C-REACTIVE PROTEIN AND PENTRAXIN 3 IN WOMEN LIVING WITH HIV WHO ALSO HAVE PRE-ECLAMPSIA

By

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PREFACE

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Optics & Imaging Centre, Doris Duke Medical Research Institute, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor Thajasvarie Naicker and Dr Pamela Pillay.

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DECLARATION

I, **Thalia Moodley** declare that:

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.
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Signed: _____ Date: ----03/08/2023----------

DEDICATIONS

To God

For removing all obstacles and guiding me throughout the year.

To my late grandfather

Thank you for consistently believing in me and encouraging me to strive for excellence. From my earliest years, you envisioned the scientist within me. Your constant support has left an indelible mark on my journey.

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2. Thalia Moodley, Pamela Pillay, Sumeshree Govender and Thajasvaree Naicker (2023). The role of C – reactive protein and Pentraxin 3 in HIV-associated pre-eclampsia. Submitted to *Inflammation Research.* Journal submission ID: dba954e7-0ee8-497c-91e0-671f81825122

➢ TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

LIST OF ABBREVIATIONS

ART	Antiretroviral therapy
ARV	Antiretroviral
BMI	Body mass index
BP	Blood pressure
CAP	Community-acquired pneumonia
CRP	C-reactive protein
CVD	Cardiovascular disease
EOPE	Early-onset pre-eclampsia
HAART	Highly active antiretroviral treatment
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IL	Interleukin
IQR	Inter quartile range
ISTI	Integrase strand transfer inhibitors
IUGR	Intrauterine growth restriction
LOPE	Late onset pre-eclampsia
LPS	Lipopolysaccharides
MFI	Median florescent intensity
NRTI/NNRTI	Non-nucleoside reverse transcriptase inhibitors
PE	Pre-eclampsia
PI	Protease inhibitors
PTX 3	Pentraxin 3
SAP	Serum amyloid p
TGF	Transforming growth factor
TLR	Toll like receptor
TNF	Tumour necrosis factor- α
VEGF	Vascular endothelial growth factor

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

ABSTRACT- ENGLISH

ABSTRACT:

Background:

Patients with pre-eclampsia exhibit elevated levels of pentraxin 3 and c-reactive protein compared to those with normal pregnancies. In the context of pre-eclampsia co-morbid with HIV, inflammation triggers pentraxin 3 and c-reactive protein release, activating C1q and promoting C3 and C4 deposition. This cascade induces cytokine secretion, including tumor necrosis factor-α, interleukin-1, and interleukin-6, potentially contributing to trophoblast apoptosis in pre-eclampsia. This study aimed to compare the expression of inflammatory proteins, pentraxin 3 and c-reactive protein, in the context of HIV-associated normotensive and pre-eclamptic pregnancies.

Methods:

The study population (n = 80) was stratified based on HIV status (HIV-positive and HIV-negative) and pregnancy type (normotensive and pre-eclampsia). Plasma concentrations of pentraxin 3 and c-reactive protein were measured using a ProcartaPlex™ immunoassay technique.

Results:

Pregnancy type: Pentraxin 3 exhibited a significant increase in pre-eclamptic compared to normotensive pregnancies ($p < 0.0001$), regardless of HIV status. In contrast, c-reactive protein was down regulated in pre-eclamptic compared to normotensive pregnancies, irrespective of HIV status, albeit non-significant.

HIV status: The concentrations of pentraxin 3 and c-reactive protein were not significantly different based on HIV status, irrespective of pregnancy type.

Across all groups: Pentraxin 3 concentrations were significantly different between normotensive HIV-negative versus pre-eclamptic HIV-negative groups ($p = 0.0001$) and normotensive HIV-negative versus preeclamptic HIV-positive groups ($p = 0.0049$). There was no significant difference in c-reactive protein concentrations across the study population.

Conclusions:

This study showed increased concentrations of pentraxin 3 in pre-eclampsia, irrespective of HIV status when compared to normotensive pregnancies. This heightened expression results from inflammation and oxidative stress in the hypoxic pre-eclamptic microenvironment. Conversely, c-reactive protein levels were lower in pre-eclampsia compared to normotensive pregnancies, although not statistically significant. This is possibly due to its association with hepatic dysfunction, gestational age, disease severity, interleukin-6 production, and sample size. Additionally, we find no differences in pentraxin 3 and c-reactive protein concentrations by HIV status, most likely a result of anti-retroviral therapy's

immune-repair effects. The complex relationship between these inflammatory markers offers a thorough understanding of the intricate pathophysiology of pre-eclampsia associated with HIV, establishing the foundation for potential therapeutic interventions.

ABSTRACT- ISIZULU

IZIPHEPHA:

Umhlaba:

Abathengi abane-pre-eclampsia bakhombisa izinhlaka ezinamandla zepentraxin 3 kanye no-c-reactive protein kulabo abanolwazi ngamakathini. Eningizimu ye-pre-eclampsia okumelwe kucelwa nangomzimba we-HIV, i-intshukumo iyatshela izinhlaka zepentraxin 3 kanye no-c-reactive protein, ikwazi ukuyenza i-C1q ibuye isetshenzise i-C3 kanye ne-C4. Lolu hlambi luzenzakalisa ukuhlola amanzi, nokunakwa kwe-trophoblast uma kukhona i-pre-eclampsia. Lezifundo zazifuna ukukhuluma ngenqubomgomo yezinhlaka zepentraxin 3 kanye no-c-reactive protein, exhumene izinhlaka ezenzakalayo, ezifanele izigaba zezifo.

Izifundo:

Ihlabathi lizifaka (n = 80) leziqinile ngokwethulwa kwengqubomgomo ye-HIV (yesimanje kanye neyakudlula) kanye nokufana nombhaco (ngokwethulwa kwe-pre-eclampsia). Amakhasimende we-pentraxin 3 kanye no-c-reactive protein eplasini azilulwe nge-ProcartaPlex™ immunoassay technique.

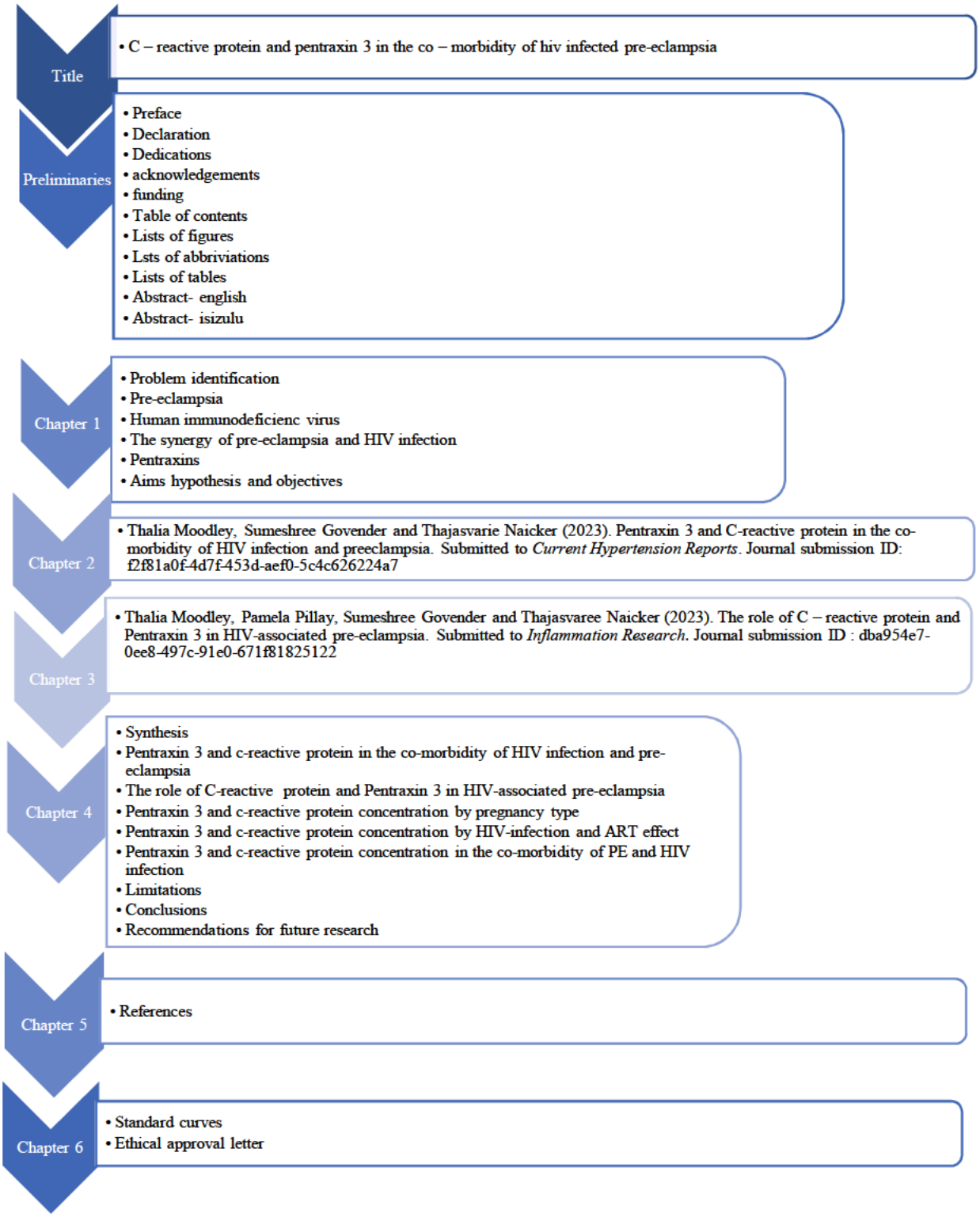
Izilokotho:

Uhlobo lwesifo: I-pentraxin 3 yayilula kakhulu e-pre-eclamptic kulowo othile ($p < 0.0001$), noma yini imqondo we-HIV. Ngaphezu kwalokho, izifinyelelo zezilokotho zesiqinile kakhulu phakathi komndeni we-HIV osimanje no-pre-eclamptic HIV osimanje ($p = 0.0001$) kanye no-pre-eclamptic HIV omubi ($p = 0.0049$). Kodwa e-pre-eclamptic pregnancies, i-c-reactive protein yayilula ngaphambi kwe-pre-eclamptic pregnancies, noma kube yiyo imqondo we-HIV, kodwa le nto ayihlukile. Izinzuzo zepentraxin 3 kanye no-c-reactive protein zazikhomba ukulula phakathi komndeni, kodwa ayizifanelelwe ekwelewele.

Isimo:

Lezifundo lathi luthola izinzuzo ezifikelele ze-pentraxin 3 e-pre-eclampsia, noma yini imqondo we-HIV, uma kufananishwa nokubhukuda kwemizazi. Lolu hlambi lwenza izinhlaka ezinamandla uma kukhona isifo kanye nokuthintana kwesiko ne-oxidative stress ezindaweni zemicroenvironment ye-pre-eclampsia ethinta amanzi. Kodwa, izinzuzo zesilokotho se-c-reactive protein zazizinyulu e-pre-eclampsia ikhona, kodwa akudingeki kwezenzakalayo. Kukhona kusikhathalele ukuthi lokhu kube nesithombe kuyo kwezintsha ezimpilweni, iminyaka yohlobo lwemfuyo, ukuphucula kwesifo, ukwenza i-interleukin-6, kanye nesayizi lezithombe. Nokho, asingakwazi ukubona inkinga ezifana nezinzuzo zepentraxin 3 kanye no-c-reactive protein ngesimo we-HIV, kungaba njengezinkinga yokufiphaza komkhuhlane-retroviral therapy. Uhambo oluphambene lwezinzuzo ezifuna impahla eyengeziwe kwe-HIV kanye no-pre-eclampsia lukhomba amathemu okukhulu, luhamba phambili ngezinkinga ezithile.

