The interplay between magnesium and hypertensive disorders of pregnancy in a pregnant, Black South African population

By

Naeera Abdul

220004494

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

to

School of Laboratory Medicine and Medical Sciences, College of Health Sciences,
University of KwaZulu-Natal

August 2025

DECLARATION

I, Naeera Abdul, declare as follows:

i. The research reported in this dissertation, except where otherwise indicated, is my original work.

ii. This dissertation has not been submitted for any degree or examination at any other university.

iii. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

iv. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

   a. Their words have been re-written, but the general information attributed to them has been referenced;

   b. Where their exact words have been used, their writing has been placed inside quotation marks, and referenced.

v. This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signature [redacted]

Naeera Abdul

Student number: 220004494

Date: 18th August 2025

SUPERVISORS' DECLARATION

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

Signature: 

Dr V Dorsamy

Supervisor

Date: 18th August 2025

Signature 

Dr C Bagwandeen

Co-supervisor

Date: 18th August 2025

FUNDING

This study was 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. Further, there was no input from these organisations in the interpretation of the result.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to:

All study participants who partook in this study. Their participation not only provided valuable information but also allowed for this study to be conducted.

My supervisors, for all the time and effort they put towards guiding me throughout this thesis.

Dr Anushka Ajith, for assisting with peer review of the search strategy.

Ms Fazana Desai, from the Clinical Medicine Laboratory, for all her assistance with sample preparation and handling.

The National Research Foundation, SA Medical Research Council and the University of KwaZulu-Natal for funding this study.

My friends and family for their support throughout this degree.

DEDICATION

To my father, for being the reason I've gotten this far.

ACRONYMS

ANOVA	Analysis of Variance
ART	Antiretroviral Therapy
DNA	Deoxyribonucleic Acid
HAART	Highly Active Antiretroviral Therapy
PRISMA-P	Preferred Reporting Items for Systematic Review and Meta-analysis Protocols

ABBREVIATIONS

ARIC	Atherosclerosis Risk in Communities
BMI	Body mass index
DOAJ	Directory of Open Access Journals
EOPE	Early-onset pre-eclampsia
First VisitNT	First Visit Normotensive
GDM	Gestational diabetes mellitus
HDP	Hypertensive disorders of pregnancy
HICs	High-income countries
HIV	Human immunodeficiency virus
IUGR	Intrauterine growth restriction
LMICs	Low to middle-income countries
LOPE	Late-onset pre-eclampsia
Mg	Magnesium
$MgSO_4$	Magnesium sulphate
PE	Pre-eclampsia
PEO	Population, Exposure, Outcome
PIH	Pregnancy-induced hypertension
SA	South Africa
SDG	Sustainable Development Goal
SGA	Small for gestational age
SSA	Sub-Saharan Africa
TermNT	Term Normotensive
TRMP6	Transient Receptor Potential Cation Channel Subfamily M Member 6
RCT	Randomised control trial

LIST OF TABLES

Chapter Two

LIST OF FIGURES

Chapter One

Chapter Two

LIST OF APPENDICES

ABSTRACT

Background

South Africa, a low- to middle-income country, faces a high burden of maternal and neonatal morbidity and mortality, with hypertensive disorders of pregnancy— particularly pre-eclampsia— contributing substantially to these outcomes. Magnesium plays a critical role in vascular regulation, endothelial function, and blood pressure control. Although magnesium sulphate is well established in the management of severe pre-eclampsia, the relationship between circulating maternal magnesium levels and the development of pre-eclampsia remains poorly understood, especially in populations with high rates of obesity and HIV.

Aim

To investigate the association between maternal serum magnesium concentrations and pre-eclampsia, and to examine the influence of HIV status and obesity on this relationship in a South African antenatal population.

Methods

This cross-sectional study included 252 pregnant Black South African women, categorised into: First Visit Normotensive (First VisitNT), Term Normotensive (TermNT), early-onset pre-eclampsia (EOPE), and late-onset pre-eclampsia (LOPE). Serum magnesium was measured using atomic absorption spectrophotometry. Differences between groups were assessed using analysis of variance (ANOVA) with Bonferroni post-hoc tests, while associations with pre-eclampsia were examined using chi-square tests and multinomial logistic regression, adjusting for body mass index, HIV status, and gestational age.

Results

Among normotensive women, serum magnesium levels declined significantly from the first antenatal visit to term (0.78 ± 0.08 mmol/L vs. 0.72 ± 0.10 mmol/L; $p < 0.001$). This decline was not observed in women with pre-eclampsia, whose magnesium levels remained stable or slightly elevated. Hypomagnesaemia (<0.66 mmol/L) was most prevalent in the TermNT group (23.9%) and was associated with lower odds of pre-eclampsia (odds ratio [OR] = 0.216, 95% confidence interval [CI]: 0.065–0.721, $p = 0.013$). Obesity was a strong predictor of both early-onset pre-eclampsia and late-onset pre-eclampsia, whereas HIV status showed no significant association with magnesium levels or pre-eclampsia risk.

Conclusion

In this cohort, normotensive pregnancies showed a physiological decline in serum magnesium, a pattern absent in pre-eclampsia. The inverse association between hypomagnesaemia and pre-eclampsia suggests altered magnesium regulation in hypertensive pregnancies rather than a protective effect of low magnesium. These findings highlight the complexity of magnesium's role in pre-eclampsia pathophysiology and underscores the need for longitudinal studies to clarify causality and inform targeted antenatal interventions.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

The introductory chapter of this dissertation provides the rationale for the study, namely the possible association between circulating maternal magnesium levels and pre-eclampsia, and the need to investigate this more definitively, in light of the current literature. The methodology employed is provided . In addition, the format of the thesis is elucidated.

1. Background and Context

Hypertensive disorders of pregnancy (HDPs), particularly pre-eclampsia (PE), remain a leading cause of maternal and perinatal morbidity and mortality globally[1,2]. These disorders are especially burdensome in low to middle-income countries (LMICs) such as South Africa, where the dual burden of infectious diseases such as HIV and non-communicable conditions, including (but not limited to), obesity create a complex obstetric landscape[3-5]. Pre-eclampsia typically arises after 20 weeks of gestation and is characterised by new-onset hypertension with proteinuria or end-organ dysfunction[1,2,6]. Whilst the exact aetiology of PE remains elusive, endothelial dysfunction, immune dysregulation, and angiogenic imbalance have been implicated[7].

Magnesium (Mg) is a mineral and electrolyte that the body needs in relatively large amounts to function properly[7].

It plays key roles in:

- Enzyme activity – acts as a cofactor in over 300 biochemical reactions.
- Muscle and nerve function – helps transmit nerve signals and regulate muscle contractions.
- Blood pressure regulation – supports normal vascular tone.
- Energy production – essential in converting food into usable energy (ATP).
- Bone health – works with calcium and vitamin D to maintain strong bones[7].

There has been a resurgence of research examining Mg, particularly in light of its roles in blood pressure homeostasis, enzymatic catalysis, and attenuation of inflammatory effects[8–10]. Magnesium sulfate ($MgSO_4$) is already a cornerstone in the management of severe PE and eclampsia, yet the role of maternal Mg status in the development or progression of HDP remains inadequately explored[11]. This gap is particularly concerning in LMIC settings, where nutritional deficiencies, limited access to antenatal care, and a high prevalence of comorbidities may exacerbate maternal vulnerability[3,5,12,13].

1.1. Problem Statement

Despite the widespread therapeutic use of $MgSO_4$ in PE, relatively little is known about the baseline Mg status of pregnant women in resource-limited settings[11.14]. Most existing studies have been conducted in high-income countries (HICs), often excluding women with comorbidities such as HIV or obesity[14-16]. Furthermore, while some observational studies suggest a correlation between hypomagnesaemia and the risk of HDP, others report no significant association or even elevated Mg levels in affected women1[7-19]. The underlying mechanisms, timing, and directionality of this relationship remain unclear.

This knowledge gap is particularly relevant for South Africa, where HIV prevalence among pregnant women remains high and obesity is an emerging public health challenge[20,21]. These conditions may independently affect Mg metabolism, yet their potential to confound or modify the Mg-HDP relationship is seldom addressed. As such, there is a pressing need to generate locally relevant evidence to guide antenatal screening and intervention strategies.

1.2. Justification and Significance of the Study

In South Africa, HDPs contribute significantly to maternal mortality; The 2017–2019 Saving Mothers report identified the condition as a leading cause of maternal death, often exacerbated by delays in diagnosis and inadequate antenatal risk stratification[22]. Understanding the role of magnesium in HDP could support the development of screening tools or risk indices tailored to LMIC contexts. Additionally, elucidating how obesity and HIV affect Mg status could inform more personalised approaches to antenatal care.

The findings of this study may have direct implications for public health policy and clinical practice. If maternal hypomagnesaemia is confirmed as a modifiable risk factor for PE, it could become a target for nutritional intervention or early clinical surveillance. This is particularly pertinent in settings where sophisticated diagnostic tools are scarce, and Mg assessment may offer a cost-effective adjunct to existing protocols.

1.3. Study Aim and Objectives

The overall aim of this study is to evaluate the association between maternal serum Mg levels and HDPs, and to explore how HIV status and obesity may modify this relationship within the South African context.

The specific objectives are to:

1. Compare serum Mg levels among women with normotensive preganacies, and early and late onset PE.

2. Determine the influence of HIV infection and obesity on serum Mg levels.

3. Assess the association between serum Mg levels and adverse pregnancy outcomes.

2. Literature Review

2.1. Introduction

Hypertensive disorders of pregnancy remain one of the most significant causes of maternal and perinatal morbidity and mortality worldwide. The World Health Organization WHO) estimates that HDP complicates approximately 5–10% of all pregnancies, with PE accounting for the majority of severe cases and related maternal deaths[14]. Globally, PE is estimated to cause between 50,000 and 75,000 maternal deaths annually, with the vast majority occurring in LMICs[1,2]

2.1.1. Global and South African Burden

In LMICs, including South Africa, the public health impact of PE is compounded by constrained healthcare resources, delays in diagnosis, and limited access to tertiary care facilities. The Saving Mothers 2017–2019 report identified HDP, particularly PE, as a leading cause of maternal mortality, accounting for approximately 17% of maternal deaths nationally[22]. The report highlighted systemic challenges such as inadequate antenatal screening, inconsistent application of clinical guidelines, and late presentation of women with advanced disease.

From a perinatal perspective, PE is strongly associated with preterm birth, intrauterine growth restriction (IUGR), and small-for-gestational-age (SGA) infants, which in turn contribute to high neonatal morbidity and mortality rates[4]. These outcomes have long-term implications for child health, including impaired neurodevelopment and increased risk of chronic diseases later in life[23].

2.2. Complex Pathophysiology and the Role of Micronutrients

Pre-eclampsia is widely recognised as a complex, multisystem disorder with a multifactorial aetiology. Proposed mechanisms include abnormal placentation, immune maladaptation, oxidative stress, and endothelial dysfunction[6,24]. While much of the research focus has been on angiogenic and inflammatory pathways, growing evidence suggests that micronutrient status — including calcium, vitamin D, and magnesium — may modulate risk.

Magnesium in particular has emerged as a micronutrient of interest because of its vasodilatory, anti-inflammatory, and calcium-antagonistic properties[10,25] Magnesium sulphate ($MgSO_4$) is already the standard of care for preventing eclamptic seizures in women with severe PE[14]. However, the potential role of baseline maternal Mg status — before the onset of disease — in influencing PE risk is poorly understood. This represents a potentially modifiable factor that

could be addressed through dietary interventions or supplementation, especially in high-risk populations.

2.2.1. The South African Context

South Africa presents a unique epidemiological profile for studying the Mg–PE relationship. The country faces a dual burden of disease: persistent undernutrition alongside a high prevalence of obesity (estimated at 46% among women of reproductive age) and one of the highest antenatal HIV prevalence rates globally (~27%)[20,21]. Both obesity and HIV can independently affect Mg metabolism — obesity through chronic inflammation and altered renal handling, and HIV (plus certain antiretroviral regimens) through gastrointestinal malabsorption and urinary Mg loss[26-28].

The interplay of these comorbidities with Mg status in pregnancy is under-researched, yet potentially critical in understanding PE pathophysiology in this setting. Moreover, dietary patterns in South Africa, characterised in many communities by low consumption of magnesium-rich foods (nuts, legumes, green leafy vegetables), may contribute to marginal Mg status even before pregnancy[13] .

2.2.2. Purpose for the Review

Despite international recognition of Mg's physiological importance, evidence linking Mg status to PE is inconsistent. High-income country (HIC) studies often find lower Mg levels in women with PE compared to controls, whereas African studies, including those from South Africa, report mixed results — some finding no association, others observing higher Mg in PE[19,29]. Methodological differences, lack of pregnancy-specific reference ranges, and inadequate adjustment for confounders (e.g., BMI, HIV, diet) contribute to these discrepancies.

This literature review aims to critically appraise global and regional evidence on magnesium physiology in pregnancy, its relationship with HDP, and the influence of comorbidities prevalent in South Africa. By identifying methodological gaps and inconsistencies, it builds the scientific rationale for the present study, which seeks to generate context-specific evidence to inform antenatal care guidelines in LMICs.

2.3. Physiology of Magnesium in Pregnancy

2.3.1. Magnesium Overview

Magnesium is the fourth most abundant cation in the body and the second most prevalent intracellular cation after potassium[17]. Approximately 50–60% of total body Mg is stored in

bone, about 20% in skeletal muscle, and less than 1% in the extracellular fluid, where it is measured clinically[25]. Circulating Mg exists in three fractions: ionised (active) Mg, protein-bound Mg (mostly albumin), and Mg complexed with anions such as phosphate or citrate. Ionised Mg is physiologically active and directly involved in enzymatic and cellular processes.

The adult human body contains around 21–28 g of Mg, and daily requirements for women are estimated at 310–320 mg, increasing to 350–360 mg during pregnancy to meet the needs of the growing fetus and maternal physiological adaptations[7]. Primary dietary sources include nuts, seeds, legumes, whole grains, and green leafy vegetables. However, intake in many LMIC populations falls below recommended levels[13]

2.4. Magnesium Absorption, Transport, and Regulation

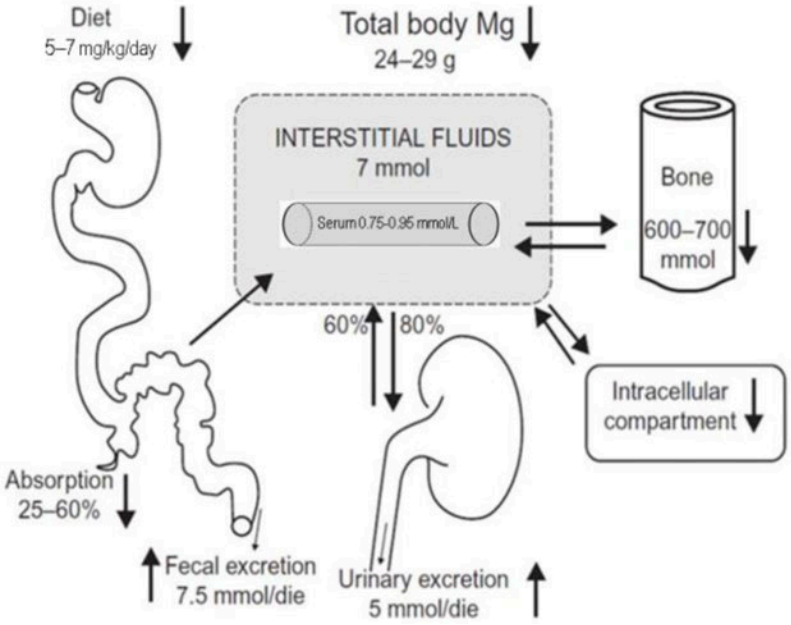


Figure 1. *Diagram illustrating magnesium homeostasis[30]*

Intestinal magnesium absorption occurs mainly in the small intestine via two mechanisms: Passive paracellular transport in the jejunum and ileum, driven by electrochemical gradients. Active transcellular transport in the distal small intestine, mediated by Transient Receptor Potential Melastatin 6 and 7 (TRPM6 and TRPM7) channels, which are regulated by dietary Mg status, vitamin D, and hormonal influences[17].

Once absorbed, Mg is transported in plasma and distributed to tissues. Renal regulation plays a central role in maintaining Mg homeostasis, with around 70% of filtered Mg reabsorbed in the thick ascending limb of the loop of Henle , 10–15% in the distal convoluted tubule, and the

remainder excreted in urine. The kidneys respond dynamically to Mg intake and status: deficiency prompts enhanced reabsorption, while excess promotes urinary excretion[25].

2.4.1. Physiological Changes During Pregnancy

Pregnancy induces substantial changes in Mg metabolism:

Haemodilution occurs by expansion of plasma volume by 40–50%, diluting circulating Mg concentration. Glomerular filtration rate (GFR) rises by approximately 50%, increasing Mg filtration and potentially urinary Mg loss. In terms of fetal demands, Mg is actively transported across the placenta, especially in the third trimester, to support fetal skeletal development and enzyme systems. Furthermore hormone modulation, such as elevated oestrogen and progesterone, may influence magnesium distribution and renal handling[8] .

These physiological changes typically produce a gradual decline in serum Mg concentration as gestation advances, even in healthy pregnancies. However, distinguishing between physiological and pathological hypomagnesaemia is challenging due to the absence of pregnancy-specific reference ranges, particularly for African populations[15].

2.5. Magnesium's Role in Maternal Physiology

Magnesium plays multiple roles in pregnancy that extend beyond its contribution to fetal growth. It regulates vascular tone by acting as a natural calcium antagonist, inhibiting calcium influx into vascular smooth muscle cells and thereby promoting vasodilation and reducing peripheral resistance[31]. Adequate Mg also supports endothelial function through enhanced nitric oxide synthesis and reduction of oxidative stress at the endothelium[24]. At the neuromuscular level, Mg modulates acetylcholine release at the neuromuscular junction, influencing uterine contractility, while in energy metabolism, it is essential for all ATP-dependent reactions, including those required for effective placental nutrient transfer. These physiological functions provide a rationale for the hypothesis that Mg insufficiency could contribute to elevated blood pressure, increased vascular reactivity, and other clinical features of HDP.

Assessment of Mg status in pregnancy typically relies on serum Mg concentration, yet this measure is limited because it reflects less than 1% of total body Mg and is maintained within a narrow range by homeostatic mechanisms, often masking early deficiency. Serum levels may remain normal until substantial depletion of body stores has occurred. Alternative assessments include erythrocyte Mg as an intracellular measure, 24-hour urinary Mg excretion to reflect dietary intake and renal handling, and the Mg loading test, which evaluates retention after

parenteral administration. However, these methods are rarely implemented in large-scale pregnancy studies in LMICs due to cost and logistical constraints[18].

In contexts where dietary Mg intake is already suboptimal, such as many LMICs, the normal physiological decline in Mg during pregnancy may more easily tip women into clinically significant deficiency. This concern has prompted trials of Mg supplementation in pregnancy, although results have been inconsistent[15].

2.6. Pathophysiology of Pre-eclampsia

Pre-eclampsia is a multisystem disorder defined by new-onset hypertension after 20 weeks of gestation, accompanied by proteinuria and/or evidence of maternal organ dysfunction (e.g., hepatic, renal, neurological, or hematological) or uteroplacental dysfunction[32].The condition is unique to human pregnancy and remains a major cause of maternal and perinatal morbidity and mortality worldwide.

2.6.1. Two-Stage Model of Pre-eclampsia

The two-stage model explains how abnormal placentation (Stage 1) precipitates the maternal endothelial syndrome (Stage 2) of PE.

Stage 1 – Abnormal placentation: In early pregnancy, defective remodeling of the maternal spiral arteries by extravillous trophoblasts results in narrow, high-resistance vessels. This limits the capacity of the uteroplacental circulation to meet increasing fetal demands.

Stage 2 – Maternal syndrome: The ischemic placenta releases a variety of antiangiogenic, inflammatory, and oxidative stress mediators into the maternal circulation, leading to widespread endothelial dysfunction and the clinical features of PE.

While this model captures the core sequence of events, it is now recognized that PE is heterogeneous, with early-onset and late-onset forms differing in placental pathology, maternal risk profiles, and underlying mechanisms[33].

2.6.2. Endothelial Dysfunction as a Central Mechanism

Endothelial dysfunction is considered the final common pathway in PE pathogenesis. It manifests as reduced nitric oxide bioavailability, increased endothelin-1 production (a potent vasoconstrictor), enhanced vascular permeability, leading to oedema and proteinuria, and pro-thrombotic changes, including platelet activation and microangiopathy[34-36].

Magnesium may interact at several points in this pathway. Its role as a calcium antagonist can reduce vascular smooth muscle contractility, while its influence on nitric oxide synthesis and oxidative stress could counteract some features of endothelial injury[24]. However, whether these effects are protective before disease onset remains uncertain.

2.6.3. Angiogenic–Antiangiogenic Imbalance

In PE, the balance between proangiogenic factors (vascular endothelial growth factor [VEGF], placental growth factor [PlGF]) and antiangiogenic factors (soluble fms-like tyrosine kinase-1 [sFlt-1], soluble endoglin [sEng]) is disturbed. Elevated sFlt-1 binds VEGF and PlGF, reducing their bioavailability and impairing endothelial repair[37]. This angiogenic imbalance is more pronounced in early-onset PE. Some animal studies suggest magnesium status may influence angiogenesis through modulation of VEGF expression, though human evidence is scarce[38]. This represents a potentially unexplored mechanistic link between micronutrient status and placental vascular development.

2.6.4. Oxidative Stress and Inflammation

Placental ischemia–reperfusion injury leads to the generation of reactive oxygen species (ROS), lipid peroxidation, and activation of inflammatory cascades. Elevated oxidative stress is observed in both placenta and maternal circulation in PE[39]. Magnesium has been shown to modulate oxidative stress responses by influencing antioxidant enzyme activity (e.g., superoxide dismutase, glutathione peroxidase) and reducing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–mediated ROS generation[40]. In Mg-deficient states, pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are elevated, which could exacerbate endothelial activation and vascular dysfunction[41].

2.6.5. Immunological Maladaptation

Pregnancy requires maternal immune tolerance toward the semi-allogeneic fetus. In PE, there is evidence of impaired regulatory T-cell function, increased T helper 1 and 17 responses, and activation of innate immune pathways[42]. These immune shifts can impair trophoblast invasion and contribute to abnormal placentation. Although Mg's role in immune regulation is less studied in pregnancy, experimental data suggests it influences lymphocyte proliferation and cytokine production[43]. Given the high burden of infections such as HIV in South Africa, interactions between immune status, magnesium, and PE warrant further exploration.

2.6.6. Metabolic and Cardiovascular Risk Factors

Obesity, insulin resistance, and dyslipidaemia are established maternal risk factors for PE. They are associated with chronic low-grade inflammation, oxidative stress, and endothelial dysfunction — mechanisms overlapping with PE pathogenesis[44]. Obesity may alter Mg status through increased urinary excretion, lower dietary quality, and sequestration in adipose tissue [26]. This may amplify vascular reactivity and worsen hypertensive responses during pregnancy.

2.6.7. Heterogeneity in Clinical Presentation

While early-onset PE (<34 weeks) is typically driven by severe placental insufficiency and fetal growth restriction, late-onset PE (>34 weeks) often arises from a maternal predisposition to endothelial dysfunction in the context of metabolic stressors [33]. This heterogeneity has implications for Mg research.

In early-onset PE, low Mg could reflect impaired placental transfer or maternal depletion due to chronic ischemia. In late-onset PE, Mg status might be more strongly linked to maternal metabolic risk profiles. Few studies have stratified Mg–PE associations by onset type, leading to inconsistent results[45-47].

2.6.8. Critical Evaluation of Mechanistic Evidence

Theoretical pathways suggest Mg could influence PE risk via vasodilation and reduced vascular tone through calcium antagonism, improved endothelial function via nitric oxide modulation and reduced oxidative stress and anti-inflammatory effects mitigating immune activation[10,36,48].

However, much of the mechanistic evidence comes from animal models or non-pregnant populations. Human pregnancy data remain inconsistent, with some studies showing lower Mg levels in PE and others showing no difference or even higher levels[19,49]. Variability in measurement methods (total vs. ionised Mg), timing of sampling, and failure to control for confounders likely contribute to these discrepancies.

2.6.9. Implications for Research in the South African Context

In South Africa, where antenatal HIV prevalence is high and obesity is common, the interplay between these conditions, Mg metabolism, and PE pathophysiology is underexplored. HIV-associated immune activation, antiretroviral therapy, and micronutrient malabsorption could alter Mg status, potentially modifying PE risk. Obesity-related metabolic inflammation could further exacerbate this interaction.

Given these gaps, context-specific research, such as the present study, is essential to clarify whether Mg deficiency is a modifiable risk factor for PE in LMIC populations and whether interventions could reduce the burden of HDP.

2.6.10. Global and Regional Evidence on Magnesium and Pre-eclampsia Overview of the Evidence Base

The relationship between maternal Mg status and the risk of PE has been examined in a variety of study designs — cross-sectional, case–control, cohort studies, and clinical trials. However, the evidence is highly inconsistent, with some studies suggesting an inverse association (low Mg linked to higher PE risk), others showing no relationship, and a few reporting higher Mg levels in women with PE. These conflicting results complicate clinical recommendations and raise questions about whether observed differences are causally linked or merely reflect secondary changes in Mg metabolism during disease progression. These findings are expanded on below.

2.7. Findings from High-Income Countries

In high-income settings, dietary magnesium intake tends to meet or exceed recommended dietary allowances (RDA) in most populations, but subclinical deficiencies can occur, especially in women with poor diet quality, obesity, or gastrointestinal disorders.

2.7.1. Inverse association findings

Frølich et al. (1992) reported that both pregnancy-induced hypertensive pregnancies and uncomplicated pregnancies experienced declines in Mg compared to non-pregnant women [50]. Jain et al. (2010) found in a Canadian study a significant inverse correlation between ionised Mg and both systolic blood pressure and proteinuria severity, suggesting that active Mg fraction may be more relevant than total Mg[51].

2.7.2. Null or inconsistent findings

The Aspirin for Evidence-based Preeclampsia Prevention (ASPRE) trial, although primarily designed to assess aspirin for PE prevention, included biochemical sub-studies in which magnesium levels were not significantly different between PE and non-PE groups[52]. Richards et al. (2013) reported no consistent differences in serum magnesium across PE severity categories in Australia, concluding that serum Mg may not be a useful biomarker in well-nourished populations[19].

Possible reasons for discrepancies in HIC data include:

Nutritional adequacy may blunt the impact of Mg variability.

There are measurement differences, where some studies measure total Mg, while others use ionised Mg or erythrocyte Mg.

Timing of sampling is important as Mg status may differ if measured in early pregnancy vs. at diagnosis of PE.

2.8. Findings from Low- and Middle-Income Countries

In LMICs, Mg status is influenced by multiple dietary, infectious, and metabolic factors. Staple diets often rely on refined grains low in Mg, and diets may lack variety. High prevalence of infections (HIV, TB) and inflammatory states may also affect micronutrient metabolism[53].

2.8.1. Null findings

A comparative study conducted at Korle-Bu Teaching Hospital, Ghana found no statistically significant difference in serum Mg levels between pre-eclamptic and normotensive women[54].

2.8.2. Inverse association findings

Eslamzadeh et al. (2023) found significantly lower mean serum Mg in PE cases compared to controls in an Iranian study. They suggested Mg deficiency could impair endothelial function and potentiate oxidative stress[49]. In Nigeria, Akinloye et al. (2010) observed reduced serum Mg in PE patients, with levels correlating inversely with blood pressure[55]. They concluded that Mg supplementation might have preventive potential in resource-limited settings. A larger case–control study from the Cape Coast region reported significantly lower serum MG levels in women with PE and pregnancy-induced hypertension compared to healthy pregnant controls[29]. Similarly, Idogun et al. (2007) found reduced extracellular Mg in pre-eclamptic women in another Nigerian study[56]. However, evidence remains inconsistent, as highlighted by a Cochrane review of supplementation trials that showed no clear preventive effect[15]. This heterogeneity emphasizes the need for context-specific studies in African settings, where comorbidities such as HIV, obesity, and renal disease may modify the relationship between Mg and PE.

2.8.3. The African Context: Unique Considerations

In African LMICs, including South Africa, the interplay of nutritional deficiencies, infectious diseases, and rising non-communicable conditions creates a unique risk profile for both Mg

deficiency and pre-eclampsia. Diets in many regions are dominated by maize meal and refined staples, which are typically low in Mg-rich components such as legumes, nuts, and green leafy vegetables. This dietary pattern contributes to chronically low Mg intake[57].

Several African studies provide evidence that Mg status may influence PE risk. In Ghana, Ephraim et al. (2014) found significantly lower serum Mg levels among women with pre-eclampsia compared to normotensive pregnant women[29]. Similarly, studies from Nigeria reported reduced plasma Mg concentrations in pre-eclamptic women, suggesting a potential mechanistic role for Mg in blood pressure regulation and endothelial stability[56,58]. A study from Sudan also demonstrated that low Mg status was associated with an increased likelihood of PE, reinforcing the hypothesis that Mg deficiency contributes to vascular dysfunction in pregnancy[59].

Compounding these nutritional risks are high burdens of HIV infection and obesity in South Africa, both of which may alter Mg metabolism and vascular health. Antiretroviral therapy (ART) use has been associated with gastrointestinal side effects and altered micronutrient absorption, whilst obesity and metabolic syndrome have been linked to lower serum Mg concentrations and greater systemic inflammation[60,61]. Antenatal micronutrient supplementation programs in the region traditionally focus on iron and folate, with Mg rarely included, and renal comorbidities such as HIV-associated nephropathy may further alter Mg handling[62].

Together, these factors complicate the interpretation of Mg–PE associations in African populations and underscore the urgent need for locally relevant, well-designed studies that account for these overlapping risk factors.

2.9. Methodological Challenges in the Literature

A critical review of the literature reveals several methodological limitations that likely contribute to the inconsistent associations reported between Mg status and PE.

2.9.1. Measurement variability

Most studies rely on total serum Mg, which reflects less than 1% of total body Mg and is tightly regulated by homeostatic mechanisms. This measure may therefore underestimate true deficiency. Ionised Mg, the physiologically active fraction, is rarely assessed despite its stronger association with vascular reactivity[67]. Erythrocyte Mg provides a more stable indicator of intracellular status but has only been applied in a limited number of pregnancy-related

studies, mostly outside Africa[68]. The absence of pregnancy-specific reference ranges further complicates interpretation, particularly in LMIC contexts where nutritional intake is more variable.

