The impact of low-dose aspirin on haemoglobin levels during pregnancy

by

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A thesis submitted in manuscript format in fulfilment of the requirements for the degree of Master in Medical Science

to

School of Laboratory Medicine and Medical Sciences, College of Health Sciences,

University of KwaZulu-Natal

9th July 2025

DECLARATION

I, Nokwethemba Ngcobo, declare as follows:

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SUPERVISORS' DECLARATION

We hereby confirm that we have read the contents of this dissertation and approve its submission.

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FUNDING

Funding for this project was made possible through the University of KwaZulu-Natal (UKZN) College of Health Sciences (CHS) scholarship.

The institution listed has no vested interest in the study. Further, there was no input from this organisation in the interpretation of the results.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Vinogrin Dorsamy, my supervisor, for his invaluable guidance and support throughout this study. I equally extend my heartfelt thanks to Dr. Chauntelle Bagwandeen for her expert advice and encouragement as my co-supervisor.

Special thanks to Dr Zinhle Mkhize and Prof Moodley, my co-investigators, for their collaboration and dedication to this research. Their expertise and unwavering support were integral to the success of this study, and I am deeply appreciative of all their contributions.

DEDICATION

I dedicate this work to my friends and family for their unwavering support and encouragement throughout my research journey. Their love, understanding, and belief in me have been a constant source of strength, helping me overcome challenges and stay focused. Thank you for being my rock.

ACRONYMS

WHO: World Health Organization

LMICs: Low to middle-income countries

HIC: High income countries

LDA: Low-dose aspirin

PE: Preeclampsia

HDP: Hypertensive disorders of pregnancy

PPH: Postpartum haemorrhage

RCT: Randomised controlled trial

ART: Antiretroviral therapy

BMI: Body mass index

HIV: Human immunodeficiency virus

SSA: Sub-Saharan Africa

PRISMA-P: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols

eNOS: Endothelial nitric oxide synthase

HELLP: Hemolysis, Elevated Liver Enzymes, and Low Platelet Count

IUGR: Intrauterine growth restriction

FIGO: International Federation of Gynecology and Obstetrics

UKZN: University of KwaZulu-Natal

PMMH: Prince Mshiyeni Memorial Hospital

ABBREVIATIONS

Hb: Haemoglobin

Plts: Platelets

NO: Nitric Oxide

TXA_2: Thromboxane A_2

COX-1: Cyclooxygenase-1

IL-6: Interleukin-6

CRP: C-Reactive protein

GA: Gestational age

BP: Blood pressure

ANC: Antenatal care

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ABSTRACT

Background: Low-dose aspirin, initiated between 12 and 28 weeks of gestation, is recommended for the prevention of preeclampsia, a major hypertensive complication of pregnancy. However, its influence on maternal haemoglobin levels—and its possible role in modifying anaemia risk, remains unclear. Anaemia continues to pose a significant burden in maternal health, contributing to increased morbidity and mortality.

Aim: This study investigated the effect of low-dose aspirin on maternal haemoglobin levels and explored the association between haemoglobin changes and hypertensive disorders of pregnancy.

Methods: This secondary analysis included 249 pregnant women selected from a larger randomised controlled trial, in which participants were randomised to receive 162 mg low-dose aspirin daily or standard care. Haemoglobin levels at enrolment were retrieved from trial records, while haemoglobin measurements at birth were extracted retrospectively from hospital maternity charts and the National Health Laboratory Service. Maternal outcomes, including hypertensive disorders of pregnancy, were analysed in relation to haemoglobin changes.

Result: Of the total participants, 111 showed a decline in haemoglobin levels from enrolment to birth, while 138 demonstrated an increase. Repeated measures analysis of variance revealed a significant group-by-time interaction ($p = 0.002$, $\eta^2 = 0.088$) amongst those with declining haemoglobin. Participants in the low-dose aspirin group experienced a smaller decrease in haemoglobin ($\Delta Hb = -0.902$ g/dL) compared to the control group ($\Delta Hb = -1.405$ g/dL). Furthermore, a decline in haemoglobin was associated with higher odds of developing hypertensive disorders of pregnancy (odds ratio = 2.21 vs 0.45, $p = 0.021$). Within this subgroup, those receiving low-dose aspirin had a 76% lower risk of hypertensive disorders of pregnancy (relative risk = 0.24, 95% confidence interval [0.11–0.53], $p < 0.001$).

Conclusion: Low-dose aspirin attenuated the decline in maternal haemoglobin levels, suggesting a novel haematological benefit beyond preeclampsia prevention. This therapeutic agent may offer value for integration into public health strategies aimed at improving maternal and fetal outcomes—particularly in low to middle-income countries, where anaemia is prevalent. A reduction in haemoglobin levels may also serve as an early predictor of hypertensive disorders of pregnancy.

CHAPTER ONE: INTRODUCTION

The introductory chapter of this thesis provides the rationale for the study, namely, to investigate the impact of low-dose aspirin on haemoglobin levels during pregnancy and its potential implications for maternal and fetal health. The chapter concludes with the outline of the rest of the dissertation.

1.1 Background

Preeclampsia (PE) remains one of the most serious complications of pregnancy, particularly in low to middle-income countries (LMICs) where healthcare resources are limited (1,2). In South Africa (SA), hypertensive disorders of pregnancy (HDP) are the leading direct cause of maternal mortality, accounting for approximately 18% of maternal deaths (3). This has prompted extensive research into effective, accessible interventions that can prevent these conditions.

Acetylsalicylic acid, when given in low doses, has emerged as a promising therapeutic agent for PE prevention. The US Preventive Services Task Force (USPSTF), American College of Obstetricians and Gynecology (ACOG), and World Health Organization (WHO) recommend low-dose aspirin (LDA) for women at high risk of developing PE, due to its anti-inflammatory and antiplatelet properties (4–6). Low-dose aspirin is particularly suitable for resource-limited settings because of its low cost, wide availability, and ease of administration (6). However, comprehensive evaluation of any medication used during pregnancy requires an understanding of both its benefits and potential risks. One such area requiring further investigation is how LDA might affect maternal haemoglobin (Hb) levels during pregnancy—an issue with important clinical implications for both maternal and fetal health.

The relationship between anaemia and PE appears to be bidirectional; PE can worsen anaemia through inflammation, oxidative stress, and endothelial dysfunction, while anaemia can compromise PE outcomes by reducing oxygen delivery to the placenta (7–9). This complex interaction creates a cycle in which each condition may amplify the other, increasing risks to maternal and fetal health.

Understanding how LDA affects maternal Hb levels may shed light on this relationship and inform the development of more targeted management strategies for pregnant women, particularly in countries where anaemia prevalence is high.

Preeclampsia develops when the placenta fails to implant properly early in pregnancy, resulting in poor blood flow and widespread, progressive inflammation (10). Research has shown that starting LDA before 16 weeks of gestation can help prevent PE by promoting better placental development and improving blood vessel function (11–13). Low-dose aspirin works by modulating the cyclooxygenase (COX) pathway, helping to restore the balance between pro- and anti-thrombotic factors—a balance that is crucial for maintaining healthy placental blood flow (14,15).

However, this is where complications arise. Anaemia, defined as Hb levels below 11 g/dL in the first or third trimester of pregnancy, is already a major health concern globally, especially in LMICs where iron deficiency is the most common cause (16,17). In SA, routine iron supplementation (200 mg ferrous sulphate daily equivalent to approximately 65 mg elemental iron) is standard antenatal practice to prevent anaemia (18).

The problem is that iron supplementation is not equally effective for all women. Some struggle to absorb iron efficiently from the gastrointestinal (GI) tract, which limits the benefit of supplementation (19,20). On the other hand, giving iron to all pregnant women regardless of their iron status can sometimes cause iron overload, particularly in women who already have adequate iron stores (21,22). Iron overload brings its own problems—it can make blood thicker, reduce blood flow to the placenta, and increase oxidative stress, which may contribute to the development of PE (23). This situation creates a U-shaped risk curve, where both iron deficiency and excess can negatively impact pregnancy outcomes (24).

Emerging research suggests several mechanisms through which LDA might influence maternal iron status. Firstly, when aspirin is metabolized, it forms aspirin-chelating metabolites (ACMs) that have a high iron-binding affinity, potentially contributing to iron depletion (25,26). Then, LDA has been associated with GI bleeding during pregnancy which may directly reduce Hb levels through chronic or acute blood loss (27). Additional concerns have been raised about an increased risk of postpartum haemorrhage (PPH) in women taking LDA (28–30), as PPH-related blood loss often exceeds the body's capacity for red cell replenishment (31). Moreover, pre-existing anaemia may increase the risk of PPH, potentially creating a vicious cycle of worsening anaemia and haemorrhage-related complications (32).

1.2 Rationale

In resource-limited settings like SA, where healthcare budgets are constrained and maternal mortality rates remain high, interventions must be both safe and cost-effective. The Sustainable Development Goals (SDGs) Target 3.1 have established the ambitious target of reducing global maternal mortality to less than 70 per 100,000 live births by 2030, requiring interventions that can be implemented at scale (33). This goal not only saves lives but also fosters healthier future generations, promotes economic stability, and reduces health disparities, creating a cycle of well-being that supports sustainable development.

Low-dose aspirin has been shown to lower the risk of PE and is associated with improved pregnancy outcomes, including a decreased incidence of fetal growth restriction, premature

delivery, and low birth weight (11). However, broader implementation requires careful evaluation of its potential risks.

The question of how LDA affects maternal Hb levels is particularly relevant in the South African context, where anaemia prevalence amongst pregnant women ranges from 29% to 43% (20,34,35). It is essential to ensure that efforts to prevent PE do not inadvertently worsen anaemia status, as adequate maternal Hb is critical for oxygen transport from mother to fetus, and a decline may result in placental ischemia or fetal hypoxia (36–38).

This question has gained additional relevance following a recent randomised controlled trial (RCT) amongst Black South African pregnant women, which showed that daily LDA use reduced the risk of HDP, including PE, by 75% compared to the control group (39). While these findings are encouraging, the complete mechanisms by which aspirin reduces pregnancy complications remain incompletely understood, and potential risks to the local population require a more thorough investigation.

This secondary analysis of the data emerging from the study addresses this knowledge gap by investigating how LDA affects Hb levels in the original cohort.

1.3 Research Question

How does administration of therapeutic LDA affect Hb levels during pregnancy?

1.4 Aims

To determine the impact of LDA on maternal Hb levels and how changes in Hb levels altered adverse pregnancy outcomes, including HDP.

1.5 Specific Objectives

- To ascertain the magnitude of change in Hb levels between the intervention and control groups at two time points: enrolment and birth.
- To determine the association between mean Hb change (ΔHb) and adverse maternal outcomes (HDP).
- To assess the association between ΔHb and covariates including human immunodeficiency virus (HIV) and body mass index (BMI).

1.6 Overview of Methodology

This study is a secondary analysis of data from a RCT that was conducted at Prince Mshiyeni Memorial Hospital (PMMH) in Durban, SA (39). The original trial was designed to test whether LDA could effectively prevent HDP, including PE, and adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (40).

The original study recruited normotensive Black pregnant women aged 18 years or older, with singleton pregnancies between 12 and 20 weeks of gestation. Participants were randomly assigned to receive either 162 mg of LDA daily (intervention) until 36 weeks of gestation or standard care (control). For this study, a sample size calculation using G*Power 3.1 determined that 249 participants were required, based on an effect size of 0.3 (small-to-medium), an alpha level of 0.05, and 80% power (41). This included 125 participants in the intervention group and 124 in the control group.

The original trial collected haematological parameters, including Hb levels, at enrolment as part of routine care. For this study, follow-up Hb levels at birth were retrieved from hospital charts or National Health Laboratory Service (NHLS) records.

Hypertensive disorders of pregnancy were defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria (42). The analysis plan included descriptive statistics to summarise baseline characteristics, repeated measures ANOVA to analyse ΔHb from enrolment to birth, and logistic regression to examine associations between ΔHb and HDP. Complete case analysis was performed to account for participants with missing Hb data at birth.

Ethical approval for this secondary analysis was obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC/00007156/2024) (Appendix1). Informed consent was obtained from all participants at the time of enrolment in the original trial (Appendix 2). Since this analysis utilised existing data only, additional consent was not required. Data security was maintained throughout the process.

1.7 Thesis Structure

This thesis follows the University of KwaZulu-Natal's College of Health Sciences guidelines for a master's thesis by manuscript (Appendix 3). It is organized into five chapters, each contributing to the overall aim of investigating the impact of LDA on Hb levels during pregnancy. Although the guidelines permit submission of a single article, three manuscripts developed during the study are included, each in the format in which they were submitted to peer-reviewed journals. As a result, some repetition of the background and rationale is unavoidable.

Chapter One provides the background, rationale, research question, aims, objectives, and a methodological overview of the study. It also outlines the structure of the remaining chapters.

Chapter Two presents the protocol for a systematic review on the impact of LDA on Hb levels during pregnancy, submitted to BioMed Central Systematic Reviews.

Although a systematic review and meta-analysis was originally planned (as outlined in Chapter Two), it was felt that the importance of the findings from a preliminary scan of the literature warranted formalising. Thus, the narrative review was conducted to avoid delays in thesis submission while awaiting journal feedback. Chapter Three contains a comprehensive literature review exploring the mechanisms by which LDA may influence Hb levels, potentially increasing the risk of anaemia, as well as its effects on maternal health and associated birth outcomes. This review has been submitted to South African Family Practice.

Chapter Four presents the main research manuscript as submitted to the European Journal of Obstetrics and Gynecology. It reports the study's findings on the impact of LDA on Hb levels and examines the association between ΔHb and HDP.

The key findings from the preceding chapters, focusing on the effects of LDA on Hb levels during pregnancy are presented as an integrated discussion in Chapter 5 and also includes the study's limitations, implications, and conclusions. Recommendations for clinical practice and future research directions are also provided.

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CHAPTER TWO: A PROTOCOL FOR A SYSTEMATIC REVIEW AND META-ANALYSIS

This chapter presents a protocol for a systematic review and meta-analysis investigating the impact of low-dose aspirin during pregnancy. The protocol was developed and formatted in accordance with the BioMed Central Systematic Reviews guidelines for authors. The article is currently under review

INVESTIGATING THE IMPACT OF LOW-ASPIRIN ON HAEMOGLOBIN LEVELS DURING PREGNANCY: A PROTOCOL FOR A SYSTEMATIC REVIEW AND META-ANALYSIS

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In Review: BioMed Central Systematic Reviews

Manuscript Number: SYSR-D-24-01179R2

Abstract

Background: Aspirin, a cyclooxygenase inhibitor with anti-inflammatory and antiplatelet properties, is widely used during pregnancy to prevent preeclampsia and intrauterine growth restriction. Despite its benefits, aspirin use has been associated with an increased risk of obstetric haemorrhage, particularly postpartum haemorrhage, and it has iron-chelating effects which may indirectly influence haemoglobin levels. However, its potential impact on haemoglobin levels and related pregnancy outcomes remains underexplored. This systematic review protocol aims to evaluate the effect of low-dose aspirin on haemoglobin levels during pregnancy.