SCHEMATIC LAYOUT OF THESIS


Title
• C – reactive protein and pentraxin 3 in the co – morbidity of hiv infected pre-eclampsia
Preliminaries
• Preface
• Declaration
• Dedications
• acknowledgements
• funding
• Table of contents
• Lists of figures
• Lsts of abbriviations
• Lists of tables
• Abstract- english
• Abstract- isizulu
Chapter 1
• Problem identification
• Pre-eclampsia
• Human immunodeficienc virus
• The synergy of pre-eclampsia and HIV infection
• Pentraxins
• Aims hypothesis and objectives
Chapter 2
• Thalia Moodley, Sumeshree Govender and Thajasvarie Naicker (2023). Pentraxin 3 and C-reactive protein in the co-morbidity of HIV infection and preeclampsia. Submitted to *Current Hypertension Reports*. Journal submission ID: f2f81a0f-4d7f-453d-aef0-5c4c626224a7
Chapter 3
• Thalia Moodley, Pamela Pillay, Sumeshree Govender and Thajasvaree Naicker (2023). The role of C – reactive protein and Pentraxin 3 in HIV-associated pre-eclampsia. Submitted to *Inflammation Research*. Journal submission ID : dba954e7-0ee8-497c-91e0-671f81825122
Chapter 4
• Synthesis
• Pentraxin 3 and c-reactive protein in the co-morbidity of HIV infection and pre-eclampsia
• The role of C-reactive protein and Pentraxin 3 in HIV-associated pre-eclampsia
• Pentraxin 3 and c-reactive protein concentration by pregnancy type
• Pentraxin 3 and c-reactive protein concentration by HIV-infection and ART effect
• Pentraxin 3 and c-reactive protein concentration in the co-morbidity of PE and HIV infection
• Limitations
• Conclusions
• Recommendations for future research
Chapter 5
• References
Chapter 6
• Standard curves
• Ethical approval letter

CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

1.0 PROBLEM IDENTIFICATION

The top four causes of mortality in South Africa are non-pregnancy-related infections; obstetric haemorrhage; hypertensive disorders of pregnancy and medical and surgical disorders (Department of Health South Africa, 2022). Pre-eclampsia (PE) accounts for 83% of maternal deaths from hypertensive disorders of pregnancy (Department of Health South Africa, 2021a).

Pre-eclampsia is defined as hypertension (a blood pressure of 140mmHg systolic and/or 90mmHg diastolic) after 20 weeks of gestation as well as either proteinuria; uteroplacental dysfunction; or maternal end-organ dysfunction (Magee *et al.*, 2022). To-date there is no known cure for PE, however, delivery of the placenta is the only ameliorative therapy (Magee *et al.*, 2022).

In South Africa, 8.45 million people live with Human immunodeficiency virus (HIV) representing 13.9% of the overall population. Moreover, 24% of women aged 15-49 years are HIV positive (Department of Statistics South Africa, 2022; UNAIDS, 2022). The province of KwaZulu-Natal in South Africa is considered the epicentre of the global HIV pandemic (Thurlow *et al.*, 2009; Johnson and Dorrington, 2023). Notably due to antiretroviral therapy (ART), there has been an increase in life expectancy concomitant with a decrease in indirect maternal mortality (Department of Health South Africa, 2021b). Since both conditions enhance immune response a study of inflammatory markers in this co-morbidity needs investigation.

Also, considering the high prevalence of both conditions in SA, it is vital that extensive research be conducted to facilitate a conceptual framework of inflammatory mediators in the duality of PE and HIV infection. Hence, this study will focus on the comparative evaluation of two inflammatory mediators, pentraxin 3 and c-reactive protein, by pregnancy type (PE versus normotensive pregnancy) as well as HIV status (HIV-positive versus HIV-negative) whilst taking into account ARTs.

1.1 Pre-eclampsia

Pre-eclampsia was evident in women as early as the 20th century (Gyselaers, 2020; Robillard *et al.*, 2018). A review article written by Robillard *et al* 2018 stated that there are seven major findings regarding the understanding of the pathophysiology of preeclampsia in the 21st century. The first is the inflatable armband to measure blood pressure (Robillard *et al.*, 2018). New onset hypertension was the key clinical symptom for the diagnosis of PE development (Roberts and Gammill, 2005). In 1967 Robertson et al (1967) observed shallow trophoblast invasion in PE. This response was a compensatory reaction to the elevated blood pressure and resultant non-physiological conversion of spiral arteries that caused inadequate nutrient supply to the developing foetus (Robillard *et al.*, 2018; Robertson *et al.*, 1967). By the end of the 1980s, it was found that symptoms experienced by pregnant women with PE

are caused by endothelial cell injury, specifically the glomerulus (in the kidneys), hepatic Kupfer cells (in the liver), and cerebral choroid plexus cells (in the brain). Thereafter, Redman et al (1999) observed widespread endothelial cell dysfunction with a hyperinflammatory response in the mother.

1.1.1 Definition of pre-eclampsia

Pre-eclampsia is defined by the new onset of gestational hypertension (≥ 140/90 mmHg), as well as significant proteinuria (0.3g/24 hours); uteroplacental dysfunction; and maternal end-organ dysfunction after 20 weeks of gestation (Magee *et al.*, 2022).

1.1.2 Epidemiology of Pre-eclampsia

Globally, PE shows diverse epidemiological patterns. It affects roughly 2-8% of pregnancies worldwide. Pre-eclampsia is estimated to occur in 13% of pregnancies in Africa, where its prevalence is typically higher. With reported rates ranging from 8-12% of pregnancies, hypertensive disorders of pregnancy are the most common direct cause of maternal mortality, accounting for 18% of all maternal deaths in South Africa (World Health Organization, 2011; Moodley *et al.*, 2019; Jikamo *et al.*, 2023). The significance of these geographic variations in understanding and reducing the effects of pre-eclampsia on maternal health is highlighted by these statistics.

1.1.2.1 Classifications of pre-eclampsia

Pre-eclampsia may be classified by gestational age and severity. Based on gestational age PE may be divided into early-onset; occurring before 34 weeks of gestation and late-onset; occurring at or after 34 weeks of gestation (Wadhwani *et al.*, 2020). Notably, early-onset PE is associated with impaired placental development whilst late-onset PE is associated with maternal endothelial dysfunction (Aneman *et al.*, 2020). Both early-onset and late-onset PE result in an increased inflammatory response hence adverse maternal and fetal complications (Aneman *et al.*, 2020).

1.1.3 Pathophysiology of pre-eclampsia

During normal pregnancy, spiral arteries undergo a physiological transformation whereby their luminal diameter increases 4 to10-fold. The smooth muscle and the inner elastic lamina of the vessel walls are lost, leading to the formation of flaccid tubes. However, during PE cytotrophoblast invasion is shallow and vessels remodelling is limited to the superficial portion of the decidua (Roberts and Gammill, 2005). Therefore, the spiral arteries are narrow resulting in a restricted blood flow that is insufficient to meet the nutrient and oxygen supply to the fetus (Naicker *et al.*, 2021). This pathology is followed by widespread endothelial cell dysfunction leading to an increase in inflammatory response (Raymond and Peterson, 2011). PE is associated with fetal distress and fetal growth restriction and in some cases may cause fetal death (Miller *et al.*, 2022).

1.1.3.1 Stages of pre-eclampsia development

Roberts and Gammil (2005) proposed PE as a two-stage disorder.

1. The first stage, known as the placental stage, involves impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries, leading to reduced placental perfusion which causes hypoxia and culminates in ischaemia. The concomitant oxidative stress leads to the release of various factors causing endothelial damage and the development of acute maternal syndrome with multi-organ failure (Zen *et al.*, 2020; Pankiewicz *et al.*, 2021).
2. The second stage, referred to as the maternal stage, is characterized by the onset of clinical symptoms in the mother, such as hypertension and/or proteinuria, which are believed to be the result of oxidative stress and the release of various factors into circulation (Staff, 2019). This stress is a common endpoint of several processes in stage 1 and can be triggered by different causes and timing of placental malperfusion (Owaki *et al.*, 2021).

1.1.3.2 Risk factors for pre-eclampsia development

Pre-eclampsia is characterized by elevated blood pressure, the presence of protein in the urine, and swelling in the hands, feet, and legs. The factors contributing to the risk of PE include a prior diagnosis of PE; maternal age of 35 years or older; chronic hypertension; diabetes mellitus; low haemoglobin levels; and an elevated Body mass index (BMI) >30 (Rana *et al.*, 2019). Additional risk factors encompass gestational diabetes, and a history of hypertension. Moreover, specific medical conditions like migraines, rheumatoid arthritis, lupus, urinary tract infections, and polycystic ovarian syndrome are associated with developing PE (Natalia *et al.*, 2022).

1.1.4 Treatment of pre-eclampsia

Table 2 Treatments of pre-eclampsia as recommended by the World Health Organisation

Prophylaxis and treatments recommended by WHO (World Health Organization, 2011)
Calcium supplementation during pregnancy (1.5 – 2.0 g elemental calcium/day) is recommended for the prevention of PE in all women, but especially those at high risk of developing PE.
Low-dose acetylsalicylic acid (aspirin, 75-150 mg) is recommended for the prevention of PE in women at high risk of developing PE.
**Women with severe hypertension during pregnancy should receive treatment with anti-hypertensive drugs.
*Magnesium sulphate is recommended for the prevention of eclampsia in women with severe PE in preference to other anticonvulsants.
**Induction of labour is recommended for women with severe PE at a gestational age when the foetus is not viable or unlikely to achieve viability within one or two weeks.
**In women with severe PE at term, early delivery is recommended.
**In women treated with anti-hypertensive drugs antenatally, continued anti-hypertensive treatment postpartum is recommended.
**Treatment with anti-hypertensive drugs is recommended for severe postpartum hypertension.

**Quality of evidence high and strongly recommended by WHO; ** Quality of evidence low but strongly recommended by WHO.*

1.2 Human immunodeficiency viral infection

Human immunodeficiency virus is an infection that primarily infects CD4+ T cells, macrophages, and dendritic cells, weakening a body's immunity against opportunistic infections (Ekabe *et al.*, 2022; World Health Organization, 2023a). HIV infects the aforementioned cells by binding to the CD4+ receptors via gp120, a viral envelop protein, facilitating viral entry through the interaction of the virus's gp41 protein with co-receptors (Ekabe *et al.*, 2022).

Treatment of HIV consists of a combination of antiretroviral (ARV) drugs, which work by stopping the replication of the virus (World Health Organization, 2023a).

1.2.1 Epidemiology

In 2022, approximately 36.4 million people were living with HIV globally (World Health Organization, 2023b). Notably, 20.6 million of those infected reside in Eastern and Southern Africa. Moreover, more than half of those infected are women in their reproductive years with 90% having access to antiretroviral therapy (ART) (UNAIDS, 2022). HIV-infected pregnant women at term receiving ART are at risk for low mitochondrial DNA expression in the placenta (Mahendrata *et al.*, 2022).

1.2.2 Anti-retroviral therapy

There are three classes of antiretroviral drugs available: nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). First-line therapy utilizes a single NNRTI together with an NRTI while second-line therapy utilizes PI together with a NRTI, employing two new agents that have not been previously used to minimize the development of cross-resistance (Gilks *et al.*, 2006).

Side effects of ART include abnormal dreams, cholelithiasis, depression, diabetes mellitus, diarrhoea, dizziness, drug-induced bone-marrow suppression, elevated creatinine, fat maldistribution and body habitus changes, hepatic toxicity, hyperglycaemia, hyperlipidaemia, insulin resistance, ischemic stroke, lactic acidosis, myocardial infarction, nausea, nephrolithiasis, osteoporosis and osteonecrosis, osteopenia, pancreatitis, peripheral neuropathy, proximal renal tubulopathy and elevated creatinine, skin rashes and hypersensitivity reactions, suicidal ideation (Dlamini *et al.*, 2022). Antiretroviral therapy during pregnancy may be associated with adverse birth outcomes, including preterm delivery and low birth weight. This is more likely to occur in women who undergo ART prior to conception (Uthman *et al.*, 2017).

ART may be associated with teratogenic effects (Gilks *et al.*, 2006). Teratogenic refers to substances that have the potential to cause developmental abnormalities or birth defects in a developing foetus during pregnancy. Nucleoside reverse transcriptase inhibitors (present in both first and second-line therapy) have been shown to have low toxicity, however, NNRTIs such as nevirapine can cause severe skin reactions and are hepatotoxic (liver toxicity) (Gilks *et al.*, 2006).

1.3 The synergy of pre-eclampsia and HIV infection

HIV treatment remains the same for both pregnant and non-pregnant women, however, the risk of vertical transmission may be reduced by ART throughout pregnancy (Landi *et al.*, 2014). PE was found to be more prominent in HIV-positive patients in a South African cohort, albeit not statistically significant (de Groot *et al.*, 2003). However, evidence supports the fact that ART use prior to and throughout pregnancy predisposes PE development due to its immuno-restorative effect (Hall *et al.*, 2014; Sikhosana *et al.*, 2022).

PE and HIV can both be regarded as inflammatory conditions (Gilks *et al.*, 2006) with a variety of soluble factors contributing to the mediation of inflammation (Fig 1.1), one being pentraxins.