2.9.2. Timing of measurement

The timing of Mg measurement varies considerably across studies. Early pregnancy levels may capture baseline status, whereas late-pregnancy or postpartum assessments may instead reflect disease-related changes, introducing the possibility of reverse causality. For example, a study in Ghana demonstrated that lower Mg was observed at the time of diagnosis of PE but could not determine whether this was causal or a secondary effect[29].

2.9.3. Confounding

Few studies adjust adequately for confounding variables such as dietary intake, renal function, HIV status, body mass index, or concurrent micronutrient deficiencies. In South Africa, these confounders are particularly relevant given the high prevalence of HIV and obesity[21,57]. Without appropriate adjustment, observed associations may be misleading.

2.9.4. Sample size limitations

Many African studies involve relatively small samples, often fewer than 100 participants per group[56]. This restricts statistical power and limits the ability to stratify analyses by key subgroups, such as early-onset versus late-onset PE.

Taken together, these methodological challenges highlight why findings across settings are heterogeneous. Addressing these gaps—particularly through the use of physiologically relevant Mg markers, early pregnancy sampling, and robust adjustment for confounding—will be critical in establishing the true relationship between Mg status and PE in African populations.

2.10. Rationale for the Study

2.10.1. The Public Health Significance of Pre-eclampsia

Pre-eclampsia remains a leading cause of maternal and perinatal morbidity and mortality globally, with a disproportionate burden in LMICs. In sub-Saharan Africa, HDPs contribute to up to 25% of maternal deaths[63]. In South Africa specifically, the 2017–2019 triennial report on confidential enquiries into maternal deaths identified hypertensive disorders — particularly PE — as a major contributor to preventable maternal mortality[22].

Beyond maternal outcomes, PE is associated with significant perinatal risks, including preterm birth, fetal growth restriction, and stillbirth. Survivors of PE-related complications face long-term sequelae, such as increased risk of chronic hypertension, cardiovascular disease, and chronic kidney disease in mothers, and potential neurodevelopmental impairments in children[64]. These long-term health implications underline the urgent need for effective prevention strategies that are feasible in resource-limited settings.

2.10.2. Limitations of Current Prevention Strategies

Current preventive measures for PE, such as low-dose aspirin and calcium supplementation, have shown benefit but are not universally effective. Aspirin reduces PE risk mainly in women at high risk and when started before 16 weeks' gestation[30]. Calcium supplementation is beneficial in populations with low dietary calcium intake, but Mg supplementation has not been widely studied in this preventive role — despite compelling physiological plausibility[65].

Magnesium sulphate remains the gold standard for the prevention and treatment of eclampsia[11]. However, this use is therapeutic rather than preventive, and it addresses seizures rather than the underlying pathogenesis of PE. The potential for Mg nutritional optimisation to reduce PE incidence has not been adequately explored, particularly in African populations where baseline dietary Mg intake is often low.

2.10.3. Physiological Plausibility

Magnesium's role in vascular tone regulation, endothelial function, and oxidative stress modulation provides a compelling biological rationale for its potential in PE prevention. As reviewed above, Mg acts as a natural calcium antagonist, inhibits vascular smooth muscle contraction, supports nitric oxide synthesis, and participates in over 300 enzymatic reactions, many of which are critical to cardiovascular health[17].

Experimental studies demonstrate that Mg deficiency can induce vasoconstriction, increase vascular reactivity to vasopressors, and promote endothelial dysfunction[66]. Given that these pathophysiological processes are central to PE, maintaining adequate Mg status could theoretically mitigate disease onset or progression.

This thesis addresses key gaps by examining magnesium levels in a South African antenatal population with high HIV prevalence and a wide BMI range. By focusing on both early- and late-onset PE, the study can explore whether associations differ by disease subtype. The findings will provide much-needed evidence on whether magnesium status is a meaningful

biomarker or modifiable risk factor for PE in LMIC settings, and whether supplementation strategies might be justified.

2.11. Summary of the Findings of the Literature Review

The existing literature supports the hypothesis that Mg plays a role in the pathogenesis of PE, but methodological shortcomings and contextual differences have hindered definitive conclusions. South Africa's high burden of PE, combined with common nutritional deficiencies and comorbidities that affect Mg metabolism, makes it an ideal setting to investigate this association. The findings from this study could fill critical knowledge gaps, inform targeted antenatal nutritional interventions, and contribute to reducing the persistent maternal and perinatal mortality associated with hypertensive disorders of pregnancy in LMICs.

This thesis therefore proceeds to investigate the association between maternal Mg status and pre-eclampsia in a South African antenatal cohort, with the overarching goal of generating evidence that is locally relevant, methodologically robust, and globally significant.

3. Detailed Study Design and Methods

3.1. Study Design and Setting

The study employed a retrospective cross-sectional observational design, conducted at a large regional public-sector hospital in KwaZulu-Natal, South Africa. This hospital serves as a referral centre for surrounding district hospitals and provides comprehensive maternity services, which allowed access to a broad spectrum of pregnancy-related conditions, including both normotensive and pre-eclamptic cases. The retrospective design was chosen to make use of existing clinical and laboratory data. This design is particularly suited to resource-constrained settings and enables the study of relatively rare outcomes such as early-onset PE.

3.2. Population, Sampling Strategy and Group Definitions

The study population consisted of pregnant women managed at the hospital between 2018 and 2022. The initial sampling frame included 260 women, purposively selected from maternity records and laboratory databases based on their clinical presentation and availability of serum samples. These women were grouped as follows:

1. Normotensive at antenatal booking: Recruited between 12 and 28 weeks' gestation at their first antenatal visit and confirmed normotensive throughout pregnancy.

2. Early-onset pre-eclampsia (EOPE): Diagnosed with pre-eclampsia prior to 34 weeks' gestation.

3. Late-onset pre-eclampsia (LOPE): Diagnosed with pre-eclampsia at or after 34 weeks' gestation.

4. Normotensive at delivery: Recruited upon admission for delivery at term (≥37 weeks), confirmed to be normotensive at the time of delivery.

3.3. Sample Size

The study used 260 randomly selected samples. Random selection resulted 163 normotensive samples and 97 pre-eclamptic samples. After selection, the samples were further categorised into the groups: normotensive at antenatal booking, EOPE, LOPE and normotensive at delivery based on existing records.

3.4. Inclusion and Exclusion Criteria

Women included in the study had to have:

- Singleton pregnancies
- Availability of serum sample for magnesium analysis
- Known gestational age confirmed by ultrasound
- Documented blood pressure measurements
- Known HIV status and weight at booking

Women were excluded due to:

- Chronic hypertension or renal disease diagnosed prior to pregnancy
- Multiple gestation
- Missing key outcome variables (e.g., birth weight, BP)
- Magnesium assay errors or haemolysed samples

3.5. Research Instrument

Sociodemographic, obstetric, and anthropometric data were collected using a structured questionnaire (Appendix 1) designed for the parent study. The questionnaire captured variables including maternal age, gravidity, parity, educational level, employment status, and household income, as well as relevant obstetric history. Anthropometric measurements included height, weight, and mid-upper arm circumference, recorded using calibrated equipment following standardised protocols. Clinical information, including HIV status, gestational age at recruitment, and blood pressure measurements, was extracted from participants' antenatal records.

3.6. Data Collection and Clinical Variables

For the original study, trained research assistants conducted interviews using the structured questionnaire and performed physical measurements at the time of recruitment. The participants had their blood pressure measured to determine their pre-eclamptic status. Urine dipstick tests were also administered to determine if they had proteinuria. Serum samples were collected either at antenatal booking or on admission to labour ward. Venous blood samples were drawn under aseptic conditions and collected in clot activator tubes (BD Vacutainer® blood collection tubes, BD Biosciences) for serum isolation by qualified nursing staff, then transported to an accredited laboratory for biochemical analysis. The blood samples were then spun at 3000g for 12 minutes. After being centrifuged, serum samples were labelled with their

pre-eclamptic and HIV status. In the present secondary analysis, relevant biochemical data (serum Mg levels) and clinical information were retrieved from the parent study database. Where necessary, missing laboratory values or delivery outcomes were obtained from hospital records at Prince Mshiyeni Memorial Hospital (PMMH) or from the National Health Laboratory Service (NHLS) database. Diagnosis of PE followed International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria, and anaemia and obesity were defined using WHO standards[32,69].

3.7. Data Analysis

After samples were selected in the present secondary analysis, 500μl of serum per sample were aliquoted into Eppendorf tubes and then transferred into Cobas Pro c 503 analytical unit (Roche Diagnostics, F. Hoffmann-La Roche Ltd.) compatible tubes[70]. Samples were processed within 2 hours of collection using atomic absorption spectrophotometry in an automated process. Before tests to determine Mg levels were conducted, the Cobas Pro c 503 analytical unit was calibrated. Once ready, serum samples were loaded onto the machine's carousel for testing. The test itself is a colorimetric endpoint test which took 10 minutes to conduct. At the start of the procedure, the serum sample was added alongside TRIS/6-aminocaproic acid buffer, EGTA and xylidyl blue. The test involved Mg in the serum sample reacting with xylidyl blue in an alkaline solution to form a purple complex. The solution itself contained EGTA to conceal calcium. The concentration of Mg was then measured photometrically at a wavelength of 505/600 nm through the decrease in xylidyl blue absorbance[71]. Magnesium categories followed standard cutoffs: <0.66 mmol/L (hypomagnesaemia), 0.66–1.07 mmol/L (normal), and >1.07 mmol/L (hypermagnesaemia). The data was then analysed using IBM SPSS Statistics (version 29, IBM Corp., Armonk, NY, USA) [72]. Descriptive statistics were computed for all variables, with continuous data expressed as means ± standard deviations (for normally distributed data) or medians with interquartile ranges (for skewed data). Categorical variables were summarised as frequencies and percentages. Between-group comparisons were performed using Student's t-test or ANOVA for normally distributed variables and Mann–Whitney U or Kruskal–Wallis tests for non-normally distributed variables. Associations between categorical variables were assessed using the chi-square test or Fisher's exact test as appropriate. Multivariate logistic regression models were constructed to determine independent predictors of PE, adjusting for potential confounders such as body mass index (BMI), HIV status, and maternal age. A p-value <0.05 was considered statistically significant. Further information about the rationale for the statistical tests chosen are given in Appendices 2 and 3.

3.8. Data Management and Storage

Data from the original study was stored in a secure, password-protected electronic database with access restricted to authorised research team members. For this secondary analysis, data were de-identified to ensure participant confidentiality and transferred to a separate, encrypted file for analysis. All physical questionnaires and laboratory reports are archived in a locked filing cabinet within the research offices at the University of KwaZulu-Natal (UKZN), in accordance with institutional data retention policies. The data will be stored for five years, as mandated by the KwaZulu-Natal Provincial Department of Health, and both electronic and physical data will be responsibly destroyed once this period has elapsed.

3.9. Measures to ensure a scientifically rigorous study

3.9.1. Validity and Reliability

Validity of the research instrument was ensured through prior piloting of the questionnaire in a similar population during the main study, allowing refinement of wording and sequencing of questions. Reliability was promoted by the use of standardised data collection procedures, training of research assistants, and calibration of anthropometric equipment before each measurement session. Laboratory analyses were conducted using validated assays, with internal quality control measures in place. Double data entry was implemented for the original database to minimise transcription errors.

3.10. Bias and Limitations

3.10.1. Selection Bias

Selection bias was minimised by applying clearly defined inclusion and exclusion criteria across all recruitment groups. Purposive sampling was used to ensure adequate representation of normotensive women and those with early- and late-onset PE. Recruitment was consecutive within each category to limit investigator selection preferences.

3.10.2. Control for Confounding

Potential confounding variables were identified *a priori* based on literature evidence and biological plausibility. These included maternal age, parity, gestational age at sampling, BMI, HIV status, and hypertensive status. Where available, anaemia status and use of micronutrient supplementation were also considered. Multivariable logistic regression models were used to estimate the association between serum Mg levels and PE while adjusting for these potential confounders. Variables were retained in the model if they were associated with both the exposure and outcome or if their inclusion altered the effect estimate by more than 10%.

3.10.3. Limitations

The main limitation of this secondary analysis was the absence of some birth measurements, which reduced the final sample size and statistical power. Despite efforts to retrieve missing data from PMMH and NHLS records, complete follow-up was not possible for all participants. Furthermore, as the study was conducted at a single regional hospital in Durban, the findings may not be generalisable to other South African settings or to populations with different demographic or epidemiological profiles. Additionally, serum Mg measurements were taken at single time points, limiting assessment of longitudinal trends. This aspect is discussed in greater detail in the synthesis chapter.

4. Ethical Considerations

4.1. Institutional Ethical Review Board

This secondary analysis was conducted following approval by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN). A full proposal and ethics application were submitted for review and granted expedited ethical clearance (Appendix 4).

4.2. Permissions

Ethical approval was obtained under BREC reference number BREC/00007156/2024 (Appendix 5). Additional permission to access and analyse the original dataset was obtained from the principal investigator of the parent study.

4.3. Informed Consent and Participant Information

Informed consent for participation and biological sample collection was obtained from all participants during the original study (Appendix 6). As this was a secondary analysis involving no direct contact with participants and using de-identified data, additional informed consent was not required. All data handling complied with the ethical principles outlined in the Declaration of Helsinki and the Protection of Personal Information Act (POPIA) of South Africa.

5. Theoretical Framework

The conceptual foundation for this study is illustrated through an epidemiological triad model, which positions low maternal Mg status as the central agent in the causal pathway to PE. Magnesium deficiency, particularly when present early in pregnancy, may impair vascular tone regulation, compromise endothelial function, and heighten oxidative stress, thereby predisposing to the development of hypertensive disorders. The host component of the triad captures biological and health-related factors that can modify this risk, including HIV infection and ART use, obesity, chronic hypertension, and renal disease. These conditions may influence Mg metabolism, alter vascular reactivity, or exacerbate underlying endothelial dysfunction.

Environmental influences further interact with both the agent and host to shape overall susceptibility. In the South African context, these include chronically low dietary Mg intake due to reliance on refined staples, poor socioeconomic conditions that limit access to nutrient-rich foods, restricted availability of antenatal care services, and seasonal fluctuations in food availability that may influence nutritional status. Together, these interdependent elements can amplify the likelihood of developing PE, with potentially stronger effects for early-onset disease, where placental dysfunction plays a more prominent role.

This framework directly informs the research approach by emphasising the need to measure Mg status early in pregnancy, adjust for key environmental and host confounders, and assess effect modification by HIV and obesity. Stratifying analyses by early- and late-onset PE will enable a more targeted interpretation of the observed associations within this high-burden South African context.

In summary, the literature demonstrates plausible biological pathways linking low maternal magnesium status to the development of pre-eclampsia, yet findings remain inconsistent due to variation in study designs, timing of measurements, and population characteristics. Within the South African context, the interplay of nutritional deficiencies, high HIV prevalence, and increasing obesity underscores the need for targeted investigation. By applying the epidemiological triad as a guiding framework, this study aims to clarify whether Mg deficiency is an independent, modifiable risk factor for PE and to inform context-appropriate preventive strategies.

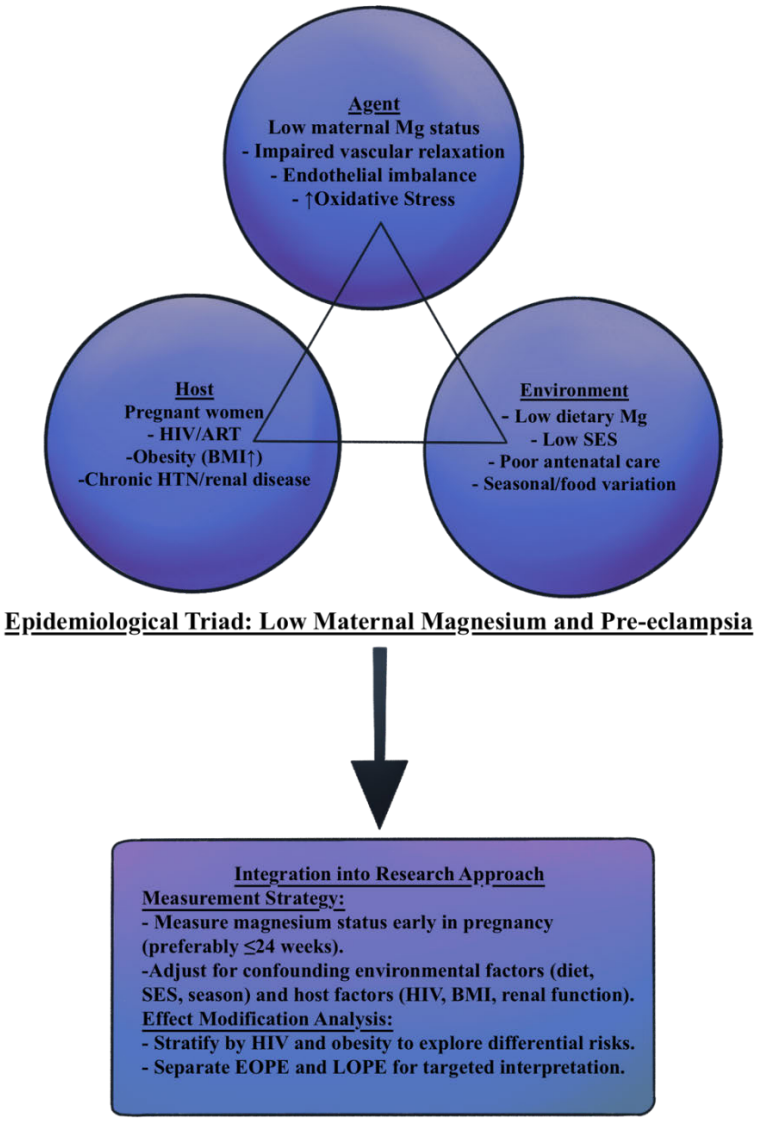


Figure 2. *Conceptual framework of the epidemiological triad illustrating the hypothesised relationships between low maternal magnesium status (Agent), host susceptibility factors (HIV/ART use, obesity, chronic hypertension, and renal disease), and environmental influences (low dietary magnesium intake, poor socioeconomic conditions, limited access to antenatal care, and seasonal variation in food availability).*

These interacting elements may increase the risk of Pe, with potentially stronger effects for early-onset disease. The framework underpins the study's objective to measure PE status early in pregnancy, account for confounding and effect modification, and clarify its role in PE risk within a South African context. This conceptual framework (Figure 2) directly informed our *a priori* hypothesis that circulating serum magnesium would differ between normotensive and pre-eclamptic pregnancies, with potential modification by host factors such as obesity and HIV.

6. Structure of the Thesis

This thesis follows the University of KwaZulu-Natal's College of Health Sciences guidelines for a master's thesis by manuscript. It is presented in three chapters.

Chapter 1 details the background, rationale, aim, and objectives of the study, and outlines the format for successive chapters. A summary of the relevant literature enhances the understanding for the background and rationale for the study. The methodology and ethical considerations are also elucidated.

The manuscript that answers the research question is presented in Chapter 2. It is the primary paper that describes the methods and results of the observational study investigating the relationship between maternal serum Mg levels and PE. This has been accepted for publication by South African Family Practice, and is presented in the format as per the instructions to authors. The study design, methods, and results are detailed, and an in-depth analysis of serum Mg trends and their association with PE is provided. Additionally, it must be noted that since sections are not numbered in the paper, this is presented as such in the Table of Contents of the thesis.

The research findings, in relation to the relevant literature, are synthesised in Chapter 3. It integrates the main findings with existing literature, critically evaluates their implications, and outlines the strengths, limitations, and potential future research directions.

7. Conclusion

This study is grounded in the recognition that PE remains a leading cause of maternal and perinatal morbidity and mortality globally, with a disproportionate impact in LMICs such as South Africa. Despite decades of research, the underlying pathophysiology of PE remains incompletely understood, and the contribution of electrolyte disturbances—particularly alterations in Mg homeostasis—has received relatively limited attention in African populations. By focusing on a South African cohort, this work addresses important gaps in the literature relating to context-specific epidemiology, nutritional status, comorbidities such as obesity and HIV, and their potential interactions with Mg regulation during pregnancy. The findings have the potential to refine local antenatal risk assessment strategies, inform nutritional and clinical guidelines, and contribute to the global discourse on PE pathophysiology.

8. References

1. Dimitriadis E, Rolnik DL, Zhou W, Estrada-Gutierrez G, Koga K, Francisco RPV, et al. Pre-eclampsia. Nature Reviews Disease Primers [Internet]. 2023 Feb 16;9(1):1–22. Available from: https://www.nature.com/articles/s41572-023-00417-6?utm_source=twitter&utm_medium=social&utm_content=organic&utm_campaign=NRRJ_2_SJB_nrdp_editorial_tweet

2. Chappell LC, Cluver CA, Kingdom J, Tong S. Pre-eclampsia. The Lancet [Internet]. 2021 May;398(10297):341–54. Available from: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)32335-7/fulltext

3. Escobar MF, Benitez-Díaz N, Blanco-Londoño I, Cerón-Garcés C, Peña-Zárate EE, Guevara-Calderón LA, et al. Synthesis of evidence for managing hypertensive disorders of pregnancy in low middle-income countries: a scoping review. BMC Pregnancy and Childbirth. 2024 Oct 1;24(1).

4. Moodley J, Soma-Pillay P, Buchmann E, Pattinson RC. Hypertensive disorders in pregnancy: 2019 National Guideline. South African Medical Journal [Internet]. 2019 Sep 13;109(9):12723. Available from: https://pubmed.ncbi.nlm.nih.gov/31635598/

5. Gizamba JM, Davies J, Africa C, Choo-Kang C, Goedecke JH, Madlala HP, et al. Prevalence of obesity, hypertension and diabetes among people living with HIV in South Africa: A systematic review and meta-analysis. BMC Infectious Diseases. 2023 Dec 7;23(1).

6. Torres-Torres J, Espino-y-Sosa S, Martinez-Portilla R, Borboa-Olivares H, Estrada-Gutierrez G, Acevedo-Gallegos S, et al. A narrative review on the pathophysiology of preeclampsia. International Journal of Molecular Sciences [Internet]. 2024 Jan 1;25(14):7569. Available from: https://www.mdpi.com/2864834

7. National Institutes of Health. Magnesium [Internet]. National Institutes of Health. 2022. Available from: https://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional/

8. Dalton LM, Ní Fhloinn DM, Gaydadzhieva GT, Mazurkiewicz OM, Leeson H, Wright CP. Magnesium in pregnancy. Nutrition Reviews. 2016 Jul 21;74(9):549–57.

9. Shukla V, Parvez S, Fatima G, Singh S, Magomedova A, Batiha GE, et al. Micronutrient interactions: Magnesium and its synergies in maternal–fetal health. Food Science & Nutrition. 2024 Jul 17;12(10):6913–28.

10. Shaikh K, Das CM, Baloch GH, Abbas T, Fazlani K, Jaffery MH, Devrajani BR, Shah SZ. Magnesium associated complications in pregnant women. World Applied Sciences Journal. 2012;17(9):1074-8.

11. Karrar S, Hong PL. Preeclampsia [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570611/

12. Gordon T, Booysen F, Mbonigaba J. Socio-economic inequalities in the multiple dimensions of access to healthcare: The case of South Africa. BMC Public Health [Internet]. 2020 Mar 4;20(1):1–13. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-020-8368-7

13. Olusanya NR, Kolanisi U, Ngobese NZ, Mayashree C. Perceptions of ujeqe and amaranthus, a potential supplement for improved food and nutrition security of South Africa. African Journal of Food Agriculture Nutrition and Development. 2024 Feb 29;24(2):25489–507.

14. World Health Organization. WHO recommendations on maternal health guidelines approved by the WHO Guidelines Review Committee second edition [Internet]. World Health Organisation; 2025 May. Available from: https://iris.who.int/bitstream/handle/10665/380666/9789240080591-eng.pdf?sequence=1

15. Makrides M, Crosby DD, Shepherd E, Crowther CA. Magnesium supplementation in pregnancy. Cochrane Database of Systematic Reviews. 2014(4).

16. Spätling L, Spätling G. Magnesium supplementation in pregnancy. A double-blind study. British Journal of Obstetrics and Gynaecology. 1988 Feb 1;95(2):120–5.

17. Jahnen-Dechent W, Ketteler M. Magnesium basics. Clinical Kidney Journal [Internet]. 2012 Feb 1;5(Supplement 1):i3–14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4455825/

18. Ahmed F, Mohammed A. Magnesium: The forgotten electrolyte—A review on hypomagnesemia. Medical Sciences [Internet]. 2019 Apr 4;7(4):56. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6524065/

19. Richards D, Lindow S, Carrara H, Knight R, Haswell S, Van der Spuy Z. A comparison of maternal calcium and magnesium levels in pre-eclamptic and normotensive pregnancies: An observational case-control study. British Journal of Obstetrics and Gynaecology. 2013 Sep 16;121(3):327–36.

20. World Health Organization. Obesity [Internet]. World Health Organization. 2025. Available from: https://www.who.int/health-topics/obesity#tab=tab_1

21. UNAIDS. South Africa [Internet]. UNAIDS; 2023. Available from: https://www.unaids.org/en/regionscountries/countries/southafrica

22. National Department of Health. Saving Mothers 2017-2019: Seventh triennial report on confidential enquiries into maternal deaths in South Africa [Internet]. National Department of Health. 2020. Available from: https://www.health.gov.za/wp-content/uploads/2023/11/Saving-Mothers-2017-2019-Technical-Report-FINAL-2-1.pdf

23. Fanni D, Gerosa C, Nurchi VM, Manchia M, Saba L, Coghe F, et al. The role of magnesium in pregnancy and in fetal programming of adult diseases. Biological Trace Element Research. 2020 Dec 14;199(10).

24. Boeldt DS, Bird IM. Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia. Journal of Endocrinology [Internet]. 2017 Jan;232(1):R27–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5115955/

25. Schwalfenberg GK, Genuis SJ. The importance of magnesium in clinical healthcare. Scientifica. 2017 Sep 28;2017(1):1–14.

26. Piuri G, Zocchi M, Della Porta M, Ficara V, Manoni M, Zuccotti GV, et al. Magnesium in obesity, metabolic syndrome, and type 2 diabetes. Nutrients [Internet]. 2021 Jan 22;13(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7912442/

27. Finkelstein JL, Mehta S. Micronutrients and perinatal outcomes in HIV-infected pregnant women. Nutrition and HIV: Epidemiological Evidence to Public Health [Internet]. 2018 May 15;207–42. Available from: https://www.ncbi.nlm.nih.gov/books/NBK572221/

28. Kassu A, Yabutani T, Mulu A, Tessema B, Ota F. Serum zinc, copper, selenium, calcium, and magnesium levels in pregnant and non-pregnant women in Gondar, Northwest Ethiopia. Biological Trace Element Research. 2008 Jan 17;122(2):97–106.

29. Ephraim RKD, Osakunor DNM, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy-induced hypertension: A case–control study in the Cape Coast metropolis, Ghana. BMC Pregnancy and Childbirth. 2014 Nov 20;14(1).

30. Barbagello M, Veronese N, Dominguez, LJ, Creative Commons. File:Magnesium balance.webp [Internet]. Nutrients. 2022. Available from: https://commons.wikimedia.org/wiki/File:Magnesium_balance.webp#

31. Cunha AR, Umbelino B, Correia ML, Neves MF. Magnesium and vascular changes in hypertension. International Journal of Hypertension [Internet]. 2012;2012(1):754250.. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3299255/

32. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. Pregnancy Hypertension [Internet]. 2018 Jul;13(13):291–310. Available from: https://reader.elsevier.com/reader/sd/pii/S2210778918301260?token=BA7AE7EFA0CE5

5173A8278420E4297E5E26629317B27F6DA630C85A3296F6E8B7AAD197A3B14382E397FA94057D4BD5C

33. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy Hypertension. 2022 Mar;27(27):148–69.

34. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. Basic Research in Cardiology [Internet]. 2008 Jul 3;103(5):398–406. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2705866/

35. Preiser JC. Oxidative stress. Journal of Parenteral and Enteral Nutrition. 2012 Feb;36(2):147–54.

36. Konukoglu D, Uzun H. Endothelial dysfunction and hypertension. Advances in Experimental Medicine and Biology [Internet]. 2016;956:511–40. Available from: https://link.springer.com/chapter/10.1007%2F5584_2016_90

37. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. The New England Journal of Medicine [Internet]. 2004;350(7):672–83. Available from: https://www.ncbi.nlm.nih.gov/pubmed/14764923?access_num=14764923&link_type=MED

38. Shimosawa T, Takano K, Ando K, Fujita T. Magnesium inhibits norepinephrine release by blocking N-type calcium channels at peripheral sympathetic nerve endings. Hypertension. 2004 Dec;44(6):897–902.

39. Poston L, Igosheva N, Mistry HD, Seed PT, Shennan AH, Rana S, et al. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. The American Journal of Clinical Nutrition. 2011 May 25;94(suppl_6):1980S1985S.

40. Nielsen FH. Magnesium deficiency and increased inflammation: Current perspectives. Journal of Inflammation Research [Internet]. 2018 Jan 18;11:25–34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5783146/

41. Zheltova AA, Kharitonova MV, Iezhitsa IN, Spasov AA. Magnesium deficiency and oxidative stress: An update. BioMedicine [Internet]. 2016 Nov 17;6(4). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5112180/

42. Hladunewich M, Karumanchi SA, Lafayette R. Pathophysiology of the clinical manifestations of preeclampsia. Clinical Journal of the American Society of Nephrology [Internet]. 2007 Apr 4;2(3):543–9. Available from: https://cjasn.asnjournals.org/content/2/3/543

43. Tam M, Gómez S, González-Gross M, Marcos A. Possible roles of magnesium on the immune system. European Journal of Clinical Nutrition [Internet]. 2003 Sep 24;57(10):1193–7. Available from: https://www.nature.com/articles/1601689

44. Roberts CK, Hevener AL, Barnard RJ. Metabolic syndrome and insulin resistance: Underlying causes and modification by exercise training. Comprehensive Physiology [Internet]. 2013 Jan;3(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4129661/

45. Bakrania BA, Spradley FT, Drummond HA, LaMarca B, Ryan MJ, Granger JP. Preeclampsia: Linking placental ischemia with maternal endothelial and vascular dysfunction. Comprehensive Physiology [Internet]. 2020 Dec 9;11(1):1315–49. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7959189/

46. Robillard PY, Dekker G, Scioscia M, Bonsante F, Iacobelli S, Boukerrou M, et al. Increased BMI has a linear association with late-onset preeclampsia: A population-based study. Xie L, editor. PLOS One. 2019 Oct 17;14(10):e0223888.

47. Ornaghi S, Tyurmorezova A, Algeri P, Giardini V, Ceruti P, Vertemati E, et al. Influencing factors for late-onset preeclampsia. The Journal of Maternal-Fetal & Neonatal Medicine. 2013 Apr 12;26(13):1299–302.