Methods: Following the Preferred Reporting Items for Systematic Review and Meta-analysis for Protocols guidelines, comprehensive searches will be conducted in PubMed, Cochrane Library, Embase, Scopus, Web of Science, and ClinicalTrials.gov. Additional grey literature sources, such as Google Scholar and conference abstracts, will also be searched. Studies will include randomised controlled trials, cohort studies, and case-control studies reporting haemoglobin levels in pregnant women treated with low-dose aspirin. Independent reviewers will screen, extract, and appraise data for quality. Data will be synthesized using meta-analysis where feasible, with subgroup analyses to explore heterogeneity. Narrative synthesis will summarize findings from studies unsuitable for pooling.

Results: The systematic review will provide pooled evidence on the impact of low-dose aspirin on haemoglobin levels and related pregnancy outcomes, such as preeclampsia and fetal growth, potentially influencing clinical guidelines.

Conclusions: Findings may inform clinical recommendations on low-dose aspirin use in pregnancy, aiming to optimize maternal and fetal health outcomes while addressing risks of anaemia and haemorrhage.

Registration: PROSPERO CRD42024556220.

Keywords: Low-dose aspirin, haemoglobin, pregnancy, preeclampsia, obstetric haemorrhage, systematic review, meta-analysis.

Background

The prophylactic use of low-dose aspirin (LDA), spans an array of conditions including cardiovascular diseases, dementia, and certain cancers, making it one of the most universally prescribed medications globally (1). Aspirin is a cyclooxygenase (COX) inhibitor with anti-inflammatory and antiplatelet properties. During pregnancy, LDA is commonly used to prevent preeclampsia (PE) and intrauterine growth restriction (IUGR) (2,3). Preeclampsia is a hypertensive disorder of pregnancy (HDP) characterized by increased blood pressure and proteinuria (4). If left untreated, it can lead to organ damage, fetal growth restriction, and preterm birth (5,6). The therapeutic rationale for LDA lies in its capacity to alter the biochemical pathways implicated in the pathogenesis of PE. Aspirin modulates the COX enzyme pathway, leading to a balanced production of thromboxane and prostacyclin, substances that play critical roles in placental blood flow regulation (7,8). Preeclampsia is associated with a more coagulable state marked by significant platelet activation (9,10). Low-dose aspirin inhibits the secretion of platelet-derived thromboxane A2 (TXA2), reducing vasoconstriction and platelet aggregation in the placenta, but does not change the secretion of prostacyclin 2 (PGI2) which is the most effective endogenous inhibitor of platelet aggregation (11). This is a key step in preventing the early onset of PE. However, its impact on haemoglobin (Hb) levels and, by extension, anaemia, during pregnancy remains underexplored. Maintaining optimal Hb levels during pregnancy is vital to ensure an adequate oxygen supply for both maternal and fetal well-being (12). A decrease in Hb or red blood cells results in anaemia, a condition characterized by a reduced capacity of the blood to transport oxygen (13). Globally, 37% of pregnant women and 30% of women 15–49 years of age are estimated to be affected by anaemia (14).

Rationale

The use of LDA during pregnancy is associated with an increased risk of postpartum and intrapartum bleeding, postpartum haematoma, and intracranial haemorrhage (15). This may result from its antithrombotic effect or excessive thromboxane inhibition as the effect of aspirin on COX-dependent prostaglandin is dose dependent. Aspirin's primary metabolite, salicylic acid, chelates iron, forming a complex that reduces iron bioavailability and can contribute to iron deficiency anaemia (IDA) (16–18). Haemorrhage is the leading direct cause of maternal death worldwide and more than two-thirds of these cases are classified as postpartum haemorrhage (PPH) (19,20). Postpartum haemorrhage can cause a decrease in Hb levels due to significant blood loss (21,22). A rapid decrease in Hb levels occurs because the body's ability

to replenish red blood cells is slower than the rate of blood loss during the PPH (23). Globally, PPH accounts for 19.7% of maternal death, with 8% occurring in developed regions and nearly 20% occurring in developing regions (20). Therefore, when advocating for the use of aspirin during pregnancy, the risk needs to be weighed against the potential benefits.

Aims

The aim of this systematic review is to elucidate the impact of LDA on Hb levels in pregnancy and its subsequent effects on pregnancy outcomes such as PE, fetal growth, and birth weight, thereby allowing for evidence-based recommendations for the refinement of anaemia management strategies, should LDA be found to be associated with an increased risk for anaemia, or there is exacerbation of existing anaemia. This research has the potential to significantly influence public health policies and practices aimed at reducing the burden of anaemia and pregnancy-related complications and thus optimizing maternal and fetal well-being in low to middle-income countries (LMICs).

Objectives

The specific objectives of this study are to:

- Investigate the associations between the use of aspirin and maternal Hb levels.
- Identify the incidence of anaemia in pregnant women treated with aspirin.
- Determine whether changes in Hb levels influence specific pregnancy outcomes, such as the incidence of PE, fetal growth, and birth weight.

Methodology

This protocol adheres to the recommendations outlined in the Preferred Reporting Items for Systematic Review and Meta-analysis for Protocols (PRISMA-P) guidelines. The results will be reported following the PRISMA 2020 statement, and the article screening and selection process will be depicted through a PRISMA 2020 flow diagram (24).

Eligibility of the Research Question

The eligibility of the research question was determined using the Population Intervention Comparator Outcome (PICO) framework, as illustrated in Table 1.

Table 1: PICO framework for the eligibility of the research question

Population	Pregnant women
Intervention	Women prescribed LDA
Comparator	Placebo or no treatment
Outcomes	*Primary Outcome* Change in Hb levels during pregnancy. *Secondary Outcome* Incidence of PE Fetal growth rate Birth weight

Inclusion criteria

Studies will be included if they investigate the effect of LDA, defined as 75–162 mg daily, on Hb levels or related maternal haematological outcomes during pregnancy. Eligible populations include pregnant women at or before 28 weeks' gestation, regardless of parity or pre-existing medical conditions.

Both randomised controlled trials (RCTs) and observational studies (cohort and case-control) will be considered. Studies must report at least one relevant maternal outcome, including:

- Change in Hb levels
- Incidence or severity of anaemia
- Hypertensive disorders of pregnancy (e.g., gestational hypertension, PE)

Only full-text articles published in English will be included.

Titles and abstracts retrieved through the search strategy will be screened independently by two reviewers (NN and VD). Full-text articles of potentially relevant studies will be assessed using the inclusion criteria. Disagreements will be resolved through discussion or by consulting a third reviewer (CB).

Studies will also be considered eligible if they meet the following PICO-based criteria:

- **Population**: Pregnant women of any age and parity, with gestational age ≤28 weeks at the time of initiating low-dose aspirin. No restrictions will be applied based on anaemia or hypertensive disorder risk.
- **Intervention**: Use of LDA (75–162 mg daily) initiated between 12- and 28-weeks' gestation during pregnancy. Studies must specify the dose, timing of initiation, and duration of aspirin therapy.

- **Comparator**: Placebo, standard antenatal care, or no treatment.
- **Outcomes**: At least one of the following maternal outcomes must be reported:
    1. Change in Hb levels from baseline (typically in the first or second trimester) to follow-up (third trimester or delivery)
    2. Incidence or severity of anaemia, based on clinical or laboratory definitions
    3. Occurrence of HDP
- **Setting**: No restriction will be placed on country, healthcare system, or level of care.
- **Study design**: Eligible studies include RCTs, cohort studies (prospective or retrospective), and case-control studies.
- **Language**: Articles published in languages other than English will be translated during screening and data extraction using Google Translate or, where necessary, professional translation services.

Exclusion criteria

Studies will be excluded if they:

- Are not available in full text, despite attempts to contact the authors
- Involve non-pregnant populations
- Are published as conference abstracts, case reports, reviews, or editorials
- Do not include an intervention group treated with low-dose aspirin and reporting on haemoglobin outcomes
- Lack a comparison or control group

Information sources

A comprehensive search will be conducted using the following electronic databases: PubMed, CINAHL, EMBASE, EBSCO, Ovid Maternity and Infant Care, the Cochrane Database of Systematic Reviews, Google Scholar (for grey literature), Web of Science, SCOPUS, PsycINFO, and ClinicalTrials.gov.

In addition to electronic databases, we will also search the reference lists of included articles (backward citation chaining) and use forward citation tracking via Google Scholar to identify additional relevant studies.

Articles published in languages other than English will be translated using tools such as Google Translate during screening and data extraction. Where necessary, clarification will be sought from native speakers or professional translation services.

Search strategy

The search terms and their synonyms have been identified using Medical Subject Headings (MeSH). The search strategy was piloted in PubMed using the following search string:

PubMed: (("Aspirin"[MeSH Terms] OR "low-dose aspirin" OR "acetylsalicylic acid") AND ("Anemia"[MeSH Terms] OR "haemoglobin" OR "hemoglobin" OR "blood iron levels" OR "iron deficiency"[MeSH Terms]) AND ("Pregnancy"[MeSH Terms] OR "pregnant women" OR "maternal" OR "pregnancy complications"[MeSH Terms] OR "hypertensive disorder of pregnancy"[MeSH Terms] OR "preeclampsia"[MeSH Terms] OR "pregnancy outcomes"[MeSH Terms] OR "obstetric hemorrhage"[MeSH Terms] OR "postpartum hemorrhage"[MeSH Terms])) as illustrated in Table 2 of Appendix A. This search strategy will be adapted for other electronic databases such as Cochrane Library, Embase, and Scopus.

This approach enhanced the precision and recall of search results (25). To ensure comprehensive coverage, grey literature sources will be included, such as ClinicalTrials.gov, OpenGrey, ProQuest Dissertations, and conference abstracts for obtaining a complete view of available evidence, particularly on the impact of LDA on Hb levels during pregnancy, where published studies are limited (26).The search strategy will undergo peer review by an information specialist following the Peer Review of Electronic Search Strategies (PRESS) guideline checklist to ensure precision and comprehensiveness (27).

Study records and data management

All study records will be consolidated in a Microsoft® Excel® spreadsheet to ensure systematic organisation and ease of access. This spreadsheet will include all relevant data items, as outlined in the "Data Items" section. Data collected using a customised charting tool developed in Google® Forms will be exported to Excel. The specific data elements to be extracted are referenced in Table 3 of Appendix B. All search results will be exported into Zotero v5.0.81 for reference management. Duplicate records will be identified and removed using Zotero's built-in duplicate detection tool, followed by manual verification. A Zotero library will be created for the review and structured according to the screening stages.

Two reviewers (NN and VD) will independently perform title and abstract screening using the Google Forms tool. Full-text screening will be conducted for all potentially eligible studies. In cases where abstracts are unavailable, full articles will be assessed directly. Any disagreements arising during the screening process will be addressed through mutual discussion or, if necessary, by seeking input from a third reviewer (CB).

Before formal screening, calibration exercises will be conducted using a random sample of 10 studies to ensure consistency in study selection, data extraction, and risk of bias assessment. Inter-rater reliability will be measured using Cohen's Kappa statistic (κ) (28). Based on the results of the calibration, necessary adjustments will be made to screening and extraction tools to improve consistency and reliability.

Screening decisions and outcomes will be documented systematically. A PRISMA 2020 flow diagram (Figure 1) will be used to illustrate the study selection process, including reasons for exclusion at each stage.

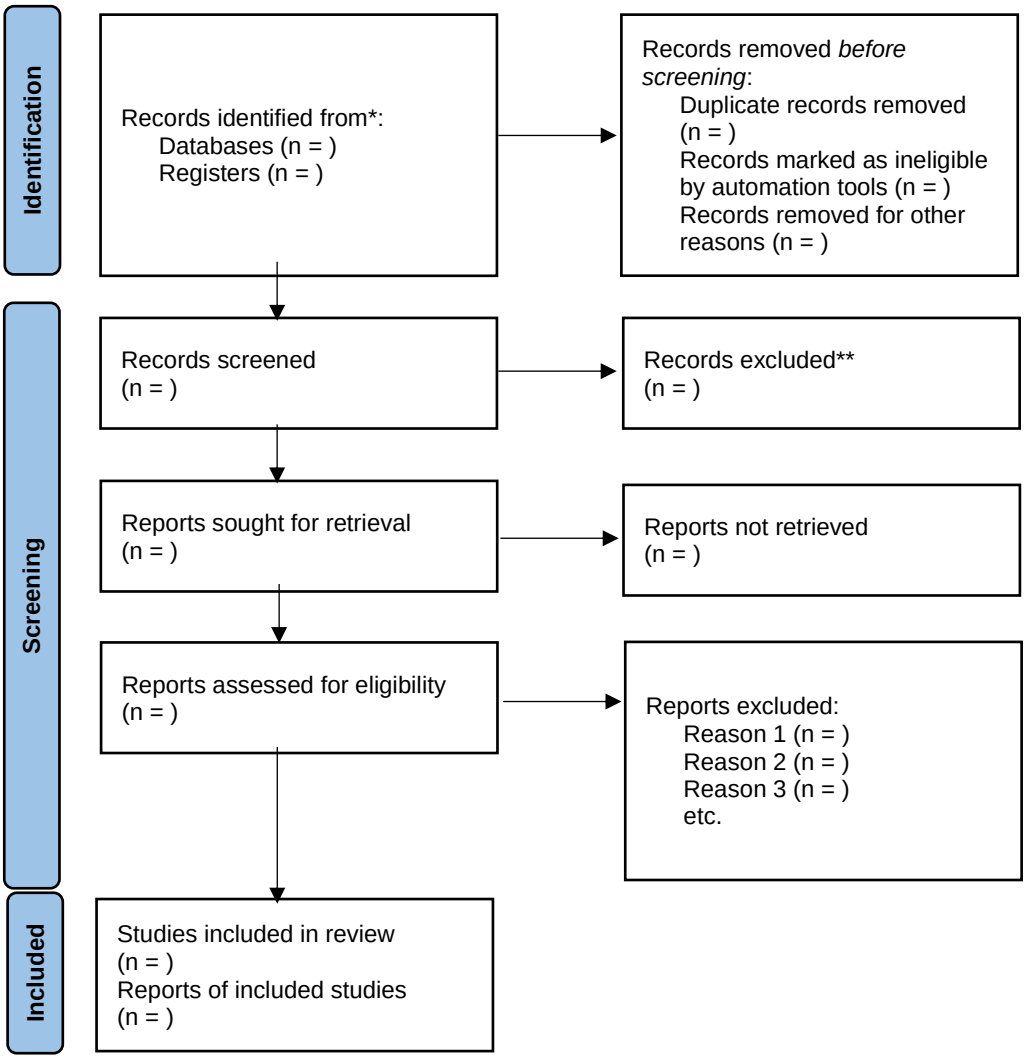

Figure 1: PRISMA 2020 flow diagram describing selection of studies for systematic review of the impact of LDA on maternal haemoglobin levels (24).

Data collection process

As illustrated in Table 3 of Appendix B, a data charting tool (Google forms) will be employed to extract and process information from each selected study. To ensure comprehensive data collection on relevant aspects of the study, the form will be piloted, and continually updated should the need arise.

