in PLWH on ART compared with HIV negative. A: ALP levels in PLWH on ART compared with HIV-negative individuals, based on study design. B: ALP levels in PLWH on ART compared with HIV-negative individuals in terms of the continent of publication. C: ALP levels in PLWH on ART compared with HIV-negative individuals based on gender. D: ALP levels in PLWH on ART compared with HIV-negative individuals based on sample size. E: ALP levels in PLWH on ART compared with HIV-negative individuals based on class of ART regimens. The solid line shows the line of no effect, the dashed line shows the effect size, the gray block shows the weight of the study, the horizontal line crossing the gray block shows the confidence intervals, diamond plot shows the combined effect size. NRTIs: nucleoside reverse transcriptase inhibitors, NNRTIs: non-nucleoside reverse transcriptase inhibitors, NtRTI: nucleotide reverse transcriptase inhibitors, PIs: protease inhibitors, INSTIs: integrase-nucleoside strand transfer inhibitors.

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