48. Prabhu K, Dastidar RG, Aroor AR, Rao M, Shetty S, Poojari VG, et al. Micronutrients in adverse pregnancy outcomes. F1000Research [Internet]. 2024 Jun 21;11:1369–9. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11131564/

49. Eslamzadeh S, Taghinezhad H, Khorasani M. Circulating levels of magnesium in preeclamptic versus normotensive pregnancies: A meta-analysis. Galen Medical Journal. 2023;12:e3151.

50. Frølich A, Rudnicki M, Storm T, Rasmussen N, Hegedüs L. Impaired 1,25-dihydroxyvitamin D production in pregnancy-induced hypertension. European Journal of Obstetrics, Gynecology, and Reproductive Biology [Internet]. 1992 Autumn;47(1):25–9. Available from: https://pubmed.ncbi.nlm.nih.gov/1426508/

51. Jain S, Sharma P, Kulshreshtha S, Mohan G, Singh S. The role of calcium, magnesium, and zinc in pre-eclampsia. Biological Trace Element Research. 2009 Jun 23;133(2):162–70.

52. Rolnik DL, Wright D, Poon LCY, Syngelaki A, O'Gorman N, de Paco Matallana C, et al. ASPRE trial: Performance of screening for preterm pre-eclampsia. Ultrasound in Obstetrics & Gynecology [Internet]. 2017 Aug 24;50(4):492–5. Available from: https://www.fetalmedicine.org/var/pdf/publications/1096.pdf

53. Friis H, Frahm Olsen M, Filteau S. Macronutrient supplementation to HIV and TB patients during treatment [Internet]. Mehta S, Finkelstein JL, editors. PubMed. New York (NY): CRC Press; 2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK572227/

54. Owusu Darkwa E, Antwi-Boasiako C, Djagbletey R, Owoo C, Obed S, Sottie D. Serum magnesium and calcium in preeclampsia: A comparative study at the Korle-Bu Teaching Hospital, Ghana. Integrated Blood Pressure Control. 2017 Aug;Volume 10:9–15.

55. O Akinloye, OJ Oyewale, OO Oguntibeju. Evaluation of trace elements in pregnant women with pre-eclampsia. African Journal of Biotechnology [Internet].

2019;9(32):5196–202. Available from: https://www.ajol.info/index.php/ajb/article/view/92151

56. Idogun ES, Imarengiaye CO, Momoh SM. Extracellular calcium and magnesium in preeclampsia and eclampsia. African Journal of Reproductive Health [Internet]. 2007 Aug;11(2):89–94. Available from: https://pubmed.ncbi.nlm.nih.gov/20690291/

57. Vorster HH. An introduction to the revised food-based dietary guidelines for South Africa. South African Journal of Clinical Nutrition. 2013 Aug 14;26(3)(Supplement):S5–12.

58. Obi SN, Ifebunandu NA, Onyebuchi AK. Nutritional status of HIV-positive individuals on free HAART treatment in a developing nation. The Journal of Infection in Developing Countries [Internet]. 2010 Aug 18;4(11):745–9. Available from: https://www.researchgate.net/publication/49772051_Nutritional_status_of_HIV-positive_individuals_on_free_HAART_treatment_in_a_developing_nation

59. Sidahmed MAE, Abubaker NE, Elfadil GA. Serum total calcium, magnesium, sodium and potassium in Sudanese women with preeclampsia. International Journal of Advanced Research [Internet]. 2017 Feb;5(2). Available from: https://www.researchgate.net/publication/314222209_Serum_total_calcium_magnesium_sodium_and_potassium_in_Sudanese_women_with_preeclampsia

60. de Pee S, Semba RD. Role of nutrition in HIV infection: Review of evidence for more effective programming in resource-limited settings. Food and Nutrition Bulletin. 2010 Dec;31(4 Supplement 4):S313–44.

61. Costello RB, Elin RJ, Rosanoff A, Wallace TC, Guerrero-Romero F, Hruby A, et al. Perspective: The case for an evidence-based reference interval for serum magnesium: The time has come. Advances in Nutrition [Internet]. 2016 Nov 10;7(6):977–93. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5105038/

62. Atta MG, De Seigneux S, Lucas GM. Clinical pharmacology in HIV therapy. Clinical Journal of the American Society of Nephrology. 2018 May 29;14(3):435–44.

63. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller AB, Daniels J, et al. Global causes of maternal death: A WHO systematic analysis. The Lancet Global Health [Internet]. 2014;2(6):e323-33. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25103301

64. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: Systematic review and meta-analysis. British Medical Journal [Internet]. 2007 Nov 8;335(7627):974. Available from: https://www.bmj.com/content/335/7627/974.short

65. Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L, Torloni MR. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems [Internet]. The Cochrane Database of Systematic Reviews. 2014. Available from: https://pubmed.ncbi.nlm.nih.gov/24960615/

66. Touyz R M. Magnesium in clinical medicine. Frontiers in Bioscience. 2004;9(1-3):1278.

67. Elin RJ. Assessment of magnesium status for diagnosis and therapy. Magnesium Research [Internet]. 2010;23(4):S194-8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20736141

68. Kisters K, Barenbrock M, Louwen F, Hausberg M, Rahn KH, Kosch M. Membrane, intracellular, and plasma magnesium and calcium concentrations in preeclampsia. American Journal of Hypertension. 2000 Jul 1;13(7):765–9.

69. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. World Health Organisation; 2011. Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.1

70. Roche Diagnostics. Cobas c 311 Analyzer [Internet]. Roche Diagnostics; 2020. Available from: https://www.labservis.com/sites/default/files/2020-02/Cobas_c311_automated_chemistry_analyzer.pdf

71. Schutten JC, Gomes-Neto AW, Navis G, Gansevoort RT, Dullaart RPF, Kootstra-Ros JE, et al. Lower plasma magnesium, measured by nuclear magnetic resonance spectroscopy, is associated with increased risk of developing type 2 diabetes mellitus in women: Results from a Dutch prospective cohort study. Journal of Clinical Medicine. 2019 Feb 1;8(2):169.

72. BM Corporation. IBM SPSS statistics 29 documentation [Internet]. IBM Corporation; 2024. Available from: https://www.ibm.com/docs/en/spss-statistics/29.0.0

CHAPTER 2: A CROSS-SECTIONAL STUDY DETERMINING THE ASSOCIATION BETWEEN CIRCULATING MATERNAL SERUM MAGNESIUM LEVELS AND PREGNANCY OUTCOMES IN PREGNANT BLACK SOUTH AFRICAN WOMEN.

A paper written in accordance with the guidelines for South African Family Practice, which has been accepted for publication, is presented in Chapter 2. The study focused on determining the levels of serum magnesium present in a cohort of Black South African women during pregnancy and considered the participants' body mass index, HIV status and pre-eclamptic status. The conclusion was that magnesium was lower in cases of normotensive pregnancy, with no association found between magnesium and either co-morbidity. Therefore, magnesium isolation cannot be considered a sufficiently strong predictive marker of PE.

Additional results that could not be included in this paper due to limitations imposed by word count, are attached as Appendix 7.

THE ASSOCIATION BETWEEN CIRCULATING SERUM MAGNESIUM LEVELS AND HYPERTENSIVE DISORDERS OF PREGNANCY IN BLACK SOUTH AFRICAN WOMEN: A CROSS-SECTIONAL STUDY

Naeera Abdul, Vinogrin Dorsamy, Chauntelle Bagwandeen

The following article was submitted to South African Family Practice on the 20th of February 2025 and was accepted for publication on the 6th of August 2025.

Attached below is an email from South African Family Practice confirming publication.

SAFP 6140: Manuscript Accepted for Publication, Sent to Editing

A

aosis@safpj.co.za <aosis@safpj.co.za>

Thursday, 07 August 2025 at 13:21

To: Naeera Abdul (220004494); Vino Dorsamy; Chauntelle Bagwandeen

****
Ref. No.: 6140
Manuscript title: The association between circulating serum magnesium levels and hypertensive disorders of pregnancy in Black South African women: A cross-sectional study
Journal: South African Family Practice
****

Dear Naeera Abdul, Vinogrin Dorsamy, Chauntelle Bagwandeen

We are pleased to confirm your manuscript's acceptance for publication on 06-Aug-25.

We can also confirm that the Submission and Review Department released your manuscript to our Finalisation Department to commence the various editing processes to secure online publication within the next 90 days (if not sooner).

Kindly note:
1. If you need to make contact with AOSIS Publishing during the finalisation stage of your manuscript, kindly contact us per email or phone.
2. The finalisation procedure works as follows: (a) The first stage is the language editing that is returned to the corresponding Author for review. This will be the final opportunity for the corresponding Author to make text changes to the manuscript. (b) At a later stage, the editorial staff will send the corresponding author one set of galley proofs, at which time the Author will have two working days to mark any typographical errors.
3. Manuscript tracking is available on the submitting authors' journal profile. The submitting Author could visit their home page frequently to assess the stage of the manuscript.

Thank you for your continued patience and support, and we hope you have joined our online community by signing up to our RSS alerts and X (formerly Twitter) page.

Kind regards,
Ms Van Blerk
AOSIS

Abstract

Background

Given South Africa's heavy burden of maternal mortality, understanding modifiable risk factors such as magnesium deficiency in pregnancy is critical. Magnesium levels progressively decline during normal pregnancies; however, their relationship with pre-eclampsia remains poorly understood, especially in populations with high maternal mortality, obesity, and HIV prevalence.

Methods

This cross-sectional study involved 252 pregnant Black South African women categorised into: First Visit Normotensive (First VisitNT), Term Normotensive (TermNT), Early-onset pre-eclampsia, and Late-onset pre-eclampsia. Serum magnesium levels were measured using atomic absorption spectrophotometry. Statistical analysis, including chi-square tests, and multinomial logistic regression, was performed to identify predictors of pre-eclampsia.

Results

Magnesium levels declined significantly during normotensive pregnancies (First VisitNT vs. TermNT, $p<.001$). However, in those women with pre-eclampsia, magnesium levels remained stable or slightly elevated. Hypomagnesemia was most prevalent in the TermNT group (23.9%) and was significantly associated with reduced odds of pre-eclampsia (OR = 0.216, $p = 0.013$). Obesity was a strong predictor of pre-eclampsia, whilst no significant relationship was found between magnesium levels and HIV status.

Conclusion

Physiological adaptations decrease magnesium levels in normotensive pregnancies and are absent in pre-eclampsia, suggesting altered magnesium regulation. This highlights magnesium's complex role in the pathophysiology of pre-eclampsia and the need for nutritional and clinical interventions targeting magnesium insufficiency in populations with high maternal morbidity. Future longitudinal studies are needed to explore the causal relationships between magnesium levels and pre-eclampsia.

Contribution

This study adds to the limited literature on magnesium dynamics in pregnancy and highlights the need for population-specific strategies to reduce maternal mortality.

Keywords

Magnesium, pre-eclampsia, maternal health, HIV, obesity, South Africa

Introduction

Magnesium sulphate is widely used in the management of pre-eclampsia (PE) to prevent seizures and progression to eclampsia, highlighting its established role in maternal care [1]. However, the potential benefit of magnesium (Mg) supplementation earlier in pregnancy to prevent the development of PE remains underexplored. Understanding whether magnesium insufficiency in early pregnancy contributes to the pathophysiology of PE is particularly relevant in high-risk populations, such as those in South Africa. As a country with persistently high maternal mortality rates, South Africa categorises maternal mortality as a distinct burden of disease, alongside infectious and non-communicable conditions [2]. Therefore, investigating accessible and cost-effective interventions like magnesium supplementation could yield significant public health benefits. Magnesium is an essential micronutrient involved in vascular tone, endothelial function, blood pressure regulation and cellular function. [3,4] During pregnancy, Mg supports fetal development and maternal well-being through its role in over 600 enzymatic reactions [3,4]. Physiological adaptations during gestation, such as haemodilution, increased renal excretion, and heightened fetal demands, contribute to a progressive decline in circulating Mg levels [5]. These changes can predispose vulnerable individuals to hypomagnesaemia, particularly those with marginal nutritional intake, and may exacerbate the risk of adverse pregnancy outcomes, including hypertensive disorders of pregnancy (HDP) [6].

The South African context presents a unique setting to explore Mg's role in pregnancy. The country paradoxically experiences a dual burden of malnutrition: undernutrition persists alongside a high prevalence of obesity, which affects nearly 46% of adult women[7]. Obesity is a well-established risk factor for PE, and it is often associated with systemic inflammation and poor dietary diversity, including reduced intake of Mg-rich fools such as whole grains, nuts, and leafy greens [8-10]. Additionally, South Africa has one of the world's highest HIV prevalence rates, with approximately 4.8 million women affected [11]. Both HIV infection and antiretroviral therapy (ART) have been linked to altered micronutrient metabolism, further complicating maternal nutritional status and pregnancy outcomes [12].

Despite the established biological relevance of Mg in maternal physiology, there is a paucity of population-specific data on circulating Mg levels and their association with PE in South African women. This gap in the literature limits the ability to identify at-risk subgroups and develop targeted interventions.

This study aims to evaluate the relationship between serum magnesium levels and hypertensive disorders of pregnancy in a cohort of pregnant Black South African women. Specifically, we aim to: (1) compare magnesium levels across different pregnancy groups, including normotensive and pre-eclamptic women; (2) assess the prevalence of hypomagnesaemia and its association with PE; and (3) identify potential predictors of PE, including obesity, HIV status, and gestational age, in a population facing intersecting burdens of malnutrition, chronic disease, and limited healthcare resources. By identifying modifiable nutritional risk factors such as magnesium deficiency, this research contributes to the development of evidence-based public health strategies aimed at reducing maternal morbidity and mortality in South Africa and similar settings.

Methods

Study Design and Setting

This was a hospital-based cross-sectional analytical study conducted at a regional hospital in Durban, KwaZulu-Natal. The study aimed to assess the association between circulating serum magnesium levels and HDP including early-onset and late-onset pre-eclampsia (EOPE and LOPE), in a cohort of pregnant Black South African women. Data collection occurred between June 2017 to January 2020.

Study Design Justification

Although birth outcomes such as neonatal weight and gestational age at delivery were included in the analysis, the study maintained a cross-sectional design. Each participant was assessed once during pregnancy for clinical and biochemical data, and delivery outcomes were retrospectively extracted from medical records. No longitudinal follow-up or repeated measurements were performed. This approach aligns with cross-sectional designs commonly used in perinatal research that incorporate administrative or clinical outcome data [13,14].

Participants

A total of 260 pregnant women aged ≥18 years, attending routine antenatal care at the study hospital, were recruited using a convenience non-probability consecutive sampling. Only participants who provided informed written consent were included.

Participants were categorised into four groups:

1. First Visit Normotensive (First VisitNT): women attending their first antenatal visit between 12–28 weeks gestation, with normal blood pressure.
2. Term Normotensive (TermNT): women confirmed normotensive at term (≥37 weeks).

3. Early-onset pre-eclampsia (EOPE): onset of pre-eclampsia before 34 weeks.
4. Late-onset pre-eclampsia (LOPE): onset of pre-eclampsia at or after 34 weeks.

The diagnosis of PE was based on the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria: new-onset hypertension (≥140/90 mmHg on two occasions at least 4–6 hours apart) after 20 weeks' gestation, with one or more of the following: proteinuria, renal dysfunction, liver involvement, neurological features, or haematological disturbances [15].

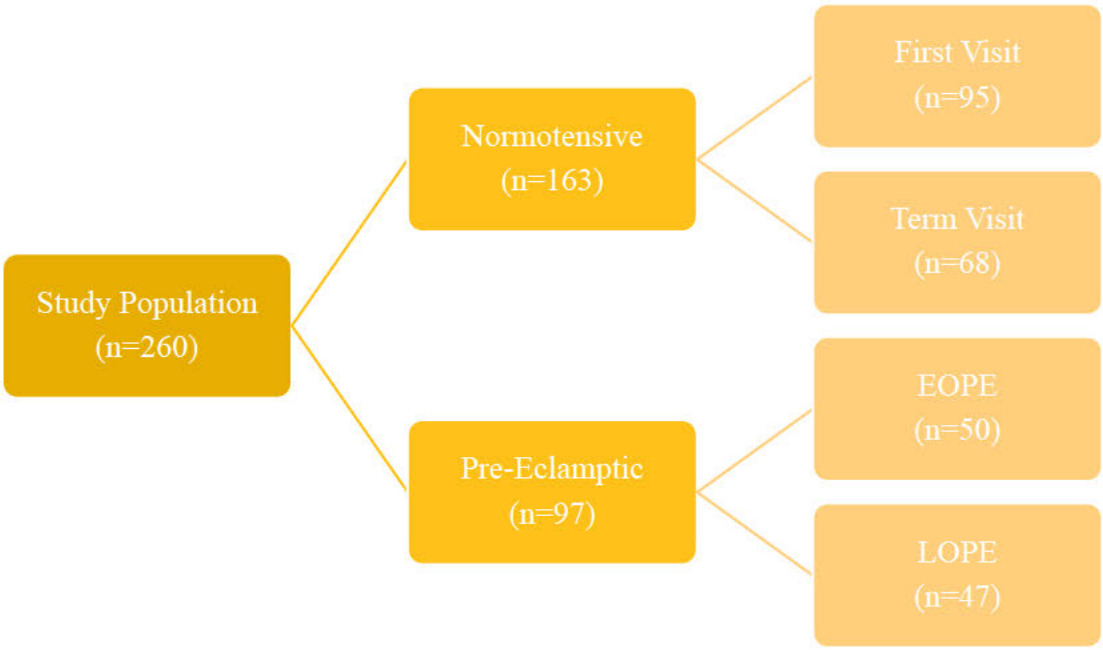


Figure 1: Flow chart illustrating the sample size per group

Inclusion and Exclusion Criteria

This study focused on pregnant Black South African women who were 18 and older. Participants who were willing to give informed consent who had a gestational age between 12 and 42 weeks were included. Participants with pre-existing chronic hypertension, diabetes mellitus, or renal disease, who were diagnosed with gestational diabetes, had known multiple pregnancies or were using antihypertensives, magnesium supplements, aspirin, warfarin, or anti-inflammatory medications were excluded.

Data Collection and Laboratory Procedures

A structured data collection form was used to obtain sociodemographic, clinical, and obstetric history during antenatal visits. Trained nurses measured blood pressure, recorded gestational age, and body mass index (BMI) from measured weight and height. Gestational age was

confirmed using a combination of last menstrual period (LMP) and first-trimester ultrasound when available. Baby weight was recorded at delivery using a standard neonatal scale.

Sample Collection

Venipuncture was performed to obtain blood samples, which were collected in BD Vacutainer® tubes. Serum was isolated via centrifugation (3000g for 12 minutes) and stored in Eppendorf tubes at −80°C until analysis. Urine dipstick tests were conducted to confirm proteinuria.

Magnesium Sample Collection and Measurement

Venous blood was collected into BD Vacutainer® clot activator tubes and allowed to clot for 30 minutes before centrifugation at 3000g for 12 minutes. Serum was separated and stored at −80°C in Eppendorf tubes until analysis. Serum Mg levels were quantified using atomic absorption spectrophotometry (Cobas Pro c 503 ,Roche Diagnostics, F. Hoffmann-La Roche Ltd). The process involved a 10-minute colourimetric endpoint assay where Mg reacted with xylidyl blue in an alkaline buffer to form a purple complex. Concentrations were photometrically measured at 505/600nm [16]. Quality controls and calibrations were performed before analysis to ensure accuracy. Results were expressed in mmol/L. Hypomagnesaemia was defined as serum Mg <0.66 mmol/L, based on National Health Laboratory Service (NHLS) reference values.

Variables

- Primary exposure: Serum magnesium concentration (continuous and categorical: normal vs. hypomagnesaemia).
- Primary outcome: Hypertensive status (normotensive, EOPE, or LOPE).
- Covariates: Age, BMI (categorised as normal, overweight, obese per WHO), HIV status (positive/negative), gestational age at delivery [7].

Statistical Analysis

Data were analysed using IBM® SPSS® Statistics Version 29.0 (IBM Corp., Armonk, NY). Continuous variables were tested for normality using the Shapiro–Wilk test. Descriptive statistics included means (±SD) for continuous variables and frequencies (%) for categorical variables. Baseline characteristics (e.g., age, BMI, gestational age) were summarized as means (±SD) for continuous variables and frequencies (%) for categorical variables. Following normality testing, analysis of variance (ANOVA) was conducted to test the differences in

magnesium levels across groups and followed by post-hoc Bonferroni comparisons. Chi-square tests were used to evaluate the association between magnesium status (normal vs. hypomagnesaemia) and study groups. Multinomial logistic regression was conducted to identify predictors of PE (EOPE and LOPE) relative to normotensive groups, adjusting for covariates such as BMI, HIV status, and gestational age.

Listwise deletion was used for cases with missing values on variables included in multivariable models. The extent of missing data for each variable was reported descriptively. No imputation techniques were used due to the relatively low proportion of missing data (<10% for all key variables). A p-value <0.05 was considered statistically significant.

Ethical Considerations

Ethical clearance was obtained from a sub-committee of the Biomedical Research Ethics Committee of the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BREC/00007005/2024). Written informed consent was obtained from all participants. Confidentiality was maintained by anonymising data, and participation was voluntary. The study was conducted in accordance with the Declaration of Helsinki[17].

Results

Study Population and Baseline Characteristics

Out of 260 enrolled participants, 252 were included in the final analysis after excluding eight with incomplete records. Participants were categorised into four groups: First Visit Normotensive (First VisitNT, n = 94), Term Normotensive (TermNT, n = 67), Early-onset pre-eclampsia (EOPE, n = 49), and Late-onset pre-eclampsia (LOPE, n = 42). Table 1 summarises the baseline characteristics of each group.

The mean age of participants ranged from 27.2 ± 5.6 years (First VisitNT) to 28.9 ± 6.9 years (LOPE), with no significant age differences between groups. Body mass index was notably higher in the pre-eclamptic groups, with the highest mean BMI recorded in the LOPE group (36.7 ± 8.6 kg/m²), followed by EOPE (33.6 ± 7.9 kg/m²), TermNT (33.0 ± 6.2 kg/m²), and First VisitNT (29.1 ± 5.0 kg/m²). The TermNT group had the highest mean baby weight (3.17 ± 0.51 kg), whereas the EOPE group had the lowest (2.32 ± 0.73 kg).

Table 1. Baseline Characteristics of Study Participants by Group

Characteristic	First VisitNT (n=94)	TermNT (n=67)	EOPE (n=49)	LOPE (n=42)
Age (years)	27.23 ± 5.56	28.46 ± 6.48	28.33 ± 6.14	28.88 ± 6.85
Gestational Age at Admission (weeks)	18.65 ± 5.52	37.94 ± 1.80	26.35 ± 5.40	37.05 ± 3.79
BMI (kg/m²)	29.05 ± 5.02	33.00 ± 6.17	33.60 ± 7.93	36.74 ± 8.56
Haemoglobin (g/dL)	11.31 ± 1.61	11.01 ± 1.57	11.04 ± 0.95	11.32 ± 1.26
Baby Weight (kg)	3.07 ± 0.58	3.17 ± 0.51	2.32 ± 0.73	2.81 ± 0.53
Magnesium Level (mmol/L)	0.78 ± 0.08	0.72 ± 0.10	0.78 ± 0.12	0.76 ± 0.09

Values are presented as mean ± standard deviation.

BMI = Body Mass Index; EOPE = Early-onset pre-eclampsia; LOPE = Late-onset pre-eclampsia; NT = Normotensive.

Serum Magnesium Levels Across Groups

Mean serum magnesium levels differed significantly between groups (ANOVA, $F(3,248) = 6.0$, $p < 0.001$, $\eta^2 = 0.076$). Among normotensive participants, a significant decline in magnesium levels was observed between First VisitNT (0.78 ± 0.08 mmol/L) and TermNT (0.72 ± 0.10 mmol/L) groups ($p < 0.001$). This decline was not observed in the PE groups.

Participants with EOPE and LOPE had mean magnesium levels of 0.78 ± 0.12 mmol/L and 0.76 ± 0.09 mmol/L, respectively. No significant differences were found between either PE group and the First VisitNT group. However, EOPE participants had significantly higher magnesium levels than those in the TermNT group ($p = 0.003$). The decline in serum magnesium levels observed between the First VisitNT and TermNT groups was not present in the EOPE and LOPE groups, where magnesium levels remained stable (Figure 2).

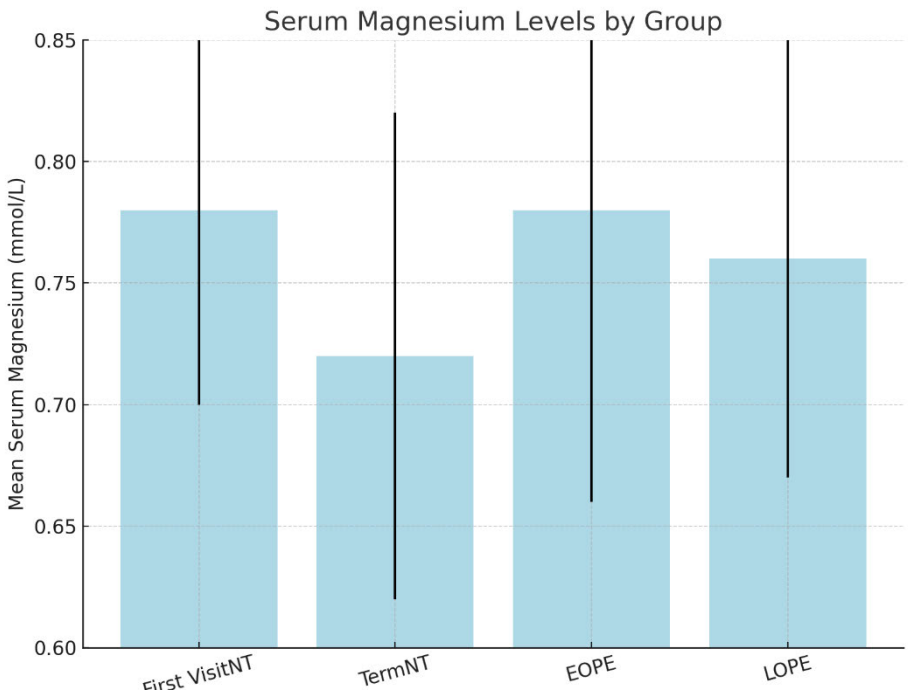


Figure 2. Mean serum magnesium levels (± standard deviation) by group.

Magnesium levels declined significantly from FirstVisitNT to TermNT pregnancies. This decline was not observed in the EOPE and LOPE groups, where levels remained stable.

Prevalence of Hypomagnesaemia

Using the National Health Laboratory Service reference range (hypomagnesaemia defined as <0.66 mmol/L), the overall prevalence of hypomagnesaemia was highest in the TermNT group (23.9%), followed by LOPE (9.5%), EOPE (12.2%), and First VisitNT (4.3%).

A chi-square test revealed a statistically significant association between magnesium status (normal vs. hypomagnesaemia) and group classification ($\chi^2(3) = 14.64$, $p = 0.002$). Notably, hypomagnesaemia was associated with significantly reduced odds of PE (OR = 0.216, 95% CI: 0.065–0.721, p = 0.013), suggesting an inverse relationship between low magnesium levels and PE in this sample.

HIV Across the Groups

In the normotensive groups, HIV affected 51,1% and 40.3% of the FirstVisitNT and TermVisitNT participants respectively, whereas 55,1% of the EOPE and 40.5% of the LOPE groups were affected by HIV. HIV was not shown to be significantly associated with either hypertensive group.

Predictors of PE

Multinomial logistic regression was used to identify independent predictors of EOPE and LOPE compared to normotensive participants. The model included BMI, gestational age at birth, serum magnesium status, and HIV status.

- Obesity was significantly associated with both EOPE (OR = 3.77, 95% CI: 1.35–10.53, $p = 0.011$) and LOPE (OR = 3.39, 95% CI: 1.36–8.47, $p = 0.009$).
- Gestational age at birth was strongly protective for EOPE (OR = 0.46, 95% CI: 0.35–0.61, $p < 0.001$).
- Magnesium status showed an inverse association with PE risk as noted above.
- HIV status was not significantly associated with any hypertensive group.

Discussion

This study examined the relationship between circulating serum magnesium levels and HDP in a cohort of Black South African women. Specifically, we sought to evaluate differences in serum magnesium across normotensive and pre-eclamptic pregnancies, determine the prevalence of hypomagnesaemia, and identify predictors of EOPE and LOPE. These objectives were addressed in the context of overlapping maternal health challenges in South Africa, including high rates of obesity, HIV infection, and nutritional deficiencies.

The findings confirm that serum magnesium levels decline significantly during the course of normotensive pregnancies, consistent with the expected physiological adaptations of gestation. In contrast, magnesium levels in women with PE remained stable and did not exhibit the same decline observed in normotensive groups. This pattern was particularly notable in the comparison between Term Normotensive and pre-eclamptic groups, suggesting that the regulatory mechanisms influencing magnesium homeostasis may be disrupted in PE. This aligns with previous research proposing impaired renal excretion or endothelial dysfunction as mechanisms for altered magnesium metabolism in PE [18,19].

Hypomagnesaemia was most prevalent in the Term Normotensive group and significantly less common among women with PE. Multivariate analysis showed that hypomagnesaemia was associated with a lower likelihood of both EOPE and LOPE. Whilst this inverse association may appear counterintuitive, it is consistent with findings from studies in Ghana and South Africa which have reported either no association or elevated magnesium levels in women with

HDPs[13,14]. One possible explanation is that the stable or elevated magnesium levels observed in pre-eclamptic women reflect underlying pathophysiological changes—such as endothelial damage or reduced renal clearance—rather than a protective effect of magnesium itself. It is also plausible that magnesium deficiency contributes to vascular risk only up to a threshold, beyond which systemic compensatory responses in PE alter circulating levels.

Obesity emerged as a strong independent predictor of both EOPE and LOPE in this study, consistent with literature highlighting the role of obesity-related inflammation and metabolic dysregulation in the pathogenesis of HDPs [20,21]. Given the high prevalence of obesity among South African women (estimated at 46%), this finding underscores the urgent need for preconception and antenatal interventions that address nutritional status and weight management [7]. Obesity may also mediate magnesium imbalance through increased renal magnesium loss, insulin resistance, or altered dietary intake, though further studies are needed to clarify these pathways.

HIV status was not significantly associated with serum magnesium levels or the risk of PE in our analysis. This finding contrasts with some prior studies suggesting a link between HIV infection, antiretroviral therapy (ART), and micronutrient depletion [22-24]. It is possible that the standardisation of ART regimens in South Africa and improved integration of antenatal HIV care have attenuated such associations. Alternatively, the sample size or limited granularity of ART-related data in this study may have obscured true effects.