Data items

Data will be extracted independently by two reviewers using a pretested Microsoft® Excel® spreadsheet. The following variables will be collected from each included study:

- **Study characteristics**: Title, authors, year of publication, country, study design, sample size, setting, and duration of follow-up.
- **Participant characteristics**: Maternal age, parity, gestational age at the time of aspirin initiation, baseline haemoglobin levels, Human Immunodeficiency Virus (HIV) status, and any reported risk factors for anaemia or hypertensive complications.
- **Intervention details**: Aspirin dosage (75–162 mg), timing of initiation, frequency, duration, and level of compliance (if reported).
- **Comparator**: Placebo, standard antenatal care, or no aspirin use.

Outcomes

Primary outcomes:

- Change in maternal Hb levels from baseline to follow-up (typically measured in the third trimester or at delivery), providing a direct assessment of the effect LDA on maternal haematological status.
- Incidence of anaemia during pregnancy, defined as Hb levels below 11.0 g/dL in the first and third trimesters, and below 10.5 g/dL in the second trimester, in accordance with Word health Organization (WHO) criteria (29).

Secondary outcomes:

- Development of HDP, including PE (defined as new-onset hypertension and proteinuria after 20 weeks of gestation) and gestational hypertension.
- Gestational age at delivery (measured in completed weeks).
- Fetal growth (assessed via ultrasound or birth weight percentiles).
- Birth weight.
- Maternal morbidity (e.g., need for transfusion or hospitalisation).

Outcome assessment: Method of Hb measurement, anaemia definitions used, timing of outcome measurement, and diagnostic criteria for PE or fetal growth restriction. Studies must clearly report the time points and methods of outcome assessment. These may include complete blood count results for Hb, ultrasound findings for fetal growth, clinical records for gestational age and delivery, and standard diagnostic tools for PE and anaemia.

Other variables: Source of study funding and any declared conflicts of interest, where applicable.

Pre-planned data assumptions and simplifications

For this systematic review protocol, we assume that variability in the definition of LDA will be addressed by categorizing dosages, with a focus on those ranging from 75 to 162 mg. We will document differences in Hb measurement techniques to account for variability across studies. Potential confounders, such as maternal nutrition, iron supplement use, and other medications, will be noted to understand their influence on Hb levels. Consistency in Hb measurement methods and definitions of anaemia will be crucial for effective data synthesis. We anticipate that variations in aspirin dosage and timing of administration may impact the generalisability of the results. Additionally, we will evaluate and discuss potential biases related to funding sources, particularly from pharmaceutical companies, especially if these studies present significantly different outcomes.

Risk of bias in individual studies

Risk of bias in individual studies will be assessed using validated tools appropriate for each study design:

- For randomised controlled trials, the Cochrane Risk of Bias 2.0 (RoB 2) tool will be used. This tool evaluates five domains: the randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result (30). Each domain will be rated as having low risk, some concerns, or high risk of bias, in accordance with the RoB 2.0 guidance.
- For observational studies, the Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) tool will be applied. This tool assesses bias due to confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, outcome measurement, and selection of the reported result (31). Each domain will be rated as low, moderate, serious, or critical risk of bias, or marked as having no information, based on ROBINS-I criteria.

Two reviewers (NN and VD) will independently evaluate the risk of bias for each included study. Conflicting judgments will be reconciled through discussion, and if consensus is not reached, a third reviewer (CB) will be consulted to make the final decision. This structured approach ensures a transparent and standardised evaluation of study quality across both randomised and non-randomised designs.

Effect measure

Effect estimates will be calculated separately for each outcome. For continuous outcomes (e.g., change in Hb levels, birth weight, or fetal biometric measurements such as head circumference or femur length), either the mean difference (MD) or the standardised mean difference (SMD) will be used:

- Mean difference will be applied when outcomes are reported using the same scale across studies (e.g., g/dL for Hb).
- Standardised mean difference will be used when studies assess the same outcome but report it using different measurement scales.

For dichotomous outcomes (e.g., incidence of anaemia or PE), we will calculate risk ratios (RR) or odds ratios (OR) with 95% confidence intervals (CIs). Statistical heterogeneity will be assessed using the I^2 statistic, and pooled effect estimates will be presented using forest plots.

Data synthesis and Analysis

A meta-analysis will be performed where studies are sufficiently similar in design, population, interventions, and outcomes. Randomised controlled trials and observational studies will be analysed separately to preserve methodological consistency. Pooled effect sizes will be calculated using a random-effects model in MetaXL®, which accounts for potential between-study variability.

The degree of heterogeneity will be evaluated using the I^2 statistic, with values exceeding 50% interpreted as reflecting considerable variability among studies. In cases where heterogeneity is low ($I^2 \leq 50\%$), a fixed-effect model may be considered, depending on clinical judgement.

If studies are not suitable for quantitative pooling (e.g., due to heterogeneity in outcome reporting or methodological differences), a narrative synthesis will be conducted. This synthesis will be structured around study design, population characteristics, intervention details, and the direction and magnitude of effect estimates.

Where feasible, subgroup analyses will be conducted based on the following variables:

- Study design (RCT vs. observational)
- Aspirin dose and timing of initiation
- Baseline anaemia status
- Geographic region or healthcare setting

Sensitivity analyses will be conducted to evaluate the robustness of the pooled results. This will involve excluding studies with high risk of bias, small sample sizes, or unclear reporting (32).

Meta-bias Assessment

To assess potential publication bias, the Doi plot and Luis Furuya-Kanamori (LFK) index will be used (33). These methods have demonstrated improved sensitivity over conventional funnel plots, particularly when fewer than 10 studies are included. A symmetrical Doi plot and an LFK index between –1 and +1 will be interpreted as indicating no major publication bias or small-study effects.

Confidence in cumulative evidence

The certainty of evidence for each key outcome will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. This method evaluates five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias. Certainty ratings will be classified as high, moderate, low, or very low, and results will be presented in Summary of Findings (SoF) tables generated using GRADEpro GDT.

Primary outcomes (change in Hb levels, incidence of anaemia) and key secondary outcomes (e.g., PE, birth weight) will be graded. Randomised controlled trials without significant limitations are expected to contribute higher-certainty evidence, while observational studies will generally begin at a lower certainty level due to potential confounding and risk of bias (34).

Discussion

There is a noticeable gap in research focusing on the impact of LDA on Hb levels in pregnant women, as most studies have concentrated on its efficacy in reducing PE. It is crucial to conduct active research in this vulnerable population, given the higher prevalence of anaemia during pregnancy in LMIC's. The WHO aims to halve the rate of anaemia amongst women of childbearing age within the period from 2010 to 2025. Examining how Hb levels fluctuate with

aspirin usage and assessing the risk of developing anaemia indicated by changes in Hb levels might help to achieve this goal. Understanding these fluctuations is vital to prevent adverse pregnancy outcomes related to such changes, offering valuable insights into managing anaemia. This understanding could also highlight the need for frequent monitoring of Hb levels during pregnancy to prevent anaemia and improve pregnancy outcomes. The insights gained from this research may be used to inform in the development of health policies and practises that positively impact maternal and fetal health outcomes.

Abbreviations and Acronyms

LDA: Low-dose aspirin

Hb: Haemoglobin

PE: Preeclampsia

IUGR: Intrauterine Growth Restriction

PPH: Postpartum Haemorrhage

WHO: World Health Organization

MD: Mean Difference

SMD: Standardized Mean Difference

RR: Risk Ratio

OR: Odds Ratio

LMICs: Low to middle-income countries

Registration

The details about the registration of this protocol, including the registry name (Nokwethemba Ngcobo) and PROSPERO registration number (CRD42024556220).

Amendments

Any necessary amendments to this protocol will be documented independently, including the date, nature of the change, and rationale behind it.

Support

Not applicable

Authors' Contributions

NN and VD conceptualized the study and wrote the draft protocol with input from CB. All three authors contributed to the development of the background, planned output of the research, as well as the study design.

Declaration

I hereby declare that the systematic review protocol titled 'Investigating the impact of low dose aspirin on haemoglobin levels during pregnancy: a protocol for a systematic review and meta-analysis' is my original work and has been developed in accordance with the PRISMA-P guidelines for systematic reviews. This protocol has not been previously published or submitted for publication elsewhere. All sources of information used in the development of this protocol have been properly acknowledged.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data collected or analysed during this study will be included in the published systematic review article and will be made available upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

This protocol for a systematic review is part of a larger study investigating the impact of LDA on Hb levels during pregnancy, which is funded by the University of KwaZulu- Natal (UKZN) College of Health Sciences (CHS) scholarship. VD is funded by the National Research Foundation Thuthuka Grant (TTK170508230162) in collaboration with the University of KwaZulu-Natal and Medical Research Council of South Africa (SIR Grant UNS14197). The institutions listed have no vested interest in the study and is not involved in the protocol design, analysis plan, data collection or analyses. Therefore, there will be no input from this institution in the interpretation and publication of the results.

Acknowledgments

The primary investigator would like to express gratitude to Dr Vinogrin Dorsamy and Dr Chauntelle Bagwandeen for their unwavering support and guidance in the development of this protocol.

Appendix A (Table 2): Search strategy piloted in PubMed

Database used	Search terms	Articles found
PubMed	(("Aspirin"[MeSH Terms] OR "low-dose aspirin" OR "acetylsalicylic acid") AND ("Anemia"[MeSH Terms] OR "haemoglobin" OR "hemoglobin" OR "blood iron levels" OR "iron deficiency"[MeSH Terms]) AND ("Pregnancy"[MeSH Terms] OR "pregnant women" OR "maternal" OR "pregnancy complications"[MeSH Terms] OR "hypertensive disorder of pregnancy"[MeSH Terms] OR "preeclampsia"[MeSH Terms] OR "pregnancy outcomes"[MeSH Terms] OR "obstetric hemorrhage"[MeSH Terms] OR "postpartum hemorrhage"[MeSH Terms]))	1 608

Appendix B (Table 3): Data charting table

Study Identification
Authors
Year of publication
Title of the study
Journal name
Study Characteristics
Study design
Sample size
Study location
Duration of follow-up
Participant Characteristics
Population
Inclusion and exclusion criteria
Gestational age at the start of aspirin usage
Demographics
Intervention Details
Dosage of LDA
Duration of aspirin use
Comparison group details
Outcomes Measured
Haemoglobin levels (baseline and follow-up)
Incidence of anaemia
Changes in haemoglobin levels over time
Pregnancy Outcomes
Incidence of preeclampsia
Fetal growth rate
Birth weight
Other relevant outcomes (e.g., preterm birth, stillbirth)
Results
Main findings related to haemoglobin levels
Main findings related to anaemia
Associations between aspirin usage and pregnancy outcomes

Statistical significance and effect sizes
Risk of Bias and Quality Assessment
Methodological quality of the studies
Confounding factors considered

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CHAPTER THREE: A NARRATIVE REVIEW OF THE LITERATURE

This chapter presents a comprehensive review of the literature exploring the mechanisms through which low-dose aspirin may influence haemoglobin levels, potentially contributing to anaemia, and its effects on maternal health and birth outcomes. The review has been summarised and formatted according to the submission guidelines for South African Family Practice.

LOW-DOSE ASPIRIN AND ANAEMIA RISK IN PREGNANCY: A NARRATIVE REVIEW WITH EMPHASIS ON LOW TO MIDDLE-INCOME CONTEXTS

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In Review: South African Family Practice

Manuscript ID:6195

Abstract

Background: Anaemia remains a major public health concern during pregnancy, as it is associated with increased risks of adverse outcomes such as preterm birth, low birth weight, and maternal morbidity. Low-dose aspirin is commonly prescribed to reduce the risk of preeclampsia and other hypertensive disorders of pregnancy. However, its potential impact on haemoglobin levels—a critical marker of anaemia—is not yet fully understood.

Methods: A non-systematic literature search was conducted using PubMed, Google Scholar, Scopus, Web of Science, and the Cochrane Library. Articles published up to April 2025 were considered, using search terms including "low-dose aspirin," "preeclampsia," "maternal anaemia," and "haemoglobin." Both interventional and observational studies, as well as systematic reviews and meta-analyses, were included.

Results: This review identifies multiple mechanisms through which low-dose aspirin may influence Hb levels, including anti-inflammatory effects, modulation of endothelial function, changes in iron absorption and metabolism, and gastrointestinal blood loss. Evidence from both pregnant and non-pregnant populations suggests possible benefits on haemoglobin levels, although some studies report potential risks, particularly in settings with a high burden of infection. Current evidence during pregnancy is limited and inconclusive.

Conclusion: The mechanisms by which low-dose aspirin may affect haemoglobin levels—such as gastrointestinal blood loss, altered iron absorption, and anti-inflammatory modulation of hepcidin—are biologically plausible but not yet conclusively demonstrated in pregnancy.

Contribution: Understanding the association between low-dose aspirin and maternal haemoglobin levels is essential for guiding the safe use of low-dose aspirin during pregnancy, particularly in low to middle-income countries where anaemia is highly prevalent.

Key words: Low-dose aspirin, haemoglobin, anaemia, preeclampsia, hypertensive disorders of pregnancy, gastrointestinal bleeding, obstetric haemorrhage.

Introduction

Low-dose aspirin (LDA) is increasingly being recognized for its role in the prevention of preeclampsia (PE), a hypertensive disorder of pregnancy (HDP). Globally, HDPs continue to cast a long and persistent shadow over maternal and perinatal health, accounting for a significant proportion of obstetric morbidity and mortality (1,2). Low to middle-income countries (LMICs), bear the heaviest brunt of this burden, since health systems often lack capacity, and late antenatal booking remains common (3,4). South Africa (SA) is no exception, for nearly one in five maternal deaths is attributed to HDP (5). In recent years, the use of LDA has become a more widely adopted strategy to interrupt this trajectory, offering a simple yet powerful intervention to prevent PE if prescribed at the appropriate gestational age (6,7).

Preeclampsia is a multifaceted syndrome characterised by new-onset hypertension with organ dysfunction after 20 weeks' gestation. Its origins lie in defective placentation, poor spiral artery remodelling, and systemic endothelial dysfunction—pathways that collectively compromise maternal and fetal well-being (8,9). Low-dose aspirin works primarily by irreversibly inhibiting platelet cyclooxygenase-1 (COX-1), suppressing thromboxane A_2 (TXA2)—a potent vasoconstrictor and pro-thrombotic agent—thereby restoring the delicate vascular balance needed to ensure healthy placental perfusion (10,11).

Alongside the burden of HDP is the global crisis of anaemia in pregnancy, which further erodes quality maternal health in LMICs. Defined by the World Health Organization (WHO) as haemoglobin (Hb) below 11 g/dL, anaemia affects over 35% of pregnant women globally (12). In many low-resource settings, it is driven by a complex web of causation, consisting of nutritional deficiencies, chronic inflammation, and structural barriers to care (13,14). Anaemia increases the risk of preterm birth, low birth weight, and perinatal mortality, and its consequences—though often less visible than those of PE—are no less profound (15,16).

Haemoglobin concentrations in pregnancy follow a non-linear pattern of risk (17). Both low and high levels are associated with adverse outcomes: anaemia reduces oxygen delivery to the fetus, while elevated Hb may signal inadequate plasma volume expansion or iron overload, increasing the risk of placental dysfunction and hypertension (18,19).