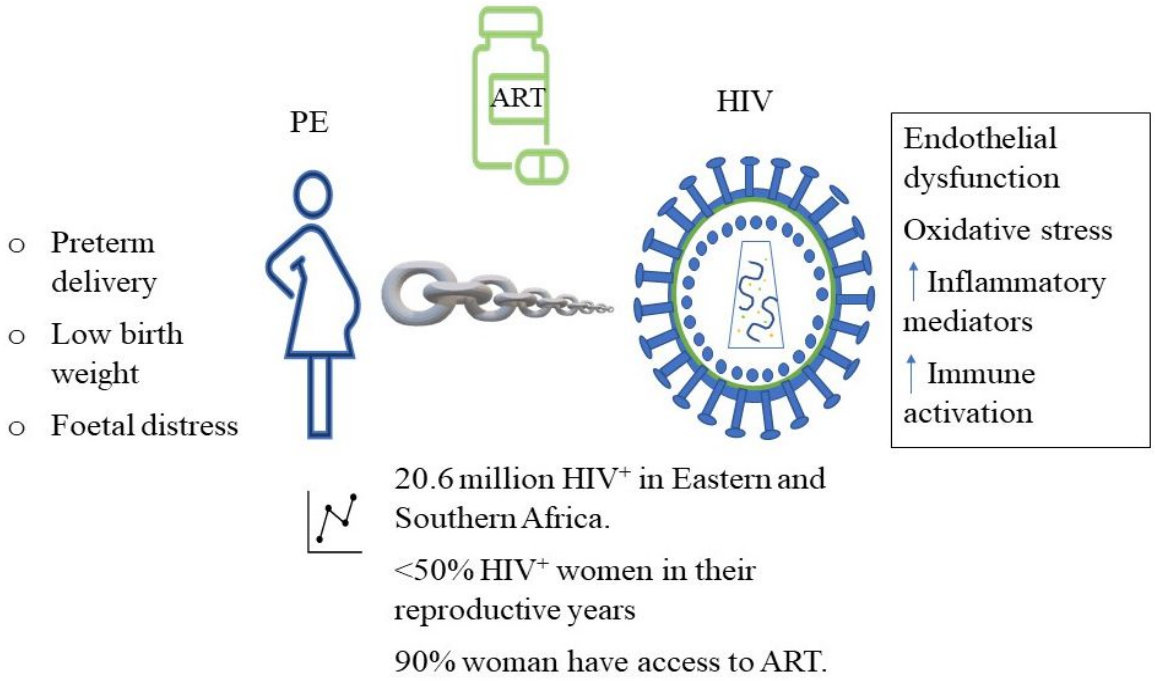


Figure 1.1 Schematic diagram highlighting the relationship between pre-eclampsia, HIV and antiretroviral therapy.

1.4 Pentraxins

Pentraxins are made from monomers arranged in pentameric structures with a discoid shape, and a 205 amino acid chain located at the c-terminal. Depending on the length of the protein sequence, pentraxins may be divided into two subgroups viz.,

a. Short pentraxins, such as C-reactive protein (CRP) and serum amyloid P component, and
b. Long pentraxins, such as neuronal pentraxin 1, neuronal pentraxin 2, neuronal pentraxin receptor, pentraxin 3 (PTX 3), and pentraxin 4.

The long pentraxins are approximately twice the size of the short pentraxins and contain a long N-terminal sequence (Wang *et al.*, 2020).

Short pentraxins regulate the human immune response, this includes triggering inflammation, removing mutant cells, and acting against pathogen invasion. Neuronal pentraxin 1 and neuronal pentraxin 2 are involved in the development of the central nervous system and neurodegenerative diseases. In contrast to pentraxin 4, PTX 3 is involved in activation of the innate immune response (Wang *et al.*, 2020).

To ensure the successful outcome of pregnancy, inflammation and activation of the innate immune system occur (Mor and Cardenas, 2010). Complex interactions that occur between the maternal immune system and fetal cells contribute to the survival and healthy development of the foetus. Fetal cells

express paternal alloantigens, which are not recognized as foreign by the mother due to the presence of physical and immunological barriers established at the maternal-fetal interface. The maternal-fetal interface plays a crucial role by promoting tolerance to the presence of paternal alloantigens whilst also maintaining the maternal immune response against potential invasion by pathogens (Martin *et al.*, 2014).

Angiogenic biomarkers were found to make a significant impact on clinical-based prediction model studies (Allen *et al.*, 2014) and therefore help in the diagnosis of PE. In light of the elevated inflammatory response in PE as opposed to the lowered response in HIV infection, this chapter focuses on two pentraxins; PTX 3 and CRP in the synergy of HIV-infected PE.

1.4.1 Pentraxin 3

Pentraxin 3 is located on chromosome 3q25 (Wang *et al.*, 2020). It is a glycoprotein consisting of a 203-amino acid-long C-terminal and a 178-amino acid-long N-terminal (Zhang *et al.*, 2022). It is produced by several cells such as monocytes; macrophages; epithelial cells; polymorphonuclear cells; endothelial cells; dendritic cells; and fibroblasts (Fig 1.2) (Larsson *et al.*, 2011; Kondo *et al.*, 2013).

1.4.1.1 Mechanism of Action

Pentraxin 3 is a marker for endothelial dysfunction and inflammation (Larsson *et al.*, 2011).in PE. It regulates inflammation by controlling the levels of pro-inflammatory and anti-inflammatory cytokines and interacting with ligands associated with inflammation. Additionally, PTX 3 plays a crucial role in regulating complement activation (Hawfield and Freedman, 2009; Zhang *et al.*, 2022).

PTX 3 is a component of humoral innate immunity, acting as an opsonin (Presta *et al.*, 2007). It affects the inflammatory process and innate immunity; both the effects are associated with endothelial cell functions (Gyselaers, 2020). Vascular endothelial growth factor (VEGF) plays a crucial role in the microcirculation of the renal system. VEGF is secreted by both the endothelium and podocytes of the glomeruli. Disruptions in the excretion of VEGF can cause proteinuria, a symptom of PE. Pentraxin 3, on the other hand, reduces the excretion of VEGF by inhibiting FGF2. This creates an anti-angiogenic environment and impairs the microcirculation within the glomerular endothelium (Turkmen *et al.*, 2015).

Certain adipose tissue hormones such as adiponectin and resistin influence PTX 3 production. In contrast to adiponectin, resistin upregulates PTX 3 synthesis in response to NF-kB (Presta *et al.*, 2007).

1.4.1.2 Pentraxin 3 in pregnancy

During normotensive pregnancy, PTX 3 levels remain stable with average values increasing slightly between each trimester. Notably, its expression is higher in the third trimester compared to the first and second trimester (Wirestam *et al.*, 2021). However, during PE, the level of PTX 3 increases significantly

(Cetin *et al.*, 2006; Hawfield and Freedman, 2009; Ali *et al.*, 2018). More specifically it is significantly higher in the first trimester indicating that PTX 3 may be a good biomarker for early PE prediction (Ali *et al.*, 2018). Of note, Wang et al (2020) reported a high sensitivity between PTX 3 and inflammation. It is known that the levels of PTX 3 are higher in patients with hypertension than those without, thus being a good predictor of PE (Akhter *et al.*, 2017). This study also found that PTX 3 levels in PE women have a negative association with gestational age at birth and infant birth weight.

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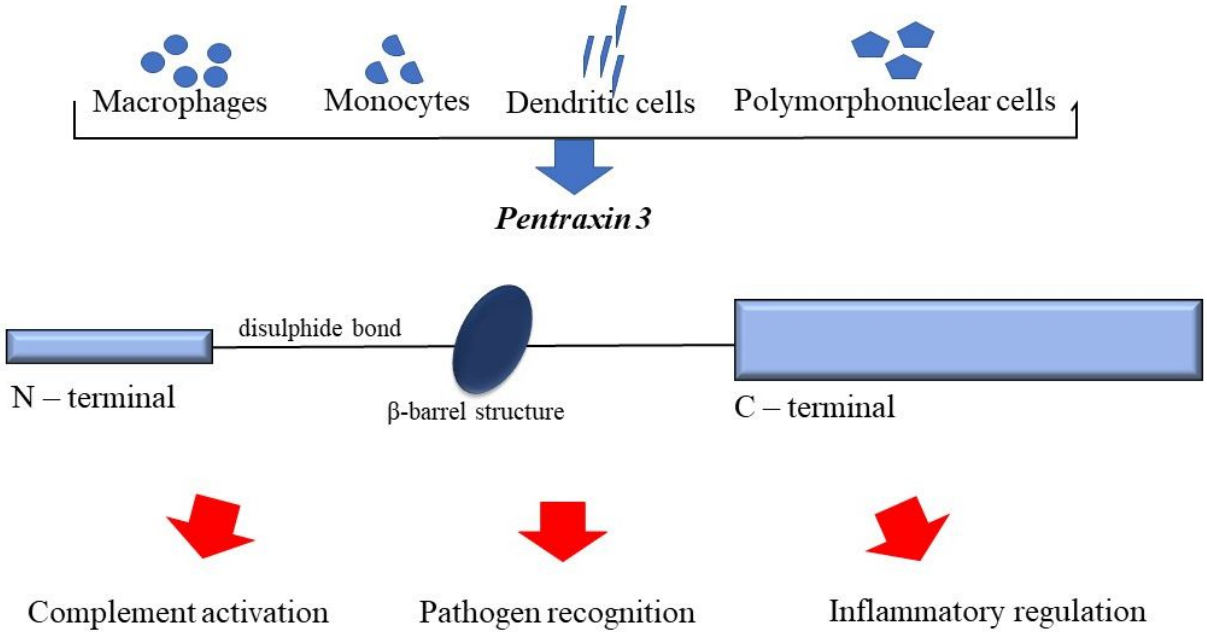

Figure 1.2 Showing the structure of pentraxin 3 and its functions.

1.4.2 C-reactive protein

C-reactive protein is a pentameric protein (Fig 1.3) present in blood plasma. Synthesized in the liver, its structural gene is situated on chromosome 1 (Adizova, 2022). Elevated CRP levels occur in reaction to various inflammatory conditions, serving as an early defence mechanism against infections (Kumari *et al.*, 2020). Its systemic concentration increases in response to inflammation (Sproston and Ashworth, 2018). The standard range for CRP is 0 to 10 mg/l (Wirestam *et al.*, 2021). In instances of inflammation, there is a substantial increase in CRP expression (Adizova, 2022). CRP levels can surge to 350-400 mg/l within a span of two hours during inflammation (Kumari *et al.*, 2020).

1.4.2.1 Mechanism of Action

Notably, CRP, like PTX 3, is associated with the complement system and contains a binding site for C1q (Du Clos, 2013). It triggers the activation of the complement pathway by directly binding to C1q, which serves as the initial component of the pathway. Each pentamer of CRP possesses a singular binding site for C1q, and a minimum of two CRP molecules is necessary for C1 activation (Du Clos, 2013). While CRP induces only minimal activation of C5-C9 (the strongest inflammatory mediators), restricted activation of complement favours opsonization without a strong inflammatory response. Similarly, CRP prevents the lysis of apoptotic cells through complement activation, promoting opsonization. Moreover, CRP has demonstrated the ability to enhance the production of anti-inflammatory cytokines, further augmenting its anti-inflammatory properties (Du Clos, 2013).

1.4.2.2 C-reactive protein expression in pregnancy

During a healthy pregnancy, CRP levels increase during the third trimester with a median value of 4.12 mg/l, significantly higher than the first and second trimesters where the levels are ≤0.3 mg/l.

In PE, the concentration of CRP is 8.6 times higher than in normotensive pregnancies (Adizova, 2022). Multiple other studies also observed an increase in CRP in PE than normotensive pregnancies (Qiu *et al.*, 2004; Arikan *et al.*, 2012; Renu *et al.*, 2022; Suliman *et al.*, 2022). However, Suliman et al (2022) reported a similar increase in both serum and plasma CRP concentrations in PE.

The level of CRP in the bloodstream indicates the severity of endothelial cell injury, which makes it a good predictor for PE severity (Renu *et al.*, 2022). CRP has been shown to have a lower sensitivity in patients with a higher BMI (Rebelo *et al.*, 2013) and thus BMI would need to be taken into consideration when investigating CRP levels. During normal pregnancy BMI in the first and second trimesters was shown to have a weak correlation, however, during the 3rd trimester p-values were significant (Wirestam *et al.*, 2021). This study also concluded that no significant differences were found between CRP and fetal birth weight in normal pregnancy.