Together, these findings support the hypothesis that magnesium plays a complex role in pregnancy and HDP pathophysiology, but also illustrate that circulating magnesium levels may be influenced by a variety of systemic factors. As such, low serum magnesium alone may not be a reliable biomarker for predicting PE without considering co-factors such as gestational age, renal function, dietary intake, and obesity status.

Strengths and Limitations

This study adds to the limited body of research on magnesium dynamics in African populations, with particular relevance to public health settings burdened by nutritional and metabolic disorders. The use of a clearly defined cohort, standardised laboratory methods, and multivariable adjustment are key strengths.

However, the findings must be interpreted in light of several limitations. First, the cross-sectional design precludes causal inference, and the temporal relationship between magnesium levels and HDP onset cannot be definitively established. Second, serum magnesium does not reflect intracellular magnesium, which may be more relevant to vascular function. Third, dietary magnesium intake, renal function, and ART regimen details were not assessed, limiting insight into potential confounders or mediators. Lastly, restricting the study population to Black South African women, though intentional for population-specific analysis, limits generalisability to other groups.

Conclusion

This study adds novel data on the profile of serum magnesium levels across gestation in a South African population, revealing a divergence in trends between normotensive and pre-eclamptic pregnancies. The inverse association between hypomagnesaemia and PE challenges prevailing assumptions and invites further scrutiny into the regulatory mechanisms of magnesium in pregnancy. As one of the few studies to report on this relationship in a resource-constrained African setting, the findings hold relevance for local antenatal screening strategies and emphasizes the importance of context-specific research in maternal health.

Acknowledgements

The authors extend gratitude towards the study's participants as their contribution was invaluable and allowed for this study to take place. This article is partially based on the author's thesis, 'The interplay between magnesium and hypertensive disorders of pregnancy in a pregnant Black South African population', towards the degree of Master of Medical Science (Physiology).

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Author contributions

NA and VD were responsible for the conception and design of the article. NA collected the data and drafted the article. VD analysed the data. Both VD and CB supervised the research process and provided guidance.

Funding

This study was 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. Further, there was no input from these organisations in the interpretation of the result.

Data availability

Data will be available from the corresponding author, NA, upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and are the product of professional research. The article does not necessarily reflect the official policy or position of any affiliated institution, funder, agency, or that of the publisher. The authors are responsible for this article's results, findings, and content

.

References

1. Chappell LC, Cluver CA, Kingdom J, Tong S. Pre-eclampsia. The Lancet [Internet]. 2021 May;398(10297):341–54. Available from: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)32335-7/fulltext

2. Samodien E, Abrahams Y, Muller C, Louw J, Chellan N. Non-communicable diseases – A catastrophe for South Africa. South African Journal of Science. 2021 May 28;117(5/6).

3. Dalton LM, Ní Fhloinn DM, Gaydadzhieva GT, Mazurkiewicz OM, Leeson H, Wright CP. Magnesium in pregnancy. Nutrition Reviews. 2016 Jul 21;74(9):549–57.

4. Shukla V, Parvez S, Fatima G, Singh S, Magomedova A, Batiha GES, et al. Micronutrient interactions: Magnesium and its synergies in maternal–fetal health. Food Science & Nutrition. 2024 Jul 17;12(10):6913–28.

5. Laires MJ. Role of cellular magnesium in health and human disease. Frontiers in Bioscience. 2004 Jan 1;9(1-3):262–76.

6. Schoenaker DA, Soedamah-Muthu SS, Mishra GD. The association between dietary factors and gestational hypertension and pre-eclampsia: a systematic review and meta-analysis of observational studies. BMC Medicine [Internet]. 2014 Sep 22;12(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4192458/

7. World Health Organisation. Obesity [Internet]. World Health Organization. 2025. Available from: https://www.who.int/health-topics/obesity#tab=tab_1

8. Piuri G, Zocchi M, Della Porta M, Ficara V, Manoni M, Zuccotti GV, et al. Magnesium in obesity, metabolic syndrome, and type 2 diabetes. Nutrients [Internet]. 2021 Jan

9. Popkin B. Ultra-processed foods' impacts on health [Internet]. 2030-Food, Agriculture and rural development in Latin America and the Caribbean; 2020. Available from: https://openknowledge.fao.org/server/api/core/bitstreams/39d6f1db-c162-416a-8629-b47b5e3a0725/content

10. National Institutes of Health. Magnesium [Internet]. National Institutes of Health. 2022. Available from: https://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional/

11. UNAIDS. South Africa [Internet]. unaids.org. UNAIDS; 2023. Available from: https://www.unaids.org/en/regionscountries/countries/southafrica

12. Finkelstein JL, Mehta S. Micronutrients and perinatal outcomes in HIV-Infected pregnant women. Nutrition and HIV: Epidemiological Evidence to Public Health [Internet]. 2018 May 15;207–42. Available from: https://www.ncbi.nlm.nih.gov/books/NBK572221/

13. Ephraim RKD, Osakunor DNM, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy-induced hypertension: a case–control study in the Cape Coast metropolis, Ghana. BMC Pregnancy and Childbirth. 2014 Nov 20;14(1).

14. Richards D, Lindow S, Carrara H, Knight R, Haswell S, Van der Spuy Z. A comparison of maternal calcium and magnesium levels in pre-eclamptic and normotensive pregnancies: an observational case-control study. British Journal of Obstetrics and Gynaecology. 2013 Sep 16;121(3):327–36.

15. Schutten JC, Gomes-Neto AW, Navis G, Gansevoort RT, Dullaart RPF, Kootstra-Ros JE, et al. Lower plasma magnesium, measured by nuclear magnetic resonance spectroscopy, is associated with increased risk of developing type 2 diabetes mellitus in women: Results from a Dutch prospective cohort study. Journal of Clinical Medicine. 2019 Feb 1;8(2):169.

16. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy Hypertension. 2022 Mar;27(27):148–69.

17. World Medical Association. WMA Declaration of Helsinki – Ethical principles for medical research involving human participants [Internet]. wma.net. World Medical

Association; 2024. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki/

18. Boeldt DS, Bird IM. Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia. Journal of Endocrinology [Internet]. 2017 Jan;232(1):R27–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5115955/

19. Wang X, Shields CA, Ekperikpe U, Amaral LM, Williams JM, Cornelius DC. Vascular and renal mechanisms of preeclampsia. Current Opinion in Physiology [Internet]. 2023 Jun 1;33:100655. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2468867323000251

20. Olson KN, Redman LM, Sones JL. Obesity "complements" preeclampsia. Physiological Genomics. 2019 Mar 1;51(3):73–6.

21. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators of Inflammation [Internet]. 2010 Jul 14;2010(Mediators of Inflammation in Obesity and its Comorbidities):1–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2913796/

22. Suy A, Martínez E, Coll O, Lonca M, Palacio M, de Lazzari E, et al. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. AIDS. 2006 Jan 2;20(1):59–66.

23. Machado ES, Krauss MR, Megazzini K, Coutinho CM, Kreitchmann R, Melo VH, et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. The Journal of Infection [Internet]. 2014 Jun 1;68(6):572–80. Available from: https://pubmed.ncbi.nlm.nih.gov/24462561/

24. Kassu A, Yabutani T, Mulu A, Tessema B, Ota F. Serum zinc, copper, selenium, calcium, and magnesium levels in pregnant and non-pregnant women in Gondar, Northwest Ethiopia. Biological Trace Element Research. 2008 Jan 17;122(2):97–106.

CHAPTER 3: SYNTHESIS AND CONCLUSION

The purpose of this chapter is to synthesise the preceding discussions and to contextualise and integrate the findings of the empirical study, in the context of the current literature, by addressing the central research question: Can serum magnesium levels serve as a predictive marker for pre-eclampsia? The study limitations, implications for future research and recommendations for clinical practise in the South African context are also presented.

1. Introduction

This thesis investigated the relationship between maternal serum magnesium (Mg) levels and the development of pre-eclampsia (PE) in a South African cohort, with a specific focus on contextual epidemiological realities, including comorbidities such as obesity and HIV infection. The rationale stemmed from the understanding that electrolyte imbalances, particularly in Mg homeostasis, may contribute to the pathophysiology of PE—a condition that remains a leading cause of maternal and perinatal morbidity and mortality globally. While international literature frequently reports reduced Mg levels in PE, limited and conflicting evidence from African settings suggests the need for population-specific studies. The aim of this work was to integrate biochemical, clinical, and sociodemographic data to generate context-sensitive insights that could inform clinical risk assessment and antenatal care strategies in low- and middle-income countries (LMICs).

2. Summary of Main Findings

This study demonstrated distinct patterns in serum Mg dynamics between normotensive pregnancies and those complicated by PE. Among normotensive women, there was a statistically significant decline in mean serum Mg concentrations from the first antenatal visit to term, suggesting a physiological downward trajectory consistent with haemodilution and increased fetal demand as gestation advances. In contrast, women with PE maintained stable or marginally elevated Mg levels across pregnancy, indicating a deviation from the expected physiological decline.

Hypomagnesaemia was most prevalent in term normotensive women, while its occurrence was significantly lower in both early-onset and late-onset PE groups. Logistic regression analysis confirmed an inverse association between hypomagnesaemia and the presence of PE, even after adjusting for confounding factors such as maternal age, parity, BMI, and HIV status. Notably, obesity emerged as a strong and independent predictor of PE, aligning with established literature linking elevated BMI to increased vascular resistance, inflammation, and endothelial dysfunction. In contrast, HIV status did not show a statistically significant relationship with PE incidence after multivariate adjustment, suggesting that in this cohort, well-managed HIV infection may not confer additional PE risk beyond that attributable to other factors.

These findings collectively suggest that the relationship between Mg and PE in this South African cohort is not characterised by deficiency, as frequently reported in other settings, but rather by altered Mg regulation or retention. The data point toward a possible compensatory

physiological mechanism in PE, potentially linked to endothelial or renal handling of Mg. The divergent Mg trajectories between normotensive and PE groups underscore the importance of considering gestational timing, comorbidities, and local nutritional patterns when interpreting biochemical markers in pregnancy.

3. Comparison with Existing Literature

The results of this study differ in important ways from much of the international literature on maternal serum Mg and PE. Several meta-analyses and observational studies from high-income settings have consistently reported lower serum Mg concentrations in women with PE compared with normotensive pregnancies [1-3]. These findings have contributed to the prevailing view that Mg deficiency may play a pathogenic role in PE, leading to recommendations in some contexts for Mg supplementation as a preventive measure.

However, African studies have produced more variable results. Case–control studies from Ghana and South Africa have reported either no significant differences in Mg between PE and normotensive groups or, in some cases, higher Mg levels in women with PE [4-6]. These findings align with the present study, which observed stable or elevated Mg in PE compared with the gestational decline seen in normotensive pregnancies.

Several methodological differences may account for these discrepancies. First, gestational timing of sampling is critical: many global studies compare third-trimester PE cases with early-pregnancy normotensive controls, potentially conflating physiological gestational changes with pathological differences. In our study, group-specific trajectories were considered, highlighting a divergent pattern rather than a simple mean difference. Second, differences in laboratory methodology, including the use of serum versus plasma Mg, the timing of centrifugation, and storage conditions, may affect comparability. Third, background dietary intake and supplementation patterns differ markedly between populations. In LMICs such as South Africa, baseline Mg intake may be lower, yet the occurrence of elevated Mg in PE could reflect impaired renal clearance secondary to endothelial dysfunction [7].

The inverse association between hypomagnesaemia and PE observed here is particularly notable, as it challenges the deficiency paradigm dominant in high-income country (HIC) literature. Instead, it aligns with the hypothesis that PE in this context may involve altered Mg handling rather than absolute depletion. Furthermore, while obesity was a strong independent predictor of PE in our cohort — consistent with both global and South African studies— HIV

status was not associated with PE risk after adjustment [8,9]. This contrasts with some reports from other African cohorts where HIV-related immune and vascular changes were linked to hypertensive disorders of pregnancy [10,11]. The difference may reflect improved HIV care and viral suppression in our setting.

Overall, the present findings contribute to a growing body of evidence that the Mg–PE relationship is not uniform across populations. They underscore the importance of context-specific research that accounts for regional nutritional patterns, comorbidity burdens, and health system differences, rather than extrapolating directly from HIC data.

4. Biological Interpretation

Magnesium acts as a physiological calcium antagonist, modulating vascular tone by promoting vasodilation and reducing vascular smooth muscle contractility [12,13]. In PE, the observed stable or elevated Mg levels may be attributable to impaired renal clearance secondary to endothelial dysfunction or to a compensatory physiological response aimed at counteracting heightened vasoconstriction [7]. This suggests that Mg dysregulation in PE is not solely a reflection of deficiency but may involve dynamic shifts in response to the disease process. Additionally, Mg metabolism may interact with other pathophysiological pathways, including oxidative stress and inflammatory cascades, further complicating its role in PE.

5. Strengths and Limitations

A major strength of this study lies in its use of a well-characterised South African cohort, with rigorous case definitions for PE and normotensive pregnancies based on standard clinical criteria. The careful grouping of participants — including both early-onset and late-onset PE — allowed for nuanced analyses that considered heterogeneity within the disease spectrum. Serum Mg measurements were performed using standardised laboratory assays with established internal quality control procedures, ensuring reliability and reproducibility of biochemical data. Additionally, the inclusion of key demographic and clinical covariates, such as BMI and HIV status, enabled multivariate analyses that reduced the likelihood of confounding.

The study's context-specific approach is another strength. By situating biochemical findings within the South African epidemiological profile — characterised by high burdens of obesity, variable nutritional adequacy, and diverse comorbidity patterns — the work offers insights that are directly relevant to antenatal care in LMICs. Furthermore, the identification of an inverse

association between hypomagnesaemia and PE risk provides a novel contribution that challenges the widely held “Mg deficiency hypothesis” derived mainly from high-income settings.

However, several limitations must be acknowledged. The cross-sectional design precludes definitive conclusions regarding temporal or causal relationships between Mg levels and PE development. While gestational timing was considered, the absence of repeated measures for each participant limits the ability to map individual Mg trajectories across pregnancy. Additionally, the study did not assess dietary Mg intake, supplement use, or markers of intracellular Mg status — factors that could have provided a more complete picture of Mg homeostasis. Renal function parameters, which could clarify whether elevated Mg in PE reflects impaired clearance, were also not measured.

Residual confounding remains a possibility, particularly from unmeasured lifestyle factors such as physical activity, dietary diversity, and micronutrient interactions (e.g., calcium or vitamin D status) that may influence Mg metabolism. Another limitation relates to external validity: although the cohort is representative of the local population attending the study hospital, findings may not be generalisable to rural populations or to other LMICs with different nutritional and disease profiles. Finally, laboratory assay differences across studies mean that direct numerical comparisons with international reference ranges should be made with caution.

Taken together, these strengths and limitations highlight both the value and the interpretative boundaries of the present findings. They also point to clear priorities for future research, including longitudinal study designs, more comprehensive biochemical profiling, and multi-centre collaborations to enhance generalisability.

6. Public Health and Clinical Implications

While serum Mg alone may have limited predictive utility for PE, its inclusion in multifactorial risk assessment models may improve the identification of at-risk women in LMICs. Public health interventions should address nutritional adequacy, weight management, and improved antenatal care access, while also considering broader determinants of maternal health. The findings also emphasise the necessity of establishing pregnancy-specific and population-specific biochemical reference ranges, as reliance on global cut-offs may not reflect local nutritional and epidemiological realities.

7. Future Research Directions

Future studies should adopt longitudinal designs to track Mg changes across pregnancy in both normotensive and PE-affected women. Investigations into genetic polymorphisms affecting Mg transport, the role of dietary patterns, and the interaction with comorbidities such as obesity and HIV are warranted. Expanding research to include intracellular Mg measurements, renal clearance assessments, and functional vascular studies could elucidate mechanistic pathways. Large-scale, multi-centre African studies are necessary to validate these findings and guide evidence-based clinical recommendations. As part of our future work, we intend to undertake a systematic review and meta-analyses of the literature, as per the protocol prepared and submitted to BMC systematic reviews (Appendix 8)

8. Conclusion

This thesis contributes to a more nuanced understanding of Mg homeostasis in pregnancy, suggesting that altered regulation rather than simple deficiency may characterise PE in the South African context. By integrating biochemical findings with local epidemiological realities, it offers evidence to support context-sensitive research and targeted interventions for improving maternal health outcomes in LMICs.

9. References

1. Tavana Z, Hosseinmirzaei S. Comparison of maternal serum magnesium level in pre-eclampsia and normal pregnant women. Iranian Red Crescent Medical Journal. 2013 Dec 5;15(12).

2. Dalton LM, Ní Fhloinn DM, Gaydadzhieva GT, Mazurkiewicz OM, Leeson H, Wright CP. Magnesium in pregnancy. Nutrition Reviews. 2016 Jul 21;74(9):549–57.

3. Kisters K, Barenbrock M, Louwen F, Hausberg M, Rahn KH, Kosch M. Membrane, intracellular, and plasma magnesium and calcium concentrations in preeclampsia. American Journal of Hypertension. 2000 Jul 1;13(7):765–9.

4. Ephraim RKD, Osakunor DNM, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy-induced hypertension: A case–control study in the Cape Coast metropolis, Ghana. BMC Pregnancy and Childbirth. 2014 Nov 20;14(1).

5. Richards D, Lindow S, Carrara H, Knight R, Haswell S, Van der Spuy Z. A comparison of maternal calcium and magnesium levels in pre-eclamptic and normotensive pregnancies: An observational case-control study. British Journal of Obstetrics and Gynaecology. 2013 Sep 16;121(3):327–36.

6. Maduray K, Moodley J, Soobramoney C, Moodley R, Naicker T. Elemental analysis of serum and hair from pre-eclamptic South African women. Journal of Trace Elements in Medicine and Biology. 2017 Sep;43:180–6.

7. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clinical Journal of the American Society of Nephrology. 2014 Oct 6;10(7):1257–72.

8. Olson KN, Redman LM, Sones JL. Obesity “complements” preeclampsia. Physiological Genomics. 2019 Mar 1;51(3):73–6.

9. Kintiraki E, Papakatsika S, Kotronis G, Goulis DG, Kotsis V. Pregnancy-induced hypertension. Hormones [Internet]. 2015;14(2):211–23. Available from: https://pubmed.ncbi.nlm.nih.gov/26158653/

10. Suy A, Martínez E, Coll O, Lonca M, Palacio M, Lazzari E, et al. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. AIDS. 2006 Jan 2;20(1):59–66.

11. Machado ES, Krauss MR, Megazzini K, Coutinho CM, Kreitchmann R, Melo VH, et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. The Journal of Infection [Internet]. 2014 Jun 1;68(6):572–80. Available from: https://pubmed.ncbi.nlm.nih.gov/24462561/

12. Cunha AR, Umbelino B, Correia ML, Neves MF. Magnesium and vascular changes in hypertension. International Journal of Hypertension [Internet]. 2012;2012:1–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3299255/

13. Schwalfenberg GK, Genuis SJ. The importance of magnesium in clinical healthcare. Scientifica. 2017 Sep 28;2017(1):1–14.

APPENDICES

Appendix 1: Questionnaire

The trio of anaemia, parasites and pre-eclampsia in Black African women in South Africa.

V Dorsamy

IP/OP no: ☐ **Study no:** ☐

Contact Details: _____

Category (tick): (more than 1 category may require a tick)

1. Pregnant Normotensive HIV +ve, CD4 < 350: □
   HAART □
   Triple regime (single tablet) □
2. Pregnant Normotensive HIV +ve, CD4 > 350: □
   PMTCT (dual) □
   Triple regime (single tablet) □
3. Pregnant Normotensive HIV -ve: □
4. Non-pregnant Normotensive HIV -ve: □
5. Non-pregnant Normotensive HIV +ve: □

PLEASE CHECK EXCLUSION CRITERIA LIST □

<u>THE FOLLOWING BLOODS MUST BE TAKEN FOR THE PURPOSES OF THIS STUDY. PLEASE TICK IF THE SPECIMENS WERE TAKEN</u>

1. **Sterile VAC 4ml K_3EDTA lavender tube Mother** □
2. **Sterile VAC 4ml K_3EDTA lavender tube Cord Blood** □
3. **Sterile VAC 5ml serum gel SST red yellow tube** □
4. **Sterile ESR tube black top** □
5. **Stool sample** □
6. **Urine Sample** □

Age (years)			
Area of Residence (tick)	Rural	Urban	Semi urban
Access to water	Plumbed water	Collected (rain, etc.) Treated?	River/stream
Type of residence	Formal / informal	No of people in dwelling	How long lived here
Bathing	Plumbed water	Collected (rain, etc.)Treated?	River/stream
Access to toilet	Plumbed	Pit latrine	Other
Clothes washing	Plumbed	Communal tap	Other (specify)
Cigarette smoking (y/n)		No. of cigarettes/day	
Alcohol		Quantity/day	
Recreational drugs (eg. cocaine)		Quantity/day	
Recreational drugs (eg. cocaine)		Quantity/day	

1. Do you have any illness that you are aware of?
2. For how long have you had it?
3. Are you on any treatment for the illness
4. Chronic fatigue for no apparent reason
5. Swollen or achy joints
6. Increased appetite, hungry after meals
7. Eat out at restaurants
8. Nervous or irritable
9. Restless sleep/teeth grinding while asleep
10. Night sweats
11. Blurry, unclear vision
12. Fevers of unknown origin
13. Frequent colds, flu, sore throats
14. Recurrent feelings of unwellness
15. Constipation
16. Diarrhoea in last 3 months
17. Diarrhea alternating with constipation

18. Thinning or loss of hair
19. Allergies, food sensitivities
20. Irritable bowel, irregular bowel
21. Rectal, anal itching
22. Bloating or gas
23. Abdominal or liver pain/cramps
24. Mucus in nose that is moist or encrusted
25. Dark circles under the eyes
26. Bowel urgency
27. Skin problems, rashes, hives, itchy skin
28. Vertical wrinkles around mouth
29. Kiss pets, allow pets to lick your face
30. Go barefoot outside the home
31. Travel in 3rd world countries
32. Eat lightly cooked pork/ salmon products
33. Eat raw fish or meat products
34. Swim/bathe in dams, rivers, lakes
35. History of parasitic infection
36. How long?
37. Treatment?
38. Any family members diagnosed with parasitic infections in your household
39. When diagnosed
40. Loose stools or diarrhea
41. Pale, anemic or yellowish skin
42. Foul-smelling stools
43. Low back or kidney pain
44. Indigestion, malabsorption
45. Sexually transmitted diseases
    a. Do you have excessive vaginal discharge
    b. Diagnosed with syphilis
    c. HIV CD4 count
    d. When diagnosed
    e. Treatment type
    f. Duration of treatment
    g. Continuing treatment?

46. Previous history of anaemia
    a. When diagnosed
    b. Are you on / have you been on treatment?
    c. Have you had a blood transfusion
    d. Are you taking iron tablets?
    e. Have you noticed blood in your stools
47. Non pregnant: Menstrual History: Normal or heavy

General hospital information

Admission date		PMMH no	

Comments:

Maternal treatment

Type of Treatment	**Yes**	**No**	**If Yes, no. of days**
Magnesium sulphate			
Aldomet			
Monoohydralazine			
Nifedipine			
Dihydralazine (nepresol)			
Labetalol			
Others			

Clinical data

Parity	P: G:			Weeks gestation on admission				
Reason for previous pregnancy loss (If any)								
Highest BP	Systolic: Diastolic:							
Maternal weight		Maternal height			BMI			
Oedema (tick)	ankle	Up to knee	Up to groin	Generalised (facial)				
Lab results (or attach copy of results)	Proteinuria	Dipstick						
		Lab 24hr protein (if done)						
		Creatinine clearance (if done)						
	Full blood count	Red cell count		White cell count				
		Haemoglobin		Neutrophils				
		Haematocrit		Lymphocytes				
		Mean cell volume		Monocytes				

		Mean cell Hb		Eosinophils	
		Platelets		Basophils	
		ESR		CRP	
		Procalcitonin		EPO	
	Urea and electrolyte	Sodium		Urea	
		Potassium		Creatinine	
		Chloride		Anion gap	
		CO2		Serum creatinine	
		Haem		Helminths	
	Liver function tests	Total protein		Alkaline phos	
		Albumin		AST	
		Globulin		ALT	
		Alb : Glob		LDH	
		Total bilirubin			

Antenatal fetal investigations

Type (tick)	Gestational age done		Note any abnormalities
Sonar			
Doppler		RI =	
Electronic fetal HR			

Birth details

Weeks of gestation at time of birth	
Date of birth	**Time of birth**

Sex of baby			
Indication for delivery (tick one)	Maternal interest	Fetal Distress	Combination of Maternal and fetal interest.
		CTG abnormal	
		MSL	
		IUGR	
	Explain above if relevant	Explain above if Relevant	Explain above if relevant
	Diagnosis: Eclampsia, severe abruptio infection		

Method of Delivery (tick one)	Normal vaginal		Caesarean	
	Spontaneous		Elective	
	Induced		Emergency	

Complications in labour.	Eclampsia –related (tick)	Severe pre-eclampsia	Imminent eclampsia
	Abruptio-placentae		
	Other (explain)		
Mother outcome	Admitted to ICU	Death	

Baby details at birth

APGAR	1 min		5 min	
Baby (tick)	Live		Stillborn (early neonatal death)	
	Neonatal death (up to 28 days)			

Baby weight (kgs)	
Admission to NICU	

FOLLOW UP DATA PRIOR TO DISCHARGE FROM HOSPITAL

Date:_____ Inpatient / Outpatient visit: _____

Oedema (tick)	ankle	Up to knee	Up to groin	Generalised (facial)

Any other observations/clinical data/information of relevance for mother:

(Maternal complications / morbidity)

_____
_____
_____
_____
_____

Baby weight: _____ Maternal BP: _____

Feeding choice	formula	Breast	flash heating	not fed	TPN
Cranial scan					

Morbidities in early NN period

Resp Distress	HMD, TTN, Pneum ?Mas, other	
CNS	Asphyxia, meningitis	
Metabolic	hypoglycaemia, electrolyte imbalance	Other
hypothermia,		
Infections	Minor	Skin,

		eye,
		umbilicus,
		Suspected sepsis
		normal WCC +CRP
	Major	Pneumonia,
		Septicaemia (positive BC),
		meningitis (positive culture
		NEC,
		susp sepsis + low wcc and raised CRP (negative culture)

All positive cultures = severe infections. CPAP and ventilalion = severe illness

Any other observations/clinical data/information of relevance for child:

(Neonatal complications / morbidity)

_____
_____
_____
_____
_____

FOLLOW UP DATA AFTER DISCHARGE FROM HOSPITAL

Date:_____ Inpatient / Outpatient visit: _____

Oedema (tick)	Ankle	Up to knee	Up to groin	Generalised (facial)

Baby weight: _____ Maternal BP: _____

Any other observations/clinical data/information of relevance for mother or child:

_____
_____
_____
_____
_____

_____

HIV status of baby 6 weeks post delivery	HIV +ve (PCR)	HIV –ve
CD4 count		
Baby NVP and AZT		(7 days or 28 days)
Bactrim yes/no		

Late morbidities

Neurological impairment	
BPD	
ROP	
Nutritional	

Outcomes

Alive well	
Alive ill - record morbidities as above	Minor infections, HIV related infections, ROP and Audiology if small babies (<34 weeks), feeding choices

Lab investigations

BP	Systolic Diastolic			
Blood	Iron studies CRP			
Stool	Microscopy			
Urine	MSU			
	Haem			
	Helminths			

Additional Notes

Appendix 2: Statistical Methods and Analytical Justification

Appendices 2 and 3 provide a detailed justification for the statistical methods used in the study, outlining how each research objective was operationalised, the corresponding analytical approach, the rationale for test selection, and how assumptions and potential confounding were addressed.

Linking Research Objectives to Statistical Tests

Research Objective	Variables Analysed	Statistical Test	Rationale for Choice	Assumption Checks / Notes
Compare serum magnesium levels among women with EOPE, LOPE, and normotensive pregnancies	Continuous DV: Serum magnesium (mmol/L) Categorical IV: Pregnancy group (4 categories)	One-way ANOVA with Bonferroni post-hoc comparisons	Compares means across >2 independent groups when DV is continuous and approximately normally distributed	Normality tested with Shapiro–Wilk; homogeneity of variances tested with Levene's test; Bonferroni used to adjust for multiple comparisons
Determine the influence of HIV infection and obesity on serum magnesium levels	Continuous DV: Serum magnesium Categorical IVs: HIV status (binary), BMI category (normal, overweight, obese)	One-way ANOVA (BMI) and independent samples t-test (HIV status)	Allows comparison of means between two or more groups when DV is continuous	Same normality and variance homogeneity checks as above
Assess association between serum magnesium status (normal vs. hypomagnesaemia) and hypertensive status	Categorical DV: Pregnancy group (4 categories) Categorical IV: Magnesium status (binary)	Chi-square test of independence	Tests association between two categorical variables	Checked that expected cell counts ≥5 for ≥80% of cells
Identify predictors of EOPE and LOPE relative to normotensive pregnancies	Categorical DV: Hypertensive status (EOPE, LOPE, normotensive) IVs: Magnesium status, BMI, HIV status,	Multinomial logistic regression	Suitable for nominal DV with >2 categories; allows adjustment for multiple covariates simultaneously	Checked multicollinearity (VIF < 2), independence of observations, linearity of logit for continuous predictors

	gestational age at delivery			
Compare birth outcomes (e.g., baby weight) across groups	Continuous DV: Baby weight (kg) Categorical IV: Pregnancy group (4 categories)	One-way ANOVA	Compares means across >2 independent groups when DV is continuous	Same normality and variance homogeneity checks as above

Narrative Justification of Analytical Choices

Continuous variables were assessed for normality using the Shapiro–Wilk test. Variables meeting normality assumptions were summarised using means ± SD; non-normally distributed variables were explored with medians and IQRs but were transformed where appropriate. One-way ANOVA was selected for comparisons involving a continuous dependent variable and a categorical independent variable with >2 groups. Levene's test checked homogeneity of variances, and Bonferroni post-hoc adjustments controlled type I error. Chi-square tests examined relationships between categorical variables, with expected cell count assumptions confirmed. Multinomial logistic regression was used to identify predictors of EOPE and LOPE relative to normotensive pregnancies, adjusting for BMI, HIV status, and gestational age. Missing data were handled via listwise deletion (<10% missingness), and results are reported with p-values and effect sizes.