These conditions—PE and anaemia—do not operate in isolation. Instead, they often coexist in a physiological tug-of-war. Preeclampsia can suppress red cell production through systemic inflammation and oxidative stress, while anaemia exacerbates placental hypoxia and maternal cardiovascular strain (20–22). This is particularly relevant in LMICs, where coexisting burdens

such as Human Immunodeficiency Virus (HIV), tuberculosis, malaria, and obesity intersect with sub-optimal maternal nutrition, further complicating management.

As LDA becomes embedded into global antenatal guidelines, the yet to be resolved question is whether it might influence Hb levels—especially in anaemia-prone populations. While LDA's vascular benefits are well-established, emerging concerns include its potential to cause subclinical gastrointestinal bleeding, interfere with iron absorption, or modulate inflammatory pathways that affect erythropoiesis.

This narrative review synthesizes current evidence on the relationship between LDA, Hb levels, and anaemia risk in pregnancy, with a particular focus on LMICs. Drawing on mechanistic and clinical data, we explore how aspirin may affect iron metabolism and red cell parameters, identify key knowledge gaps, and highlight implications for antenatal care in resource-limited settings—particularly where the dual burden of HDP and anaemia necessitates careful risk–benefit evaluation, yet the benefits of LDA should not be overlooked.

Methods

Study design

This article presents a narrative review of the literature on the association between LDA use and anaemia risk, with a particular focus on pregnancy and PE risk in LMICs. A narrative approach was selected to integrate findings across mechanistic, clinical, and contextual domains, as well as to identify gaps in the existing research.

Literature search strategy

A comprehensive literature search was conducted across five major biomedical databases, including PubMed, Google Scholar, Scopus, Web of Science, and the Cochrane Library. The search included articles published up to April 2025. No language or study design restrictions were applied to maximise inclusion of relevant evidence. Search terms included combinations of Medical Subject Headings (MeSH) and free-text keywords, such as:

- “low-dose aspirin”
- “preeclampsia”
- “maternal anaemia”
- “haemoglobin”
- “pregnancy”
- “low to middle-income countries”

Boolean operators (AND, OR) were used to refine the search and capture relevant studies. Reference lists of included articles were also screened to identify additional pertinent publications.

Inclusion and exclusion criteria

Inclusion criteria

- Studies reporting on the effects of LDA on anaemia or Hb levels in women (pregnant and non-pregnant).
- Research conducted in LMICs or in populations with similar sociodemographic characteristics.
- Both interventional and observational studies, as well as systematic reviews and meta-analyses.

Exclusion criteria

- Studies not involving human participants.
- Editorials, commentaries, or opinion pieces.
- Studies lacking adequate data on anaemia or Hb-related outcomes.

Data extraction and synthesis

Data were extracted on study design, population characteristics, LDA dosage and timing, and relevant outcomes related to anaemia or Hb concentrations. Where reported, contextual factors such as HIV status, nutritional factors, co-existing infections, and genetic influences were noted.

Given the heterogeneity in study populations, interventions, and outcomes, findings were synthesized narratively rather than through formal meta-analysis. Evidence was grouped thematically into mechanistic pathways, clinical outcomes, and contextual factors relevant to antenatal care in LMICs.

Physiological, Pharmacological, and Contextual Pathways

This section synthesizes the literature on LDA use and anaemia risk in pregnancy, beginning with physiological changes in Hb and iron metabolism during gestation. It then examines the pharmacological effects of LDA and the plausible biological pathways linking its use to maternal anaemia. Finally, it considers contextual factors—such as HIV status, nutritional deficiencies, co-existing infections, and genetic influences—that may modify susceptibility to anaemia.

Physiological changes in Hb and iron metabolism during pregnancy

Pregnancy is marked by profound haematological adaptations that support fetal growth, maternal metabolism, and placental function. One of the most prominent changes is a progressive expansion of plasma volume, which begins early in gestation and peaks in the second trimester. Although red blood cell (RBC) mass also increases, the disproportionate rise in plasma volume leads to a physiological haemodilution, resulting in low Hb concentrations (23). This dilutional anaemia is considered a normal adaptation, enhancing placental perfusion, and reducing blood viscosity, but it complicates the diagnosis of true iron deficiency anaemia (IDA). Recent studies have suggested that traditional diagnostics thresholds may underestimate the prevalence of anaemia when these physiological changes are not accounted for, prompting calls for context specific reference ranges in pregnancy (24).

Iron plays a central role in erythropoiesis and is essential for maternal, fetal and placental development. As gestation progresses, maternal iron requirements increase substantially, particularly in the second and third trimesters, to support expanded maternal blood volume, fetal demands, and placental iron demands (25). To accommodate this, maternal physiology adapts through suppression of hepcidin, the liver-derived hormone that regulates systemic iron homeostasis (26). Suppressed hepcidin levels enhance intestinal iron absorption and promote the mobilisation of iron from stores, especially in women with low baseline iron status (27,28).

However, the relationship between iron status and pregnancy outcomes is complex and nonlinear. A growing body of evidence suggests a U-shaped association: both iron deficiency and iron overload are linked to adverse outcomes for mother and fetus (29). Iron-deficiency anaemia is associated with impaired oxygen delivery, placental hypoxia, and increased risk of preterm birth and low birth weight (30). Conversely, excessive iron supplementation or dysregulated iron metabolism can lead to oxidative stress, increased blood viscosity, and reduced uteroplacental perfusion, all of which can exacerbate PE risk (19).

These physiological dynamics highlight the need for careful assessment and individualised management of iron status during pregnancy. Over-reliance on single Hb thresholds without considering plasma volume changes, inflammation, or underlying comorbidities may lead to misclassification and inappropriate interventions. In contexts where iron deficiency is prevalent, but inflammation and infection are also common, as in many LMICs, accurate interpretation of iron biomarkers remains challenging but essential.

Pharmacological effects of LDA and potential mechanisms linking LDA to anaemia in pregnancy

Potential mechanisms by which LDA may influence anaemia

Although the potential benefits of LDA in preventing PE are well established, its influence on maternal Hb concentrations and iron homeostasis remains incompletely understood (31,32). Building on the concepts introduced earlier, this section explores specific biological pathways through which LDA may plausibly affect iron status—either adversely or beneficially—particularly in anaemia-prone populations.

1. Aspirin and iron chelation

A speculative but emerging hypothesis is that aspirin may act as a mild iron chelator. Salicylates have been shown to form iron-binding complexes in the gastrointestinal tract, potentially reducing the bioavailability of dietary or supplemental iron (33,34) . While clinical data in pregnant populations are lacking, this mechanism may be relevant in LMIC contexts where baseline iron stores are often marginal. Chronic interference with iron absorption, even at a subclinical level, could contribute to cumulative iron deficiency.

2. Gastrointestinal blood loss and microbleeds

Aspirin's ability to compromise gastrointestinal (GI) mucosal integrity is well recognised, even at low doses. Micro-erosions or subclinical GI bleeding—while rarely causing overt haemorrhage—may lead to insidious iron loss over time (35). This is particularly concerning in women with limited nutritional reserves or pre-existing anaemia. A rare but severe case of upper GI bleeding in a pregnant woman on LDA was recently reported by Plancha *et al*. (2024), exhibiting the potential for clinically significant events (36). Moreover, data from a large Swedish cohort demonstrated an increased risk of postpartum bleeding (adjusted OR 1.23) and haematoma formation in aspirin users, although causality remains uncertain (37). Postpartum hematomas typically occur in the vulvar, vaginal, or pelvic regions; however, specific sites were not detailed in that study (38,39).

3. Risk of obstetric haemorrhage

Several observational studies have raised concerns about an elevated risk of obstetric haemorrhage associated with LDA, particularly around the time of delivery (40,41). However, findings from large randomised controlled trials (RCTs) ad systematic reviews, including the Aspirin for Evidence-Based PE Prevention (ASPRE) trial and Cochrane analyses, generally do not support a significant increase in clinically meaningful bleeding events (7,42,43).

Nonetheless, in low-resource settings where anaemia is common and blood transfusion services may be limited, even a modest rise in bleeding risk could have serious implications for maternal health.

4. Inflammation, hepcidin and erythropoiesis

Conversely, LDA may have protective effects on iron metabolism through its anti-inflammatory properties. Chronic inflammation—common in PE, HIV, tuberculosis, and other LMIC-relevant comorbidities—disrupts erythropoiesis by stimulating hepatic hepcidin production, which limits iron absorption and mobilisation (44,45). Low-dose aspirin has been shown to reduce systemic inflammation, including levels of interleukin-6 (IL-6), a key driver of hepcidin synthesis. In a recent study by Xiao *et al.* (2023), LDA administration downregulated placental expression of Activating Transcription Factor 2 (ATF2) and its pro-inflammatory gene targets (IL-6, IL-8, MMP-2), potentially facilitating improved iron availability (46). Additionally, LDA promotes the production of 15-epi-lipoxin A4, a lipid mediator involved in resolving inflammation and preserving endothelial function (47). These pathways may support red cell production and mitigate anaemia of chronic disease.

Taken together, these mechanisms illustrate the dual potential of aspirin to either exacerbate or alleviate anaemia, depending on underlying physiological and environmental contexts. A closer examination of how endothelial health and placental perfusion interact with iron regulation may help clarify these divergent effects.

5. Endothelial Function, Placental Perfusion, and Iron Homeostasis

Endothelial dysfunction lies at the heart of PE pathophysiology, contributing to vasoconstriction, inflammation, and impaired uteroplacental blood flow (48). In the normal pregnancy state, vascular adaptation involves a shift in the balance between TXA2 and prostacyclin toward increased prostacyclin activity, leading to reduced platelet aggregation and enhanced vascular perfusion. Preeclampsia disrupts this equilibrium, resulting in widespread endothelial activation and a pro-thrombotic state (9).

Low-dose aspirin restores vascular balance by irreversibly inhibiting cyclooxygenase-1 in platelets, reducing TXA2 production while preserving endothelial prostacyclin synthesis (11). This action supports vasodilation and helps to maintain placental perfusion, particularly when LDA is initiated before 16 weeks' gestation—during the critical window for spiral artery remodelling (7).

Importantly, impaired endothelial function and reduced placental perfusion not only increase the risk of hypertension and fetal growth restriction but also have downstream effects on iron metabolism. Placental hypoxia triggers oxidative stress, activates hepcidin, and disrupts the mobilisation of iron to the fetal compartment (27). Moreover, endothelial dysfunction is associated with increased circulating levels of inflammatory cytokines, including IL-6, which further amplify hepcidin production and suppress erythropoiesis (49,50).

By improving endothelial health and reducing systemic inflammation, LDA may indirectly improve iron availability and mitigate anaemia of inflammation. These vascular effects may be especially relevant in high-risk populations where comorbid infections or chronic inflammation are common.

The degree to which these physiological benefits are realised, however, depends heavily on population-level factors such as nutritional status, infection burden, and the timing of aspirin initiation—all of which vary across LMICs.

Population-specific factors influencing LDA, anaemia, and pregnancy outcomes in LMICs

In LMICs, the interaction between LDA, anaemia, and HDP is shaped by a unique and often complex set of contextual factors. These include a high burden of infectious diseases, nutritional deficiencies, sociodemographic inequalities, and limited health system capacity—all of which can influence both the safety and efficacy of LDA in pregnancy (51–53).

Infectious Diseases: HIV, Tuberculosis, and Malaria

Chronic infections such as HIV, tuberculosis, and malaria remain prevalent in many LMICs and have direct effects on both iron metabolism and vascular health (50,54). In HIV, systemic inflammation, and antiretroviral therapy (ART) contribute to functional iron deficiency and anaemia of chronic disease. Chronic inflammation disrupts iron metabolism via hepcidin dysregulation, while ART may exacerbate anaemia through bone marrow suppression or mitochondrial toxicity (55). However, evidence for altered aspirin metabolism in HIV is limited, though aspirin's antiplatelet effects may be modified by HIV-associated immune activation (56,57). Tuberculosis induces a pro-inflammatory state and is associated with suppressed erythropoiesis, while malaria disrupts red blood cell turnover and increases iron demand through haemolysis (58,). In malaria-endemic regions like the Democratic Republic of Congo, malaria infection modified the impact of LDA on pregnancy outcomes, particularly perinatal mortality suggesting that infectious disease influences aspirin's efficacy during

pregnancy (59). In such settings, the inflammatory environment may blunt LDA's vascular benefits or shift its haematologic impact in unpredictable directions.

Nutrition and Obesity: A Dual Burden

Although nutritional inadequacy—particularly iron, folate, and vitamin B12 deficiency—remains a dominant contributor to maternal anaemia in LMICs, at the same time, rising rates of obesity, especially in urban centres, contributes to a concurrent and dual burden of morbidity (60,61). Obesity is associated with chronic low-grade inflammation, insulin resistance, and altered lipid metabolism, all of which may interfere with iron absorption and increase hepcidin levels (62). It can alter the pharmacokinetics and efficacy of LDA (63,64).

Obese pregnant women may also have a heightened baseline risk for PE, further complicating the interpretation of Hb changes in the context of LDA use (65).

Genetic Factors: Sickle Cell Trait, Thalassaemia, and G6PD Deficiency

Inherited haematologic conditions such as sickle cell trait, thalassaemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency are prevalent in parts of sub-Saharan Africa, the Indian subcontinent, and Southeast Asia (66,67). These conditions may alter baseline Hb levels, increase haemolysis, and affect responses to aspirin or iron supplementation. For example, individuals with G6PD deficiency may be more susceptible to oxidative stress, and theoretical concerns exist around the use of non-steroidal anti-inflammatory drugs in this context—though evidence specific to LDA remains limited (68).

Socioeconomic and Health System Considerations

Late antenatal booking, inconsistent iron supplementation, and poor continuity of care are common challenges in LMIC health systems (69). These structural barriers may delay the initiation of LDA, limit monitoring of Hb trends or reduce opportunities to tailor iron therapy based on individual risk. Furthermore, stockouts of iron supplements or aspirin, poor adherence due to side effects, and limited diagnostic capacity for anaemia subtyping can all influence maternal outcomes in subtle but significant ways (70).

Understanding these contextual layers is essential for interpreting clinical evidence on LDA use in pregnancy. Without consideration of population-specific variables, studies conducted in high-income settings may yield conclusions that are poorly generalisable to LMICs—underscoring the need for contextually grounded research and locally relevant guidelines.

As we turn to the literature, these contextual considerations offer an important lens through which to evaluate the evidence base on aspirin, anaemia, and pregnancy outcomes.

Clinical Evidence: LDA and Hb in Pregnancy

While LDA is well established as an effective intervention to prevent PE, few studies have directly examined its impact on Hb levels or anaemia risk in pregnancy. This gap in the evidence base is particularly relevant for settings where anaemia is common and multiple contextual factors—such as comorbid infection, nutritional deficiency, or limited antenatal care—may modify physiological responses to aspirin.