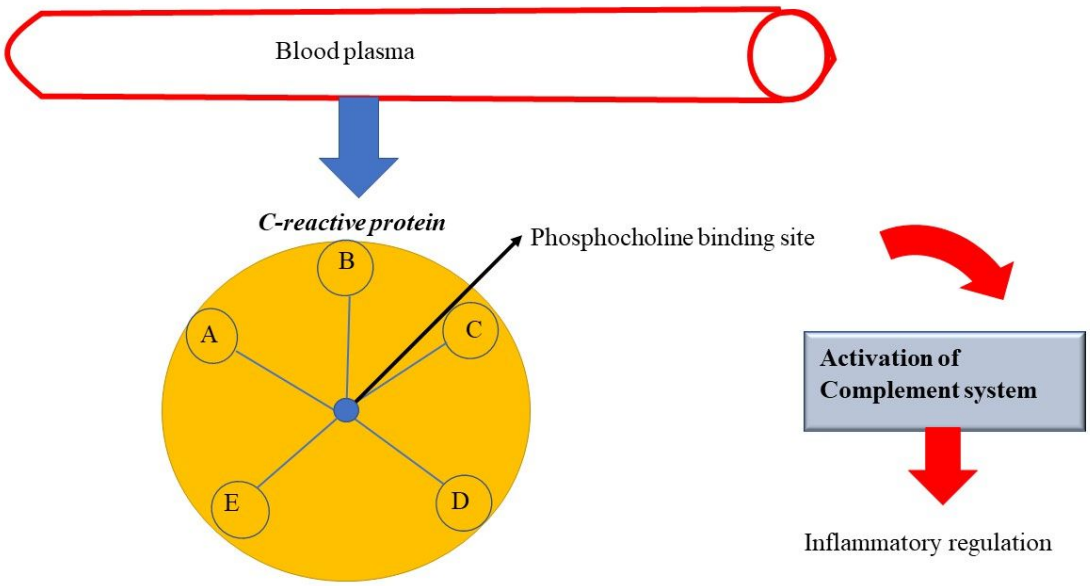

Figure 1.3 Showing the structure of c-reactive protein and its functions.

1.5 Aims, hypothesis and objectives

1.5.1 Aim of study

The aim of this study is to compare the effect of pentraxin 3 and c-reactive protein in women living with HIV who also have pre-eclampsia.

1.5.2 Specific objectives

The objectives of the study are as follows:

1. To investigate the concentration of pentraxin 3 and c-reactive proteins by pregnancy outcome (normotensive versus pre-eclampsia)
2. To investigate the concentration of pentraxin 3 and c-reactive proteins by HIV status (HIV-positive versus HIV-negative)
3. To compare the concentration of pentraxin 3 and c-reactive proteins across the study population (normotensive HIV-negative; normotensive HIV-positive; pre-eclamptic HIV-positive; pre-eclamptic HIV-negative)
4. To correlate pentraxin 3 and c-reactive protein concentration with clinical data

CHAPTER 2

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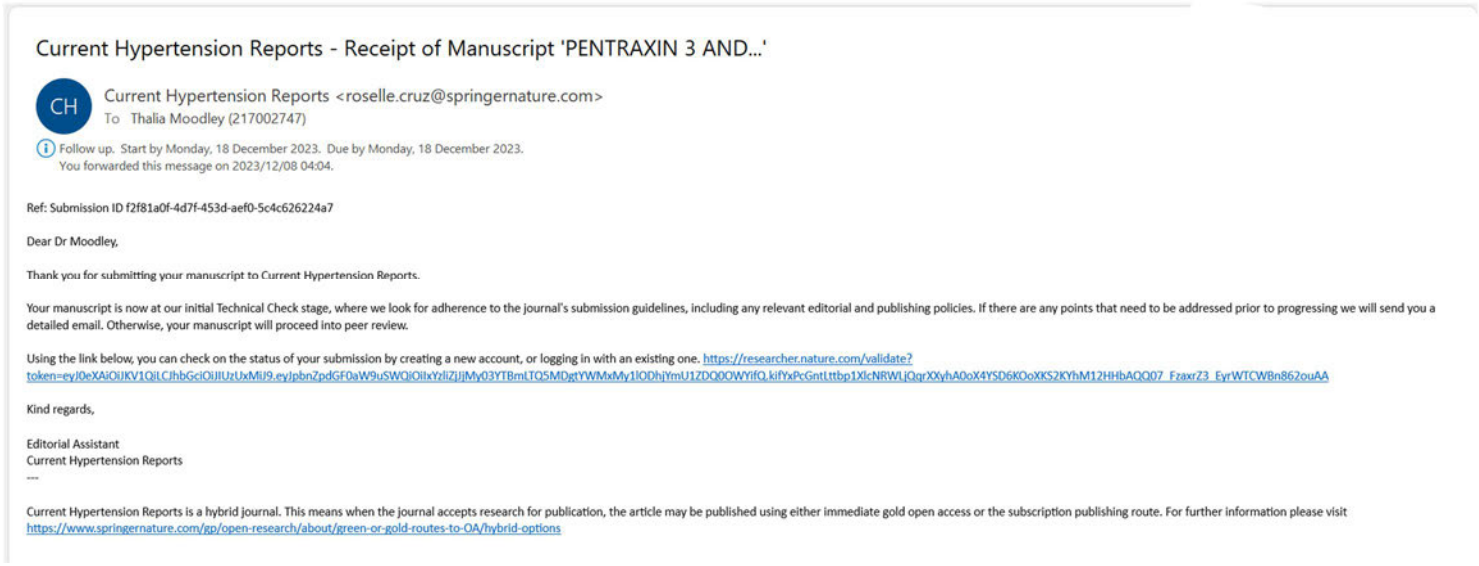

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PENTRAXIN 3 AND C-REACTIVE PROTEIN IN THE CO-MORBIDITY OF HIV INFECTION AND PRE-ECLAMPSIA

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ABSTRACT

Purpose of Review

The purpose of this narrative style review is to evaluate inflammatory markers, c-reactive protein and pentraxin 3 in the synergy of pre-eclampsia comorbid with HIV infection.

Recent Findings

Patients with pre-eclampsia have higher levels of pentraxin 3 and c-reactive protein compared to those with normal pregnancies. Pentraxin levels vary based on gestational age, across trimesters of pregnancy, and by obesity/body mass index.

A significant association was observed between the duration of antiretroviral therapy and reduced levels of pentraxins in individuals with HIV. However, initiating antiretroviral therapy only restores some inflammatory markers to normal levels. Hence pentraxin levels are still elevated during HIV infection. In the case of pre-eclampsia co-morbid with HIV infection, inflammation triggers pentraxin 3 and c-reactive protein release, activating C1q release and promoting C3 and C4 deposition. This provokes the secretion of cytokines, such as tumor necrosis factor- α, interleukin-1 and interleukin-6. The elevation of pentraxin 3 and c-reactive protein may account for the raised trophoblast apoptosis in pre-eclampsia.

Summary

This review explores the elevated levels of pentraxin 3 and c-reactive protein in the hyperinflammatory milieu of HIV-associated pre-eclampsia. Inflammation triggers pentraxin 3 and c-reactive protein release, activating C1q and facilitating C3 and C4 deposition. This cascade prompts cytokine secretion by macrophages, T cells, and adipocytes, causing endothelial dysregulation, permeability, and injury. Elevated pentraxin 3 and c-reactive protein levels may contribute to increased trophoblast apoptosis in pre-eclampsia. Pentraxin concentrations vary based on gestational age, pregnancy trimesters, and body mass index. In HIV infection, antiretroviral therapy initiates immune restoration and has the potential to counteract the effects of pentraxin 3 and c-reactive protein. However, while levels remain elevated, they are notably lower than those observed in patients not undergoing antiretroviral therapy.

Keywords

Pentraxin 3; c-reactive protein; pre-eclampsia; human immunodeficiency virus; inflammation

Statements and Declarations

Competing interests

The authors declare that they have no conflicts of interest.

Pentraxins

Pentraxins are an evolutionary old family of proteins consisting of monomers that are organized into pentameric structures. They have a discoid shape comprising a 205 amino acid chain located at the C-terminal. Pentraxins may be categorized based on their protein sequence length as either short or long pentraxins (which are approximately twice the size of short pentraxins) [1].

a. Short pentraxins include C-Reactive Protein (CRP) and serum amyloid P component. Short pentraxins have a regulatory role in the human immune system; they initiate inflammation, eliminate mutant cells, and counteract pathogen invasion.
b. Long pentraxins include neuronal pentraxin 1, neuronal pentraxin 2, neuronal pentraxin receptor, Pentraxin 3 (PTX 3), and pentraxin 4 [2]. Neuronal pentraxin 1 and neuronal pentraxin 2 are responsible for the development of the central nervous system, with abnormal development leading to neurodegenerative diseases while, PTX 3 is associated with the activation of the innate immune system (Wang et al., 2020).

Due to the complex interaction between the maternal immune system and fetal cells, the activation of innate immunity is imperative to ensure a successful pregnancy. Of-note, fetal cells contain paternal alloantigens, which the mother's body does not recognize as foreign due to the protective physical and immunological barriers at the maternal-fetal interface [3].

Notably, pre-eclampsia (PE) is a disorder unique to human pregnancy that manifests as severe hypertension and/or proteinuria in a previously healthy woman on or after the 20th week of gestation [4]. In PE, abnormal cytotrophoblast invasion precipitates a lack of physiological transformation of myometrial spiral arteries. This pre-empts a reduction in blood flow resulting in the release of anti-angiogenic factors and other inflammatory mediators into circulation, causing poor endothelial function [5].

HIV infection remains a global health challenge [6]. In low to middle-income countries, both PE and HIV infection are a major cause of maternal morbidity [7]. Furthermore, there are currently 8.45 million people living with Human Immunodeficiency Virus (HIV) infection in South Africa with almost 25% being HIV-positive women in their reproductive ages [8]. It is an obstetric dilemma that 40% of pregnant women in SA are HIV-positive [9]. Therefore, it is vital to understand inflammatory markers involved in the hyperinflammatory milieu in the synergy of PE and HIV infection in the antiretroviral therapy (ART) era. This paper is a narrative review focusing on PTX 3 and CRP in the synergy of PE co-morbid with HIV infection.

Pentraxins 3

Pentraxin 3 is a glycoprotein that plays a crucial role in innate immunity, and inflammation and is directly involved in endothelial dysfunction [10, 11]. It is produced by monocytes, macrophages, dendritic cells, neutrophils, endothelial cells, epithelial cells and fibroblasts. More specifically, it activates and interacts with the classical pathway of the complement system leading to the elimination of pathogens (Fig. 1) [12]. Here it binds with C1q which promotes increased C3 and C4 deposition [13]. This regulation of C1q triggers abnormal placental growth in PE whilst in the PE co-morbid with HIV infection it is up-regulated with subsequent opsonization and removal of healthy cells, thereby leading to amplification and increased dissemination of the virion [14].

Pentraxin 3 in Pregnancy

During normal pregnancy, the concentration of PTX 3 remains stable, with a small increase in both serum and plasma levels across trimesters [15]. Despite different techniques of analysis and sample type both Larsson et al (2011) and Wirestam et al (2021) observed similar PTX 3 levels between the first two trimesters, whilst in the third trimester it was significantly higher than the first two trimesters [15, 16].

A Brazilian study noted PTX 3 levels in pre-term and term patients. They reported that 91.5% of the term amniotic fluid samples had slightly increasing levels across gestation, albeit non-significantly. However, 88.0% of women with spontaneous pre-term labour had higher PTX 3 than those not in labour. They demonstrated a 1% increase in the risk of preterm labour for each $1x10^{-6}$ mg/l rise in PTX 3 level within amniotic fluid [3].

The vascular bed is involved in inflammatory conditions [17]. Due to the high maternal systemic inflammation, endothelial dysfunction is a significant characteristic of PE development [17]. Both early-onset pre-eclampsia (EOPE) and late-onset pre-eclampsia (LOPE) are associated with an elevated inflammatory microenvironment that culminates in adverse maternal and fetal complications [18]. A meta-analysis study has revealed that PTX 3 has the potential to serve as a novel molecular marker with predictor test value for the early diagnosis of PE [19]. EOPE is associated with impaired trophoblast invasion [20]. A study conducted in Sweden observed that PTX 3 levels were higher in EOPE than LOPE women compared to normal multiparous pregnancies rather than nulliparous women [20]. Similarly, they showed that plasma PTX 3 levels of primiparous women were higher in the EOPE than in LOPE. Additionally, the latter study reported a significant association between PTX 3 and the anti-angiogenic sFlt-1 implicating the downstream effect of endothelial dysfunction. Similarly, Hamad et al (2012) also observed an up-regulation of PTX 3 in EOPE compared to LOPE, supporting the hyperinflammatory milieu in the aforementioned sub-type [21]. Notably, EOPE rather than LOPE is characterized by defective placentation (Roberts et al 2021).