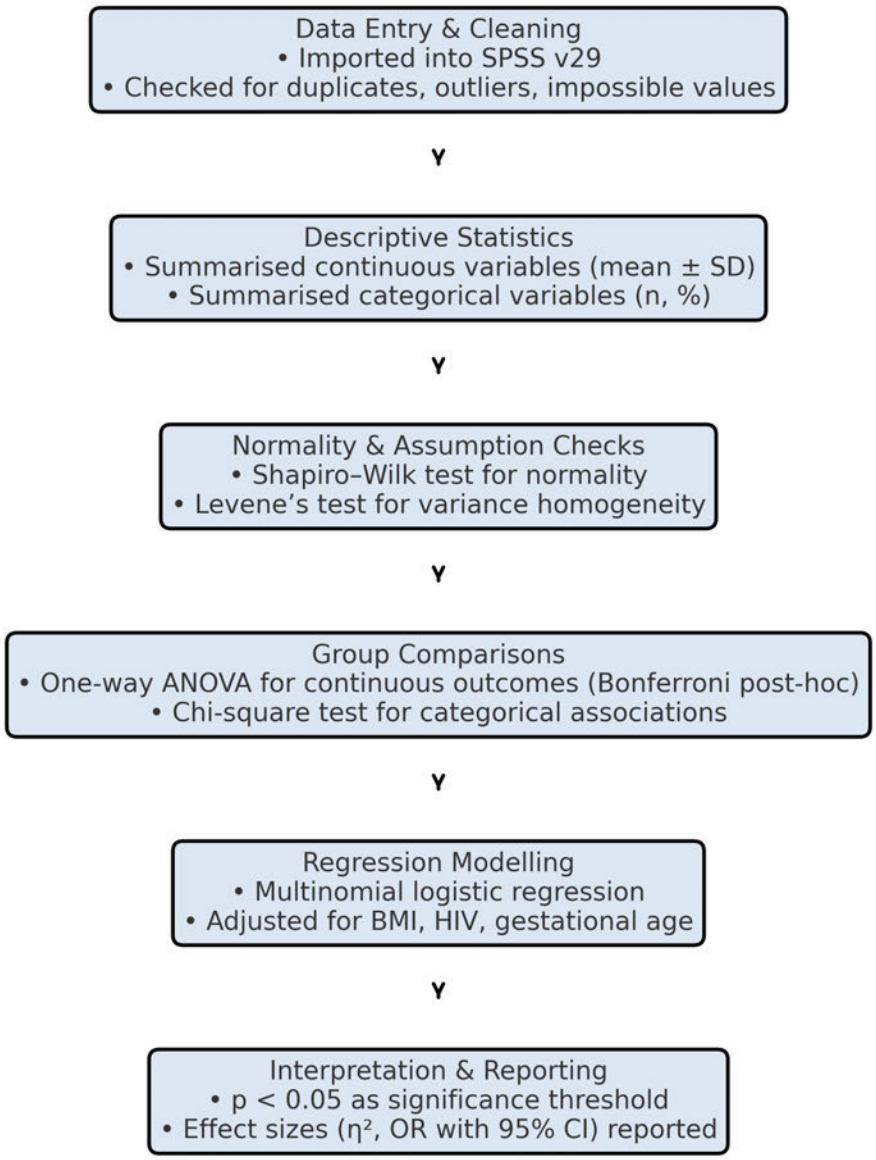
Handling of Confounders

Confounders were identified from literature and clinical plausibility: BMI, HIV status, and gestational age at delivery. These variables were included in multivariable models to isolate the independent effect of serum magnesium status on PE risk. No adjustment was made for potential mediators such as birth weight to avoid attenuating the association of interest.

Statistical Limitations

Limitations include the cross-sectional design preventing causal inference, residual confounding from unmeasured variables (e.g., dietary magnesium intake, renal function), potential power limitations in subgroup analyses, and limited generalisability due to the single-site, population-specific sampling.

Appendix 3: Statistical Analysis Flow Diagram



Flow of statistical analysis from raw dataset to final interpretation. Data cleaning and descriptive summaries were followed by assumption checks (normality and variance homogeneity), appropriate bivariate analyses (BMI, chi-square), and multivariable modelling (multinomial logistic regression) with adjustment for key confounders (BMI, HIV status, gestational age). Significance was set at $p < 0.05$, with effect sizes reported alongside p-values to ensure both statistical and clinical relevance.

Appendix 4: Research Proposal

Research Project Proposal:

Magnesium levels and its association with pre-eclampsia in a Black, South African population

By

Naeera Abdul (220004494)

Supervisors:

Dr V Dorsamy

Dr C Bagwandeen

Discipline of Human Physiology, School of Laboratory Medicine, and Medical Sciences.

College of Health Sciences

Table of Contents

1. Background

Pre-eclampsia (PE) is a multi-system, placental disorder which manifests at >20 weeks of gestation [1]. PE has been studied intensively over many decades due to its high morbidity. Despite this, its etiology remains unknown and inadequate diagnosis and management of pre-eclampsia can escalate to eclampsia, which has high risk of death [2]. Hypertension is commonly seen as a predictive marker of pre-eclampsia [2,3], with factors such as kidney disease, diabetes mellitus and obesity contributing to the risk of PE. Furthermore, PE risk is increased in first pregnancies, pregnancies containing multiples, and in subsequent pregnancies of previously pre-eclamptic women [4].

According to Dimitriadis, E. *et al.* (2023), pre-eclampsia affects 4 million women annually worldwide. Hypertensive pregnancy disorders are responsible for approximately 14% of maternal deaths, with PE causing about >70 000 maternal deaths and 500 000 infant deaths. The incidence of PE varies between populations; however, the 2023 study noted that low-income and middle-income countries (LMIC) had less instances of PE in comparison to high-income countries (HIC). The only exception was sub-Saharan Africa, an LMIC, which had the highest instances of PE. It was assumed that limited accessibility to pre-natal facilities contributed to the low PE incidence report rates in LMICs [3]. Despite this conclusion, a study conducted by Nathan, H.L. *et al. (2018)* in South African tertiary facilities involving 1598 pre-eclamptic women showed that 1% of the women had passed away, 9.5% of the women had developed eclampsia, 0.3% of the women suffered from strokes and 17.6% experienced kidney injury. In 1589 births, 21% resulted in perinatal births whereas 84.5% resulted in stillbirths. In the remaining 1308 livebirths, 70% were delivered at <37 weeks of gestation and 41.7% were delivered at <34 weeks of gestation. The study had concluded that despite tertiary facilities being accessible, South Africa's instances of PE were higher than other LMICs [5]. This indicates that South African women may be more pre-disposed to PE, which is supported by studies showing that PE is most seen in sub-Saharan Africa from all LMICs [2,3,6]. The high incidence of PE manifestation in South Africa calls into question whether South African socio-demographic factors have an influence on PE. Preliminary data from an ongoing study involving 671 women, that was conducted from May 2017 to February 2020, had shown that 46% of the participants were HIV-positive and 56% were obese [7]. This gives indication that both HIV and obesity have high incidence in South Africa. This is be supported by data from UNAID showing that approximately 8 million South Africans live with HIV, with approximately 4.8 million of these South Africans being women [8,9]. A different study noted that the incidence of obesity is high in South Africa, affecting 27% of the population. 42% of this general obese population are women, with the study noting that the prevalence of obesity in South African is amongst the highest in the world [10]. As these conditions are endemic in South Africa, it

is imperative that studies conducted on PE factor in the role they play in its development. Based on this, it is worthwhile to investigate the influence both obesity and HIV potentially have on PE. Furthermore, South Africans, due to socioeconomic challenges, may be susceptible to nutritional insufficiency. A recent systematic review by Dorsamy et (2022) reports that iron deficiency is common among pregnant women. It stands to reason that there may be other elements critical to survival that are likewise depleted due to the insufficiency. An important element to consider is magnesium.

Magnesium is an element found in high quantities in the human body. Despite its abundance, it is not given the same priority as iron in terms of routine diagnostics. Cases of hypermagnesemia have often been observed in pre-eclamptic women who have received Mg^{2+}treatment. Other than that, cases of hypomagnesemia have been observed in 15% of a general population, particularly in cases of diabetes mellitus, hypertension and metabolic disease [11,12]. Due to how widespread magnesium's physiological involvement is[13] further investigation of magnesium levels in pre-eclamptic women is warranted, particularly in cases of hypomagnesemia manifesting in cases of hypertension and hypermagnesemia manifesting during magnesium supplementation in cases of PE. Overall, the relationship between PE and magnesium, alongside confounding factors such as HIV and obesity, is little understood. This project hopes to establish how these factors can influence PE manifestation, as well as determine if said factors can be used as predictive markers for cases of PE in a South African population.

1.1 Problem Statement

Pre-eclampsia is a hypertensive disorder of pregnancy, about which much remains unknown. Levels of circulating magnesium are speculated to contribute to the manifestation of pre-eclampsia.

1.2 Research Question

What is the association between circulating maternal serum magnesium levels and the prevalence of pre-eclampsia?

1.3 Hypothesis

The levels of serum magnesium may vary between pre-eclamptic and normotensive pregnancies.

1.4 Significance

The aetiology of pre-eclampsia remains poorly understood. This study will strengthen the body of knowledge of magnesium levels during pregnancy. Furthermore, it could help

ascertain if magnesium levels are associated with PE and can be used as a predictive marker for pre-eclampsia. It could be used as a mechanism of screening and subsequently aid in earlier identification and better management of pre-eclampsia, thus contributing to decreasing both maternal and infant morbidity and mortality.

1.5 Aims and Objectives

The aim of this study is to determine the association between serum magnesium levels and pre-eclampsia as well as to determine if maternal factors such as obesity and HIV are associated with magnesium levels and pre-eclampsia.

The objectives of this study are:

- To measure the circulating levels of magnesium in pre-eclamptic women and normotensive women
- To determine if there is a relationship between circulating magnesium levels and the prevalence of PE
- To determine if there is a relationship between magnesium and HIV
- To determine if there is a relationship between magnesium and obesity
- To determine if HIV and obesity influence PE prevalence

2. Literature Review

2.1 Introduction

Pre-eclampsia is a disorder that is still not widely understood; however, its defining characteristics and clinical presentation have remained unchanged over the last decade. It has been observed that women with a history of pre-eclampsia have a significantly increased risk of PE manifesting [3,4,6] and studies have shown that medical conditions such as diabetes mellitus, chronic hypertension, and obesity can increase the risk of PE [3,6].

It is generally accepted that LMICs will have higher reports of PE compared to HICs, due to reduced accessibility to care facilities [3,6] and facilities being under-equipped to manage PE [5]. Countries in sub-Saharan Africa are considered to have higher instances of PE compared to most LMICs [2,3,6], as observed in the South African study spanning 2015-2016 [5]. In South Africa, obesity and HIV are prevalent in the population and could be considered as confounders in determining causation of PE. Considering that obesity has been observed to increase the risk of PE [14] alongside other health conditions, it would be worthwhile to observe the effects of obesity on PE in a South African population. This applies to HIV as well. HIV is a medical issue with a population prevalence of 4.8 mill in South African women [8,9]. Due to how often PE manifests, it would be beneficial to investigate the relationship between HIV and PE as well.

2.2 Pre-eclampsia

Pre-eclampsia is a placental disorder characterized by abnormal placental placement. It can result in restricted fetal growth as well as stillbirth and it is one of the leading causes of maternal and infant morbidity and mortality [1,3]. In mothers, it has been shown to lower life expectancy, as well as shown an increased likelihood of development of chronic conditions such as cardiovascular disease, diabetes, and strokes post-delivery. In infants, premature delivery and perinatal death may be increased; however, the child may also experience issues with neurological development as well as an increased chance of metabolic and cardiovascular diseases manifesting during the life cycle [3]. Pre-eclampsia can be further classified into 2 phenotypes, namely early-onset pre-eclampsia (EOPE) and late-onset pre-eclampsia (LOPE). EOPE is associated with delivery before 34 weeks of gestation, as well as abnormal placental development and restricted growth of the fetus [1,3,15]. LOPE is associated with dysfunction of the maternal endothelium, as well as delivery after 34 weeks of gestation [3,15]. LOPE is the most common phenotype of PE, with approximately 80-95% of PE cases being

LOPE. Early onset pre-eclampsia is the rarer phenotype, despite EOPE having higher infant mortality rates, as well as greater rates of maternal morbidity [15].

2.3 Diagnosis and Treatment of Pre-eclampsia

Hypertension around >20 weeks of gestation alongside other symptoms such as proteinuria or maternal organ dysfunction may be characterized as PE. In PE, systolic blood pressure will be >140 mmHg and diastolic blood pressure will be ≥90 mmHg and protein concentration are around 30mg a day [2,3,6,16]. Late diagnosis and poor management of PE can cause it to escalate to eclampsia. To prevent this, magnesium sulphate is administered to alleviate symptoms of eclampsia and prevent seizures [17]. Despite the ongoing research, no definitive cure other than delivery of the baby, and as a result, the placenta have been established as a treatment for PE, often prematurely. Ideally this should only be carried out in circumstances that benefit both infant and maternal outcomes , but this may not always be the case[3,4,6].

2.4 The Physiology of Magnesium and Magnesium Homeostasis

Magnesium is one of the most common metals found in high quantities throughout the body and is involved in a myriad of physiological functions, as well as acts as both a cofactor of enzymatic reactions and as an activator of enzymes. It is utilized in several reactions of physiological importance, such as in nucleic acid and protein synthesis, muscle contraction, neurological function, vascular tone regulation, and adenosine triphosphate (ATP) formation [13,18,19,20, 21,22]. The total amount of magnesium in the body is estimated to be 24g [18], and it can exist both intracellularly, and extracellularly. Intracellular magnesium forms 99% of the body's magnesium concentration and is stored in the bones, muscles, and the soft tissues, whereas extracellular magnesium only makes up about 1% of the total magnesium and is found in the serum and red blood cells (RBCs); with 0.3% specifically being found in the serum [19,22,23]. Intracellular magnesium is typically found in concentrations of 5 to 20 mmol/L, with 1-5% of it being ionized. The remaining magnesium will bind to proteins, ATP, and molecules with a negative charge [22]. Extracellular magnesium can also be categorized into ionized and protein-bound magnesium, but it can also be further classified as complexed magnesium, and is commonly seen with phosphate, sulphate, or bicarbonate [22, 23]. Normal levels of serum magnesium are 0.7–1.1 mmol/L [18, 22], with the range ionized magnesium being approximately 0.55–0.75 mmol/L and the RBC magnesium concentration being 1.65–2.65 mmol/L [22].

Magnesium homeostasis is maintained by the kidney, the bones, and the small intestine, with the kidneys being the most involved component [11,12,13,18,22,23]. Magnesium is absorbed by the small intestine and then subsequently stored in the bone. All excess magnesium is filtered and excreted by the kidney [18,22,23]. In the kidney, approximately 90-95% of filtered magnesium is reabsorbed. Only 3-5% gets excreted. 15-20% of magnesium reabsorption is carried out in the proximal tubule, with 65-75% of reabsorption occurring in the thick ascending limb (TAL) of the loop of Henle [23]. Errors in magnesium homeostasis are generally caused by problems with the absorption, storage or the reabsorption of magnesium [23], and they result in hypermagnesemia and hypomagnesaemia [11,12,13,18, 22,23]. Hypermagnesemia typically manifests as a consequence of ingestion of Epsom salt or administration of magnesium-based treatments [18]. It has also been observed in pre-eclamptic women who have received magnesium supplementation [12,18]. It is characterized by hypotension, nausea, and neurological issues [18]. Hypomagnesemia has been seen to manifest in approximately 15% of a generalized population [11,12,18,23], and can be caused by insufficient magnesium consumption, impaired redistribution of magnesium from and extracellular format to an intracellular format, or due to problems with the kidney [23]. Hypomagnesemia has been known to appear asymptomatic in some cases, but it has been associated with characteristics such as hypertension, muscle weakness, seizures, diabetes mellitus and metabolic disease [11,12, 18,23].

2.5 Magnesium in Pregnancy and Pre-eclampsia

Not much is understood about how magnesium levels can influence a pregnancy, other than hypermagnesemia has been seen in some pre-eclamptic women who were receiving magnesium supplementation as well as hypomagnesemia being associated with hypertension, which is associated with PE originating [11,12,18,22,23]. However, several studies have shown that magnesium levels are generally lower in pregnant women compared to non-pregnant women [19,20]. Laires, M., Monteiro, C. and Bicho, M. (2004) were able to show that the intracellular magnesium in the myometrium was low, and that the subsequent urine magnesium concentration matched the levels seen in the myometrium [20]. However, most studies into magnesium levels in pregnancy look at serum magnesium specifically. One such study involved a test group of untreated but normotensive pregnant women, and a test group of pre-eclamptic women whose serum, cellular and erythrocyte magnesium levels were assessed and compared to a control group. The results showed that the levels of magnesium in both the pre-eclamptic and normotensive test groups were significantly lower than the control group levels with the pre-eclamptic test group having lower serum and intracellular magnesium levels overall.

It was speculated that the decrease in magnesium levels contributed to hypertension manifesting [24]. Another study carried out by Tavanaand Hosseinmirzaei (2013) observed a control group and a group of pre-eclamptic women over the course of their pregnancies. The pre-eclamptic test group's serum magnesium was also lower than the study's control group, and serum magnesium had further decreased by the time the diagnosis was confirmed. It was concluded that magnesium levels decreased as gestational age increased, ascertaining that magnesium could be used as a potential predictive marker for development of PE [16].

2.6 Magnesium and Pre-eclampsia in Obesity

Women with a BMI of 35 kg/m^2 are more at risk of developing PE, with the risk of PE-manifestation being increased by 30% [10]. Multiple studies have concluded that a body mass index (BMI) of >30kg/m^2 is considered a predictive marker of PE and have listed obesity as a risk factor of PE [2,3,6,10]. The general trend of magnesium in PE has shown that serum magnesium levels are lower than normal [16,19,20,24], which makes observation of magnesium in pregnant women with raised BMI crucial as it potentially can be a predictive marker for PE. Magnesium deficiency is commonly seen in diabetic patients as well as patients with metabolic syndrome [25]. Magnesium is important due to its involvement in blood pressure reduction [25]. About 2.5-15% of normal, healthy people have reported low levels of magnesium due to insufficient magnesium consumption based on their dietary intake. It has been speculated that poor magnesium consumption is the result of foods being overly processed, which subsequently decreases their magnesium content. This indicates that poor dietary intake is associated with reduced magnesium consumption [19,25]. Obesity is associated with poor diets where the caloric intake exceeds the amount of nutrients absorbed, leaving most cases of obesity with low levels of magnesium [25].

2.7 Magnesium and Pre-eclampsia in HIV

Despite not much being known about PE's aetiology, studies have noted that endothelial dysfunction is commonly seen in PE [3,15,26], as a result of an exaggerated inflammatory response [26]. HIV has been seen to impact both inflammatory response and endothelial dysfunction [26]. One of the characteristics of HIV is reduced functioning of the immune system. Infection has been shown to decrease manifestation of PE. However, the incidence of PE has been seen to increase in cases of women undergoing highly active antiretroviral therapy (HAART), which conflicts with the notion that there are currently no indications that PE manifestation differs between treated and untreated cases of HIV [26,27,28]. Despite HAART contributing to

increased risk of PE manifestation, it is still the recommended treatment for HIV due to its positive effects on life expectancy as well as decreasing the risk of HIV transmission from mother to infant during pregnancy, upon delivery and through breastfeeding [26]. The concept of HIV providing protection against PE was seen in a study observing the manifestation of gestational hypertension and PE in both HIV-positive and HIV-negative women. The study population was divided into HIV-positive and HIV-negative, with African American Back women, forming a further sub-division. In the study, only 2.38% of the HIV-positive patients developed PE and gestational hypertension, whereas 10% of the HIV-negative patients developed PE and gestational hypertension. In the African American sub-division, 4.7% of the HIV-positive patients developed PE and gestational hypertension, compared to 16.7% of the HIV-negative patients developing PE and gestational hypertension [29]. This indicates that HIV-positive women display lower chances of PE manifestation compared to women who are HIV-negative, with Black women being seemingly more predisposed to PE manifestation.

In studies involving PE, low levels of magnesium have been seen across study populations [19,20]. However, few studies have focused on magnesium levels in HIV. There was a study that investigated the trace elements in both pregnant and non-pregnant, HIV-positive women. The study had concluded that pregnant women, regardless of their HIV status, had lower levels of serum magnesium in comparison to non-pregnant women [19,20,30]. The study had also found that magnesium deficiency in pregnant, HIV-positive women (52.4%) was much higher than magnesium deficiency in the pregnant, HIV-negative population (22.2%) [30]. Another study showed that serum magnesium in HIV-positive people was lower than the serum magnesium levels in controls, and that CD4 cell levels could potentially contribute to the concentration of magnesium in HIV [31]. In 2012, a study involving 80 HIV-positive people was conducted. The test group consisted of 70 participants on HAART in conjunction with immune boosters, whilst the remaining 10 were only using immune boosters. Twenty healthy participants with no history of HIV formed the control group. The age and gender of the participants were noted. The study revealed that the concentration of magnesium in infected women was 0.99 ± 1.70, compared to normal magnesium levels being 0.70-0.98. this indicates that magnesium in HIV-positive women is higher than normal [32]. Looking at this in relation to the prior-mentioned studies, this potentially supports the speculation that PE manifestation is decreased in HIV-positive women. This could be because hypomagnesemia is typically associated with hypertension, which is characteristic of PE manifestation [2,3,11,12,18,23].

3. Methodology

3.1 Introduction of study group

This study utilizes stored blood samples that were collected from a larger study (BE552/16) investigating anaemia in pregnancy that was conducted from May 2017 to February 2020. Participants were recruited from women seeking antenatal care at a regional hospital in Durban south region in KwaZulu-Natal, following informed consent. Their blood pressure was measured to diagnose pre-eclampsia, and urine dipstick analysis conducted to determine proteinuria.

Two hundred serum samples will be selected at random from the original study and grouped according to participants pre-eclamptic status and gestational age (100 each from the pre-eclamptic and normotensive groups. Demographic details, HIV status and anthropometric measurements will be recorded for the selected participants. All pre-eclamptic participants will be further categorized into EOPE and LOPE.

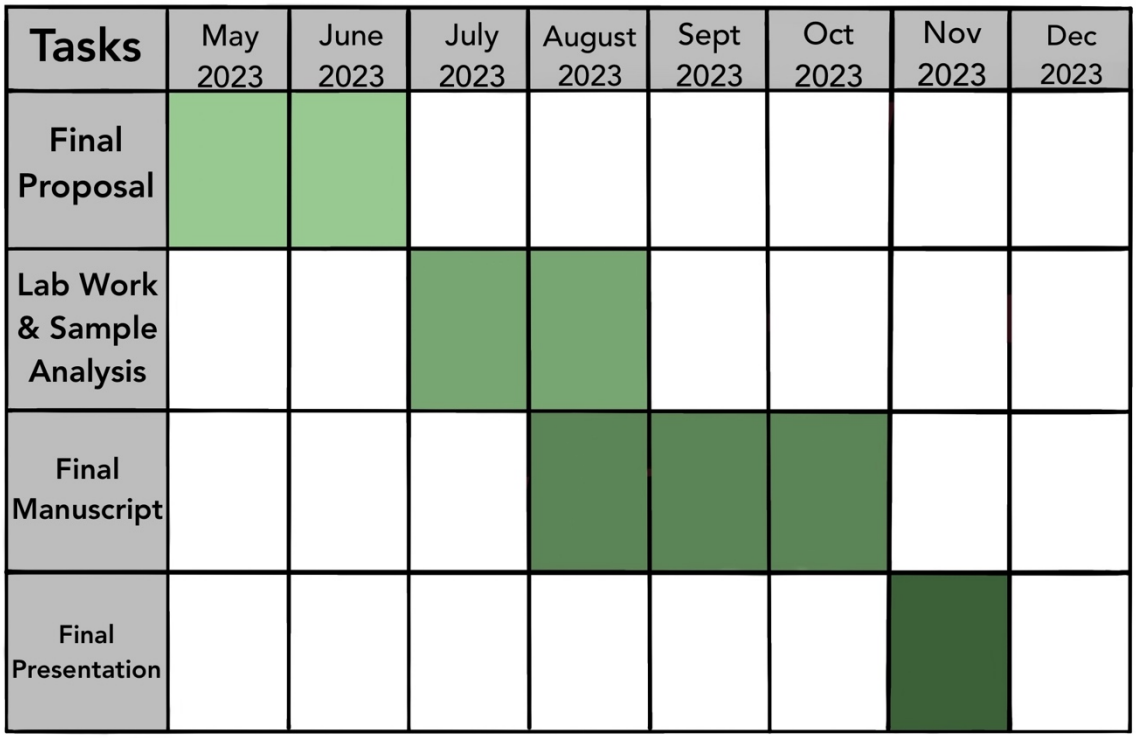
3.2 Samples and Method

Serum collected using vacutainer tubes and spun down at 3000G for 10 minutes was aliquoted into 2ml Eppendorf tubes and frozen. These samples will be thawed and 100uL will be used to assess maternal serum magnesium concentration using atomic absorbance spectrophotometry.

3.3 Statistical Analysis

All data will be captured into a database and analysed using SPSS® ver. 27 (IBM, Armonk, NY). The frequencies, means and standard deviation for each variable of interest will be reported. ANOVA (parametric) and Kruskal Wallis tests (non-parametric) will be employed (where appropriate) to determine circulating levels of Mg and between group differences. A p-value of .05 will be taken as statistically significant. Chi-square analysis of association will be conducted to determine if there are any associations between the study groups and two factors: HIV status and obesity (determined using BMI)

4. Project Timeline



5. Budget

Consumables – R5000

Atomic Absorbance Spectrophotometry – R12800:

R66 x 200 samples

6. References

1. Chappell, L.C. *et al.* (2021) 'Pre-eclampsia', *The Lancet*, 398(10297), pp. 341–354. Available at: https://doi.org/10.1016/S0140-6736(20)32335-7
2. Al-Jameil, N. *et al.* (2014) 'A Brief Overview of Preeclampsia', *Journal of Clinical Medicine Research*, 6(1), pp. 1–7. Available at: https://doi.org/10.4021/jocmr1682w
3. Dimitriadis, E. *et al.* (2023) 'Pre-eclampsia', *Nature Reviews Disease Primers*, 9(1), pp. 1–22. Available at: https://doi.org/10.1038/s41572-023-00417-6
4. Vest, A.R. and Cho, L.S. (2014) 'Hypertension in Pregnancy', *Current Atherosclerosis Reports*, 16(3), p. 395. Available at: https://doi.org/10.1007/s11883-013-0395-8
5. Nathan, H.L. *et al.* (2018) 'Maternal and perinatal adverse outcomes in women with pre-eclampsia cared for at facility-level in South Africa: a prospective cohort study', *Journal of Global Health*, 8(2), p. 020401. Available at: https://doi.org/10.7189/jogh.08-020401
6. Uzan, J. *et al.* (2011) 'Pre-eclampsia: pathophysiology, diagnosis, and management', *Vascular Health and Risk Management*, 7, pp. 467–474. Available at: https://doi.org/10.2147/VHRM.S20181
7. Dorsamy, V. (2021) 'The Trio of Anaemia, Pre-eclampsia and Parasites in Pregnant Black South African Women'.
8. Freer, J. and Mudaly, V. (2022) 'HIV and covid-19 in South Africa', *BMJ*, 376, p. e069807. Available at: https://doi.org/10.1136/bmj-2021-069807.
9. *South Africa* (no date). Available at: https://www.unaids.org/en/regionscountries/countries/southafrica (Accessed: 26 April 2023).
10. Samodien, E. *et al.* (2021) 'Non-communicable diseases - a catastrophe for South Africa', *South African Journal of Science*, 117(5–6), pp. 1–6. Available at: https://doi.org/10.17159/sajs.2021/8638.
11. Ray, E. *et al.* (2023) 'Physiology of a Forgotten Electrolyte-Magnesium Disorders', *Advances in Kidney Disease and Health*, 30(2), pp. 148–163. Available at: https://doi.org/10.1053/j.akdh.2022.12.001.
12. Wolf, M.T.F. (2017) 'Inherited and acquired disorders of magnesium homeostasis', *Current Opinion in Pediatrics*, 29(2), pp. 187–198. Available at: https://doi.org/10.1097/MOP.0000000000000450.

13. Viering, D.H.H.M. *et al.* (2017) 'Genetic causes of hypomagnesemia, a clinical overview', *Pediatric Nephrology (Berlin, Germany)*, 32(7), pp. 1123–1135. Available at: https://doi.org/10.1007/s00467-016-3416-3

14. Olson, K.N., Redman, L.M. and Sones, J.L. (2019) 'Obesity "complements" preeclampsia', *Physiological Genomics*, 51(3), pp. 73–76. Available at: https://doi.org/10.1152/physiolgenomics.00102.2018

15. Aneman, I. *et al.* (2020) 'Mechanisms of Key Innate Immune Cells in Early- and Late-Onset Preeclampsia', *Frontiers in Immunology*, 11. Available at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.01864

16. Tavana, Z. and Hosseinmirzaei, S. (2013) 'Comparison of Maternal Serum Magnesium Level in Pre-eclampsia and Normal Pregnant Women', *Iranian Red Crescent Medical Journal*, 15(12), p. e10394. Available at: https://doi.org/10.5812/ircmj.10394.