Evidence from RCTs

Several large RCTs have evaluated LDA in high-risk pregnancies, although haematologic endpoints were infrequently reported:

- The ASPRE trial, which administered 150 mg of aspirin nightly from 11–14 weeks until 36 weeks' gestation, demonstrated a significant reduction in preterm PE but did not systematically assess Hb or iron parameters (7).
- The American College of Obstetricians and Gynecologists (ACOG) observed no significant increase in maternal haemorrhagic complications associated with LDA use and recommends 81 mg daily for PE prophylaxis when initiated between 12 and 28 weeks of gestation (6). Other LDA-related hypertension prevention meta-analyses and systematic reviews also did not report anaemia outcomes (71,72).
- A recent Cochrane review and the US Preventive Services Task Force (USPSTF) meta-analysis confirmed the safety and efficacy of LDA in reducing PE risk, without finding a statistically significant increase in maternal bleeding. However, neither the review explored Hb levels or anaemia as outcomes (38,39).

In the Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes (JPAD) trial conducted in a non-pregnant population with type 2 diabetes, long-term LDA use was associated with an increased risk of GI bleeding which may contribute to a slight reduction in Hb levels over time, attributed to chronic GI blood loss (73). Although not directly transferable to pregnancy, such findings raise concerns about subtle, cumulative effects on iron status with prolonged LDA use.

Observational and Real-World Evidence

Evidence from observational studies is more varied. In a retrospective study from Sweden, LDA was associated with a modest increase in postpartum bleeding and haematoma formation (35). However, the clinical significance in terms of antenatal anaemia remains unclear.

A more contextually relevant South African secondary analysis of an RCT found no significant difference in overall mean Hb levels between aspirin and control group (74). This aligns with the findings from a secondary analysis of the ASPIRIN trial (75). However, amongst women who experienced a decline in Hb during pregnancy, those in the aspirin group had a less pronounced reduction, suggesting a potential protective effect. Notably, this study accounted for HIV status and body mass index (BMI), neither of which significantly influenced Hb outcomes. Limitations included a small sample size and the absence of nutritional status assessment, both of which could confound the results.

Overall, real-world findings suggests that while LDA may not substantially alter average Hb levels in pregnancy, it may attenuate Hb decline in specific subgroups. Few studies have stratified outcomes by modifiers such as HIV, obesity, timing of LDA initiation, or iron status—factors particularly relevant in LMICs.

Studies in Non-Pregnant Populations

Evidence from non-pregnant cohorts suggests a potential link between long-term LDA use and mild reductions in Hb. These findings are most commonly observed in older adults or individuals with comorbidities such as diabetes or cardiovascular disease, where cumulative GI losses are more likely (76). While such data offer mechanistic plausibility, their applicability to pregnancy—characterised by unique physiological changes—remains limited. Additionally, several studies in non-pregnant populations have consistently demonstrated an association between chronic aspirin use and an increased risk of major gastrointestinal and cerebral bleeding complications (77,78).

There is a clear need for prospective studies that are powered and designed specifically to evaluate the effects of LDA on haematologic indices in pregnancy, particularly in LMIC settings where baseline anaemia is prevalent and health system constraints may amplify even modest risks.

Knowledge Gaps and Research Priorities

Although LDA is now widely recommended for the prevention of PE, its effects on Hb and iron status in pregnancy remain poorly understood. The current evidence base is limited by the absence of haematologic outcomes in most clinical trials, underrepresentation of LMICs, and insufficient consideration of individual-level risk factors that may influence response to LDA.

Several critical knowledge gaps remain:

- Lack of haematologic endpoints in major LDA trials: most studies assessing the efficacy of LDA for PE prevention do not include Hb concentration, iron status, or anaemia classification as predefined outcomes.
- Insufficient data from LMICs: given the high prevalence of anaemia, undernutrition, and infectious comorbidities in LMICs, the effects of LDA on haematologic parameters may differ substantially from those observed in high-income settings. Yet very few studies originate from or focus on these populations.
- Limited stratification by comorbidities: Conditions such as HIV, tuberculosis, malaria, obesity, and inherited haemoglobinopathies are common in LMICs and may significantly alter iron metabolism and aspirin pharmacodynamics. These variables are rarely accounted for in current studies.
- Unclear role of LDA timing, dose, and duration: while early initiation of LDA improves vascular outcomes, it remains uncertain whether timing or cumulative exposure modifies its effect on Hb or iron balance.
- Lack of mechanistic studies in pregnancy: much of the current understanding of aspirin's effects on iron status is derived from non-pregnant populations or in vitro models. Studies specifically addressing inflammation, hepcidin, gastrointestinal losses, and iron absorption in pregnancy are urgently needed.
- Poor integration with anaemia classification and management: research seldom differentiates between iron deficiency anaemia, anaemia of inflammation, or mixed aetiologies—each of which may interact differently with LDA exposure and require distinct management strategies.

Future studies should prioritise context-sensitive designs that reflect real-world antenatal care in LMICs, include haematologic endpoints, and apply stratified analyses based on nutritional status, comorbidity profiles, and gestational age at LDA initiation. In parallel, mechanistic research is needed to clarify how LDA interacts with maternal iron metabolism during pregnancy.

Developing such evidence will be critical for tailoring guidelines to diverse populations and ensuring that the benefits of LDA are not undermined by unanticipated haematologic consequences.

Conclusion

Low-dose aspirin has transformed the landscape of PE prevention and is increasingly recommended in antenatal guidelines worldwide. Robust evidence from RCTs and meta-analyses has demonstrated its efficacy in reducing early-onset PE, with no consistent signal of serious bleeding or haematologic harm in well-nourished populations.

However, the extent to which these findings apply to LMICs where anaemia is highly prevalent and health system constraints may limit individualised care—remains unclear. The mechanisms by which LDA could influence iron status, including gastrointestinal blood loss, altered iron absorption, and anti-inflammatory modulation of hepcidin, are biologically plausible but not yet conclusively demonstrated in pregnancy.

Given the well-established efficacy of LDA in reducing PE risk, its continued use is strongly supported in appropriate clinical settings. However, in populations where anaemia is common and comorbid conditions like HIV or tuberculosis are prevalent, there is a need for greater vigilance, routine iron status monitoring, and tailored supplementation.

Targeted research—including mechanistic studies and haematologically focused trials in LMICs—is essential to ensure that the full benefits of LDA are realised while monitoring for potential haematologic impacts in vulnerable populations.

Acknowledgements

Competing interest

The authors declare that they have no financial or personal relationships that could have inappropriately influenced the writing of this article.

Authors' contribution

N.N. and V.D. contributed to the conceptualization and writing of the manuscript. C.B. was responsible for reviewing and providing critical feedback. All authors read and approved the final manuscript.

Ethical consideration

This article adheres to ethical standards for research involving no direct contact with human or animal subjects.

Funding information

This narrative review forms part of a broader research project examining the effects of low-dose aspirin on haemoglobin levels during pregnancy. The study is supported by the National Research Foundation through the Thuthuka Grant (TTK170508230162), in collaboration with the University of KwaZulu-Natal and the South African Medical Research Council (SIR Grant UNS14197). The funding bodies were not involved in the analysis, interpretation of findings, or the preparation of this manuscript.

Data availability

Data sharing is not applicable to this article, as no new data were created or analysed.

Disclaimer

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CHAPTER FOUR: THE IMPACT OF LOW-DOSE ASPIRIN ON HAEMOGLOBIN LEVELS IN PREGNANCY: A SECONDARY ANALYSIS OF A RANDOMIZED CONTROLLED TRIAL FOR PREVENTION OF HYPERTENSIVE DISORDERS OF PREGNANCY.

This chapter presents a manuscript prepared according to the author guidelines of the International Journal of Gynecology & Obstetrics (IJGO), currently under peer review, and includes the aims, methodology, and results of a secondary analysis of a randomised controlled trial, which serves as the main research component of this thesis.

The study concluded that low-dose aspirin may have a protective effect against haemoglobin decline during pregnancy, in addition to its established role in reducing the risk of hypertensive disorders of pregnancy. This potential dual benefit may be particularly relevant in low to middle-income countries, where the burden of both anaemia and hypertensive disorders in pregnancy is high.

THE IMPACT OF LOW-DOSE ASPIRIN ON HAEMOGLOBIN LEVELS IN PREGNANCY: A SECONDARY ANALYSIS OF A RANDOMISED CONTROLLED TRIAL FOR PREVENTION OF HYPERTENSIVE DISORDERS OF PREGNANCY

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In Review: International Journal of Gynecology & Obstetrics

Manuscript number: IJG-D-25-02079

Abstract

Objectives: To evaluate the impact of low-dose aspirin on haemoglobin levels during pregnancy and investigate the association between changes in haemoglobin levels and pregnancy outcomes, including hypertensive disorders of pregnancy.

Methods: This secondary analysis of a randomised controlled trial comprised 249 pregnant women, who were attending antenatal clinic at a regional hospital in Durban, South Africa. Participants were randomised to receive either 162 mg of low-dose aspirin daily (intervention) or standard care (control). Haemoglobin levels were measured at enrolment and at birth. Data on maternal outcomes, including hypertensive disorders of pregnancy were analysed in relation to changes in haemoglobin levels to assess potential associations.

Results: At enrolment, the majority of participants (51.4%) had mild anaemia (9.0-10.9g/dL) with no major differences between the intervention (45.6%) and control (57.3%) groups. By birth, 43.4% remained mildly anaemic, while 44.2% had normal haemoglobin levels (11–13 g/dL). Amongst participants who experienced a decline in haemoglobin, the reduction was significantly mitigated in the intervention compared to the control group ($\Delta Hb = -0.902$ vs. -1.422, $p = 0.002$, $\eta^2 = 0.088$). Declining haemoglobin levels increased the odds of developing hypertensive disorders of pregnancy compared to increasing haemoglobin (OR = 2.21 vs 0.45, $p = 0.021$). However, the intervention group within this subgroup had 76% reduction in risk of developing hypertensive disorders of pregnancy (RR = 0.24, 95% CI [0.11–0.53], $p < 0.001$), compared to the control group.

Conclusion: Low-dose aspirin attenuated the decline in haemoglobin levels, suggesting a novel haematological benefit beyond preeclampsia prevention. A reduction in haemoglobin levels may serve as an early predictor of hypertensive disorders of pregnancy.

Key words: Low-dose aspirin, haemoglobin, anaemia, preeclampsia, hypertensive disorders of pregnancy.

Introduction

Low-dose aspirin (LDA), initiated between 12 and 28 weeks of gestation, is recommended to prevent hypertensive disorders of pregnancy (HDP), which includes gestational hypertension, preeclampsia (PE), eclampsia - major contributors to adverse maternal and fetal outcomes (1). While LDA's role in reducing the risk of HDP is well supported, its potential effects on maternal haemoglobin (Hb) levels remains unclear. Anaemia during pregnancy, defined as Hb levels below 11 g/dL in the first or third trimester, is a significant global health issue, especially in low to middle-income countries (LMICs), with iron deficiency the most common underlying cause (2). In South Africa (SA), an estimated 31% of pregnant women are affected, largely due to poor nutrition, infections, and limited micronutrient intake (3).

There is growing recognition of a bidirectional relationship between anaemia and HPD; HDP-induced inflammation and oxidative stress may exacerbate anaemia, while anaemia-associated hypoxia may impair placental function, increasing the risk of hypertensive complications (4–6). This interplay highlights the need to assess whether LDA, while reducing HDP risk, might inadvertently compromise maternal Hb levels.

Low-dose aspirin remains a cost-effective and widely accessible intervention, particularly valuable in resource limited settings. However, its potential iron-chelating properties and association with gastrointestinal blood loss have raised safety concerns. For instance, Plancha *et al.* (2024) reported a rare case of severe gastrointestinal bleeding in a pregnant woman taking LDA for HDP prevention (7). Furthermore, aspirin's metabolites may bind iron, potentially reducing iron availability (8,9).

Understanding LDA's effect on Hb levels is essential, especially in sub-Saharan Africa (SSA) where both PE and maternal anaemia are highly prevalent (10–12). A prior randomised controlled trial (RCT) in a South African cohort of Black women reported a 75% reduction in HDP risk following LDA administration between 12–20 weeks gestation (13). However, the trial did not explore haematological outcomes. This secondary analysis aimed to address this gap by evaluating whether LDA influences Hb levels in pregnancy and how these changes correlated with HDP development. Clarifying this relationship could inform safer clinical practices and maternal health strategies in high-risk populations.

Methods

Study Design and setting: This secondary analysis is based on data from an RCT conducted at a regional hospital in Durban, SA, which evaluated the efficacy of LDA in preventing HDP (13). Pregnant women were randomly assigned to receive either LDA (intervention) until 36 weeks of gestation or standard care alone (control). Haemoglobin data for this analysis were extracted from hospital records and participants were selected from the RCT, which followed Consolidated Standards of Reporting Trials (CONSORT) guidelines (14). Haemoglobin levels were classified per WHO criteria: normal ($11 \leq Hb \leq 13$ g/dL), mild anaemia ($9 \leq Hb \leq 10.9$ g/dL), moderate anaemia ($7 \leq Hb \leq 8.9$ g/dL), and high (Hb>13g/dL) which we report descriptively as 'iron overload'; not a confirmed diagnosis (2).

Study Population: Normotensive Black pregnant women aged 18 years or older with singleton pregnancies between 12 and 20 weeks of gestation were recruited into the initial study. Further, confirmation of a fetal heartbeat and gestational age (GA) within the specified range was required. Exclusion criteria included multiple pregnancies, known fetal abnormalities, pre-existing hypertension, chronic diseases, prior aspirin use, or contraindications to aspirin.

Randomisation and Sample Size: Participants in the RCT (13) were randomised by coin toss with alternate assignment, ensuring relatively equal group sizes. For this study , a sample size of 249 was determined using G*Power 3.1 based on an effect size of 0.3 (small-to-medium), an alpha of 0.05, and 80% power (125 in the intervention group and 124 in the control group) (15).

Data Collection: Baseline data, including demographic, BMI (indicator of obesity), and Hb levels, were collected at enrolment as part of the original trial. Follow-up Hb levels at birth were retrieved from hospital maternity case records or National Health Laboratory Service (NHLS). Maternal outcomes, including HDP (defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria), gestational hypertension (new-onset hypertension after 20 weeks of pregnancy with organ involvement), PE (hypertension with proteinuria or organ dysfunction), and PE with severe features such as headache, nausea, vomiting, or eclampsia were collected (16).

Missing data: Of the 423 participants enrolled in the RCT, data were analysed for 249 participants. A total of 174 were excluded due to missing Hb measurements at birth. As the missing cases were evenly distributed between the intervention and control groups, a complete

case analysis was conducted, including only participants with available Hb data at birth. This approach helped reduce potential bias from missing data.

Data Analysis: Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 29 (IBM Corp, Armonk, NY, USA). Descriptive statistics including frequencies and percentages for categorical variables, were used to summarise baseline characteristics while mean ± standard deviation (SD) summarised changes in Hb levels. Repeated measures ANOVA was used to evaluate mean change in Hb levels (ΔHb) between enrolment and birth. This method allowed for the assessment of both within-subject changes in Hb over time and between-group differences (intervention vs. control), including their interaction. Subgroup analyses compared participants with declining versus increasing Hb levels. Logistic regression examined the association between ΔHb and HDP, with risk compared between intervention and control groups. The assumption of normality was assessed using the Shapiro Wilks test and visual inspection of Q-Q plots. A p-value < 0.05 was considered statistically significant. As the data were approximately normally distributed, parametric methods were applied.