The association between higher plasma PTX 3 levels and inflammation in normotensive pregnancy compared to PE is $2.20\ x10^{-3}$ and $1.38\ x10^{-2}$ mg/l respectively during the third trimester of pregnancy [17]. Similarly, serum PTX 3 levels are also progressively elevated in normal pregnancy compared to PE across trimesters with a further significant increase during labour [22]. These results were further corroborated by Ali et al (2018) who demonstrated that serum PTX 3 levels were significantly higher in the third trimester, with an increase from $9.26\ x10^{-2}$ mg/l in the first trimester to 0.15 mg/l in the third trimester [23]. The vast range of PTX 3 concentration between aforementioned studies could be indicative of disease severity.

More specifically, PTX 3 promotes Th1 cell differentiation and impairs the recognition of late apoptotic cells, which could inhibit the recognition function of dendritic cells [19]. This may have a potential impact on the onset of PE by affecting the function of vascular endothelial cells. PE is characterized by increased apoptosis of trophoblast cells [24]. The elevation of PTX 3 may be a plausible explanation for the raised trophoblast apoptotic dysregulation in PE.

Based on disease severity, PTX 3 levels in Turkish women are higher in severe compared to mild PE [25]. They attributed the lack of correlation between PTX 3 and proteinuria as they calculated the protein/creatinine ratio in spot urine samples rather than in 24-hour urine collections [25].

Pentraxin 3 in Other Pathologies

Kidney disease: In PE, endothelial injury and podocyturia result in impaired glomerular filtration, and subsequent kidney disease [26]. A study conducted using two independent cohorts [Swedish patients with stage 5 chronic kidney disease and Turkish patients with type 2 diabetes], correlated PTX 3 with proteinuria and normal renal function. PTX 3 levels were significantly higher in patients with chronic kidney disease [5.3 $x10^{-3}$ and 1.80 $x10^{-3}$ mg/l] compared to the control [7.40 $x10^{-3}$ and 1.40 $x10^{-3}$ mg/l] suggesting a link with podocyturia, proteinuria, endothelial dysfunction and inflammation, via PTX 3 signalling, transduction, translation and protein expression [10]. Additionally, they demonstrated a strong and positive correlation between PTX 3 and urinary protein excretion that suggests an association of PTX 3 with proteinuria, endothelial dysfunction, podocyturia and inflammation. The role of podocytes in the early detection of PE is established [27].

Rheumatoid arthritis: PTX 3 is also dysregulated in patients with active rheumatoid arthritis and microbial infections, with levels of 1.68 $x10^{-3} \pm 0.18$ $x10^{-3}$ and 0.47 $x10^{-3} \pm 0.08$ $x10^{-3}$ mg/l, respectively. These levels suggest that the rise in PTX 3 is specific, as it does not show an increase in circulating levels among individuals with acute infection [22].

Myocardial function: PTX 3 is an early and strong prognostic marker of mortality in a cohort of women with myocardial infarction and unstable angina pectoris [21].

Community-acquired pneumonia (CAP): This is the most frequently occurring infectious disease in the world associated with high morbidity and mortality rates. Kao et al (2013) reported that plasma PTX 3 levels were elevated in CAP compared to uninfected patients. However, after the initiation of antibiotic treatment, PTX 3 levels were significantly down-regulated. They also observed significantly higher levels of CRP, white blood cells and neutrophils in CAP pre-treatment compared to post-treatment. They concluded that plasma PTX 3 has a higher sensitivity than CRP and hence may be used to diagnose the severity of CAP [28].

Table 1 provides an overview of how PTX 3 is regulated in various pathologies as documented in the literature mentioned in this review.

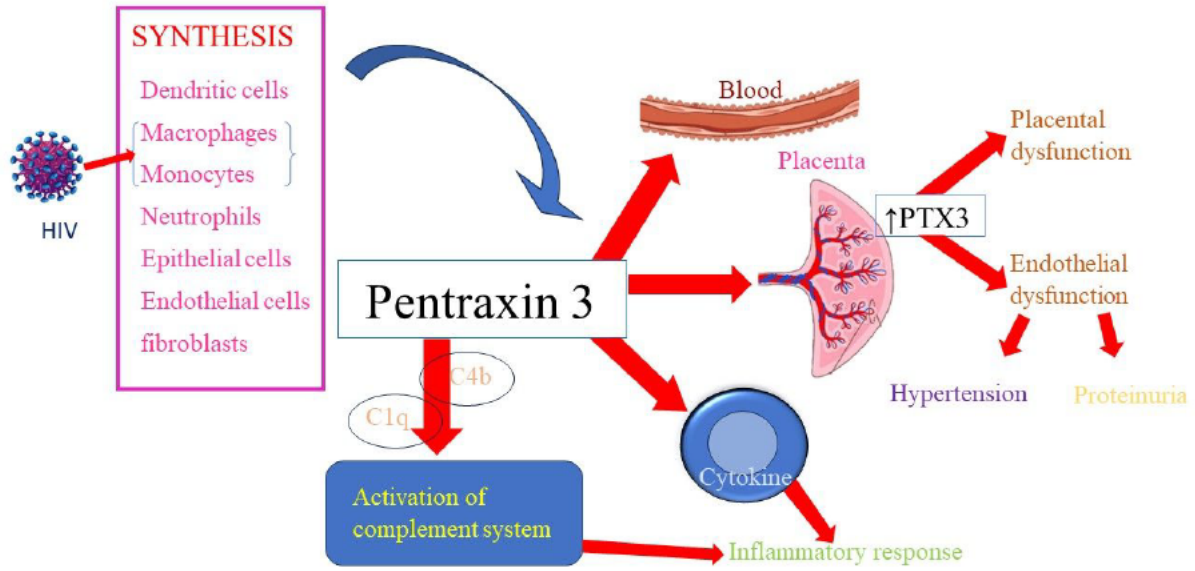

Fig 1. ***Showing the synthesis and pathways of pentraxin 3. HIV affects macrophages and monocytes, which are responsible for synthesizing PTX 3 in the bloodstream. PTX 3 activation stimulates the complement system and the release of cytokines, initiating an inflammatory response. Elevated PTX 3 levels in the placenta have the potential to induce dysfunction in both placental and endothelial tissues. Endothelial dysfunction, in turn, contributes to hypertension and proteinuria.***

PTX 3 - pentraxin 3; c1q- component 1q; c4b - C4b-binding protein; HIV – human immunodeficiency virus.

Table 1. ***Showing the regulation of PTX 3 in different pathologies.***

Pathology	PTX 3 Expression	Author (year)
PE	↑	Cetin *et al.*[17]
PE; rheumatoid arthritis	↑	Rovere-Querini *et al.* [22]
Kidney disease; type 2 diabetes	↑	Suliman *et al.* [10]
EOPE *vs* LOPE	↑	Boij *et al.* [20]
EOPE *vs* LOPE	↑	Hamad *et al.*,.[21]
HIV infection	↑	High *et al.* [29]
Community-acquired pneumonia	↑	Kao *et al.*[28]
Preterm	↑	Martin *et al.*[3]
PE	↑	Turkmen *et al.* [25]
PE	↑	Ali *et al.* [23]
EOPE *vs* LOPE	↑	Aneman *et al.* [18]
PE, kidney disease	↑	Kattah,[26]
HIV infection; ART	*↑	Minami et al [30]
PE	↑	Xiong *et al.*[19]

↑ - *Upregulation; PE - pre-eclampsia; EOPE - early on-set pre-eclampsia; LOPE - late on-set pre-eclampsia; HIV - human immunodeficiency virus; ART – anti-retroviral therapy; * - Although PTX 3 is upregulated in HIV patients on ART they have a lower concentration of PTX 3 than those who are not on ART.*

C-Reactive Protein

C-reactive protein is a plasma protein synthesized in the liver (Fig 2.) [31]. Since the half-life of CRP remains consistent regardless of disease severity, the concentration of CRP in circulation is influenced by its rate of synthesis [32]. Its rate of synthesis directly mirrors the intensity of the underlying pathological processes that stimulate its production [32]. Notably, plasma CRP levels may increase from around 1 mg/l to over 500 mg/l within 24 to 72 hours of severe tissue damage [33]. More specifically, the level of CRP increases in a wide range of acute and chronic inflammatory conditions, including bacterial, viral or fungal infections, rheumatic and other inflammatory diseases as well as in malignancy and tissue injury [34]. This elevation is a result of the secretion of inflammatory cytokines, such as IL-1 and IL-6 by macrophages, T cells, and adipocytes [34]. More specifically, CRP binds to Fc receptors on immune cells [33]. This activates the release of pro-inflammatory cytokines, which promotes vasodilation and increases vascular permeability and the recruitment of new immune cells to the site of injury or infection. However, the release of pro-inflammatory cytokines may also amplify the inflammatory response via the activation of other immune cells [33].

The primary physiological function of CRP is to bind to lysophosphatidylcholine which is expressed on the surface of dying cells and pathogens [34]. Similar to PTX 3, CRP is also activated by C1q, which promotes phagocytosis

by macrophages, facilitating the clearance of necrotic and apoptotic cells as well as pathogens (Fig 2.) [34]. Therefore, CRP serves as an early defence mechanism in innate immunity protecting against HIV infection.

C-reactive Protein in Pregnancy

During uncomplicated pregnancy, CRP is elevated in women experiencing premature uterine contractions and are at an increased risk of developing preterm labour and delivery [35]. Elevated levels of CRP occur before the onset of PE development [36]. Numerous studies have suggested that CRP may also be produced in the placenta [36, 37]. The up-regulation of CRP pre-empts its binding to phosphocholines, which are then transferred to neurokinin B and neuropeptide which promote the activation of the neurokinin 3 receptor. This process results in organ damage and the onset of hypertension [36].

The following looks at CRP in PE *vs* normotensive pregnancies:

Severity: mild vs severe pre-eclampsia – Based on PE severity, serum CRP levels are higher in mild and severe PE [> 6 mg/l] compared to normal pregnancies [< 6 mg/l]. These elevated levels in PE suggest its predictor risk indicator test value for placental vasculopathy that occurs before the onset of clinical PE [38]. Similarly, Suliman et al (2023), reported that CRP levels were significantly different and correlated with mean arterial blood pressure between mild and severe PE compared to normotensive pregnant women [39]. A Turkish cohort in their third trimester reported increased CRP levels in mild (albeit non-significant) and severe PE compared to normotensive pregnant women [40]. More recently, Adizova (2022) observed high serum CRP levels in severe PE [45.12 mg/l] versus mild PE [23.00 mg/l]. In PE, serum CRP are 8.6 times higher than in normotensive pregnancies [31]. This study was similar to that of Pitiphat *et al*. 2005.

Gestational age: Early-onset pre-eclampsia vs late-onset pre-eclampsia – In normotensive primigravida women, serum CRP levels may be categorized with initial values of either > 5 mg/l or < 5 mg/l The highest mean CRP level was observed in the > 5 mg/l groups, measuring at 14 mg/l in contrast to 2.30 mg/l in the <5 mg/l group [34]. Kumari et al (2020) outlined the potential of serum CRP for the early prediction of adverse pregnancy outcomes suggesting its value as a prognostic indicator of subtle and/or exaggerated inflammatory responses in pregnancy, even in the absence of infection [34].

Delivery: pre-term vs term – Based on the time of delivery, plasma CRP levels are higher preterm compared to term delivery [4.80 mg/l and 2.50 mg/l] respectively. Moreover, they reported that singleton pregnancies were associated with high plasma CRP in early pregnancy and correlated with an increased risk of preterm delivery [41]. Pitiphat et al (2005) observed that the risk of preterm delivery increased when the amount of CRP in PE patients exceeded 10 mg/l. Hence the establishment of a baseline value for CRP would assist in the early identification of, perinatal and obstetric complications[41]. In addition, Savvidoua et al (2002) reported no difference in serum CRP levels in PE compared to those who had uneventful pregnancies between 23–25 weeks of gestation [42].

Obesity – C-reactive protein levels are linked to both obesity and PE development [43]. Obesity is a recognized risk factor for PE development, and CRP serves as an indicator of systemic inflammation, often elevated in individuals with obesity. In a study involving pregnant women, it was observed that first-trimester CRP levels

were notably higher in PE compared to normotensive pregnant women. However, when BMI was taken into consideration, the relation between CRP and PE was not statistically significant suggesting that inflammation is implicated in the obesity pathogenic pathway and contributes to PE development [43].

A South African study indicated a correlation between HIV infection and obesity suggesting these HIV-positive patients receiving ART are more likely to be overweight or obese [44]. The study also observed elevated levels of CRP in HIV-positive patients. Their results conform to the findings of Wolf et al (2001) who] alluded that the increased CRP levels in obese individuals may, in part, be attributed to the high inflammation and alterations in lipid and glucose metabolism that occur in HIV infection [44, 45].

Body mass index –Plasma CRP levels were evaluated in healthy pregnant Swedish women with a BMI of 18-25 kg/m^2 at the time of conception and across their first, second, and third trimesters. They reported significantly higher CRP levels during the third trimester, compared to the first and second trimesters. Additionally, a significant but weak correlation was observed between BMI and CRP levels in the first and second trimesters, whilst no significant correlation occurred in the third trimester [16].