17. Rozenberg, P. (2006) '[Magnesium sulphate for the management of preeclampsia', *Gynecologie, obstetrique & fertilite*, 34(1), pp. 54–59. Available at: https://doi.org/10.1016/j.gyobfe.2005.06.025

18. Blaine, J., Chonchol, M. and Levi, M. (2015) 'Renal Control of Calcium, Phosphate, and Magnesium Homeostasis', *Clinical Journal of the American Society of Nephrology*, 10(7), p. 1257. Available at: https://doi.org/10.2215/CJN.09750913

19. Dalton, L.M. *et al.* (2016) 'Magnesium in pregnancy', *Nutrition Reviews*, 74(9), pp. 549–557. Available at: https://doi.org/10.1093/nutrit/nuw018

20. Laires, M., Monteiro, C. and Bicho, M. (2004) 'Role of cellular magnesium in health and human disease', *Frontiers in bioscience : a journal and virtual library*, 9, pp. 262–76. Available at: https://doi.org/10.2741/1223

21. Laurant, P. and Touyz, R.M. (2000) 'Physiological and pathophysiological role of magnesium in the cardiovascular system: implications in hypertension', *Journal of Hypertension*, 18(9), p. 1177

22. Jahnen-Dechent, W. and Ketteler, M. (2012) 'Magnesium basics', *Clinical Kidney Journal*, 5(Suppl 1), pp. i3–i14. Available at: https://doi.org/10.1093/ndtplus/sfr163

23. Ahmed, F. and Mohammed, A. (2019) 'Magnesium: The Forgotten Electrolyte—A Review on Hypomagnesemia', *Medical Sciences*, 7(4), p. 56. Available at: https://doi.org/10.3390/medsci7040056

24. Kisters, K. *et al.* (2000) 'Membrane, intracellular, and plasma magnesium and calcium concentrations in preeclampsia', *American Journal of Hypertension*, 13(7), pp. 765–769. Available at: https://doi.org/10.1016/S0895-7061(00)00240-5

25. Piuri, G. *et al.* (2021) 'Magnesium in Obesity, Metabolic Syndrome, and Type 2 Diabetes', *Nutrients*, 13(2), p. 320. Available at: https://doi.org/10.3390/nu13020320

26. Naidoo, N., Moodley, J. and Naicker, T. (2021) 'Maternal endothelial dysfunction in HIV-associated preeclampsia comorbid with COVID-19: a review', *Hypertension*

Research, 44(4), pp. 386–398. Available at: https://doi.org/10.1038/s41440-020-00604-y

27. Suy, A. *et al.* (2006) 'Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy', *AIDS*, 20(1), p. 59. Available at: https://doi.org/10.1097/01.aids.0000198090.70325.bd

28. Machado, E.S. *et al.* (2014) 'HYPERTENSION, PREECLAMPSIA AND ECLAMPSIA AMONG HIV-INFECTED PREGNANT WOMEN FROM LATIN AMERICA AND CARIBBEAN COUNTRIES', *The Journal of infection*, 68(6), pp. 572–580. Available at: https://doi.org/10.1016/j.jinf.2013.12.018

29. Landi, B. *et al.* (2014) 'HIV Infection in Pregnancy and the Risk of Gestational Hypertension and Preeclampsia', *World Journal of Cardiovascular Diseases*, 2014. Available at: https://doi.org/10.4236/wjcd.2014.45034.

30. Kassu, A. *et al.* (2008) 'Serum Zinc, Copper, Selenium, Calcium, and Magnesium Levels in Pregnant and Non-Pregnant Women in Gondar, Northwest Ethiopia', *Biological Trace Element Research*, 122(2), pp. 97–106. Available at: https://doi.org/10.1007/s12011-007-8067-6.

31. Westermann, G. *et al.* (2001) 'Magnesium in HIV-Infection.', *Advances in magnesium research: nutrition and health*, pp. 315–320.

32. Ndidi, O.-N. *et al.* (2013) 'Assay of the Level of Calcium, Magnesium and Inorganic Phosphorus in HIV Infected Patients in Owerri, Southeast Nigeria', *Journal of Clinical & Experimental Pathology*, 03. Available at: https://doi.org/10.4172/2161-0681.1000134.

Appendix 5: Ethical Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

27 May 2024

Miss Naeera Abdul (220004494)
School of Lab Med & Medical Sc
Westville

Dear Miss Abdul,

Protocol reference number: BREC/00007005/2024
Project title: Magnesium levels and its association with pre-eclampsia in a Black, South African population
Degree: MMedSc

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 27 May 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 27 May 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 (2015), 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 11 June 2024.

Yours sincerely,

[redacted]

Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor S Singh
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
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 6: Informed Consent

Study: The trio of anaemia, parasites and pre-eclampsia in Black African women in South Africa.

A PhD project conducted at the University of KwaZulu-Natal by principal investigator Mr V Dorsamy, supervised by Professor J Moodley and Professor K Mlisana

Information to participants

Dear Ms/Miss/Mrs

I am ……, a research midwife, working with Mr Dorsamy. We are trying to find out why some pregnant women have low blood in their bodies. This is sometimes due to low amounts of iron, therefore we provide you with iron tablets. Low blood levels may also be due to infections caused by germs or worms called parasites. It is not normal practice to either look for or treat such worms in South Africa, if there is no complaints such as loose stools. We are therefore investigating how many pregnant women really have worm infections. If you agree to participate in this study, it will help you in future pregnancies and may result in doctors and nurses doing this test routinely during pregnancy.

What do you have to do if you agree to <u>participate?</u>

1. We will ask you a few questions about where you live, what you eat and where you get your water from.
2. We will try to find out if your stools are loose by asking you a few questions
3. We will take an extra tablespoon of blood to see if you have any substances in your blood that show evidence of worm infection. We will take this blood at the same time as your normal bloods are taken to prevent any further discomfort and to minimise any risks.
4. We will use your blood results to see if you have low blood
5. This blood investigation also includes a test to show whether your body is more likely to become infected by worm infestations. In order to establish these investigations, we may have to test a small fraction of your blood for studies of special genes.
6. We will ask you to provide a specimen of your stool either today or give you a container to take home to bring back on your next visit
7. Once we have examined the stool and have a result, we will give you further information and advice.
8. The taking of the blood or the stools may cause you discomfort but will not harm you or your pregnancy in any way.

There will be no other investigations that will be done but I will see you at your next visit to find out how you are doing and provide you with results when available.

We will also follow up on the details of the outcome of your pregnancy by looking at your hospital records.

Please take some time to read this information and ask any questions that you may want to. I will be prepared to give you any information, either about this research study, or about pregnancy in general. Thank you.

CONSENT DOCUMENT

The trio of anaemia, parasites and pre-eclampsia in Black African women in South Africa. (Ethics reference number 552/16)

CONSENT

I (Name) have been informed about the study entitled "**The trio of anaemia, parasites and pre-eclampsia in Black African women in South Africa.**" by Mr V Dorsamy.

I understand the purpose and procedures of the study which is to find out whether there is a link between low blood, high blood pressure and worms in your body. A tablespoon of blood and a sample of stool will be taken at the same time as other standard bloods are being taken.

I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

I have been informed about any available compensation or medical treatment if injury occurs to me as a result of study-related procedures.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at Cell: [redacted], Work: 031 260 4029 and e-mail: dorsamyv1@ukzn.ac.za

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001
Durban
4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609 Email: BREC@ukzn.ac.za

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**

(Where applicable)

_____ _____

Signature of Translator **Date**

(Where applicable)

Genetic Research Studies

1.**Research Project and Title:**

The Trio of Anaemia, Parasites and Pre-eclampsia in Black African Women

Investigator(s):

Vinogrin Dorsamy (PhD Candidate) School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal

Prof J Moodley (Supervisor), Womens Health and HIV, Universtiy of kwaZulu-Natal

Prof K Mlisana (Co-supervisor) School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal

This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

2.**Purpose of the genetic research**

High blood pressure and low blood (anaemia) during pregnancy is common in South Africa. Parasite infections is also common in South African populations. Our aim is to try to understand the cause and association between these common conditions. We want to see if pregnant women who develop these conditions have anything in their DNA which makes them more likely to develop anaemia or high blood pressure, which may cause them or their babies to be sick during or after pregnancy. We have identified certain parts of the DNA (called genes) which are responsible for making certain proteins that may be linked to increasing the blood pressure or the anaemia. We have also selected certain genes that are linked to parasite infections common to Africa that may make these conditions worse. We want to see if these genes make more proteins in some people and not others, and if this then causes disease. At a future time we wish to explore more genes that may be associated with the high blood pressure and anaemia.

3.**Procedures**

In order for us to get the DNA we need to take about a teaspoon of blood. This will be collected from your arm using a needle and a tube. The needle may cause a slight discomfort during the procedure and there is very little risk of complications or discomfort thereafter and will not harm your pregnancy. The entire process will take less than a minute. The bloods will be taken to the Medical Microbiology laboratory at Inkosi Albert Luthuli Central Hospital where it will be frozen until it is ready for DNA analysis. The tube with the blood will not have any personal information on it that can identify that the sample belongs to you.

4.**Voluntary Participation**

Participation in this study is voluntary. You are under no obligation to participate and should you refuse, it will not affect your current treatment. If you choose to participate, you may decline participation at any time if you so wish. You may contact the research team and inform them of your wishes or should you have any queries. Contact information is provided below.

5.**Risks and Discomforts**

DNA will be extracted from blood cells. The blood is drawn with a needle from the inside of your elbow. About a teaspoon of blood is collected in a glass tube. You may experience discomfort when the needle is inserted and the entire process lasts about a minute and there are rarely any complications from the procedure. The procedure is similar to any other time you have had blood taken for a blood test and will not harm your pregnancy. All tubes will have a special number attached to it and no other information that can identify you. This blood will be stored for use in the future for this study or other relevant studies.

Safeguards have been established to ensure that such disclosure will not occur. For example, we have taken steps to ensure that a coded number, rather than your name, will be used to identify your sample and that your name will not be disclosed in association with the sample at any time. In addition, the results of genetic testing will not be placed in your medical record. Despite these efforts, however, we cannot guarantee you with 100% certainty that your genetic information could never be linked to you.

6.**Benefits**

The benefits of the study and any future study include furthering local knowledge about our local populations that can help health providers better understand the diseases affecting pregnant women. Samples will also be used to promote further academic study by students thereby increasing local capacity.

7.**Confidentiality**

At all times no personal identification will be put onto the samples and your name or any other information will not be included with the sample. The results of the genetic tests will not be published with any personal information that can identify you nor will it be linked to your medical records. The results of the study is not intended for any financial gain and will be used to increase knowledge about the conditions we are studying.

The samples will be stored frozen indefinitely and DNA extracted will be used to conduct further research into diseases affecting women. The samples may be used for other studies by the research team or their students. When no further tests are to be done, the remaining samples will be destroyed. All samples will not have any personal information linked to it and will remain anonymous. The principal investigator will store information in a database on the computer that may have information related to your health. That information will not be kept with the samples and will be secured by the research team. All results will be anonymous and will not be disclosed to you, your family or any third party.

8.**Commercialization**

By agreeing to participate in this study and consent to genetic research you cede any rights to potential commercial benefits that may arise out of the study

9.Please indicate the type of consent that you willing to give for the genetic research by ticking the appropriate box:

Coded* use of their biological materials for the proposed study only, with no further contact ☐

Permitted to ask for permission to do further studies ☐

Permitting coded use of their biological materials for the proposed study only, with further contact

Permitted to ask for permission to do further studies ☐

Permitting coded use of their biological materials for any study relating to the condition for which the sample was originally collected, with further contact allowed to seek permission for other types of studies ☐

Permitting coded use of their biological materials for the proposed study only and anonymised use for any kind of future study ☐

Permitting only anonymised (unlinked) use of their biological materials in research ☐

Permitting coded use of their biological materials for the proposed study and coded use for any kind of future study ☐

*coded = identifiable, traceable. Biological materials that are unidentified for research purposes but can be linked to their sources through the use of a code.

10.Signature Page

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time without jeopardising the health care you are entitled to receive. Your continued participation should be as informed as your initial consent.

You will be informed in a timely manner if information becomes available that may affect your willingness to continue participating in this study. You should also feel free to ask for clarification or new information throughout your participation. If you have further questions concerning matters related to this research, please contact:

V. Dorsamy

Phone number: [redacted]

If you have questions concerning your rights as a possible participant in this research, please contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
University of KwaZulu-Natal
Private Bag X 54001, Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2602486 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Participant's name (please print) Participant's signature

_____x
_____x

Date_____x

Investigator's name (please print) Investigator's signature

V Dorsamy_____

_____

Date

Delegate's name (please print) Delegate's signature

_____

_____

We have given you a copy of this form to keep for your records and reference.

SUBJECT'S ACCEPTANCE OF THIRD PARTY AUTHORIZATION

Because your illness (or injury) made it impossible for you to participate in the informed consent process, a third party authorization (e.g. family member) was obtained on your behalf. Your surrogate believed you would have wished to participate in this research if you had been able to express your own opinion at the beginning of the research.

The process of informed consent must be continuous throughout the research project. This means that you have the right to change your mind and, therefore, must be given opportunities to read relevant consent materials, ask questions and then agree or disagree with the decision made by your surrogate to enroll you in this research project.

If you agree with the decision made by your surrogate to enroll you, your signature will affirm your participation in this study. If you do not agree with the decision made by your surrogate to enroll, you may withdraw now or at any other time from the study. Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject.

In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time without jeopardising the health care you are entitled to receive. Your continued participation should be as informed as your initial consent. You will be informed in a timely manner if information becomes available that may affect your willingness to continue participating in this study. You should also feel free to ask for clarification or new information throughout your participation.

Please check the appropriate boxes to indicate your decision: ☐

Agree with your surrogate's decision.
☐

Wish to remain in the study. ☐

Do not agree with your surrogate's decision.
☐

Wish to withdraw from the study. ☐

Participant's name (please print) Participant's signature

_____X
_____X

Date_____X

Investigator's name (please print) Investigator's signature

V Dorsamy_____
_____

Date

Delegate's name (please print) Delegate's signature

_____

_____

We have given you a copy of this form to keep for your records and reference.

INFORMED CONSENT FORM FOR STORAGE OF HUMAN BIOLOGICAL MATERIAL FOR RESEARCH PURPOSES[1]

BIOMEDICAL RESEARCH ETHICS COMMITTEE, UNIVERSITY OF KWZULU- NATAL

The trio of anaemia, parasites and pre-eclampsia in Black African women

The Document consists of two parts:

1. Information Document (Informing the participant about the storage of his/her additional and or residual biological material/s) and a
2. Certificate of Consent (Record of the agreement)

INFORMATION DOCUMENT

INTRODUCTION

Dear Ms/ Mrs

Greetings to you.

We are performing a study to determine whether having worms (parasites) or low blood (anaemia) affects pregnancy. Having these conditions are very common in our country. We wish to study if being infected by worms or low blood may affect a pregnant woman's pressure and how it will affect the mother and the baby. We also wish to determine whether these conditions are more likely to occur in some people than others. The reason this may occur may be dependent on their genetic make-up. The DNA in everybody determines how we look, the shape of our bodies and how we handle sickness. How we deal with parasites, and anaemia and whether we develop high pressure during pregnancy may be linked to our DNA.

This research is being conducted by V. Dorsamy, a PhD student at the University of kwaZulu-Natal, under the supervision of Profs J Moodley and K Mlisana. We want to **see how many**

people suffer with anaemia or high blood pressure during pregnancy and whether conditions are affected by parasites. You have been approached to enrol in this study because you may be pregnant or may have one of these conditions and we invite you to participate in this research which may provide information that will help us understand why they happen. If you wish to participate, we require to take about a tablespoon of blood from you, and to collect a sample of your stool and urine. These samples will be used to test for certain markers of disease or to find evidence of parasites that may affect pregnancy. As we want to continue our studies of these conditions in the future, some of the samples will be stored frozen for future use. We are therefore asking for your permission to store these samples that we obtain from you.

USE AND STORAGE

The tests that we do will require us to collect many samples from lots of pregnant women and do the tests altogether. Therefore some of the samples need to be stored until we can do this. Any of the samples that are remaining will be stored indefinitely for future use by our group and students studying diseases that affect women especially during pregnancy. We expect to store about a teaspoon of each sample. We will be storing these samples in freezers located at the Department of Medical Microbiology at Inkosi Albert Luthuli Central Hospital. Only our study group or our students will have access to the samples. Some of the samples may be taken out of the country in order to examine the DNA. All information that can identify you will be removed from the sample. DNA material will be extracted from the sample and stored. Should you agree to participate you will only be required to provide samples at one time.

BENEFITS

The study will provide valuable information of the effects of parasites on anaemia and high blood pressure during pregnancy. There will not be any direct benefit to you but the information gained from the study may help us understand and better treat pregnant women in the future. The samples will be used to further our knowledge of diseases of pregnancy and will help us generate new scientific knowledge in the world. It will assist many students to gain academic qualifications. Your sample will not be used for any financial gain.

RISKS

We do not believe that there are any risks to you giving us permission to store these samples. Any name or information identifying you will be removed from the sample. Further studies will only use information about your health and pregnancy and not your personal information like your name or contact details.

CONFIDENTIALITY

This study will maintain the confidentiality of participants. When we receive the samples we will attach a study number to the samples in order to identify who they belong to. That is the only information that will be stored on the samples. No name or other personal information

will be stored with the samples. The principal investigator will have a separate spreadsheet with any identification that is associated with the samples. None of this personal identification information will be reported in the study. The results from the tests will not affect you directly in any way and none of the results will go into your patient chart.

PARTICIPANTS RIGHTS

Some people who participate in studies do not wish to have their samples stored or may not want to accept certain parts of the study. You are free to choose whether you want to provide samples for storage. If you do not wish to store samples for future use it will not affect your participation in this study. You can decide whether you wish to participate in the current study looking at parasites and anaemia but you can refuse to participate in any future study that uses the samples in storage. You can also choose which part of the study you wish to participate in or which samples you do not which to be kept. You must also ask questions about anything you are unsure about regarding the study and the storage of the samples. You can also decide at any time that you revoke your permission to store the samples or to participate in this or subsequent studies. You can contact the principal investigator: V Dorsamy, 0847447611, Nelson R Mandela School of Medicine, 719 Umbilo Road, Congella, Durban 4013.

Any future study using these samples will have to be approved by the Biomedical Research Ethics Committee or any Research Ethics Committee where the future research will be carried out.

Genetic studies will be performed in this study. The genes studied are associated with high blood pressure, parasites that affect humans, and infections that are common in pregnant women. Such investigations will determine if you have any genes that are different from other people that may make you more likely to be affected by pre-eclampsia or increase your chances of infection by parasites or give you anaemia. You may choose not to participate in the genetic part of the study. You may choose to have all your personal information removed from the sample or choose which type of genetic study you wish your samples to be used for in this study or future studies involving genetic research. There is a separate consent form for genetic studies that you may sign.

CERTIFICATE OF CONSENT

In the light of the information that I have received, and having had the opportunity to ask questions that have been answered, and if any of the biological material [specify i.e. blood, tissue, isolates] I [name of participant] have provided for this research project [specify] is unused or leftover or additional samples have been provided, I agree to participate in the research study and consent to the following:

	Yes	**No**
The samples [blood /urine/stool] to be disposed of lawfully, immediately	☐	☐
The sample/s [blood /urine/stool] to be disposed of lawfully after__ years.	☐	☐
The sample/s [blood /urine/stool] returned to me for burial/cremation.	☐	☐
The sample/s [blood /urine/stool] to be stored for ___years	☐	☐
The sample/s [blood /urine/stool] to be stored indefinitely	☐	☐
The samples [blood /urine/stool] to be exported under BREC oversight	☐	☐

AND if the sample is to be stored I consent to the following:

The sample/s collected during this study may be stored at [UKZN/IALCH]	☐	☐
The sample/s [blood /urine/stool] to be stored and used in future research for the specific purposes of this study [Pre-eclampsia, anaemia and parasite infection] approved by BREC	☐	☐
The sample/s [blood /urine/stool] to be stored and used in future research of any type which has been approved by BREC.	☐	☐
The sample/s [blood /urine/stool] to be stored and used in future research except for research about [Haematology/genetics/] approved by BREC	☐	☐
The sample/s[blood /urine/stool] to be used for teaching, quality assurances, public health surveillance, clinical audit, publications and presentations approved by BREC	☐	☐
The sample/s [blood /urine/stool] to be used by researchers for the development of commercial products without any financial benefit to me	☐	☐
The samples to be used by secondary *bona fides* researchers approved by BREC	☐	☐
The samples may be exported under BREC's oversight and approval	☐	☐

AND **Yes**

I want my identity to be removed from my sample/s [specify] ☐ ☐

I want my identity to be kept with my samples [specify]

☐ ☐

AND

I am willing to be re-contacted by the researcher/s about possible future use of my sample/s in the future ☐ ☐

I do not want to be re-contacted to provide more sample/s in the future or take pa ☐ ☐
in future studies..

I declare:

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction.

I consent voluntarily to have my samples stored in the manner and for the purpose **Yes** **No**
indicated above. I have been informed of my right to withdraw my consent to the storage and/or use of my samples at any time and without giving any reason and without prejudice to myself or my treatment. ☐ ☐

I have been informed that I will be given information from the research team concerning the progress and general results of the research studies upon my explicit request. I have also been informed that they will not communicate any individual results to me. ☐ ☐

*Name of Participant*_____

Signature of Participant _____

Date _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

*Print name of witness*_____ ***Thumb print of participant***

Signature of witness _____

Date _____ *Time*_____

Day/month/year

STATEMENT BY THE RESEARCHER/PERSON TAKING CONSENT

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Blood urine and stool samples will be collected from the participant after her consent.
2. Cord blood samples will be collected.
3. These samples will be transported and stored at a laboratory at University until such time as they are require to conduct the current study
4. Samples will be stored and used by the principal investigator/supervisor and their students when necessary to conduct further research
5. Samples will be anonymised and no personal details will be attached to the samples

I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed consent form has been provided to the participant.

*Name of Researcher/person taking the consent*_____

*Signature of Researcher /person taking the consent*_____

Date ______________________________ *Time*__________________________________

Day/month/year

Appendix 7: Additional Results

This document includes unused tables and statistical diagrams that were references in the empirical study yet were unable to be included due to the limitations on word count imposed by the journal.

Tables

Magnesium Groups			
PE Group		N	%
First VisitNT	HypoMg	4	4,3%
	Normal	90	95,7%
TermNT	HypoMg	16	23,9%
	Normal	51	76,1%
EOPE	HypoMg	6	12,2%
	Normal	43	87,8%
LOPE	HypoMg	4	9,5%
	Normal	38	90,5%

Table 1: *A table that illustrates the numerical and percentage distribution of hypomagnesemia and normal magnesium levels across the study groups.*

HIV Prevalence			
PE Group		N	%
First VisitNT	HIV-	46	48,9%
	HIV+	48	51,1%
TermNT	HIV-	40	59,7%
	HIV+	27	40,3%
EOPE	HIV-	22	44,9%
	HIV+	27	55,1%
LOPE	HIV-	25	59,5%
	HIV+	17	40,5%

Table 2: *A table that illustrates the numerical and percentage distribution of HIV across the study groups.*

Figures

Results						
	HIV+	HIV-				***Row Totals***
First VisitNT	48 (44.39) [0.29]	46 (49.61) [0.26]				94
TermNT	27 (31.64) [0.68]	40 (35.36) [0.61]				67
EOPE	27 (23.14) [0.64]	22 (25.86) [0.58]				49
LOPE	17 (19.83) [0.40]	25 (22.17) [0.36]				42
Column Totals	119	133				**252 (Grand Total)**

The chi-square statistic is 3.833. The *p*-value is .28007. The result is *not* significant at $p < .05$.

Figure 1: *A Chi-squared analysis of the relationship between HIV and pre-eclamptic status.*

Results						
	HIV+	HIV-				***Row Totals***
NormalMg	108 (104.83) [0.10]	114 (117.17) [0.09]				222
HypoMg	11 (14.17) [0.71]	19 (15.83) [0.63]				30
Column Totals	119	133				**252 (Grand Total)**

The chi-square statistic is 1.5224. The *p*-value is .217254. The result is *not* significant at $p < .05$.

Figure 2: *A Chi-squared analysis of the relationship between HIV and magnesium levels.*

Appendix 8: The impact of magnesium levels in pregnancy on adverse birth outcomes: A protocol for a systematic review and meta-analysis

The following appendix includes a protocol for a systematic review and meta-analysis. The article was originally submitted to BMC Systematic Reviews on the 9th of February 2025 and was subsequently resubmitted to the journal on the 26th of May 2025 following the editor's request for article revisions. The article is currently under review.

Attached below is an email confirming acceptance of the revised protocol.

Confirmation of revised submission to Systematic Reviews - SYSR-D-25-00192R1 - [EMID:40755290a4a2ac2f]

SR

em.sysr.0.93a6b2.2d5aea1e@editorialmanager.com <em.sysr.0.93a6b2....

on behalf of

Systematic Reviews Editorial Office <em@editorialmanager.com>

Monday, 26 May 2025 at 09:56

To: Naeera Abdul (220004494)

SYSR-D-25-00192R1
The impact of magnesium levels in pregnancy on adverse birth outcomes: a protocol for a systematic review and meta-analysis
Naeera Abdul; Vinogrin Dorsamy; Chauntelle Bagwandeen
Systematic Reviews

Dear Miss Abdul,

Thank you for the revised version of your manuscript 'The impact of magnesium levels in pregnancy on adverse birth outcomes: a protocol for a systematic review and meta-analysis' submitted to Systematic Reviews.

You may check the status of your manuscript at any time by accessing the following website:

https://www.editorialmanager.com/sysr/

If you have forgotten your username or password please use the "Send Login Details" link to get your login information. For security reasons, your password will be reset.

We will inform you of the Editor's decision as soon as possible.

Best wishes,
Editorial Office
Systematic Reviews
https://systematicreviewsjournal.biomedcentral.com/

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

Recipients of this email are registered users within the Editorial Manager database for this journal. We will keep your information on file to use in the process of submitting, evaluating and publishing a manuscript. For more information on how we use your personal details please see our privacy policy at https://www.springernature.com/production-privacy-policy. If you no longer wish to receive messages from this journal or you have questions regarding database management, please contact the Publication Office at the link below.

Abstract

Background

Magnesium plays a vital role in maternal and fetal physiology during pregnancy, influencing vascular tone, inflammation, and cellular metabolism. However, inconsistencies in magnesium level measurements and their association with adverse pregnancy outcomes limit the development of evidence-based antenatal care guidelines, particularly in low to middle-income countries (LMICs), where comorbidities such as HIV, anaemia, and obesity are common.

Objectives

To systematically evaluate the association between maternal magnesium levels and adverse pregnancy outcomes, establish trimester-specific reference ranges, and assess the modifying effects of comorbid conditions, including HIV, anaemia, and obesity.

Eligibility Criteria

Original research articles—observational (cross-sectional, case-control, cohort) and interventional studies (RCTs)—reporting quantitative maternal magnesium levels and at least one adverse maternal or neonatal outcome will be included. There will be no restrictions on language, setting, or publication year.

Information Sources

Bibliographic literature databases including PubMed, Scopus, Embase, Cochrane Library, DOAJ, LILACS, African Index Medicus, and grey literature sources such as OpenGrey and ProQuest Dissertations & Theses will be systematically searched.

Risk of Bias Assessment

Two reviewers will independently assess study quality using Hoy's tool for prevalence studies and ROBINS-I for observational studies. Disagreements will be resolved by a third reviewer.

Synthesis Methods

Data will be synthesised using random-effects or quality-effects meta-analyses. Subgroup analyses will be performed by trimester, region, comorbidities, and study design. GRADE will be used to assess the certainty of evidence for each outcome.

Discussion

This review will provide a comprehensive synthesis of the evidence on maternal magnesium levels and pregnancy outcomes, informing clinical guidelines and public health strategies. It will also highlight evidence gaps and research priorities for LMICs.

Systematic Review Registration

PROSPERO registration number: CRD42024518427

Keywords

Pregnancy, magnesium, maternal health, pregnancy outcomes, comorbidities, low to middle-income countries

Background

The role of magnesium (Mg) in pregnancy represents an area of both significant promise yet ongoing controversy, as a possible mitigating factor in the prevention of pre-eclampsia. Whilst the global consensus is that Mg is vital for maternal and fetal health, inconsistencies in the literature regarding its optimal levels and association with pregnancy outcomes hinder its integration into prenatal care guidelines. The variability in reference ranges used by different laboratories compounds the conundrum; commonly cited ranges for serum Mg include both 0.75-0.95mmol/L and 0.65-1.05mmol/L with some studies suggesting 0.85mmol/L as a suitable minimum threshold for defining adequacy[1−6]. This lack of standardization in establishing the optimum Mg range, given the conflicting results from existing studies as to what this optimum range should be, leaves critical knowledge gaps in what could be a safe and cost-effective means to ensure desired pregnancy outcomes, and thereby minimise the burden of unwanted maternal and fetal morbidity and mortality. This poses the question: should Mg therefore be incorporated into standard maternal treatment guidelines as a supplement, especially in low to middle-income countries (LMICs), where adverse pregnancy outcomes in the face of co-existing conditions are disproportionately prevalent compared to available resources?

Magnesium is an essential trace mineral that supports over 600 enzymatic reactions, including those responsible for DNA synthesis, muscle contraction, energy metabolism, and nerve function[7,8]. During pregnancy, there is an extension of these biochemical functions, directly influencing maternal and fetal health as Mg requirements increase to accommodate fetal growth and maternal physiological adaptations. Sufficient Mg levels help regulate vascular tone, reduce inflammation, and stabilise cellular energy pathways – key factors in preventing complications such as gestational diabetes mellitus (GDM), pregnancy-induced hypertension (PIH), and pre-eclampsia (PE)[9]. Magnesium sulphate, a cornerstone treatment for severe PE and eclampsia, further highlights the mineral's clinical importance[10]. However, maintaining optimal Mg levels is a delicate balance, as both hypomagnesemia and hypermagnesemia are associated with adverse maternal and neonatal outcomes[11,12].

The challenges in understanding the role of Mg are exacerbated by its complex interplay with other comorbidities, particularly those common in LMICs, such as HIV, anaemia, and obesity, which not only influence Mg metabolism but also increase pregnancy-related risks. For

example, antiretroviral therapy in HIV-positive women may disrupt Mg homeostasis, whilst anaemia and obesity independently contribute to adverse maternal and fetal outcomes[14,15].

Despite the potentially significant public health impact to be accrued, research addressing the interactions between Mg levels and these comorbidities in vulnerable populations remains limited and inconsistent. The lack of comprehensive evidence impedes the development of comprehensive, evidence based targeted interventions and clinical guidelines that consider these multifaceted challenges[16–18].

To address these critical gaps in the literature, this paper outlines a protocol for a systematic review and meta-analysis aimed at synthesising current evidence on the relationship between maternal Mg levels and adverse pregnancy outcomes. Unlike existing studies, which are often limited in scope and population specificity, this review seeks to provide a holistic and population-sensitive analysis of Mg's role in maternal and fetal health. To our knowledge, no systematic review has yet comprehensively evaluated maternal Mg levels and their association with pregnancy outcomes. This review will place a particular focus on populations most vulnerable to Mg deficiency and its complications, especially in LMICs, where dietary diversity is limited, and comorbidities such as HIV, anaemia, and obesity are prevalent. In doing so, it aims to provide actionable insights for healthcare professionals, policymakers, and researchers, ultimately improving maternal and neonatal outcomes in resource-limited settings.

Objectives

The primary objective is to evaluate the relationship between maternal Mg levels and adverse pregnancy outcomes, focusing on populations in LMICs.

Secondary objectives include:

1. Establishing trimester-specific reference ranges for maternal Mg levels.
2. To determine the impact of comorbidities (e.g., HIV, anaemia, obesity) on maternal Mg levels and adverse pregnancy outcomes.
3. To assess the potential benefit of Mg supplementation in reducing adverse outcomes in high-risk populations.