Ethical Considerations: Ethical approval was obtained from the UKZN Biomedical Research Ethics Committee (BREC reference BREC/00007156/2024). Some participants declined to enrol in the RCT, but all other eligible women provided informed consent. Data was stored securely, and the secondary analysis did not require additional consent.

Results

A total of 249 participants were analysed, with 125 (50,2%) in the intervention group and 124 (49,8%) in the control group. Their sociodemographic characteristics and baseline characteristics including gravidity, employment, marital status, Human immunodeficiency (HIV) status and body mass index (BMI) were summarised in **Table 1**.

Table 1: Summary of sociodemographic profile and baseline characteristics

Variables	Control (N = 124)	Intervention (N = 125)	Total (N = 249)
Gravidity			
Primigravid	47 (37.9%)	37 (29.6%)	84 (33.7%)
Multigravid	77 (62.1%)	88 (70.4%)	165 (66.3%)
Employment			
Unemployed	87 (70.2%)	98 (78.4%)	185 (74.3%)
Employed	16 (12.9%)	17 (13.6%)	33 (13.3%)
Student	21 (16.9%)	10 (8.0%)	31 (12.4%)
Marital Status			
Single	101 (81.5%)	97 (77.6%)	198 (79.5%)
Married/Stable	23 (18.5%)	28 (22.4%)	51 (20.5%)
HIV Status			
Negative	70 (56.5%)	59 (47.2%)	129 (51.8%)
Positive	54 (43.5%)	66 (52.8%)	120 (48.2%)
BMI Group			
< 30 (Normal)	78 (62.9%)	69 (55.2%)	147 (59.0%)
≥ 30 (Obese)	46 (37.1%)	56 (44.8%)	102 (41.0%)

Haemoglobin levels at Enrolment and at Birth

Haemoglobin levels were measured at two time points: at enrolment (12-20 weeks of gestation) and at birth (upon admission). At enrolment, 51.4% (n = 128) of participants had mild anaemia, with no major differences between the intervention (45.6%) and the control groups (57.3%). Iron overload (0.4%) and moderate anaemia (1.2%) were uncommon at enrolment, with

moderate anaemia rising to 8.0% (n = 20) and iron overload to 4.4% (n = 11) at birth. Normal Hb levels (44.2%) were the most common finding upon admission, while mild anaemia was observed in 43.4% of participants. Detailed distributions are provided in **Table 2**.

Table 2: Haemoglobin categories

Diagnostic Criteria	**Control (N = 124)**	**Intervention (N =125)**	**Total (N = 249)**
At Enrolment			
Iron Overload	1 (0.8%)	0 (0.0%)	1 (0.4%)
Normal Hb	51 (41.1%)	66 (52.8%)	117 (47.0%)
Mild Anaemia	71 (57.3%)	57 (45.6%)	128 (51.4%)
Moderate Anaemia	1 (0.8%)	2 (1.6%)	3 (1.2%)
At Birth			
Iron Overload	4 (3.2%)	7 (5.6%)	11 (4.4%)
Normal Hb	51 (41.1%)	59 (47.2%)	110 (44.2%)
Mild Anaemia	56 (45.2%)	52 (41.6%)	108 (43.4%)
Moderate Anaemia	13 (10.5%)	7 (5.6%)	20 (8.0%)

Changes in haemoglobin Levels from Enrolment to Childbirth

Overall, mean Hb levels increased slightly from enrolment to birth in both intervention and control groups though this change was not statistical significance, likely masked by opposing trends in Hb changes. Subgroup analysis revealed that amongst participants whose Hb levels declined, the decrease was significantly smaller in the intervention group (ΔHb = -0.902 g/dL; $p = 0.002$, $\eta^2 = 0.088$; cohen's d=0.45) compared to the control group (ΔHb = -1.422 g/dL). In contrast, amongst participants with increasing Hb levels, no significant differences were observed between groups over time ($p = 0.56$, $\eta^2 = 0.003$). Tables 3 & 4 provide detailed statistics.

Table 3: Change in haemoglobin levels from baseline to follow-up amongst participants whose Hb decreased by study group (n = 111)

Group	Sample Size	Baseline Hb Mean ± SD (g/dL)	Follow-Up Hb Mean ± SD (g/dL)	ΔHb (g/dL)	*p*-value (time × group interaction)
Control	55	11.111 ± 0.8359	9.689 ± 1.0367	-1.422	0.002
Intervention	56	11.020 ± 0.5616	10.118 ± 0.9092	-0.902	
Total	111	11.065 ± 0.7091	9.905 ± 0.9936	-1.160	

Note. *Hb* = haemoglobin; *SD* = standard deviation; *ΔHb* = mean change in haemoglobin from baseline to follow-up.

Repeated measures ANOVA was used to assess differences in Hb levels over time and between groups. A significant main effect of time was observed ($F(1,109) = 211.132$, $p < 0.001$, $\eta^2 = 0.660$), indicating a decrease in Hb levels from enrolment to birth. The time × group interaction was also significant ($F(1,109) = 10.571$, $p = 0.002$, $\eta^2 = 0.088$, cohen's d=0.45), suggesting that the intervention group experienced a smaller decline in Hb levels compared to the control group. Statistical significance was set at $p < 0.05$.

Table 4: Change in haemoglobin levels from baseline to follow-up amongst participants whose Hb increased by study group (n = 138)

Group	Sample Size	Baseline Hb Mean ± SD (g/dL)	Follow-Up Hb Mean ± SD (g/dL)	ΔHb (g/dL)	*p*-value (time × group interaction)
Control	69	10.346 ± 0.6917	11.677 ± 1.4432	1.331	0.56
Intervention	69	10.545 ± 0.7858	11.764 ± 0.9280	1.219	
Total	138	11.446 ± 0.740	11.720 ± 1.210	1.274	

Note. *Hb* = haemoglobin; *SD* = standard deviation; *ΔHb* = mean change in haemoglobin from baseline to follow-up.

Repeated measures ANOVA was used to evaluate changes in Hb over time and between groups. A significant main effect of time was observed ($F(1,136) = 179.990$, $p < 0.001$, $\eta^2 = 0.570$), indicating that Hb levels increased over the course of pregnancy. However, the time × group interaction was not significant ($F(1,136) = 0.345$, $p = 0.560$, $\eta^2 = 0.003$), suggesting that the pattern of Hb increase did not differ between the control and intervention groups. Statistical significance was set at $p < 0.05$.

Association between ΔHb, HDP and potential confounders

Logistic regression analysis was performed to assess the association between ΔHb and HDP, adjusting for potential confounders (BMI and HIV status). The mean Hb change remained a significant predictor of HDP, whereas HIV and BMI were non-significant. Increasing Hb levels were associated with lower odds of developing HDP (OR = 0.45, 95% CI [0.23–0.89], p = 0.021), while a decline in Hb levels increased the odds (OR = 2.21, 95% CI [1.13–4.31], p = 0.021). However, in participants with declining Hb, the intervention group had a 76% lower risk of HDP compared to the control group (RR = 0.24, 95% CI [0.11–0.53], p < 0.001). Additional regression analysis was conducted to examine whether BMI or HIV status influenced ΔHb, which showed no significant associations with either BMI (OR = 1.29, 95% CI [0.77–2.16], p = 0.343) or HIV (OR = 0.93, 95% CI [0.56–1.55], p = 0.778).

Discussion

This secondary analysis evaluated the effects of LDA on Hb changes during pregnancy and their association with HDP. Overall Hb levels did not differ significantly between the intervention and control groups, which aligns with findings from a similar analysis of the ASPIRIN trial (17). The direction of Hb change was independent of treatment allocation, BMI, and HIV status.

Stratified analysis based on Hb change (decline vs increase) provided deeper insights that may have been masked in the overall population. Amongst participants with declining Hb levels, the intervention group experienced a significantly smaller reduction (ΔHb = -0.902 g/dL) compared to the control group (ΔHb = -1.422 g/dL), suggesting that LDA may mitigate Hb loss. This protective effect of LDA may be explained by LDA's known anti-inflammatory and endothelial-supportive mechanisms (18). For instance, LDA has been shown to downregulate IL-6, lowering hepcidin levels and improving iron availability, thereby supporting erythropoiesis (19,20). Additionally, improved placental perfusion may reduce haemodilution and help stabilize Hb levels (21). However, despite established association between low Hb with adverse maternal or fetal outcome, studies specifically on the effect of LDA on Hb during pregnancy remain limited (22).

In contrast amongst participants whose Hb levels increased during pregnancy, no significant between-group differences were observed, indicating that LDA may not enhance Hb gain where sufficient iron stores are already present. Interestingly, this finding contradicts mechanistic studies proposing that aspirin metabolites facilitate iron chelation and increase iron excretion potential predisposing to iron deficiency (8,9).

The association between Hb decline and HDP was notable. Logistic regression revealed that participants with decreasing Hb had more than twice the odds of developing HDP compared to those with increasing Hb (OR = 2.21 vs OR = 0.45, p = 0.021). This supports existing literature on the relationship between anaemia and hypertensive complications (23,24). For example, Chen *et al*. (2018) demonstrated elevated HDP risk in severely anaemic women while Goodchild *et al*. (2024) found mid-pregnancy anaemia to be a predictor of prehypertension in late pregnancy amongst South African women (25,26).

Importantly, amongst participants with declining Hb, LDA was associated with a 76% reduction in HDP risk (RR = 0.24, 95% CI: 0.11–0.53, p< 0.001), underscoring a potential dual benefit: both HDP prevention and Hb preservation.

Limitations

This study had several limitations. First, its single-centre design and relatively small sample size (N=249) may affect generalizability. Second, adherence to iron and folate supplementation was not monitored, and biochemical markers of iron status, such as ferritin, were not measured. Since supplementation is routine in SA, the observed attenuation in Hb decline cannot be attributed to LDA alone; they may also reflect differences in compliance with supplementations. Therefore, the findings should be interpreted with caution and are not yet directly applicable to clinical practice in the South African context. The computed effect size (partial $\eta^2 \approx 0.088$) was small, but reporting it enhanced transparency and enabled comparison with other studies. Finally, although the study was not originally powered to assess anaemia outcomes, the use of NHLS laboratory data strengthens reliability of the Hb measurements.

Conclusion

Our findings suggest that LDA may have a protective effect against Hb decline during pregnancy in addition to its established benefit in reducing HDP risk (27,28). This dual action may be particularly relevant in LMICs with high burdens of anaemia and HDP. Further studies are needed to validate these findings and explore the underlying mechanisms.

Future Directions

To strengthen these findings, future research should incorporate longitudinal monitoring of iron indices, dietary intake assessments, and adherence to supplementation. Investigating possible interactions between LDA and micronutrient therapies may also yield insights for integrated

maternal health interventions. Pooled analyses across LMICs are also warranted to assess whether the effects of LDA vary by anaemia severity or supplementation coverage.

Financial Support

This study was funded by the University of KwaZulu-Natal (UKZN) College of Health Sciences Scholarship (CHS) and the National Research Foundation Thuthuka Grant (TTK170508230162) in collaboration with the University of KwaZulu-Natal and Medical Research Council of South Africa (SIR Grant UNS14197).

Declaration of Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We express our sincere gratitude to the Obstetrics and Gynaecology staff at Prince Mshiyeni Memorial Hospital (PMMH), Durban, SA for their assistance in data retrieval.

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CHAPTER FIVE: SYNTHESIS AND CONCLUSION

This chapter synthesizes key findings from the preceding chapters—namely, the protocol for a systematic review, and the narrative literature review presented in Chapters 2 and 3, and the secondary analysis study detailed in Chapter 4. The synthesis is primarily centred on the effect of low-dose aspirin on haemoglobin levels during pregnancy. By integrating evidence from the existing literature with the results of the empirical analysis, this chapter aims to provide a cohesive interpretation of how low-dose aspirin may influence maternal haematological profiles and its potential implications for maternal health outcomes.

Understanding the influence of aspirin as such is essential, as it is widely recommended for the prevention of preeclampsia. These interactions are explored within the broader public health context of South Africa—a setting characterized by a high disease burden and complex nutritional and socioeconomic dynamics—and integrated with existing literature on the use of low-dose aspirin during pregnancy in both low- and middle-income countries as well as high-income countries.

The chapter concludes with the study's limitations, suggestions for future research and potential implications of the findings for reassessing current maternal health policies and guidelines.

5.1 Introduction

Pregnancy is marked by complex physiological changes, including plasma volume expansion and haemodilution, which are essential for fetal development and uteroplacental perfusion (1,2). These adaptations contribute to the expected decline in haemoglobin (Hb) levels, often termed physiological anaemia of pregnancy, and is a well-established marker of normal gestational progression (3). However, in low to middle-income countries (LMICs) such as South Africa (SA), where anaemia is common due to nutritional and infectious burdens, this physiological change can become clinically significant (4,5).

Against this background, low dose aspirin (LDA) has gained recognition as a cost effective, widely accessible intervention to reduce the incidence of preeclampsia (PE), particularly amongst high-risk women (6,7). When initiated early, typically before 16 weeks of gestation, LDA has been shown to reduce the risk of PE by improving placental blood flow through anti-thrombotic and anti-inflammatory mechanisms (6,8). It is now recommended by numerous international bodies, including the World Health Organization (WHO) and US Preventive Service Task Force (USPSTF), as a standard of care in antenatal management for women at elevated risk of hypertensive disorders of pregnancy (HDP) (7,9).

While LDA's vascular benefits are well documented, its effects on haematological indices, particularly Hb levels, have received considerably less attention (10,11). In clinical practice, a woman's Hb trajectory during pregnancy is often interpreted as a proxy for nutritional status, anaemia risk, and readiness for labour (12). Understanding how LDA affects these trajectories is therefore not only of academic interest but of immediate clinical importance—especially in settings where anaemia and PE co-occur, both contributing significantly to maternal morbidity and mortality (13).

This chapter synthesises findings from the systematic review protocol (Chapter 2), narrative review (Chapter 3), and secondary data analysis (Chapter 4) to examine the relationship between LDA use and Hb levels in pregnancy. It concludes by exploring public health implications, study limitations, and recommendations for antenatal care.

5.2 Protocol for a Systematic Review and Meta-Analysis

A protocol for a systematic review and meta-analysis was developed to evaluate the impact of LDA on maternal Hb levels during pregnancy.

The key objectives were:

1. To investigate the association between LDA use and maternal Hb levels.
2. To identify the incidence of anaemia in pregnant women treated with LDA.
3. To determine whether changes in Hb levels influence pregnancy outcomes such as PE, fetal growth, and birth weight.

Although the review itself was not completed due to time constraints and delays in receiving journal feedback, the process of developing the protocol was instrumental in clarifying the research gap and shaping the thesis. It highlighted a notable gap in the literature: while LDA is widely used to prevent PE, its haematological effects are not yet fully understood.

Thus, a narrative review was undertaken to summarise the most recent available literature, and to highlight gaps and challenges. It is hoped that this can reinforce the need to explore LDA's potential influence on haematological adaptation during pregnancy in LMICs. In these settings, PE and anaemia frequently co-occur and may share a bidirectional relationship (14,15).