In contrast, a UK study reported that CRP levels did not vary across trimesters. They showed that CRP levels increased by 8.8% for each additional 1 kg/m^2 of BMI at any stage of pregnancy [46]. Similarly, CRP levels were significantly elevated in PE compared to normotensive pregnancies in Swedish and US cohorts, using pre-pregnancy BMI. Elevated CRP emanating from pre-pregnancy BMI correlates with a risk of PE development [47]. Therefore, BMI needs to be considered when assessing CRP levels in PE. This study reports that elevated CRP levels predisposes a 2.5-fold increased risk of PE development in lean women, while no similar association was found in overweight women [47]. CRP is not a significant predictor of PE after the inclusion of BMI in the multiple regression models. Additionally, maternal overweight status alone, without elevated CRP concentration, was associated with a 4.9-fold increased risk of PE development. Notably, women who are overweight and with elevated CRP concentrations are predisposed to PE development [47].

In HIV infection, weight loss in obese menopausal women leads to a decline in CRP levels. Since weight loss occurs in HIV infection. CRP serves as an independent predictor of survival, regardless of BMI [48].

A study conducted in the Netherlands assessed the variation in CRP level during the late first trimester or early second trimester of pregnancies complicated by intrauterine growth restriction (IUGR). Results show mean CRP values were significantly higher in PE compared to the control group and did not rise or fall significantly during a time range of 1 week during the first half of pregnancy [49].

Table 2 provides an overview of how CRP is regulated in various pathologies as documented in the literature mentioned in this review.

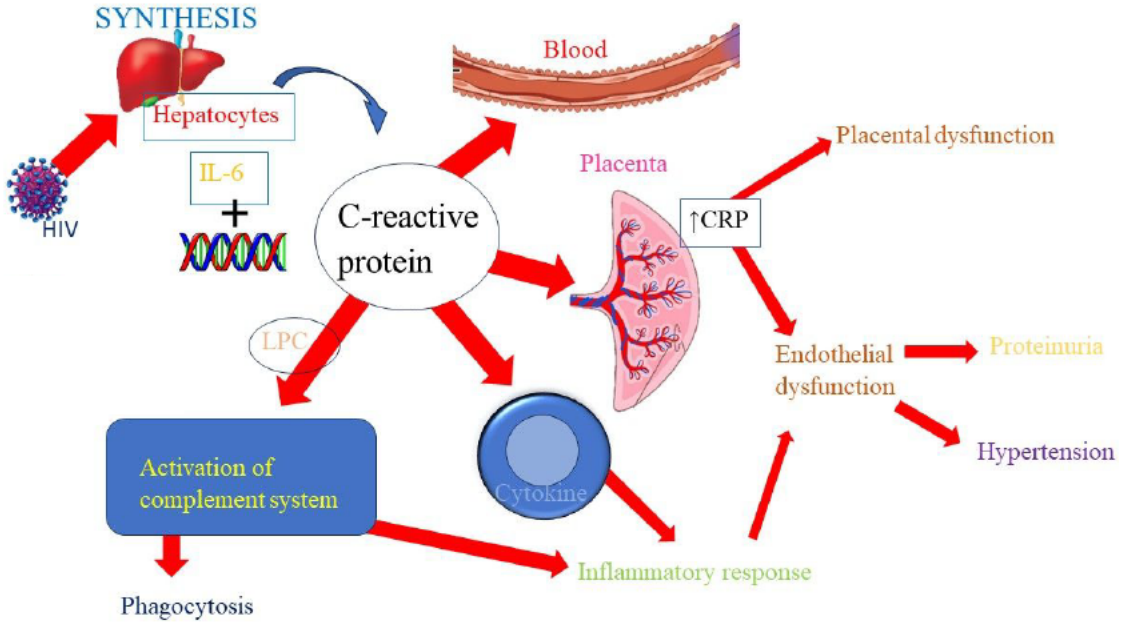

Fig 2, ***Showing the synthesis and pathways of c-reactive protein. HIV induces an increase in IL-6 and CRP levels. CRP activates the complement system by releasing LPC and prompts the release of cytokines, initiating an inflammatory response. Elevated CRP levels in the placenta have the potential to induce dysfunction in both placental and endothelial tissues. This endothelial dysfunction, in turn, contributes to hypertension and proteinuria.***

1 CRP - c-reactive protein; IL-6 -interleukin 6; LPC – lysophosphatidylcholine.; HIV – human immunodifficency virus

Table 2 ***Showing the upregulation/ downregulation of CRP under different conditions.***

Condition	CRP expression	Author (year)
PE vs uneventful pregnancy (23–25 weeks)	–	Savvidoua *et al.* [42]
HIV-1 infection	↑	Feldman *et al.* [48]
PE; IUGR	↑	Toja *et al.* [49]
PE; increased BMI	↑	Qui *et al.* [47]
Previous preterm delivery	↑	Pitiphat *et al.* [41]
PE	↑	Arikan *et al.* [40]
HIV infection	↑	High *et al.* [29]
Community-acquired pneumonia	↑	Kao *et al.*[28]
HIV infection, ART	↑	Zungsontiporn *et al.*[50]
Pregnant ladies with CRP level > 5 mg/l	↑	Kumari *et al.*[34]
HIV infection, ART	*↑	Minami *et al.*, 2020
Increased BMI	↑	Dockree *et al.*[46]
3rd trimester; increased BMI	↑	Wirestam *et al.*[16]
PE	↑	Adizova[31]
Mild *vs* Severe PE	↑	Renu *et al.*[38].
PE; kidney disease; type 2 diabetes	↑	Suliman *et al.*.[39]

↑ - *Upregulation; - remained the same; PE - pre-eclampsia; HIV - human immunodeficiency virus; IUGR - intrauterine growth restriction; BMI - body mass index; ART – anti-retroviral therapy; * - Although PTX 3 is upregulated in HIV patients on ART have a lower concentration than those who are not on ART.*

HIV, Pentraxin 3, and C-reactive protein

In HIV infection, lipopolysaccharides (LPS) bind to CD14 on monocytes and macrophages forming an LPS/CD14 complex which activates Toll-like receptor-4 (TLR4) thereby promoting pro-inflammatory cytokine production. Chronic immune activation and inflammation affect the lymphoid tissue, by triggering an increase in transforming growth factor β (TGF-β), which stimulates collagen production, thereby replacing the fibroblastic reticular network and causing a progressive loss of naïve T cells [51]. During HIV infection heightened inflammation activates the complement system (Fig.1and 2), resulting in an increase in PTX 3 and CRP levels in the blood [52].

Anti-retroviral therapy (ART)

Metabolic syndrome and chronic inflammation are prevalent in approximately 20-33% of HIV-infected patients using combination ART. [53]. Patients living with HIV are in a state of chronic inflammation as indicated by an up-regulation of cytokines and proinflammatory substances, such as interleukin-6, PTX 3, CRP, D-dimer, and cystatin C (fig. 2). Research has shown that combination ART is highly effective in immune reconstitution and suppresses viral load however, chronic immune dysfunction and inflammation persist [29].

Although the initiation of ART should lead to downregulation of inflammatory markers, patients receiving nucleoside reverse transcriptase inhibitors (NRTIs) have shown an increase in CRP concentration 24 and 96 weeks after the initiation of treatment [$p = 0.004$; and $p = 0.016$, respectively] [54].

In HIV-infected men, PTX 3 levels before ART administration are higher than in HIV-infected men post-ART administration of Protease Inhibitors (PIs) and Integrase strand transfer inhibitors (ISTIs), although the differences were not statistically significant [53]. This suggests that the initiation of ART down-regulates PTX 3 expression as noted in Tables 1 and 2. This study included a control group of HIV-negative men with no chronic or inflammatory diseases, surprisingly, PTX 3 levels were higher in the control group [4,40 $x10^{-3}$ mg/l] compared to the two HIV-positive groups [2.70 $x10^{-3}$ mg/l and 2.60 $x10^{-3}$ mg/l] [53].

The different types of ART and their action are outlined in Table 3 [55].

Table 3 ***shows the mechanism of action of ART.***

TYPE OF ART	**MECHANISM OF ACTION** [55].
NRTIs and NNRTIS	Attach to and block the action of reverse transcriptase, since HIV relies on reverse transcriptase to convert its RNA into DNA, NRTIs and NNRTIs prevent HIV replication
PIs	Blocks protease, inhibiting the transformation of immature HIV particles to mature HIV particles
INSTIs	Blocks integrase, preventing HIV replication as integrase is used to insert viral DNA into the host CD4 cell.

NNRTIs - Non-nucleoside reverse transcriptase inhibitors; NRTIs - Nucleoside reverse transcriptase inhibitors; PIs - Protease inhibitors; CCR5 – CCR5 antagonists; INSTIs -Integrase strand transfer inhibitors

Initiation of ART at an early stage leads to the restoration of several inflammatory markers to normal levels, while CRP levels decreased after initiating ART, however, they remain elevated compared to individuals who are HIV naïve [56]. This indicates that commencing ART early is not enough to eliminate the chronic inflammation linked to the risk of overall morbidity and mortality [56].

A study conducted on HIV-infected patients on ART for three months found a significant association between high levels of CRP and Serum amyloid P (a short pentraxin) [50]. However, this study concluded that elevated SAP levels are significantly associated with reduced brachial artery flow-mediated dilation even after accounting for traditional CVD risk factors. In contrast, the relationship between CRP and brachial artery flow-mediated dilation is dependent on traditional CVD risk factors, indicating SAP may reflect different pathological processes from CRP [50].

A previous study showed a significant correlation between the duration of ART and low PTX 3 levels with no correlation to the CD4+ count, and the type of ART taken. After the initiation of ART, patients with a BMI ≧ 25kg/m^2 showed a decrease in PTX 3 levels in plasma. The study concluded that lifestyle changes boost PTX 3 levels and may lead to better outcomes for people living with HIV who are on consistent ART treatment. [30].

Conclusion

This narrative review reports that both PTX 3 and CRP levels are elevated in the synergy of the hyperinflammatory co-morbidity of women living with HIV who also have PE. In both conditions inflammation triggers PTX 3 and CRP release thereby activating C1q and promoting C3 and C4 deposition. This provokes the secretion of cytokines, such as TNF- α, IL-1 and IL-6 by macrophages, T cells, and adipocytes dysregulating endothelial cell permeability and endothelial injury. The elevation of PTX 3 and CRP may also contribute to the raised trophoblast apoptosis in PE. The level of these pentraxins varies by gestational age, across trimesters of pregnancy and by obesity/BMI. In women living with HIV who also have PE, patients receiving ART undergo immune restoration. Hence, both PXT 3 and CRP are expected to be neutralised, yet they remain up-regulated albeit lower than that of ART naïve patients.

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CHAPTER 3

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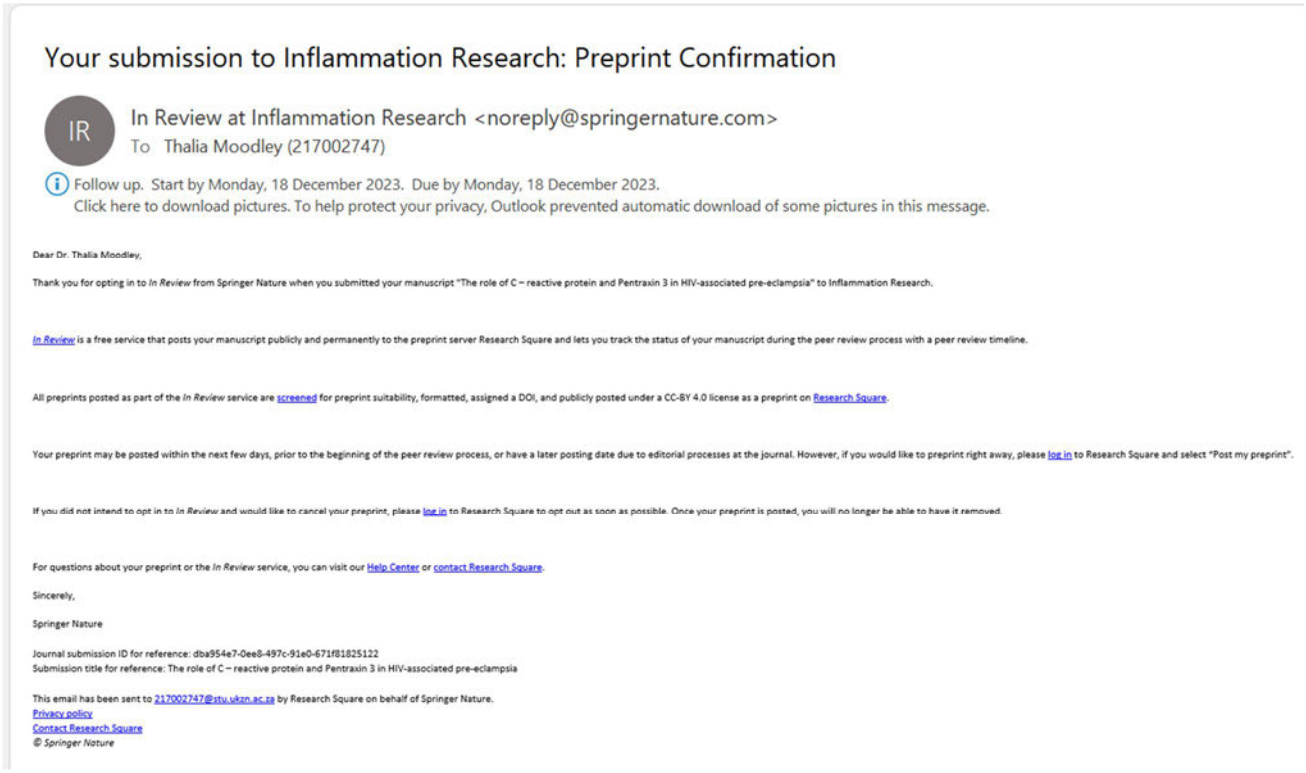

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The role of C – reactive protein and Pentraxin 3 in HIV-associated pre-eclampsia.