Methodology

This protocol for a systematic review and meta-analysis adheres to the Preferred Reporting Items for Systematic Review and Meta-analysis for Protocols (PRISMA-P) 2015 guidelines[19]. The completed PRISMA-P checklist has been submitted as a supplementary file. The final

systematic review will be reported in accordance with the PRISMA 2020 statement to ensure transparency and completeness of reporting[20].

Framing the Research Question Using the PEO Framework

This review adopts the PEO (Population, Exposure, Outcome) framework to guide the development of the research question and eligibility criteria (Table 1).

Table 1 PEO framework for eligibility of research question

Population	Pregnant women across all trimesters, including subgroups with comorbid conditions such as HIV, anaemia, or obesity
Exposure	Maternal magnesium levels assessed through validated laboratory methods (e.g., serum magnesium, RBC magnesium)
Outcomes	Adverse maternal and neonatal outcomes including PE, GDM, PIH, intrauterine growth restriction (IUGR), preterm birth, and low birth weight..

The PEO framework informed the inclusion criteria and helped ensure a focused and structured literature search.

Eligibility Criteria

Inclusion Criteria

- Original research articles reporting quantitative maternal Mg levels using validated laboratory methods.
- Studies reporting maternal or neonatal outcomes such as PE, GDM, IUGR, preterm birth, or low birth weight.
- Study designs: cross-sectional, case-control, cohort studies (prospective or retrospective), and randomised controlled trials (RCTs).

Exclusion Criteria

- Case reports, reviews, editorials, or commentaries.
- Non-human studies.
- Studies lacking extractable data on Mg levels or pregnancy outcomes.

Search Strategy

A comprehensive search will be conducted in PubMed, Embase, Scopus, Cochrane Library, DOAJ, LILACS, CINAHL, and African Index Medicus. Grey literature will be searched via OpenGrey, ProQuest Dissertations & Theses, and relevant conference proceedings. The search strategy was reviewed using the PRESS 2015 checklist by an information specialist, and was piloted on the 23rd of May 2025 to determine the appropriateness of the selected keywords and electronic databases (Table 2)[21]. The full search strategies for each database will be included in an appendix for the systematic review. No language or date restrictions will be applied. Google Translate will be used to translate non-English articles. Untranslatable articles will be listed in an appendix.

Boolean operators and keywords will incorporate variations in terminology, such as:

- Pregnancy terms: ("pregnancy" OR "gestation" OR "maternal")
- Magnesium terms: ("magnesium" OR "MgSO4" OR "hypomagnesemia" OR "hypermagnesemia")
- Outcome terms: ("pre-eclampsia" OR "pregnancy outcomes" OR "low birth weight")
- Comorbidity terms: ("HIV" OR "anaemia" OR "obesity")

Table 2 Potential search strategy on PubMed

Search	**Query**
#1	**((((((("pregnant"[All Fields]) OR ("pregnancy"[All Fields])) OR ("maternal"[All Fields])) OR ("mother"[All Fields])) OR ("gestation"[All Fields])) OR ("gestational"[All Fields])) OR (pregnancy[MeSH Terms])**
#2	**((((((((((((((((((((((((((("preeclampsia"[All Fields]) OR ("eclampsia"[All Fields])) OR ("hypertension"[All Fields])) OR ("hypertension"[MeSH Terms])) OR ("hypertensive disorder"[All Fields]))) OR ("hypertensive pregnancy"[All Fields])) OR ("hypertensive pregnancy disorder"[All Fields])) OR ("disease"[All Fields])) OR ("disorder"[All Fields])) OR ("pregnancy disorder"[All Fields])) OR ("hypertensive"[All Fields])))) OR ("pregnancy outcome"[All Fields])) OR ("pregnancy outcome"[MeSH Terms])) OR (pregnancy complication) ("hypertensive"[All Fields]))) OR ("gestational diabetes"[All Fields])) OR (gestational diabetes[MeSH Terms])) OR (gestational diabetes mellitus[MeSH Terms])) OR ("gestational diabetes mellitus"[All Fields])) OR ("gdm"[All Fields])) OR**

	("intrauterine growth restriction"[All Fields])) OR ("iugr"[All Fields])) OR ("fetal growth restriction"[All Fields])) OR ("fgr"[All Fields])) OR ("gestational hypertension"[All Fields]) OR ("pregnancy induced hypertension"[All Fields]) OR ("pih"[All Fields]) OR ("preterm labour"[All Fields])) OR (premature birth[MeSH Terms])) OR (infant, low birth weight[MeSH Terms])) OR (preeclampsia[MeSH Terms])
#3	**(((((((("magnesium"[All Fields]) OR ("magnesium"[MeSH Terms])) OR ("magnesium sulphate"[All Fields])) OR ("magnesium sulfate"[All Fields])) OR ("magnesium sulfate"[MeSH Terms])) OR ("mgso4"[All Fields])) OR ("hypermagnesemia"[All Fields])) OR ("hypomagnesemia"[All Fields])) OR ("low magnesium"[All Fields])) OR ("high magnesium"[All Fields])**
#4	**#1 AND #2 AND #3**

Study Selection and Screening

Articles will be screened in two stages - title/abstract and full-text - by two independent reviewers (NA and VD). To ensure consistency and reliability, calibration exercises will be conducted on a random sample of 10 studies prior to screening. Discrepancies will be resolved through discussion, with a third reviewer (CB) serving as arbitrator if consensus is not reached. Cohen's kappa statistic will be calculated to assess inter-rater agreement. Duplicate records will be removed using Zotero (v6.0.30). Additionally, a hand search of reference lists will be performed by NA and VD to identify any additional eligible studies. Screening outcomes will be documented in accordance with the PRISMA 2020 flow diagram[20,22].

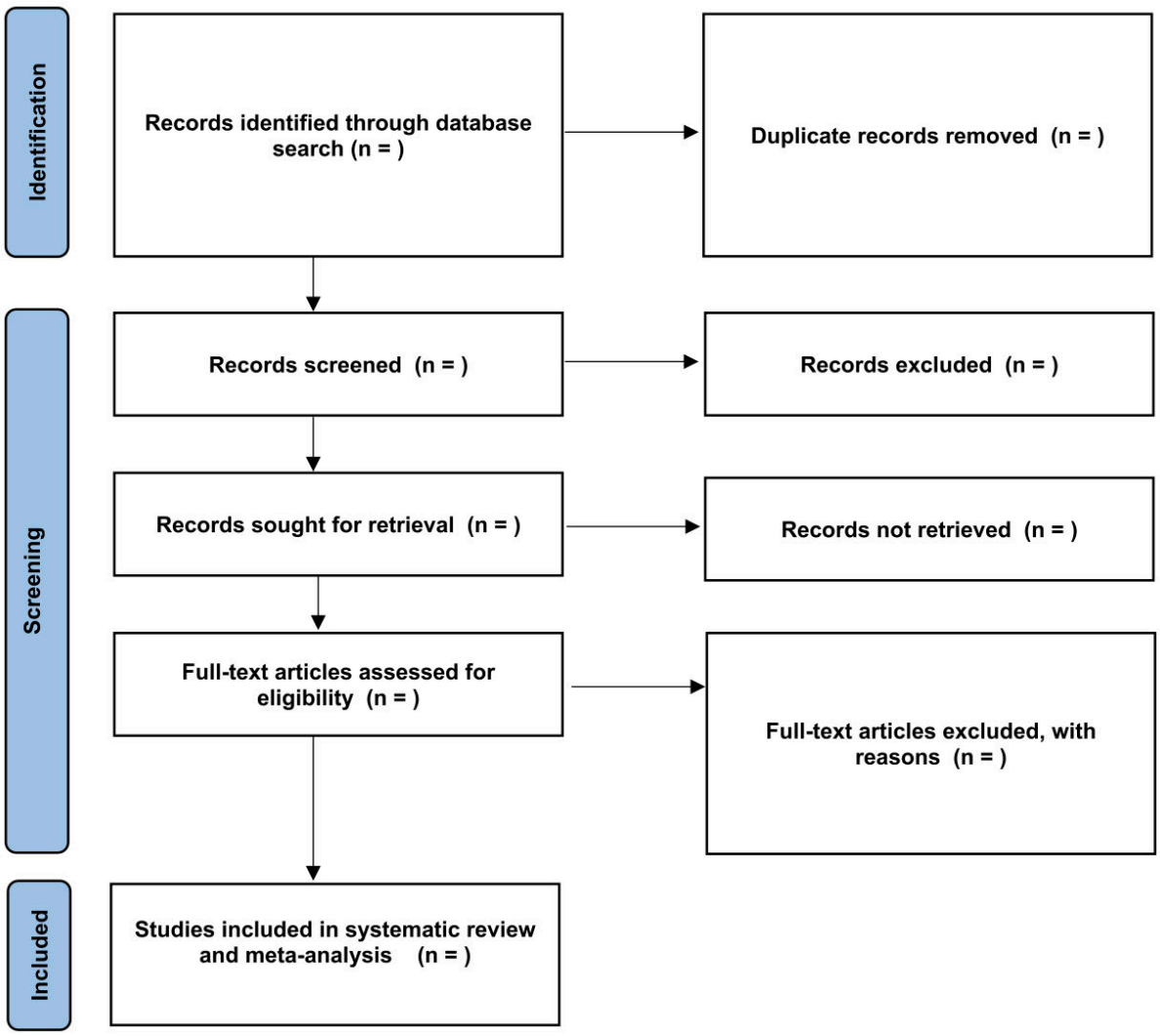


Figure 1: PRISMA flow diagram describing selection of studies for systematic review of Mg levels in pregnancies with adverse outcomes[23].

Data Extraction

A standardised data extraction form will be developed in Microsoft® Excel Version 16.97 and piloted on a subset of 10 studies[24]. Two independent reviewers (NA and VD) will extract data, and discrepancies will be resolved through discussion or by a third reviewer (CB). The data extraction form will be structured to capture key study characteristics and findings relevant to the review objectives. Data will be extracted according to the search strategy outlined in Table 2 above.

Where effect estimates are not reported, raw data will be used to compute them where feasible. Authors of included studies will be contacted for clarification if necessary. The extracted data will form the basis for quantitative synthesis and narrative summaries, as appropriate. The data will be extracted according to Table 3.

Table 3 Data to be extracted

Study Identification	Author(s), year of publication, country, journal
Study Design and Setting	Type of study, recruitment setting, sample size
Population Characteristics	Age, gestational age, parity, gravidity, BMI, socioeconomic status, HIV status, antiretroviral use, anaemia, obesity
Exposure Details	Type of magnesium measurement (e.g., serum or RBC), units, reference ranges, laboratory assay methods used
Outcomes	Maternal outcomes (e.g., PE GDM, PIH), neonatal outcomes (e.g., IUGR, preterm birth, low birth weight)
Effect Measures	Reported effect estimates (e.g., ORs, RRs, mean differences) and corresponding confidence intervals
Additional Notes	Author conclusions, study limitations, and reviewer comments

Risk of Bias and Methodological Quality Assessment

The internal validity of the included studies will be assessed using two domain-based risk of bias tools appropriate for the study designs:

- Hoy's Risk of Bias Tool will be used for prevalence studies. It evaluates domains such as sampling, data collection, and outcome measurement[25].
- ROBINS-I **(**Risk of Bias in Non-randomised Studies – of Interventions) will be used for observational studies to assess bias related to confounding, selection, measurement, and reporting[26].

Two reviewers (NA and VD) will independently assess each study, with discrepancies resolved by consensus or a third reviewer (CB). A pilot calibration exercise on a subset of studies will be conducted prior to formal assessment.

These tools will guide our overall appraisal of methodological quality and support the interpretation of findings in the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework[27].

GRADE Assessment

The certainty of evidence for each outcome will be assessed using the GRADE framework[27]. This approach evaluates the following five domains:

1. Risk of Bias – Based on assessments using Hoy's tool and ROBINS-I for individual studies[25,26].
2. Inconsistency – Assessed through heterogeneity in results across studies (e.g., I^2 > 50%).
3. Indirectness – Evaluates how directly the evidence applies to the review question in terms of population, exposure, and outcomes.
4. Imprecision – Determined by the size of the confidence intervals and the overall sample size contributing to the estimate.
5. Publication Bias – Evaluated through funnel plots and Egger's regression test when 10 or more studies are available for a pooled estimate[28].

Two reviewers (NA and VD) will perform GRADE assessments independently. Any disagreements will be resolved through consensus or consultation with a third reviewer (CB).

GRADEpro GDT software will be used to generate Summary of Findings tables[29]. These tables will summarise effect estimates, number of studies and participants, and the overall certainty of the evidence (rated as high, moderate, low, or very low).

Data Synthesis and Analysis

Meta-analyses will calculate pooled estimates using random-effects or quality-effects models, depending on heterogeneity and study quality. Heterogeneity will be quantified using the I^2 statistic, and thresholds of <25%, 25–50%, and >50% will represent low, moderate, and high heterogeneity, respectively. Forest plots will be generated for visual presentation of pooled results.

Different study designs will be handled as follows:

- Cross-sectional and prevalence studies will be synthesised using pooled means or proportions where appropriate. Effect sizes (e.g., mean differences in Mg levels) will be calculated when comparable.
- Case-control studies will be synthesised using pooled odds ratios (ORs) for the association between Mg levels and adverse pregnancy outcomes.
- Cohort studies will contribute to pooled relative risks (RRs) or hazard ratios (HRs) depending on the reported data.
- Randomised controlled trials reporting baseline Mg levels and outcomes will be analysed separately, with subgroup analyses if necessary.

To minimise bias in pooled estimates, we will conduct subgroup analyses based on:

- Trimester of pregnancy
- Geographic region
- Laboratory method used to measure Mg
- Presence of comorbidities (HIV, anaemia, obesity)
- Study design

If a sufficient number of studies is available, meta-regression will be used to explore the sources of heterogeneity. Narrative synthesis will be undertaken for studies where pooling is not feasible due to clinical or methodological heterogeneity. Sensitivity analyses will be conducted by excluding studies at high risk of bias to assess the robustness of the findings.

Discussion

This systematic review and meta-analysis will address a critical gap in maternal health research by synthesising the available evidence on maternal Mg levels and pregnancy outcomes. Given the global burden of adverse pregnancy outcomes, especially in low to middle-income countries, this review aims to clarify whether maternal Mg levels should be integrated into antenatal screening and nutritional guidelines. By generating trimester-specific reference ranges and exploring interactions with prevalent comorbidities such as HIV, anaemia, and obesity, the findings will have direct relevance for both clinical practice and public health policy.

Potential limitations may include variability in laboratory methods, definitions of outcomes, and population characteristics across studies, which could affect the comparability of results. Nonetheless, the planned subgroup analyses, quality assessments, and use of GRADE to evaluate certainty of evidence are designed to enhance the rigour and interpretability of the findings.

The review is expected to provide a strong foundation for future research and may prompt further investigation into Mg supplementation as a preventive strategy in high-risk pregnancies.

Ethics Declarations

Ethics and Dissemination

No ethical approval is required as this review involves secondary analysis of published data. Findings will be disseminated via peer-reviewed publications and academic conferences.

Conflicts of Interest

The authors declare no competing interests.

Author Contributions

NA and VD conceptualised the study and developed the protocol. NA drafted the manuscript. VD and CB critically revised the manuscript. All authors approved the final version.

Acknowledgments

The authors would like to extend thanks to Dr Anushka Ajith for assistance with the search strategy.

Additional Information

Registration

Registered with PROSPERO (CRD42024518427).

Funding

This protocol forms part of a Master's thesis, which received funding for the empirical portion of the study from the National Research Foundation's 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. Furthermore, there was no input from these organisations in the interpretation of the result.

Amendments

All protocol amendments will be documented with dates and justifications in an appendix and on the PROSPERO record.

Abbreviations

Mg	Magnesium
HIV	Human Immunodeficiency Virus
DNA	Deoxyribonucleic acid
GDM	Gestational diabetes mellitus
PIH	Pregnancy-induced hypertension
IUGR	Intrauterine growth restriction
PE	Pre-eclampsia
LMICs	Low to-middle income countries
PRISMA-P	Preferred Reporting Items for Systematic Review and Meta-analysis for Protocols
PEO	Populations Exposure Outcomes
DOAJ	Directory of Open Access Journals

References

1. Price JF. Chapter 38 - Diuretics and invasive fluid management strategies [Internet]. Jefferies JL, Chang AC, Rossano JW, Shaddy RE, Towbin JA, editors. ScienceDirect. Boston: Academic Press; 2018. p. 495–510. Available from: https://www.sciencedirect.com/science/article/abs/pii/B9780128023938000387

2. Costello RB, Elin RJ, Rosanoff A, Wallace TC, Guerrero-Romero F, Hruby A, et al. Perspective: The case for an evidence-based reference interval for serum magnesium: The time has come. Advances in Nutrition [Internet]. 2016 Nov 10;7(6):977–93. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5105038/

3. Micke O, Vormann J, Kraus A, Kisters K. Serum magnesium: Time for a standardized and evidence-based reference range. Magnesium Research [Internet]. 2021 May 1;34(2):84–9. Available from: https://pubmed.ncbi.nlm.nih.gov/34463286/

4. Devkota BP. Magnesium: Reference range, interpretation, collection and panels [Internet]. eMedicine. 2019. Available from: https://emedicine.medscape.com/article/2088140-overview?form=fpf

5. Jahnen-Dechent W, Ketteler M. Magnesium basics. Clinical Kidney Journal [Internet]. 2012 Feb 1;5(Supplement 1):i3–14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4455825/

6. Okwi AL, Bimenya GS, Tumwine LK, Othieno E, Okot J, Ibingira C. The reference range of serum magnesium substance concentration among healthy young adults at Makerere University College of Health Sciences 2012. Tanzania Journal of Health Research [Internet]. 2016 Mar 28;18(2). Available from: https://www.ajol.info/index.php/thrb/article/view/120470

7. Fanni D, Gerosa C, Nurchi VM, Manchia M, Saba L, Coghe F, et al. The role of magnesium in pregnancy and in fetal programming of adult diseases. Biological Trace Element Research. 2020 Dec 14;199(10).

8. Dalton LM, Ní Fhloinn DM, Gaydadzhieva GT, Mazurkiewicz OM, Leeson H, Wright CP. Magnesium in pregnancy. Nutrition Reviews. 2016 Jul 21;74(9):549–57.

9. Shaikh K, Das CM, Baloch GH, Abbas T, Fazlani K, Jaffrey MH, et al. Magnesium associated complications in pregnant women. World Applied Sciences Journal [Internet]. 2012;17(9):1074–8. Available from: https://www.academia.edu/1230699/Magnesium_Associated_Complications_in_Pregnant_Women

10. Karrar S, Hong PL. Preeclampsia [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570611/

11. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clinical Journal of the American Society of Nephrology. 2014 Oct 6;10(7):1257–72.

12. Wolf MTF. Inherited and acquired disorders of magnesium homeostasis. Current Opinion in Pediatrics. 2017 Apr;29(2):187–98.

13. Ali MK, Magee MJ, Dave JA, Ofotokun I, Tungsiripat M, Jones TK, et al. HIV and metabolic, body, and bone disorders. Journal of Acquired Immune Deficiency Syndromes. 2014 Sep 1;67(Supplement 1):S27–39.

14. James AH. Iron deficiency anemia in pregnancy. Obstetrics & Gynecology. 2021 Sep 9;138(4).

15. Olson KN, Redman LM, Sones JL. Obesity “complements” preeclampsia. Physiological Genomics. 2019 Mar 1;51(3):73–6.

16. Barbagallo M, Veronese N, Dominguez LJ. Magnesium—An ion with multiple invaluable actions, often insufficiently supplied: From in vitro to clinical research. Nutrients [Internet]. 2023 Jan 1;15(14):3135. Available from: https://www.mdpi.com/2072-6643/15/14/3135

17. Kintiraki E, Papakatsika S, Kotronis G, Goulis DG, Kotsis V. Pregnancy-induced hypertension. Hormones (Athens, Greece) [Internet]. 2015 Apr 1;14(2):211–23. Available from: https://link.springer.com/article/10.14310/horm.2002.1582

18. Meazaw MW, Chojenta C, Muluneh MD, Loxton D. Systematic and meta-analysis of factors associated with preeclampsia and eclampsia in sub-Saharan Africa. Spradley FT, editor. PLOS ONE. 2020 Aug 19;15(8):e0237600.

19. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: Elaboration and explanation. British Medical Journal. 2015 Jan 2;349:g7647–7.

20. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. British Medical Journal [Internet]. 2021 Mar 29;372(71). Available from: https://www.bmj.com/content/372/bmj.n71

21. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS peer review of electronic search strategies: 2015 guideline statement. Journal of Clinical Epidemiology [Internet]. 2016 Jul 1;75(1):40–6. Available from: https://www.sciencedirect.com/science/article/pii/S0895435616000585

22. Haddaway NR, Page MJ, Pritchard CC, McGuinness LA. PRISMA2020: An R Package and shiny app for producing PRISMA 2020-compliant flow diagrams, with Interactivity for Optimised Digital Transparency and Open Synthesis. Campbell Systematic Reviews [Internet]. 2022 Mar 27;18(2). Available from: https://onlinelibrary.wiley.com/doi/10.1002/cl2.1230

23. Bramer WM, De Jonge GB, Rethlefsen ML, Mast F, Kleijnen J. A systematic approach to searching: An efficient and complete method to develop literature searches. Journal of the Medical Library Association [Internet]. 2018 Oct 1;106(4):531–41. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6148622/

24. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. Journal of Epidemiology and Community Health. 2013 Aug 20;67(11):974–8

25. Hoy D, Brooks P, Woolf A, Blyth F, March L, Bain C, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. Journal of Clinical Epidemiology. 2012 Sep;65(9):934–9.

26. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. British Medical Journal [Internet]. 2016 Oct 12;355(355):i4919. Available from: https://www.bmj.com/content/355/bmj.i4919

27. Siemieniuk R, Guyatt G. What is GRADE? [Internet]. BMJ Best Practice. 2017. Available from: https://dev-bestpractice.bmjgroup.com/info/us/toolkit/learn-ebm/what-is-grade/

28. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. British Medical Journal. 1997 Sep 13;315(7109):629–34.

29. GRADEpro GDT: GRADEpro guideline development tool [Software]. McMaster University and Evidence Prime, 2025. Available from gradepro.org.

Appendix 9: Beyond magnesium sulphate: Rethinking magnesium's impact on maternal and fetal health in low-to-middle-income countries

This appendix is a paper that consolidates an expanded narrative review of the literature that was submitted to South African Family Practice on the 14th of July 2025. The article was deemed suitable for review on the 15th of July 2025, and is currently under review.

Attached below is an email confirming the article suitable for review.

SAFP Submission 6196 – Suitable for Review

A

aosis@safpj.co.za <aosis@safpj.co.za>

To: Naeera Abdul (220004494)

Tuesday, 15 July 2025 at 16:18

****
Ref. No.: 6196
Manuscript title: Beyond magensium sulphate: Rethinking magnesium's impact on maternal and fetal health in low to middle-income countries
Journal: South African Family Practice
****

Dear Miss Abdul

Thank you for submitting your manuscript to the journal. All new manuscripts are given a preliminary inspection by the editorial office to assess whether the submission is complete. We are grateful for your efforts to adhere to the author guidelines of South African Family Practice.

Your manuscript will now proceed to our blinded peer review process to undergo an assessment by our expert independent reviewers. Read our peer review process https://aosis.co.za/policies#peer_review.

Editor remarks:

Ensure to keep us informed if any of your credentials have changed during this time, to ease communication with you as your manuscript progresses through the different publication phases. If you need any assistance, kindly contact the Editorial Office at submissions@safpj.co.za with any questions or concerns.

We remind our authors that our publisher is a member of CrossChecks plagiarism detection initiative and endorses and applies the standards of the Committee on Publication Ethics which promotes integrity in peer-reviewed research publications. This journal also conforms to the accreditation requirements by both the Department of Higher Education and Training of South Africa and Scielo SA.

Thank you for your continued patience and support, and we hope you have joined our online community by signing up for our RSS alerts and Twitter https://twitter.com/safpjournal page.

Abstract

Background

Hypertensive disorders of pregnancy, particularly pre-eclampsia, are the leading cause of maternal and perinatal morbidity and mortality in low to middle-income countries, undermining progress toward reducing maternal mortality. Despite magnesium's implication in hypertensive disorders of pregnancy for prophylaxis and eclamptic seizure prevention through magnesium sulphate administration; its physiological role in vascular tone regulation, endothelial function, and oxidative stress is underexplored.

Methods

A non-systematic search of PubMed, Scopus, Web of Science, the Cochrane Library, and Google Scholar (January 2000–December 2024) using terms related to "magnesium homeostasis, "pre-eclampsia," "HIV," "obesity," and "socioeconomic status" was conducted. Peer-reviewed, English-language human studies were included; animal research was included in order to gain an increased insight into plausible mechanisms of action.

Results

Randomised controlled trials lacked standardisation, leading to varied results. Studies did not consistently chart magnesium deficiency or monitor treatment protocols, with some disregarding comorbidities in low to middle-income countries. Recurring themes included pregnancy altering magnesium homeostasis by manipulating placental transfers and intestinal and renal absorption, decreased magnesium promotes oxidative stress, inflammation and impaired endothelial dysfunction and that HIV and antiretroviral therapy, obesity, and low socioeconomic status exacerbate magnesium depletion and increase hypertensive disorders of pregnancy risk.

Conclusion

Magnesium deficiency contributes to hypertensive disorders of pregnancy and adverse perinatal outcomes in low to middle-income countries. Future research should encompass early screening for magnesium deficiency, supplementation trials for hypomagnesemic women, standardisation of magnesium formulations, stratification by co-morbidities, and the integration of biomarkers within existing antenatal care frameworks.

Contribution

This review highlighted inconsistencies in magnesium investigation and reporting in randomised control trials, a lack of treatment protocols and the non-observance of

comorbidities in some low to middle-income-based studies; underscoring the importance of magnesium investigation, adhering to treatment protocols and how comorbidities can affect magnesium.

Keywords

Hypertensive disorders of pregnancy, magnesium, pre-eclampsia, HIV, obesity, South Africa

1. Introduction

The United Nations' Sustainable Development Goal (SDG) 3—to "ensure healthy lives and promote well-being for all at all ages"—sets a target of reducing the global maternal mortality ratio to under 70 per 100 000 live births by 2030 (target 3.1)[1]. Yet, hypertensive disorders of pregnancy (HDP), most notably pre-eclampsia (PE), continue to drive a substantial share of the roughly 295 000 annual maternal deaths, over 90% of which occur in low to middle-income countries (LMICs), where health systems are often overstretched and antenatal care access uneven[2,3]. Achieving SDG 3 therefore demands not only improved access to interventions such as magnesium sulphate for seizure prophylaxis, but also a deeper understanding of magnesium's (Mg) broader physiological role in blood-vessel health and placental function.

Despite its critical importance—Mg acts as an essential cofactor in over 600 enzymatic reactions, governing vascular tone regulation, endothelial integrity, and oxidative-stress control—Mg homeostasis in pregnancy remains under-studied relative to its clinical use in eclampsia prevention[4,5]. Observational studies in diverse LMIC cohorts report that women who develop PE or gestational hypertension often have lower serum and intracellular Mg levels than normotensive peers, and inadequate dietary Mg intake correlates with higher risks of preterm birth and fetal growth restriction[5–7].

Pregnancy imposes unique demands on Mg balance: intestinal transient receptor potential cation channel subfamily M member 6-mediated (TRMP6) absorption increases to meet maternal and fetal demands; renal conservation rises to offset a 40–50% surge in glomerular filtration rate; and placental transfer mechanisms maintain fetal serum Mg at approximately 80% of maternal levels by term[8]. Disruption of any of these pathways—through inadequate intake, malabsorption, excessive urinary loss, or altered placental transport—can precipitate hypomagnesemia, leading to endothelial dysfunction, vasoconstriction, and inflammatory activation.

Whilst randomised trials of Mg supplementation have produced mixed outcomes, hampered by heterogeneity in baseline Mg status, supplement form and dose, timing of initiation, and the influence of co-morbidities such as HIV and obesity, the potential for advocating for supplementation with Mg as a simple, low-cost intervention to improve maternal and fetal outcomes aligns directly with SDG 3's mandate.

This scoping review:

1. Describes the mechanisms of Mg absorption, distribution, renal handling, and placental transfer during pregnancy.
2. Synthesises epidemiological and mechanistic evidence linking Mg insufficiency to HDP, fetal growth restriction, and preterm birth.
3. Critically appraises randomised trials of Mg supplementation, noting methodological strengths, limitations, and relevance to LMICs.
4. Examines how HIV infection, antiretroviral therapy, obesity, and socioeconomic factors intersect with Mg metabolism and HDP risk.
5. Identifies key gaps and outlines research priorities—particularly in resource-constrained settings—to inform strategies that advance SDG 3 by reducing HDP-related morbidity and mortality.

2. Search Strategy

A comprehensive, non-systematic search of PubMed, Scopus, Web of Science, the Cochrane Library, and Google Scholar (January 2000–December 2024) combined terms for Mg (e.g., "magnesium homeostasis", "magnesium supplementation"), pregnancy outcomes ("preeclampsia", "gestational hypertension", "fetal growth restriction", "preterm birth"), and modifiers ("HIV", "obesity", "socioeconomic status"). Titles and abstracts were screened for relevance; full texts of eligible studies were reviewed, and reference lists hand-searched. Only peer-reviewed, English-language human studies were included. Animal research was considered selectively to enhance mechanistic insight into the role and physiological action of Mg.

2.1. Inclusion and Exclusion Criteria

We included peer-reviewed, English-language human studies published between January 2000 and December 2024 that addressed any of the following topics:

- Magnesium homeostasis (absorption, distribution, renal handling, placental transfer) in pregnancy
- Associations between maternal Mg status and HDP (PE, gestational hypertension), preterm birth, or fetal growth restriction
- Randomised controlled trials of Mg supplementation during pregnancy

- The interplay of Mg status with HIV infection/antiretroviral therapy, obesity, or socioeconomic factors in LMIC contexts

We excluded:

- Non-human (animal or in vitro) studies, except where they provided details of Mg homeostasis explicitly referenced in the text
- Case reports, editorials, commentaries, and conference abstracts without full data
- Studies lacking quantitative measures of Mg status (e.g., purely qualitative dietary surveys)
- Articles not available in full text or not published in English

3. Magnesium Homeostasis in Pregnancy

Throughout gestation, maternal physiology adapts to safeguard Mg balance for both mother and fetus.