5.3 Insights from the Narrative Review

The narrative review explored key biological mechanisms through which LDA may influence Hb levels in pregnancy, particularly in populations with overlapping burdens of anaemia and HDP. Four major pathways were identified: inflammation regulation, vascular adaptation, gastrointestinal effects, and red cell preservation.

5.3.1 Inflammation, Hepcidin, and Iron Availability

Pregnancy is a pro-inflammatory state, often exacerbated in conditions like PE or infection. Elevated interleukin-6 (IL-6) levels stimulate hepcidin, a hormone that impairs iron absorption and recycling, leading to functional iron deficiency (16). Low-dose aspirin has been shown to reduce IL-6 and related cytokines, potentially suppressing hepcidin and enhancing iron availability for erythropoiesis (17–19). This mechanism may be particularly relevant in settings like South Africa, where chronic inflammation due to infections, undernutrition, or environmental stress may exacerbate anaemia (20–23).

5.3.2 Vascular Function, Perfusion, and Haemodilution

Low-dose aspirin selectively inhibits thromboxane A_2 while sparing prostacyclin, promoting vasodilation and improved placental perfusion (24,25). Enhanced circulation may reduce compensatory erythropoiesis and support more physiological plasma volume expansion, thereby stabilising Hb levels during gestation (6,25).

5.3.3 Gastrointestinal Effects and Iron Absorption

Though generally well tolerated, LDA may cause subclinical gastrointestinal bleeding or chelate dietary iron (26–28). However, no consistent clinical evidence has demonstrated adverse haematological outcomes in pregnant women on LDA, especially where iron supplementation is routine.

5.3.4 Platelet Dynamics and Red Cell Preservation

Finally, LDA may reduce haemolysis by modulating platelet activity and endothelial integrity—factors linked to anaemia in PE (29–31). While evidence remains indirect, this mechanism adds to the plausibility of LDA's supportive role in Hb stability during pregnancy.

5.4 Interpretation of Empirical Findings in the Context of Existing Literature

This section interprets the empirical findings of the secondary analysis in relation to the primary research question: *How does administration of therapeutic LDA affect Hb levels during pregnancy?* Results are compared with existing literature to highlight consistencies and contradictions.

5.4.1 Effect of LDA on Hb Change

Overall mean Hb levels did not differ significantly between the LDA and control groups from enrolment to birth, aligning with findings by Jessani *et al*. (2021), which reported no global impact of LDA on Hb during pregnancy (32). However, subgroup analysis based on direction of Hb change (decline vs. increase) revealed that amongst women whose Hb declined—a common physiological trend—those in the LDA group experienced a significantly smaller reduction in Hb (−0.902 g/dL) compared to controls (−1.405 g/dL) ($p = 0.002$, $\eta^2 = 0.083$). This suggests LDA may attenuate gestational haemodilution or support erythropoiesis in vulnerable pregnancies.

This result supports evidence for a plausible mechanism of action that aspirin’s anti-inflammatory effects can influence erythropoiesis and endothelial function (19,25). The absence of a significant difference in women whose Hb levels increased suggests that the

protective effect of LDA may be context-dependent, providing benefit primarily in pregnancies at risk of haemodilution or suboptimal iron status.

5.4.2 Anaemia Classification and Iron Status at birth

At birth, mild anaemia was most common in both groups, but moderate anaemia was more frequent amongst controls (10.5% vs. 5.6%). Interestingly, iron overload (Hb >13.0 g/dL) was more prevalent in the LDA group (5.6% vs. 3.2%). While rare, this may reflect LDA's role in modifying inflammation and vascular permeability, potentially enhancing iron utilisation (33).

5.4.3 Association Between Hb Changes and HDP

Declining Hb levels were associated with higher odds of HDP (OR = 2.21, p = 0.021), consistent with literature linking low Hb to placental insufficiency or HDP (34–36). Amongst these women, those receiving LDA had a lower incidence of HDP, reinforcing aspirin's known role in HDP prevention (37).While causality cannot be confirmed, the preservation of Hb may complement LDA's vascular benefits.

5.4.4 Relationship Between Hb Change, HIV Status, and Body Mass Index (BMI)

No significant associations were found between Hb changes and HIV or BMI, diverging from studies linking HIV with anaemia and obesity with poor iron status (21,38–40). These discrepancies may reflect sample size limitations, high antiretroviral therapy (ART) adherence, and low variability in BMI.

5.5 Convergence and Divergence Between the Narrative Review and Empirical Findings

The empirical findings moderately support the hypotheses for the mechanism of action of LDA proposed in the narrative review. Both strands of evidence suggest that LDA does not exacerbate anaemia during pregnancy; rather, it may attenuate Hb decline in women experiencing gestational haemodilution. This aligns with those physiological pathways involving reduced inflammation, enhanced iron mobilisation, and improved vascular adaptation (17–19,25).

Some divergence was noted. The empirical study identified a small subgroup of women in the LDA group with Hb >13 g/dL at time of giving birth, suggesting possible iron overload—an outcome not anticipated in the review. This raises questions about the safety of ubiquitous iron supplementation, as LDA may amplify iron availability.

Furthermore, no significant associations were observed between Hb changes and either HIV status or BMI in the empirical data, whereas the review identified HIV and elevated BMI as

potential risk factors for anaemia (21,22,41). This discrepancy may be due to confounding factors such as limited sample size, low variability in BMI, or high adherence to antiretroviral therapy (ART). Overall, the findings suggest that LDA's influence on Hb is context-dependent, shaped by underlying inflammation, iron status, and co-morbidities, with potential roles in both vascular and haematological adaptation during pregnancy.

5.6 Public Health Implications

The findings of this study carry important implications for antenatal care in resource-limited settings, where the maternal health landscape is shaped by overlapping burdens of anaemia, HDP, nutritional deficiencies, and infectious disease (42). Low-dose aspirin, as a low-cost, accessible intervention, may offer a dual benefit: reducing the risk of PE as well as attenuating Hb decline.

5.6.1 Optimising the Dual Role of LDA in High-Burden Settings

In the South African context, where anaemia affects a large proportion of pregnant women and PE remains one of the top causes of maternal mortality, interventions that can simultaneously mitigate both risks are invaluable (43). The potential for LDA to attenuate Hb decline—whether through modulation of inflammation, improved iron availability, or support for vascular adaptation—suggests that it may offer a dual protective effect. From a public health perspective, this could translate into reduced need for blood transfusion at delivery, fewer complications related to moderate or severe anaemia, and enhanced physiological resilience in late pregnancy.

5.6.2 Rethinking Anaemia Monitoring and Iron Supplementation Strategies

These findings also prompt reconsideration of how anaemia is managed in antenatal care. Haemoglobin is often assessed only at booking and late gestation (44,45). Yet, LDA may influence Hb levels in ways that could go undetected. Trimester-based Hb monitoring for LDA users could be a cost-effective approach to identifying either insufficient volume expansion or early signs of iron excess. In selective cases—such as women with persistent anaemia despite supplementation, or where iron overload is suspected—additional indices such as ferritin or transferrin saturation may help guide management.

5.6.3 Integrating Findings into Antenatal Guidelines

While current South African and global guidelines recommend LDA for women at risk of PE, they provide limited guidance on its implications for anaemia, partly due to a lack of data on iron and folate supplementation compliance. Our findings support the integration of LDA into

antenatal care, particularly in LMICs where iron-deficiency anaemia is prevalent. LDA may help protect against Hb decline, although this should be interpreted cautiously in the absence of supplementation data. Recognising this potential dual benefit of LDA—HDP prevention and Hb preservation—could strengthen antenatal guidelines by supporting targeted Hb monitoring, particularly among high-risk pregnancies (e.g., women with obesity, HIV, or prior PE). Any implementation of LDA prophylaxis should therefore be paired with routine anaemia screening and adequate supplementation, especially in resource-limited settings where Hb decline may be strongly influenced by iron status. Future cohort studies should assess supplement adherence and measure iron biomarkers to better evaluate LDA's effect on Hb.

5.6.4 Maternal Health Equity and Implementation Considerations

Women most likely to benefit from LDA are often those least likely to receive individualised care. Ensuring that LDA use is supported by anaemia monitoring, nutritional support, and responsive antenatal services is key to realising its full protective potential within maternal health programmes.

5.7 Study Limitations

While this synthesis offers insight into the haematological effects of LDA in pregnancy, several limitations should be acknowledged.

Firstly, the empirical analysis was secondary in nature, based on a trial originally designed to assess platelet and hypertensive outcomes—not haematological endpoints. Although statistically significant results were observed, the study was not prospectively powered to detect Hb-related effects, and findings should be interpreted with caution.

Secondly, Hb was measured only at enrolment and birth, limiting assessment of gestational Hb trajectories. Intermediate data could have clarified the timing and mechanisms of LDA's impact.

In addition, key iron and inflammatory biomarkers—such as ferritin, hepcidin, and C-reactive protein—were not collected. These could have confirmed whether LDA's effects were mediated by improved iron mobilisation or reduced inflammation.

Then, adherence to iron supplementation was not monitored, nor were dietary and socioeconomic factors controlled, which may have confounded the results.

Finally, the study used a higher LDA dose (162 mg), and was conducted at a single urban site, which may limit generalisability to other settings, especially rural or lower-risk populations.

These limitations underscore the need for larger, prospective studies with broader haematological profiling.

5.8 Conclusion

This synthesis examined whether LDA, whose role in preventing PE has already been well established, may also influence Hb levels during pregnancy—an important question both in SA, and similar LMIC settings where anaemia and HDP often coexist.

Drawing from both a review of current literature and secondary data analysis, the findings suggest that LDA may attenuate gestational Hb decline, possibly through improved plasma volume expansion, reduced inflammation, or enhanced iron mobilisation. While a small increase in iron overload was observed in the aspirin group, this underscores the importance of tailoring supplementation and monitoring strategies to individual needs.

Public health implications are promising: LDA may serve a dual function—supporting both vascular and haematological adaptation. However, to realise these benefits, its use must be embedded in responsive antenatal care frameworks that include nutritional screening and Hb monitoring.

This study contributes to growing evidence that LDA's role in pregnancy may extend beyond PE prevention. Further cohort studies should investigate the interaction between LDA use and antenatal iron status, as well as the role of infectious exposures (e.g., HIV, malaria) to confirm the attenuated effect of LDA against Hb decline. A global meta-analysis found that both low and high maternal Hb concentrations were associated with adverse maternal and infant outcomes—emphasizing that anaemia remains a dominant risk factor in pregnancy worldwide (young et al. 2023). Conducting pooled analyses across LMICs could help determine whether LDA's impact varies by anaemia severity or supplementation coverage.

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Appendices

Appendix 1: Ethical Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

02 September 2024

Miss Nokwethemba Monica Ngcobo (220024353)
School of Laboratory Medicine & Medical Science
Westville

Dear Miss Ngcobo,

Protocol reference number: BREC/00007156/2024
Project title: Investigating the impact of low dose aspirin on haemoglobin levels during the pregnancy.
Degree: MMedSci

EXPEDITED APPLICATION
APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 02 September 2024. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 02 September 2024. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on RIG on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2024), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 08 October 2024.

Yours sincerely,

[redacted]

Prof S Singh
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor S Singh
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Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Appendix 2: Consent form

UKZN BIOMEDICAL RESEARCH ETHICS COMMITTEE

APPLICATION FOR ETHICS APPROVAL
For research with human participants (Biomedical)

Information Sheet and Consent to Participate in Research (ASPIRIN)

Date:

Dear Miss/Ms/Mrs.

Good day, I am…Zinhle Mkhize……, a researcher working with a team/group of researchers doing a study to find out whether a small dose of Aspirin tablets (162 mg) can prevent high blood pressure during pregnancy. You do not have high blood pressure at the moment but it may develop as your pregnancy grows. Aspirin is a headache tablet however the dosage is low as I have mentioned and will not affect you or your baby in many ways. By random you may have tablet with aspirin or without aspirin. It has been used during pregnancy before in other countries in the World. This is the first time it will be used in Durban, South Africa.

You have been invited to take part in the study. The study will include 790 participants in this site. If you agree to take part you will be given a total of 30 tablets and you will be taking one tablet at night before you sleep. It will prevent any irritation to your stomach. You will take the tablet everyday even on weekends and holidays. Tablets will cover you until the next visit and you will also be interviewed telephonically regarding any problems you may encounter. We will stop the tablet when you reach 36 weeks of pregnancy. Please remember to bring the bottle of aspirin tablets in the next visit as we will be counting the number of tablets you took and didn't take. Also, problems you encounter when taking tablets. We would like you to take part in a study which is trying to find out why some women are suffering from high blood pressure during pregnancy.

The results of the study will not be known during your pregnancy and you and your baby will not get any treatment different from other patients who are not in the study. You will be treated the same as other patients who will not take part in the study. We will also take antenatal clinic information from your clinic records. We will again take records of your delivery and ask some questions about your breastfeeding choice and family planning.

Please note that the study will take about 3 years and all the information that we collect from you and your clinic records will be kept confidential. In all our records no names will be stated, in other words all our records will have no hospital numbers or names. This information will be kept in a safe place and will only be accessed by the main researcher (Ms P Z Mkhize) to maintain confidentiality.

We would like to inform you that your clinic records will only be used for the information that we have explained to you. It will not be used for any other purposes. You are also free to decide not to take part in the study.

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (Approval number B136/19).

In the event of any problems or concerns / questions you may contact the researcher on 0833924358,

Email: zihlanlda@yahoo.com or Prof J Moodley on 031 260 4675, or the UKZN Biomedical Research Ethics Committee, contact details as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Appendix 3: Guidelines for presentation of dissertation/theses for higher degrees

1. Purpose

The purpose of this document is to provide guidance to students and supervisors on how to prepare a dissertation/thesis for Masters by Research and PhD degrees using the manuscript or publication format.

2. Introduction

These guidelines must be read together with the College of Health Sciences (CHS) Handbook as well as the Jacobs documents on examination policies and procedures for PhD degrees. The rules on thesis format are based on modification of point 1 of the definition of terms section in the Jacobs document. In this section a thesis is defined as *"the supervised research component of all PhD degrees, whether by supervised research only, or coursework and research, or by papers that are either published or in manuscript form (the supervised research component of the PhD degree by paper(s) comprises the introduction, literature review, account of the methodology, selection of manuscripts, and conclusion)."* A dissertation is defined as "*the supervised research component of all Masters degrees, whether by supervised research only, or coursework and research, or by papers that are either published or in manuscript form (the supervised research component of the Master's degree by paper(s) comprises the introduction, literature review, account of the methodology, selection of manuscripts, and conclusion*)."

2.1 PhD thesis

In the CHS Handbook the rules for a PhD thesis are not in one place; they are stated in DR8 a i& ii, DR9 c and CHS 16. DR8 a i & ii and direct that a thesis be presented in the standard format together with one published paper or an unpublished manuscript that has been submitted to an accredited journal, arising from the doctoral research. CHS16 (thesis by publications states that the thesis may comprise of at least three published papers or in press in accredited journals; such papers must have the student as the prime author. The same CHS16 provides for a thesis by manuscripts that may have at least 3 papers with the student as the prime author that have not yet been published but are in the form of manuscripts; at least two of such papers must constitute original research. In both cases (thesis by publications and manuscripts), there must be introductory and concluding integrative material sections.