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ABSTRACT

Objectives

To compare the expression of inflammatory proteins, pentraxin 3 and c-reactive proteins in HIV-associated normotensive and preeclamptic pregnancies.

Methods

Both HIV status (HIV-positive and HIV-negative) and pregnancy type (normotensive pregnant and preeclampsia) were used to stratify the study population (n = 80). Using a ProcartaPlex™ immunoassay technique, the plasma concentrations of pentraxin 3 and c-reactive protein were measured.

Results

An up-regulation of c-reactive protein was demonstrated in pre-eclamptic versus normotensive women; HIV-positive versus HIV-negative, and across the study population. albeit non-significant. Pentraxin 3 was significantly higher in preeclamptic versus normotensive women ($p < 0.0001$), irrespective of HIV status. However, no significance was found in HIV status, regardless of pregnancy type. When compared across all groups pentraxin 3 concentrations was significantly different between normotensive HIV-negative versus pre-eclamptic HIV-negative groups ($p = 0.0001$); and normotensive HIV-negative versus pre-eclamptic HIV-positive groups ($p = 0.0049$).

Conclusions

This novel study demonstrated pentraxin 3 concentrations to be significantly higher in pre-eclampsia compared to normotensive pregnant women, emanating from oxidative stress and inflammation. C-reactive protein was higher in the normotensive pregnant women compared to pre-eclampsia. This difference may stem from hepatic dysfunction, gestational age, disease severity, interleukin-6 production, and/or sample size.

Keywords: HIV infection; Pre-eclampsia; inflammation; Pentraxin 3; C-reactive protein.

Introduction

Pre-eclampsia (PE) is a pregnancy-specific disorder, that occurs after 20 weeks of gestation and may be identified by new-onset hypertension (≥ 140/90 mmHg on two or more occasions) and/or proteinuria and multi-organ dysfunction [1]. It can also cause fetal distress and growth restriction and in some cases fetal death [2]. Globally, PE is the leading cause of mortality and morbidity in pregnancies [3]. In South Africa, PE accounts for the majority of hypertensive related maternal deaths [4]

The pathophysiology of PE is described as having abnormal placentation and endothelial injury [5]. This results from abnormal cytotrophoblast invasion and absence of myometrial spiral arteriole transformation [6]. This leads to reduced blood flow, hence insufficient nutrient and oxygen supply to the fetus [7]. Inflammatory and angiogenic mediators are released systemically as a result of this oxidative stress environment [8].

C-reactive protein (CRP) is a pentameric protein, synthesized in the liver, with its structural gene found on chromosome 1 [9]. The level of CRP rise in response to a wide range of inflammatory conditions and thus act as an early innate defence system against infections [10]. Moreover, CRP binds to specific molecules on the surface of bacteria, viruses, and damaged cells, thereby activating the complement system to remove pathogens and damaged cells from the body. Furthermore, CRP may also interact with other immune cells, such as monocytes and macrophages, thus stimulating the production of cytokines, initiating an immune response [11].

The normal level of CRP is 0 to 10 mg/l; however, inflammation causes an increase in concentration [9]. Within two hours of triggering an immune response, CRP levels can spike up to 350-400 mg/l [10]. Systemic CRP levels correlate with the severity of endothelial cell injury, which makes it a good predictor of PE [12].

Numerous cells, including monocytes, macrophages, epithelial cells, polymorphonuclear cells, endothelial cells, dendritic cells, and fibroblasts, synthesize pentraxin 3 (PTX 3), which is located on chromosome 3q25 [13, 14]. It would hence have the potential predictor test value for endothelial dysfunction and inflammation in PE [15]. Endothelial dysfunction leads to the production of inflammatory factors, such as growth factors and cytokines, inducing promoting PTX 3 synthesis [13]. The normal level of PTX 3 in women are around 2.4×10^{-3} mg/l but may rise to 0.200 mg/l to 0.800 mg/l in 6 to 8 hours following the initiation of pro-inflammatory mediators [16, 17].

Pentraxin 3 is involved in the immune response against both viral and bacterial infections by binding to virus-infected cells, thus inhibiting the replication of specific viruses, Additionally, PTX 3 is also involved in reducing inflammation and aiding in the elimination of viruses. Although its exact mechanism of action is not fully understood, it is known to be involved in the recognition of viral proteins and the activation of immune cells [11].

Human immunodeficiency virus (HIV) is an infection that primarily infects CD4+ T cells, macrophages, and dendritic cells, weakening a body's immunity against opportunistic infections [18, 19]. Globally, approximately 38 million people suffer from HIV, of which 54% reside in South Africa. [20]. Within the South African population, 24% of HIV infections occur in women between the ages of 15 to 49 years old [21]. Approximately 90% of the women in Eastern and Southern Africa have access to antiretroviral therapy (ART) [20]. HIV treatment remains the same for both pregnant and non-pregnant women, however, the risk of mother-to-child transmission may be reduced by ART throughout pregnancy [22].

In light of the high maternal mortality emanating from PE and HIV infection it is imperative that we understand the synergy of both conditions. Thus, it is crucial to identify the association of biomarkers PTX 3 and CRP within the endothelial damaged hyperinflammatory micro-environment of PE co-morbid with HIV. This understanding is essential for early detection, enabling improved and timely management of this co-morbidity.

Methods and materials

Ethics Approval

This prospective study received institutional ethics approval (BE/5735/2023) for use of retrospectively collected plasma samples (BCA338/17). Women were recruited from a large regional hospital in eThekwini, KwaZulu-Natal, South Africa.

Study Population

Sample size

The sample size was determined after consultation with an institutional biostatistician using G*Power statistical software. A sample of size of 80 pregnant women was required to detect a median effect size of 0.3 between the two larger groups: normotensive and pre-eclamptic or HIV positive and HIV negative, assuming equal groups (n=40 per group). To compare four groups: normotensive (HIV-positive versus HIV-negative) and preeclamptic (HIV-positive versus HIV-negative); assuming equal groups (n=20 per group) to detect a large effect size of 0.45. All calculations are with a probability of 95% and a power of 80%. Sample size calculated.

Irrespective of CD4 cell count, all HIV-positive study participants received ART and PMTCT therapy during pregnancy and breastfeeding. Women receiving ARV treatment were given either a single medication, such as zidovudine, often referred to as azidothymidine (AZT), or a combination of many medications, including efavirenz (EFV), tenofovir disoprovil fumarate (TDF, Viread), and emtricitabine (FTC, Emtriva). In accordance with South African National HIV guidelines, an alternate medicine combination consisting of Abacavar (ABC, Ziagen), Lamivudine (3TC, Epivir), and Efavirenz (EFV) was given to a few patients together with PMTCT (nevirapine). Nevirapine prophylaxis was given to newborns exposed to HIV for 4-6 weeks.

Inclusion criteria **-** Women diagnosed with early on-set pre-eclampsia (EOPE); with a blood pressure reading of ≥ 140/90 mmHg and new-onset of significant proteinuria. Women with normotensive pregnancy. HIV status is known; both HIV-positive and HIV-negative women were considered in the study.

Exclusion criteria **-** Women who did not consent to participate and did not provide informed consent; women without antenatal care; Women with an unknown HIV status; women diagnosed with late on-set pre-eclampsia (LOPE); and Women with the following ailments: polycystic ovarian syndrome; eclampsia; pre-existing seizure disorders; intrauterine death; abruption placentae; chorioamnionitis; chronic hypertension; gestational diabetes; chronic diabetes; systemic lupus erythematous; chronic renal disease; thyroid disease; sickle cell disease; antiphospholipid antibody syndrome; cardiac disease; connective tissue disorder; asthma was excluded from the study.

Immunoassay

Plasma samples, stored at -80°C, were used and underwent an immunoassay using ProcartaPlex™ kits [ThermoFisher, HU basic kit (EPX010-10420-901); HU CRP simplex (EPX01A-10288-901); HU PTX 3 simplex (EPX010-12281-901)]. Only CRP samples underwent a dilution of 1:500.

Statistical analysis

GraphPad Prism 5.00 for Windows was used to analyse the data. Because of the non-parametric nature of the distribution, the results are shown as the median and interquartile range (IQR). The statistical significance of each pregnancy type (normotensive versus PE) and HIV status (negative versus positive) was assessed using the Mann-Whitney U test. One-way ANOVA was performed to evaluate statistical significance for each group, and the Kruskal-Wallis test was employed. The reported statistical significance was $p < 0.05$.

Data analysis techniques

Data obtained in this study was captured using a Bio-plex 200 instrument equipped with Bio-Plex® MAGPIX™ Multiplex system and analysed using Bio-Plex Manager™ analysis software version 6.1. A standard curve of each analyte was generated using the known concentration (pg/ml) by plotting the median fluorescent intensity (MFI) signal against concentration. The standard curve was then used to interpolate the concentration of the unknown samples. This data was then imported into an Excel spreadsheet for statistical analysis and converted from pg/ml to mg/l.

Results

Patient demographics and clinical characteristics

Patient demographics and clinical characteristics (Table 1) were non-parametrically distributed hence, represented by median and interquartile range (IQR). Significant differences were observed in maternal age ($p = 0.0163$); parity ($p < 0.0001$); gravidity ($p < 0.0001$); gestational age at birth ($p = 0.0001$); systolic blood pressure ($p < 0.0001$); diastolic blood pressure ($p = 0.0043$); Body Mass Index (BMI) ($p = 0.0102$); and baby weight ($p = 0.0044$) across the study groups. Parity and gravidity differences may however reflect patient selection. There were no significant differences of placental weight ($p > 0.1000$) across the study groups.

Table 3 Patient demographics across the study group

	Normotensive HIV – (n = 20)	**Normotensive HIV + (n = 20)**	**Pre-eclamptic HIV – (n = 20)**	**Preeclamptic HIV + (n = 20)**	***p*-value**
Maternal age (years)	24 (26-19)	27 (30-23)	27.5 (30.50-19.75)	28 (30-23)	0.0163 (*)
CD4+ count	-	404 (585-253)	-	413 (514.5-292)	>0.1000 (ns)
Parity	1 (1-0)	2 (2-1)	1.5 (2.250-0)	1 (2-0)	<0.0001 (****)
Gravidity	2 (2-1)	3 (3-2)	2.5 (3-1)	2 (3-1)	<0.0001 (****)
Gestation age at birth (weeks)	40 (40.50-37.50)	39 (39.25-37.00)	31 (34.50-28)	31.5 (36.75-29.25)	0.0001 (***)
Systolic BP (mmHg)	119 (128-106)	117 (123-110)	167 (180.8-159.3)	171 (180-162)	<0.0001 (****)
Diastolic BP (mmHg)	70.5 (82-66)	72 (77-70)	102.5 (112.8-94.25)	104 (114-97)	0.0043 (**)
BMI	33 (36-31)	28 (31-26)	29.5 (33.50-25.75)	28 (36-26)	0.0102 (*)
Baby weight (kg)	3.64 (3.843-3.110)	3.16 (3.300-2.970)	1.8 (2.5-1.195)	1.4 (2.590-0.98)	0.0044 (**)
Placental weight (kg)	0.67 (0.713-0.586)	0.59 (0.650-0.500)	0.485 (0.643-0.338)	0.42 (0.568-0.318)	>0.1000 (ns)

Data is represented as the median (IQR)

BP=blood pressure; BMI = body mass index; ns= non-significant

ns = 0.1234; * -= 0.0332; ** -= 0.0021; *** < 0.0001

Table 2: Plasma concentrations of CRP and PTX 3 (ng/ml) across all study groups.