3.1. Gastrointestinal Absorption and Distribution

Early in pregnancy, the intestine increases its capacity to absorb dietary Mg. Animal and human studies demonstrate that expression of the TRPM6 Mg channel in enterocytes rises by roughly 10–20%, boosting fractional absorption to meet expanding maternal blood volume and fetal skeletal demands[5]. Once absorbed, 99% of total body Mg resides intracellularly—largely in bone, muscle, and soft tissues—at concentrations of 5–20 mmol/L, while only about 1% remains in the extracellular compartment, of which serum accounts for ~0.3% (reference range 0.75-0.95mmol/L)[8].

3.2. Renal Handling

Under non-pregnant conditions the kidney filters approximately 2 400 mg of Mg daily and reabsorbs 90–95% along the nephron: 20–25% in the proximal tubule, 60–70% in the thick ascending limb via paracellular claudin-16/19 channels, and 5–10% in the distal convoluted tubule via TRPM6/7-mediated transport[9]. Pregnancy raises the glomerular filtration rate by 40–50%, which would otherwise drive excessive Mg losses. However, enhanced TRPM6 expression in the distal nephron during gestation compensates by augmenting Mg reabsorption, thereby preserving circulating levels[8,9].

3.3. Placental Transfer and Fetal Equilibrium

The placenta acts as a gatekeeper of maternal–fetal Mg exchange. Passive diffusion across the syncytiotrophoblast predominates, but active TRPM6-mediated uptake at the maternal-fetal interface fine-tunes transfer so that, by term, fetal serum Mg approximates 80% of the maternal concentration. Upregulation of placental TRPM6 expression during gestation ensures the fetus secures an adequate Mg supply even when maternal levels transiently decline[10].

4. Epidemiological Evidence: Magnesium Insufficiency and Adverse Pregnancy Outcomes

A number of observational studies in LMIC and high-income settings have consistently linked low maternal Mg status with HDP and other adverse outcomes. In the Atherosclerosis Risk in Communities (ARIC) cohort, women whose serum Mg fell below 0.75 mmol/L in early pregnancy faced a 45% higher risk of gestational hypertension compared with those above this threshold (relative risk [RR] 1.45; 95% confidence interval [CI] 1.10-1.92)[11]. A South African case–control study then showed that pre-eclamptic women were more than twice as likely to have depleted erythrocyte Mg compared with normotensive controls (odds ratio [OR] 2.10; 95%CI 1.20-3.70)[6]. In Iran, a prospective cohort of 200 women found that those who went on to develop PE experienced a 12% greater decline in serum Mg across pregnancy than matched controls ($p<0.01$)[7].

A meta-analysis of eight cohort studies—spanning diverse populations—further estimated that each additional 100 mg/day of dietary Mg reduced the odds of preterm birth by 15% (OR 0.85; 95 % CI 0.76–0.95), and of PE by 30% (OR 0.70; 95% CI 0.60-0.82)[5].

Magnesium deficiency impairs endothelial nitric oxide synthesis, fosters oxidative stress through unchecked reactive oxygen species, and amplifies pro-inflammatory cytokine release, each of which underpins the vascular dysfunction seen in HDP and contributes to fetal growth restriction[12–14].

A growing body of observational research from LMICs has documented consistent associations between low maternal Mg status and adverse gestational outcomes. Table 1 summarises four pivotal studies illustrating these associations.

Table 1. Key epidemiological studies of maternal Mg insufficiency and adverse pregnancy outcomes

Study (Year)	*Design & Setting*	*Mg Measure & Cutoff*	*Outcome*	*Effect Size (95% CI)*
Peacock et al. (1999)[11]	Prospective U.S. cohort (n=5 000)	Serum Mg < 0.75 mmol/L	Incident gestational hypertension	RR 1.45 (1.10–1.92)
Kisters et al. (2000)[6]	Case–control, South Africa (n=120)	Erythrocyte Mg depletion	PE vs. normotensive	OR 2.10 (1.20–3.70)
Tavana & Hosseinmirzaei (2013)[13]	Prospective Iran (n=200)	Δ serum Mg: 12% greater decline	Severity/risk of PE	$p < 0.01$
Dalton et al. (2016)[5]	8 cohort studies (n≈20 000)	Dietary Mg (per 100 mg/day)	Preterm birth; PE	OR 0.85 (0.76–0.95); OR 0.70 (0.60–0.82)

As can be seen, women with serum Mg below 0.75 mmol/L faced a 45% higher risk of gestational hypertension in the ARIC cohort, whilst South African women with depleted erythrocyte Mg had more than double the odds of PE[6,11]. The Iranian cohort further demonstrated that a modest 12% decline in serum Mg was significantly associated with PE development[7]. Most compellingly, a pooled analysis across eight studies found that every 100 mg/day increase in dietary Mg was linked to a 15% reduction in preterm birth and a 30% reduction in PE risk[5].

Biologically, Mg insufficiency impairs endothelial nitric oxide synthesis, promotes oxidative stress by allowing reactive oxygen species to accumulate, and heightens pro-inflammatory cytokine production—each central to the pathogenesis of HDP and fetal growth restriction[5,14,15]. Taken together, these data suggest that even modest deficits in maternal Mg stores may meaningfully elevate risks of gestational hypertension, PE, preterm delivery, and impaired fetal growth, especially in LMIC settings.

5. Randomised Controlled Trials of Magnesium Supplementation

Randomised controlled trials (RCTs) investigating the effects of Mg supplementation during pregnancy have aimed to determine whether Mg can reduce the risk of HDP and improve perinatal outcomes. While observational studies consistently show associations between low maternal Mg status and HDP, the evidence from RCTs remains inconclusive.

A Cochrane systematic review conducted by Makrides and colleagues (2014) evaluated seven RCTs comprising a total of 2,689 participants. These trials assessed the effects of oral Mg supplementation, typically administered in doses ranging from 200 to 400 mg/day, and initiated before 25 weeks of gestation. The findings suggested that Mg supplementation may reduce the risk of preterm birth, low birth weight, and small for gestational age (SGA) infants. Specifically, the review reported a relative risk reduction for preterm birth (RR 0.73; 95% CI 0.57–0.94), low birth weight (RR 0.67; 95% CI 0.46–0.96), and SGA status (RR 0.70; 95% CI 0.53–0.93). However, when a large cluster-randomised trial was excluded in sensitivity analyses, these associations lost statistical significance. This suggests that the findings may have been heavily influenced by individual trial design and that routine Mg supplementation during pregnancy remains unsupported by high-quality evidence at present[16].

An earlier RCT by Spätling and Spätling (1988) conducted in Germany involved 997 pregnant women who were randomised to receive 15 mmol of Mg aspartate daily or placebo. This study reported fewer cases of premature birth, low birth weight, and PE among women in the magnesium-supplemented group. Although promising, the study's methodological quality, including details of blinding and allocation concealment, was not well-documented. Moreover, its findings have not been consistently replicated in later trials, limiting its influence on clinical practice[17].

A number of limitations are evident in the existing body of RCTs. Firstly, the formulation and dosage of Mg varied widely across studies. Some trials used Mg oxide, which has relatively low bioavailability, while others used Mg citrate or aspartate. The lack of standardisation in Mg formulation makes it difficult to compare outcomes across studies. Secondly, the timing of supplementation initiation was inconsistent. Many trials began supplementation in the second trimester, potentially missing the early gestational window critical for placental development and vascular remodelling. Another key issue is that most trials did not assess participants' baseline Mg status before randomisation. Without stratifying or selecting women with a documented deficiency, the potential benefit of supplementation in truly deficient populations

may have been obscured. Furthermore, many of the trials were conducted in high-income countries and included relatively low-risk populations. This limits the generalisability of findings to LMICs, where comorbidities such as HIV infection, obesity, and micronutrient malnutrition are more prevalent and may interact with Mg metabolism. Adherence monitoring and reporting of adverse events were also inconsistently performed across trials. Few studies implemented pill counts or repeated serum Mg testing to assess compliance, and reporting on tolerability or side effects—particularly gastrointestinal disturbances—was limited.

In light of these limitations, future research on Mg supplementation in pregnancy should be designed with greater methodological rigour. Trials should enrol women with confirmed Mg deficiency, either biochemically or through validated dietary assessment. A single, bioavailable Mg formulation, such as Mg citrate or chloride, should be used at a consistent daily dose, ideally initiated in the first trimester or even preconceptionally. Moreover, these trials should be conducted in LMIC settings and include populations with high prevalence of HIV, obesity, and poor dietary quality. In addition to clinical outcomes such as HDP and preterm birth, mechanistic biomarkers—such as markers of endothelial function, oxidative stress, and systemic inflammation—should be incorporated to clarify potential causal pathways.

Table 2 provides a summary of the most influential RCTs and systematic reviews examining Mg supplementation in pregnancy and their reported outcomes.

Table 2. Summary of key randomised controlled trials of magnesium supplementation during pregnancy

Study (Year, Country)	*Sample Size (n)*	*Mg Formulation & Dose*	*Gestational Age at Start*	*Primary Outcomes*	*Main Findings*	*Limitations*
Makrides et al. (2014, Cochrane Review)[16]	2,689	Various forms, 200–400 mg/day	Before 25 weeks	Preterm birth, low birth weight, small for gestational age	Reduction in adverse outcomes; significance lost when excluding cluster trial	Heterogeneity in study designs; limited high-quality trials
Spätling & Spätling (1988, Germany)[17]	997	Mg aspartate, 15 mmol/day	Not specified	Preterm birth, low birth weight, preeclampsia	Decreased incidence of adverse outcomes in Mg group	Methodological limitations; lack of replication

6. Confounding Factors: HIV, Obesity and Socioeconomic Status

6.1. HIV Infection and Antiretroviral Therapy (ART)

In many LMICs, HIV remains highly prevalent among pregnant women, and both the infection itself as well as the treatment can influence Mg metabolism. In a landmark 2006 study, Suy *et al.* observed a roughly 50% higher incidence of PE and fetal death in HIV-infected women receiving highly active antiretroviral therapy (HAART) compared with HIV-negative controls[18]. Similarly, Machado *et al.* (2014) reported elevated rates of gestational hypertension and eclampsia among ART-treated women in Latin America and the Caribbean[19]. By contrast, Landi and colleagues (2014) found that in certain African settings, HIV-positive women on ART showed lower PE rates than uninfected peers - perhaps reflecting complex immune reconstitution effects[20]. Moreover, Kassu *et al.* (2008) documented significantly lower mean serum Mg in HIV-positive pregnant women (0.60 mmol/L) than in HIV-negative controls (0.72 mmol/L; $p < 0.01$), underscoring the need to assess Mg status alongside routine HIV care[21].

6.2. Obesity

The increasing prevalence of obesity in LMICs add another layer of complexity. Excess adiposity promotes a low-grade inflammatory state and oxidative stress, both of which can deplete Mg through increased urinary excretion and impaired cellular uptake. Olson *et al.* (2019) demonstrated that obese pregnant women (BMI ≥ 30 kg/m²) had lower intracellular Mg and higher inflammatory markers than their normal-weight counterparts ($p < 0.05$), suggesting synergistic risks for endothelial dysfunction and HDP[22]. Piuri and co-workers (2021) further showed that Mg deficiency correlates with metabolic syndrome features—including hypertension and insulin resistance—indicating that obesity and Mg insufficiency may jointly elevate HDP risk[23].

6.3. Socioeconomic Status

Socioeconomic deprivation often translates into poor dietary diversity and limited access to micronutrient-rich foods or supplements. A recent review by Daran *et al.* (2023) found that women in the lowest socioeconomic quintiles of sub-Saharan Africa consumed significantly less dietary Mg and experienced higher rates of PE and preterm birth than those in wealthier groups[24]. Paradoxically, urbanisation can improve overall food security while increasing consumption of processed, nutrient-poor staples, further undermining Mg intake and exacerbating HDP risk[25].

7. Limitations and Future Directions

Pregnancy imposes a unique set of demands on Mg homeostasis—uprating intestinal absorption, conserving renal reabsorption, and fine-tuning placental transfer—to support both maternal cardiovascular health and fetal development. Observational data from LMICs demonstrate that even modest Mg shortfalls raise the risks of gestational hypertension, PE, preterm birth, and fetal growth restriction by impairing endothelial function, promoting oxidative stress, and amplifying inflammatory pathways.

To date, randomised trials of Mg supplementation have not delivered definitive prevention strategies, largely because they have:

1. Enrolled heterogeneous populations rather than targeting women with confirmed Mg insufficiency;
2. Varied widely in Mg salt formulation (oxide vs. citrate), dose (200–400 mg/day), and timing (mid- vs. late gestation);
3. Lacked rigorous adherence and safety monitoring; and

4. Rarely represented the comorbidity profiles (HIV, obesity, socioeconomic deprivation) that characterise high-burden LMIC settings.

Moving forward, research and practice should focus on:

1. Early, targeted screening: incorporate serum—and, where feasible, intracellular—Mg measurements in the first trimester, using locally validated reference ranges to identify at-risk women.
2. Deficiency-enriched trials: design supplementation studies that enrol only women with confirmed Mg insufficiency or low dietary intake, enabling a clear demonstration of benefit.
3. Standardised supplement regimens: use a single, highly bioavailable Mg salt (e.g., Mg citrate) at a consistent daily dose (300–400 mg elemental Mg) and initiate preconception or in the first trimester to support early placentation.
4. Comorbidity stratification: analyse outcomes by HIV status (and ART regimen), body-mass index, and socioeconomic indicators to tailor interventions to local needs.
5. Mechanistic and clinical integration: pair clinical endpoints with biomarker assessments (e.g., endothelial function tests, oxidative-stress markers, inflammatory cytokines) to elucidate Mg's protective mechanisms.
6. Health-system embedding: pilot antenatal food-fortification or targeted supplement delivery programs that leverage existing LMIC health infrastructures for scalable impact.

By sharpening the focus on Mg-deficient populations, harmonising intervention protocols, and embedding research within the realities of LMIC antenatal care, we can move toward low-cost, scalable strategies that align with SDG 3's aim of reducing maternal mortality and improving maternal–fetal health worldwide.

8. Acknowledgements

This article is partially based on the author's thesis, 'The interplay between magnesium and hypertensive disorders of pregnancy in a pregnant Black South African population', towards the degree of Master of Medical Science (Physiology), and was written in lieu of a systematic review and meta-analysis as the author's protocol is under review.

8.1. Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

8.2. Author's Contributions

NA was responsible for collecting data and drafting this review. Both VD and CB supervised the research process and provided guidance.

8.3. Funding information

This study was funded by the National Research Foundation Thuthuka Grant (TTK170508230162) in collaboration with a public university on the East Coast and the Medical Research Council of South Africa (SIR Grant UNS14197). The institutions listed have no vested interest in the study. Further, there was no input from these organisations in the interpretation of the result.

8.4. Data availability

Data will be available from the corresponding author, NA, upon reasonable request.

8.5. Disclaimer

The views and opinions expressed in this article are those of the authors and are the product of professional research. The article does not necessarily reflect the official policy or position of any affiliated institution, funder, agency, or that of the publisher. The authors are responsible for this article's results, findings, and content.

9. References

1. United Nations. Transforming our world: The 2030 agenda for sustainable development [Internet]. United Nations. 2015. Available from: https://sustainabledevelopment.un.org/post2015/transformingourworld/publication

2. Murray CJL, Lopez AD. Measuring the global burden of disease. New England Journal of Medicine [Internet]. 2013 Aug 369(5):448–57. Available from: https://www.nejm.org/doi/full/10.1056/NEJMra1201534

3. Dimitriadis E, Rolnik DL, Zhou W, Estrada-Gutierrez G, Koga K, Francisco RPV, et al. Pre-eclampsia. Nature Reviews Disease Primers [Internet]. 2023 Feb 16;9(1):1–22. Available from: https://www.nature.com/articles/s41572-023-00417-6

4. Schwalfenberg GK, Genuis SJ. The importance of magnesium in clinical healthcare. Scientifica. 2017 Sep 28;2017(1):1–14.

5. Dalton LM, Ní Fhloinn DM, Gaydadzhieva GT, Mazurkiewicz OM, Leeson H, Wright CP. Magnesium in pregnancy. Nutrition Reviews. 2016 Jul 21;74(9):549–57.

6. Kisters K, Barenbrock M, Louwen F, Hausberg M, Rahn KH, Kosch M. Membrane, intracellular, and plasma magnesium and calcium concentrations in preeclampsia. American Journal of Hypertension. 2000 Jul 1;13(7):765–9.

7. Tavana Z, Hosseinmirzaei S. Comparison of maternal serum magnesium level in pre-eclampsia and normal pregnant women. Iranian Red Crescent Medical Journal. 2013 Dec 5;15(12).

8. Jahnen-Dechent W, Ketteler M. Magnesium basics. Clinical Kidney Journal [Internet]. 2012 Feb 1;5(Supplement 1):i3–14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4455825/

9. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clinical Journal of the American Society of Nephrology. 2014 Oct 6;10(7):1257–72.

10. Shukla V, Parvez S, Fatima G, Singh S, Magomedova A, Batiha GES, et al. Micronutrient interactions: Magnesium and its synergies in maternal–fetal health. Food Science & Nutrition. 2024 Jul 17;12(10):6913–28.

11. Peacock JM, Folsom AR, Arnett DK, Eckfeldt JH, Szklo M. Relationship of serum and dietary magnesium to incident hypertension: The atherosclerosis risk in communities (ARIC) study. Annals of Epidemiology [Internet]. 1999 Apr 1;9(3):159–65. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1047279798000404

12. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. Basic Research in Cardiology [Internet]. 2008 Jul 3;103(5):398–406. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2705866/

13. Preiser JC. Oxidative stress. Journal of Parenteral and Enteral Nutrition. 2012 Feb;36(2):147–54.

14. Konukoglu D, Uzun H. Endothelial dysfunction and hypertension. Advances in Experimental Medicine and Biology [Internet]. 2016;956:511–40. Available from: https://link.springer.com/chapter/10.1007%2F5584_2016_90

15. Prabhu K, Dastidar RG, Aroor AR, Rao M, Shetty S, Poojari VG, et al. Micronutrients in adverse pregnancy outcomes. F1000Research [Internet]. 2024 Jun 21;11:1369–9. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11131564/

16. Makrides M, Crosby DD, Shepherd E, Crowther CA. Magnesium supplementation in pregnancy. Cochrane Database of Systematic Reviews. 2014 Apr 2;CD000937.(4).

17. Ludwig Spätling, Spätling G. Magnesium supplementation in pregnancy. A double-blind study. British Journal of Obstetrics and Gynaecology. 1988 Feb 1;95(2):120–5.

18. Suy A, Martínez E, Coll O, Lonca M, Palacio M, de Lazzari E, et al. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. AIDS. 2006 Jan 2;20(1):59–66.

19. Machado ES, Krauss MR, Megazzini K, Coutinho CM, Kreitchmann R, Melo VH, et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from

Latin America and Caribbean countries. The Journal of Infection [Internet]. 2014 Jun 1;68(6):572–80. Available from: https://pubmed.ncbi.nlm.nih.gov/24462561/

20. Landi B, Bezzeccheri V, Guerra B, Piemontese M, Cervi F, Cecchi L, et al. HIV infection in pregnancy and the risk of gestational hypertension and preeclampsia. World Journal of Cardiovascular Diseases [Internet]. 2014 May 15;4(5):257–67. Available from: https://file.scirp.org/pdf/WJCD_2014052010003935.pdf

21. Kassu A, Yabutani T, Mulu A, Tessema B, Ota F. Serum zinc, copper, selenium, calcium, and magnesium levels in pregnant and non-pregnant women in Gondar, Northwest Ethiopia. Biological Trace Element Research. 2008 Jan 17;122(2):97–106.

22. Olson KN, Redman LM, Sones JL. Obesity "complements" preeclampsia. Physiological Genomics. 2019 Mar 1;51(3):73–6.

23. Piuri G, Zocchi M, Della Porta M, Ficara V, Manoni M, Zuccotti GV, et al. Magnesium in obesity, metabolic syndrome, and type 2 diabetes. Nutrients [Internet]. 2021 Jan 22;13(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7912442/

24. Daran B, Levasseur P, Clément M. Updating the association between socioeconomic status and obesity in low-income and lower-middle-income sub-Saharan African countries: A literature review. Obesity Reviews. 2023 Jul 6;24(10).

25. Joy EJM, Young SD, Black CR, Ander EL, Watts MJ, Broadley MR. Risk of dietary magnesium deficiency is low in most African countries based on food supply data. Plant and Soil. 2012 Aug 16;368(1-2):129–37.

Appendix 10: Similarity Index

Similarity Index

ORIGINALITY REPORT

12%	10%	7%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	assets-eu.researchsquare.com Internet Source	1%
2	www.researchgate.net Internet Source	1%
3	www.science.gov Internet Source	1%
4	www.researchsquare.com Internet Source	1%
5	ijmedph.org Internet Source	<1%
6	Submitted to University of KwaZulu-Natal Student Paper	<1%
7	www.coursehero.com Internet Source	<1%
8	Hairong Xu. "Role of nutrition in the risk of preeclampsia", Nutrition Reviews, 11/2009 Publication	<1%
9	"Magnesium in Human Health and Disease", Springer Science and Business Media LLC, 2013 Publication	<1%
10	Submitted to University of York Student Paper	<1%
11	epiresearch.org Internet Source	<1%

12	theses.gla.ac.uk Internet Source	<1%
13	www.frontiersin.org Internet Source	<1%
14	Naeera Abdul, Vinogrin Dorsamy, Chauntelle Bagwandeen. "Impact of magnesium levels in pregnancy on adverse birth outcomes: a protocol for a systematic review and meta-analysis", Springer Science and Business Media LLC, 2024 Publication	<1%
15	David Mulenga. "Understanding Public Health in Africa - Issues and Cases", Routledge, 2025 Publication	<1%
16	Submitted to Maastricht University Student Paper	<1%
17	alarm.cfpc.ca Internet Source	<1%
18	nursing.lsuhsc.edu Internet Source	<1%
19	Long Ngo Hoang, Haesung Lee, Sook Jeong Lee. "Improving cognitive impairment through chronic consumption of natural compounds/extracts: a systematic review and meta-analysis of randomized controlled trials", Frontiers in Aging Neuroscience, 2025 Publication	<1%
20	www.medrxiv.org Internet Source	<1%
21	medwinpublishers.com Internet Source	<1%
22	mhealth.jmir.org Internet Source	

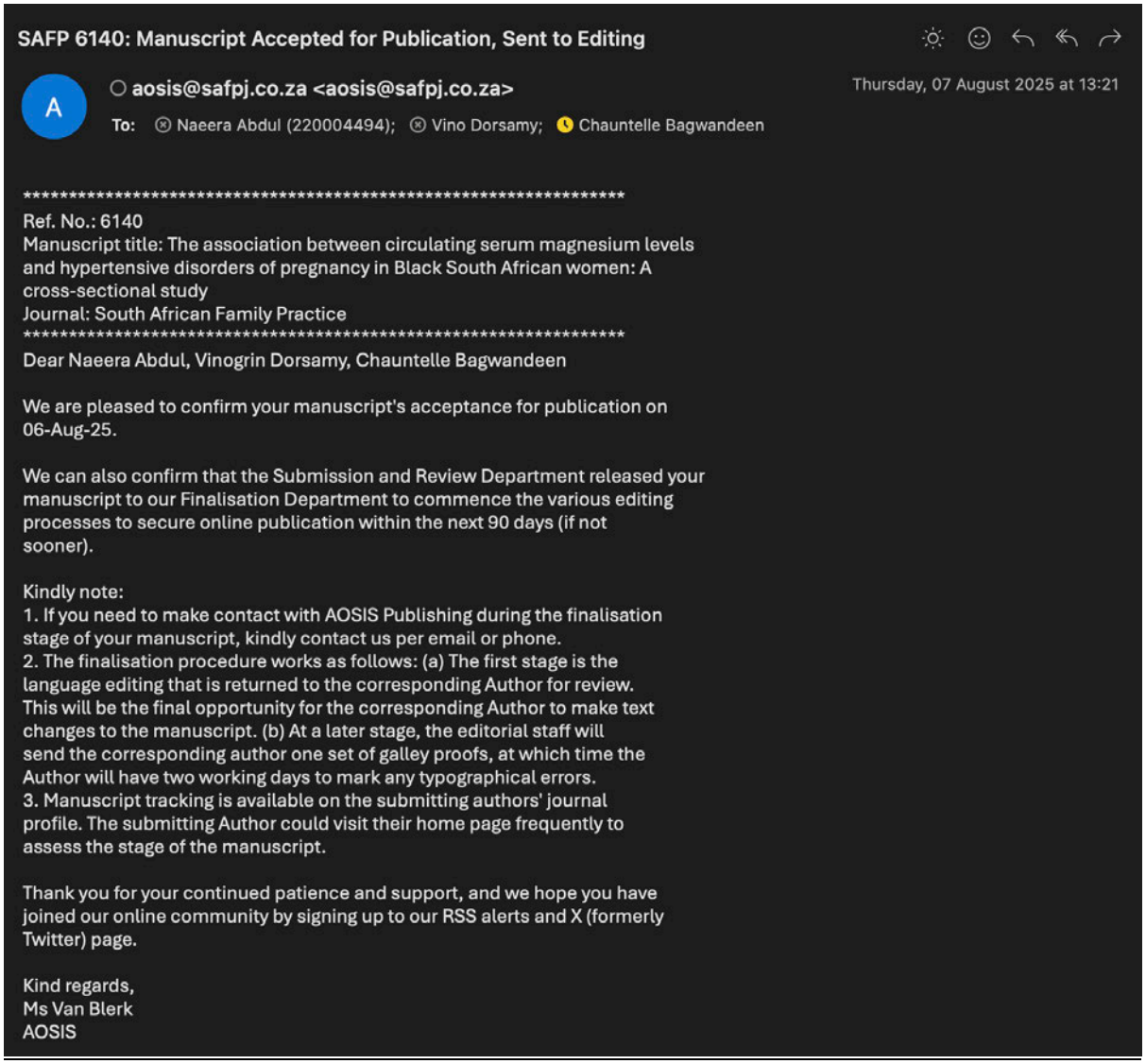
		<1%
23	ntnuopen.ntnu.no Internet Source	<1%
24	Subodh Nanavati, Pallavi Nanavati, Maulik Nanavati. "Oral Submucous Fibrosis - An Introduction", Jenny Stanford Publishing, 2025 Publication	<1%
25	Submitted to University of Sydney Student Paper	<1%
26	eprints.bournemouth.ac.uk Internet Source	<1%
27	sciety.org Internet Source	<1%
28	www.ncbi.nlm.nih.gov Internet Source	<1%
29	inplasy.com Internet Source	<1%
30	mobt3ath.com Internet Source	<1%
31	Submitted to Trinity College Dublin Student Paper	<1%
32	bmccomplementalternmed.biomedcentral.com Internet Source	<1%
33	f1000research.com Internet Source	<1%
34	repub.eur.nl Internet Source	<1%
35	www.jmir.org Internet Source	<1%
	www.nepalindata.com	

36	Internet Source	<1%
37	Olive P. Khaliq, Wendy N. Phoswa, Jagidesa Moodley. "The effectiveness of the Congo red dot paper test in hypertensive disorders of pregnancy in women of African ancestry", Medicine: Case Reports and Study Protocols, 2022 Publication	<1%
38	ijcht24.tbd.edu.vn Internet Source	<1%
39	worldwidescience.org Internet Source	<1%
40	Makiko Okazaki, Natsuko Sekiguchi, Yuki Sahashi, Hisashi Noma, Takahiro Mihara. "Effects of individual components of remote monitoring for implantable cardioverter defibrillators or cardiac resynchronisation therapy defibrillators: protocol for a systematic review and component network meta-analysis", Cold Spring Harbor Laboratory, 2025 Publication	<1%
41	bmjopen.bmj.com Internet Source	<1%
42	pmc.ncbi.nlm.nih.gov Internet Source	<1%
43	qpsi.azurewebsites.net Internet Source	<1%
44	www.crsociety.org Internet Source	<1%
45	Submitted to St George's Hospital Medical School	<1%

	Student Paper	
46	Submitted to University Of Tasmania Student Paper	<1 %
47	Submitted to University of Ulster Student Paper	<1 %
48	eprints.nottingham.ac.uk Internet Source	<1 %
49	link.springer.com Internet Source	<1 %
50	Daniel Onobun, Gerald Hean, Ajibola Adebisi, Ethel Ojo et al. "Evaluating Surgical Strategies for Adolescent Idiopathic Scoliosis: A Meta-Analysis of Posterior Spinal Fusion and Vertebral Body Tethering", Springer Science and Business Media LLC, 2025 Publication	<1 %
51	journals.plos.org Internet Source	<1 %
52	core.ac.uk Internet Source	<1 %
53	reproductive-health-journal.biomedcentral.com Internet Source	<1 %
54	www.health.gov.bc.ca Internet Source	<1 %
55	www.hivpregnancyhotline.org Internet Source	<1 %
56	"Posters", Diabetic Medicine, 3/2008 Publication	<1 %
57	dspace.unza.zm Internet Source	<1 %

58	scholar.valpo.edu Internet Source	<1 %
59	www.jscimedcentral.com Internet Source	<1 %
60	www.mdpi.com Internet Source	<1 %
61	www.sciencegate.app Internet Source	<1 %

Exclude quotes	On	Exclude matches	< 12 words
Exclude bibliography	On		



SAFP 6140: Manuscript Accepted for Publication, Sent to Editing

A

aosis@safpj.co.za <aosis@safpj.co.za>

Thursday, 07 August 2025 at 13:21

To: Naeera Abdul (220004494); Vino Dorsamy; Chauntelle Bagwandeen

****
Ref. No.: 6140
Manuscript title: The association between circulating serum magnesium levels and hypertensive disorders of pregnancy in Black South African women: A cross-sectional study
Journal: South African Family Practice
****

Dear Naeera Abdul, Vinogrin Dorsamy, Chauntelle Bagwandeen

We are pleased to confirm your manuscript's acceptance for publication on 06-Aug-25.

We can also confirm that the Submission and Review Department released your manuscript to our Finalisation Department to commence the various editing processes to secure online publication within the next 90 days (if not sooner).

Kindly note:
1. If you need to make contact with AOSIS Publishing during the finalisation stage of your manuscript, kindly contact us per email or phone.
2. The finalisation procedure works as follows: (a) The first stage is the language editing that is returned to the corresponding Author for review. This will be the final opportunity for the corresponding Author to make text changes to the manuscript. (b) At a later stage, the editorial staff will send the corresponding author one set of galley proofs, at which time the Author will have two working days to mark any typographical errors.
3. Manuscript tracking is available on the submitting authors' journal profile. The submitting Author could visit their home page frequently to assess the stage of the manuscript.

Thank you for your continued patience and support, and we hope you have joined our online community by signing up to our RSS alerts and X (formerly Twitter) page.

Kind regards,
Ms Van Blerk
AOSIS