The standard type of thesis is being phased out in many African countries in favour of the other options that originate from the Scandinavian countries. While this format ensures that all details

of the work done for the doctoral degree are captured and thoroughly interrogated, they often remain as grey literature, which is mainly useful to other students, usually within the same university, although with digitization of theses, such work may become more accessible beyond the source university. Apart from the risk of losing good work because of it not being on the public domain, as students rarely publish such work after graduating, this approach denies the college additional productivity units (PUs) emanating from publications.

The thesis by publication encourages students to publish key aspects of their doctoral research as they will not graduate if the papers are not published or in press. This approach ensures that the work of the student enters the public domain before the thesis is examined, providing the examiner with some assurance of prior peer review. The thesis must constitute a full study of the magnitude expected of a PhD with the papers providing a sound thread or storyline. Furthermore, the college maximizes the students' work as PUs are awarded for the papers as well as for graduating. However, this approach may negatively affect throughput and frustrate students as they cannot graduate unless all the papers are published or in press, in addition to the synthesis chapter demonstrating the story line of the thesis.

The option of a thesis by manuscripts ensures that students make efforts to start publishing. The risk of not passing because of failure to publish all papers (as in the thesis by publication) does not exist under this option. However, the PUs emanating from publications from the doctoral work are not guaranteed as the submitted papers may eventually be rejected. Thus, there is a possibility of the doctoral work remaining on the university library shelves as is the case for the standard thesis format. The standard thesis does have the advantage that more details of the doctoral work are usually included.

In view of the above, the best option for the college is that of a thesis by publication. However, in the interim, the attractive option is that of thesis by manuscripts, as it provides the possibility of publication without putting the student at risk of delayed graduation when some of the manuscripts are not published/accepted, which also disadvantages the college in terms of PU earnings. The standard thesis option should ultimately be phased out for the stated reasons and students are not encouraged to present their theses in that format. Consequently, this document does not describe the standard thesis.

2.2 MSc dissertation

The rules on presentation of MSc dissertations are presented in CR13 (course work), CHS 14 (course work) and MR9 (research) in the CHS Handbook. CR13 c and MR9 c direct that a dissertation "may comprise one or more papers of which the student is the prime author, published or in press in peer-reviewed journals approved by the relevant college academic affairs board or in manuscripts written in a paper format, accompanied by introductory and concluding integrative material." Such a dissertation should include a detailed description of the student's own distinct contribution to the papers. Both CHS14 and CR13 specify that reviews and other types of papers in addition to original research paper/s may be included, provided they are on the same topic.

3. *Length of thesis and dissertation by word count*

Table 1 provides a guide of the length of a thesis or dissertation by word count excluding preliminary pages and annexes.

Table 1: Thesis length by word count

Sections				
	Minimum	Maximum	Minimum	Maximum
Introduction	2700	2700	2000	2000
Chapters	10000	25000	6000	11000
synthesis	2000	2000	1700	1700
bridging	300	300	300	300
Total	15000	30000	10000	15000

4. *Intention to submit*

A written intention to submit a thesis or dissertation should be submitted to the appropriate postgraduate office with endorsement of the supervisor at least three months before the actual date of submission which should be before November if the student intends to graduate in the following year. The actual submission will under normal circumstances require approval of the supervisor.

5. Format for theses/dissertation

There is little variation in the actual format of the PhD thesis and Masters dissertation for the various types described above. The box below summarises the outline of a thesis/dissertation for the thesis by manuscripts and thesis by publication.

Box 1: Outline of thesis

Preliminary pages

i. Title page

ii. Preface and Declaration

iii. Dedication

iv. Acknowledgements

v. Table of contents

vi. List of figures, tables, and acronyms (separately presented)

vii. Abstract

Main Text

1. Chapter 1: Introduction

Introduction including literature review

Research questions and/or objectives

Brief overview of general methodology including study design

2. Chapter 2

First manuscript/publication

3. Chapter 3

Second manuscript/publication

4. Chapter n

Final manuscript/publication

5. Chapter n+1: Synthesis

Synthesis

Conclusions

Recommendations

6. References Appendices

NB. Between the manuscripts or publications there must be a 1 page (maximum) bridging text

to demonstrate the link between them

6. Details for thesis/dissertation subheadings

This section summarizes what is expected under each subheading shown in Boxes 1 and indicates where there might be variations between a Masters Dissertation and PhD Thesis.

6.*1 Title Page*

The officially approved title that is concise (Fewest words that adequately describe the contents of the thesis/dissertation – usually 15 or fewer words) is presented at the top. This should be followed by the candidate's name in a new line. At the bottom, the thesis statement should be presented. The thesis statement may be stated as "*Submitted in fulfilment of the requirements for the degree of ____ in the School of _____, University of KwaZulu-Natal"* for a PhD/Masters by Research thesis. In the case of a Masters Dissertation it should be stated as "Submitted as the dissertation component in partial fulfilment (% stated) for the degree of _____ in the School of_____, University of KwaZulu-Natal". For both Masters and PhD the date of submission must be stated.

6.2 Preface (Optional)

The preface merely states the reason (motivating factors) why the study was conducted without getting into details of what was investigated.

6.3 Declaration

This must be structured as follows:

I, Dr/Mr _____, declare as follows:

1. That the work described in this thesis has not been submitted to UKZN or other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party.

   Where a colleague has indeed prepared a thesis based on related work essentially derived from the same project, this must be stated here, accompanied by the name, the degree for which submitted, the University, the year submitted (or in preparation) and a

   concise description of the work covered by that thesis such that the examiner can be

   assured that a single body of work is not being used to justify more than one degree.
2. That my contribution to the project was as follows:

   This is followed by a concise description of the candidate's personal involvement in and contribution to the project, in sufficient detail that the examiner is in no doubt as to the extent of their contribution.

3. That the contributions of others to the project were as follows:
   This is followed by a list of all others who contributed intellectually to the project, each accompanied by a concise description of their contribution. This does not include people who ordinarily would be "acknowledged" as opposed to considered for authorship.
4. Signed _____ Date_____

6.4 Dedication

This is an optional section. Should it be included it must be very brief merely indicating to whom the work is dedicated. Avoid anything too flowery

6.5 Acknowledgements

This section acknowledges all individuals, groups of people or institutions that the candidate feels indebted to for the support they rendered. The funding source for the work should also be acknowledged.

6.6 Table of contents

Table of contents must be inserted after the preliminary sections and must capture all major sections of the thesis at the various levels (primary, secondary, tertiary subheadings). It should be electronically generated and should be able to take the reader to specific headings in the thesis.

6.7 Lists of figures, tables, and acronyms

These lists must be presented separately. All titles of figures presented in the thesis/dissertation must be listed indicating on what page they appear. Similarly for tables the titles must be presented indicating on what page they appear. In the case of acronyms, the acronym is stated and all the words describing the acronym are presented. Only key acronyms should be stated. In some cases, they may not be listed as long as full text is presented whenever the acronym is used for the first time.

6.8 Abstract

The abstract should summarize the thesis mainly stating the purpose of the study, highlights of chapters and the new knowledge contributed by the thesis. The abstract must be approved by the

supervisor of the thesis and should not be more than 350 words in length.

6.9 Introduction

The introductory chapter for both types of thesis is similar. The section should include literature review and have the following information. Headings are used as appropriate and need not correspond exactly to the following.

i. Background and the context of the study

ii. Description of the core research problem and its significance

iii. A comprehensive, critical, coherent overview of the relevant literature leading to clearly defined knowledge gaps

iv. A coherent problem statement highlighting the nature and magnitude of the problem, the discrepancy, knowledge gaps therein and possible factors influencing the problem.

v. Clear and SMART research questions, objectives, and hypothesis and/or theoretical framework

vi. A conceptual framework (optional)

vii. Description of the study area and general methodology *(in a standard thesis this should be a stand-alone section)*

viii. Layout of the thesis (thesis structure) indicating what chapters are presented in the thesis and how they address the objectives.

6.10 Literature review

This section is subsumed in the introduction within the stipulated word count for a thesis or dissertation.

6.11 Methodology

A standalone section is not needed as the methods are adequately described in each manuscript/publication.

6.12 Data chapters/manuscripts/publications

The full published paper or manuscript submitted for publication should be presented as published or submitted to the journal. The actual published paper should be scanned and inserted in the chapter. There should be a separator page between chapters that has text linking the previous chapter to the next and providing details of the next manuscript/publication indicating publication status.

6.13 General discussion/Synthesis chapter

This is a general discussion that demonstrates the logical thread that runs across the various manuscripts/publications (synthesis). There should be no doubt that the manuscripts/publications complement each other and address the original objectives stated in the general introduction of the thesis. The general discussion/synthesis chapter should end with a conclusion and recommendations where necessary.

6.14 References

Only references cited in the introduction and synthesis chapters should be listed as all other references should be within the manuscripts presented under data chapters.

6.15 Annexes

All information (questionnaires, diagrams, ethics certificates, etc) considered important but not essential for inclusion in the actual thesis is put in this section as reference material. In addition papers that emanated from the work but not directly contributing to the thesis may be included.

7. Thesis formatting

For standardisation of thesis the following formatting specifications should be followed.

7.1 Font

Times New Roman 11pt should be used throughout the thesis. However, major headings may be made bigger (12pt) but using the same font type

7.2 Paper size and margins

A4 (297 x 210 mm) should be used and in the final thesis both sides of the paper should be used. However, the loose bound copy submitted for examination should be printed on only one side. The recommended margins are 30mm for all the left, right, top, and bottom margins.

7.3 Line spacing

The copy submitted for examination should have 1.5 line spacing, but the final copy should have single line spacing. Paragraphs should be separated by a blank line. Published or submitted manuscripts should remain in their original format in all aspects as they are inserted in their published format in appropriate places.

7.4 Headings

A consistent numbering system and captions should be maintained with first level being in CAPS and centred, second level being **normal bold** font and third level being ***italics bold***. If there is need for 4th level it should be *normal italics*.

7.5 Pagination

Page numbers should be centred at the bottom of the page. All preliminary pages should be numbered in lower case Roman numerals and subsequent pages should be numbered as indicated in the Box The title page should not be numbered. The body of the thesis (chapter 1 onwards) should be numbered consecutively with Arabic numerals. The numbers should continue consecutively from the introduction through the through the publications or submitted manuscripts and subsequent sections. The published papers will therefore bear two numbers: a set specific to the manuscript (it is recommended to place these in the upper right-hand corner) or published paper, as well as the consecutive numbers belonging to the thesis as a whole. Care must be taken to distinguish these in terms of position and font.

7.6 Referencing

Supervisors have the freedom to decide the type of citation of references but there must be consistency. This is mainly applicable to the standard type of thesis. In the case of thesis by manuscripts or publications, individual papers will maintain the reference system of the journal but the supervisor can decide on the type of referencing for the introductory and synthesis chapters.

8. Final thesis submission

The thesis should be submitted for examination in a loose bound form accompanied by a PDF copy. After the examination process the final version PDF copy of the thesis must be submitted to PG office for onward submission to the library. It is not a requirement to submit a copy fully bound in leather cloth or similar material.

Appendix 4: Similarity Index

Turnitin Thesis (3).docx

ORIGINALITY REPORT

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9 pubmed.ncbi.nlm.nih.gov
Internet Source

10 pdfs.semanticscholar.org
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11 researchprotocols.org
Internet Source

12 Marzieh Azizi, Elham Ebrahimi, Zahra Behboodi Moghadam, Zohreh Shahhosseini, Maryam Modarres. "Pregnancy rate, maternal and neonatal outcomes among breast cancer survivors: A systematic review", Nursing Open, 2023
Publication

13 Ravi Shankar, Fiona Devi, Xu Qian. "Mindfulness-Based Interventions using Artificial Intelligence: A Systematic Review Protocol", Cold Spring Harbor Laboratory, 2025
Publication

14 Vinogrin Dorsamy, Chauntelle Bagwandeen, Jagadesa Moodley. "The prevalence, risk factors and outcomes of anaemia in South African pregnant women: a protocol for a systematic review and meta-analysis", Systematic Reviews, 2020
Publication

15 journals.lww.com
Internet Source

16 zaguan.unizar.es
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17 gizi-fema.ipb.ac.id

18 publichealthinafrica.org
Internet Source

19 researchspace.ukzn.ac.za
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20 www.tjoddergisi.org
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21 Submitted to University of Southampton
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22 www.ics.org
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23 Submitted to University of Ghana
Student Paper

24 Submitted to University of Nottingham
Student Paper

25 d.docksci.com
Internet Source

26 www.obgproject.com
Internet Source

27 "Poster Day 1 - Oxidative Stress", Hypertension in Pregnancy, 7/2006
Publication

28 inplasy.com
Internet Source

29 jcpsp.pk
Internet Source

30 pdffox.com
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31 Lynda Wyld, Ramsey Cutress, Jenna Morgan. "50 Landmark Papers every Breast Surgeon Should Know", CRC

32 www.jmir.org
Internet Source

33 M. A. Rifat, Mahashweta Chakrabarty, Syeda Saima Alam, Md. Masum Ali et al. "Effectiveness of interventions on early initiation of breastfeeding in South Asia: A systematic review and meta-analysis of randomized controlled trials", Springer Science and Business Media LLC, 2024
Publication

34 Oluwatosin Olu-Abiodun, Aderinsola Faturoti, Akinmade Adepoju, Davies Adeloye, Akindele Adebiyi, Olumide Abiodun. "Effectiveness and Challenges of Digital Tools Implementation for Enhancing Infectious Disease Surveillance Data Quality in Low- and Middle-Income Countries: A Systematic Review Protocol", Cold Spring Harbor Laboratory, 2025
Publication

35 ro.uow.edu.au
Internet Source

36 www.cmamforum.org
Internet Source

37 Submitted to Universiteit Utrecht
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38 pmc.ncbi.nlm.nih.gov
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42 Submitted to University for Development Studies
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43 repository.uantwerpen.be
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44 discovery.researcher.life
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45 etheses.bham.ac.uk
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47 www.nice.org.uk
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48 www.spandidos-publications.com
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49 Submitted to University of KwaZulu-Natal
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50 assets.researchsquare.com
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51 repub.eur.nl
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52 researchonline.jcu.edu.au
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53 scholar.archive.org
Internet Source

54 "Machine Learning for Preeclampsia Prediction: Enhancing Screening in Primary Health Care", Kesmas Jurnal Kesehatan Masyarakat Nasional (National Public Health Journal), 2025
Publication

55 Submitted to City University
Student Paper

56 Yaser Mohammed Al-Worafi. "Handbook of Complementary, Alternative, and Integrative Medicine - Education, Practice and Research, Volume 6: Education, Practice and Research Around the World", CRC Press, 2025
Publication

57 Submitted to Higher Education Commission Pakistan
Student Paper

58 Submitted to University Of Tasmania
Student Paper

59 Submitted to University of Melbourne
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60 koreascience.kr
Internet Source

61 spiral.imperial.ac.uk
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62 Submitted to Adtalem Global Education
Student Paper

63 Clifford O. Odimegwu, Yemi Adewoyin. "The Routledge Handbook of African Demography", Routledge, 2022
Publication

64 Submitted to University of Edinburgh
Student Paper

65 Submitted to University of Greenwich
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