	Normotensive pregnancy		**Pre-eclamptic pregnancy**	
	HIV –	**HIV +**	**HIV –**	**HIV +**
CRP (mg/l) (n = 80)	43.30 (43.30-2.661)	44.13 (70.61-2.650)	38.58 (38.58-4.176)	37.54 (37.54-6.234)
PTX 3 (mg/l) (n = 80)	0.040 (0.061-0.028)	0.076 (0.202-0.047)	0.258 (0.833-0.119)	0.299 (0.519-0.062)

All values are represented as median (IQR); CRP = c-reactive protein; PTX 3 = pentraxin 3; HIV= human immunodeficiency virus

Plasma concentration of Pentraxin 3

Pregnancy type: Pentraxin 3 concentrations were significantly higher in PE [0.279 mg/l; IQR = 0.651-0.103 mg/l] compared to normotensive [0.049 mg/l; IQR = 0.111-0.039 mg/l] groups, irrespective of HIV status [Mann-Whitney U = 353; p = <0.0001; FIGURE 1A]

HIV status PTX 3 concentrations showed no significance in HIV-negative [0.100 mg/l; IQR = 0.289-0.039 mg/l] compared to HIV-positive [0.148 mg/l; IQR = 0.4594-0.04750 mg/l] groups, irrespective of pregnancy type [Mann-Whitney U = 635; p = 0.371; FIGURE 1B]

Across all groups: PTX 3 concentrations was significantly different between normotensive HIV-negative [0.040 mg/l; IQR = 0.061-0.028 mg/l] versus PE HIV-negative [0.258 mg/l; IQR = 0.833-0.119 mg/l] groups (p = 0.0001); and normotensive HIV-negative [0.040 mg/l; IQR = 0.061-0.028 mg/l] versus PE HIV-positive [0.299 mg/l; IQR = 0.519-0.062 mg/l] groups (p = 0.0049) [Kruskal-Wallis statistic = 20.05; FIGURE 1C]

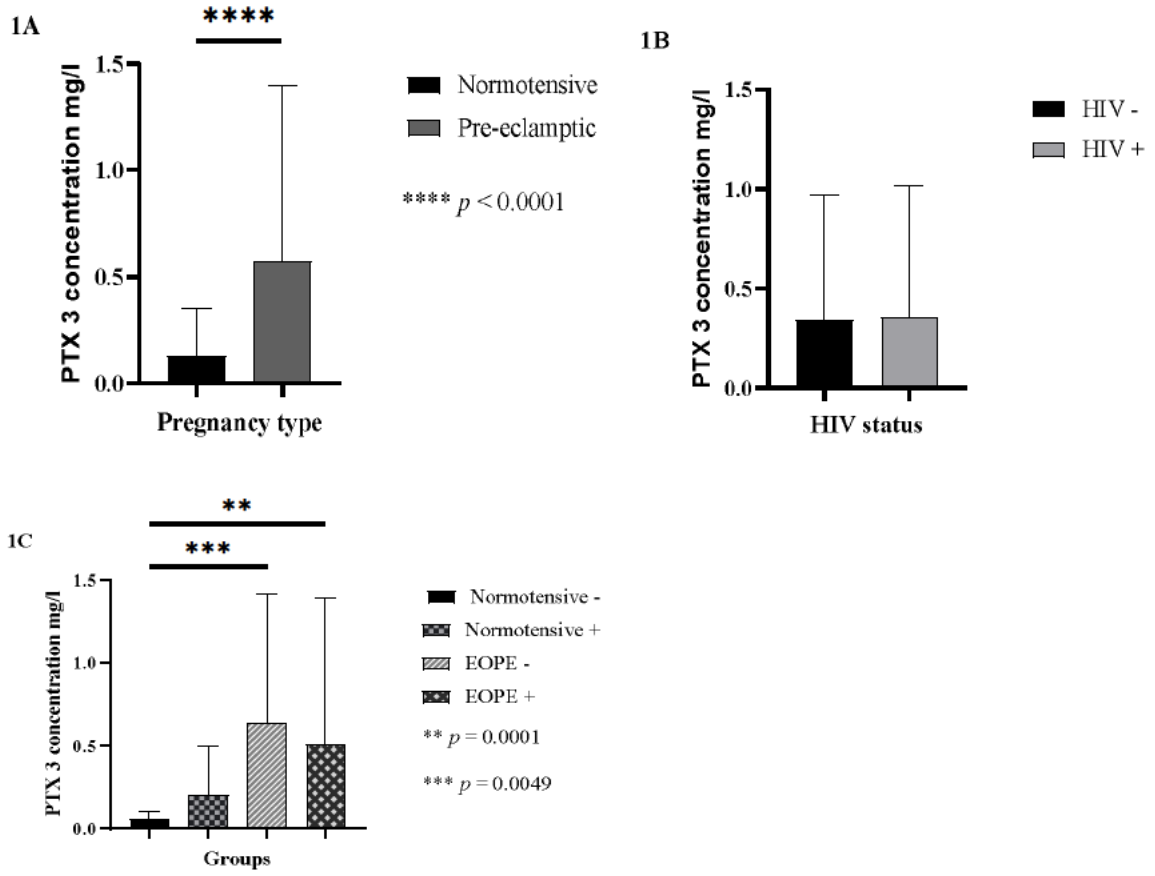

Figure 1(A-C): ***PTX 3 concentrations: (A)*** *Normotensive versus Preeclamptic groups,* ***(B)*** *HIV infected versus HIV uninfected groups, and* ***(C)*** *across all groups.* H

Plasma concentration of c-reactive protein

Pregnancy type: C-reactive protein concentrations was not statistically different between normotensive pregnant [43.30 mg/l; IQR = 63.78-2.650 mg/l] and PE [37.540 mg/l; IQR = 38.58-5.3 mg/l] groups, irrespective of HIV status [Mann-Whitney U = 602; p = 0.055; FIGURE 2A].

HIV status: Based on HIV status, CRP concentrations was not significantly different between HIV-negative [38.580 mg/l; IQR = 43.30-3.600 mg/l] versus HIV-positive [37.540 mg/l; IQR = 62.34-3.176 mg/l] groups, irrespective of pregnancy type [Mann-Whitney U = 769; $p = 0.767$; FIGURE 2B].

Across all groups: C-reactive protein concentrations did not differ across the study population; Normotensive HIV-negative [43.300 mg/l; IQR = 43.30-2.661 mg/l]; normotensive HIV-positive [44.130 mg/l; IQR = 70.61-2.650 mg/l] groups, PE HIV-negative [38.580 mg/l; IQR = 38.58-4.176g/l]; and PE HIV-positive [37.540 mg/l; IQR = 37.54-6.234 mg/l] groups [Kruskal-Wallis statistic = 4.264; $p = 0.230$; FIGURE 2C]

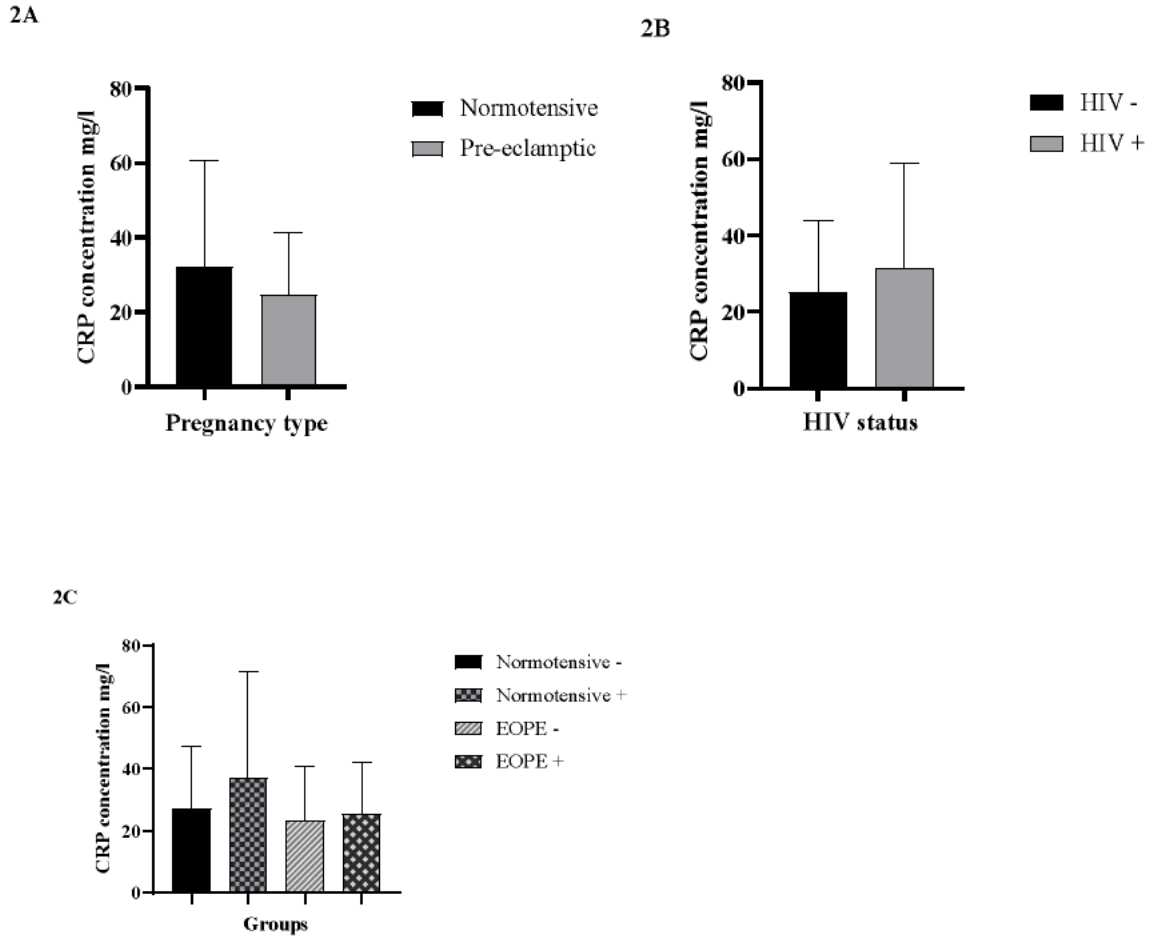

Figure 2 (A-C): ***CRP concentrations: (A)*** *Normotensive versus Preeclamptic groups,* ***(B)*** *HIV infected versus HIV uninfected groups, and* ***(C)*** *across all groups.*

Discussion

Pentraxin 3

A main finding of this study was a significant up-regulation of PTX 3 in PE compared to normotensive pregnancy, regardless of HIV status ($p < 0.0001$) and across the study population ($p = 0.0002$). These findings are consistent with previous studies [23-25]. Our results are also corroborated by Cetin et al (2009) who observed a significantly higher PTX 3 level in PE compared to normotensive pregnancy ($p = 0.0046$). Furthermore, a meta-analysis study

highlighted that PTX 3 concentration in EOPE or severe PE is up-regulated compared to LOPE or mild PE (p = 0.01) [25].

In PE a failure to establish adequate uteroplacental blood flow causes hypoxia, promoting a state of oxidative stress and exaggerated inflammation [26]. This excessive inflammation increases the levels of pro-inflammatory factors such as interleukin-1 (IL-1) and TNF- α, which synthesize PTX 3 and activate vascular and placental endothelial cells thereby leading to endothelial dysfunction [25].

Pentraxin 3 is produced at sites of inflammation, and it is intimately linked to endothelial dysfunction [27]. Since PE is a hyperinflammatory condition concomitant with endothelial injury it is expected that the concentrations of this inflammatory marker as observed in our study would be exacerbated in the synergy of PE co-morbid with HIV infection. The complement system is also activated during endothelial dysfunction, PTX 3 directly binds to C1q, which serves as the initial component of the pathway. Inflammation causes PTX 3 release in PE co-morbid with HIV infection, which activates C1q release and boosts C3 and C4 deposition. This induces the release of cytokines, including IL-1, IL-6, and TNF-α. [28, 29].

Furthermore, opsonization of apoptotic cells by PTX 3 affects the clearance of lipid-loaded macrophages and foam cells [25]. PTX 3 may promote T lymphocyte transformation into type 1 T helper cells and hinder the recognition ability of dendritic cells on late apoptotic cells. This could result in an imbalance of apoptosis, which may contribute to the development of PE. As a result, it is suggested that PTX 3 may increase the risk of PE by impairing the function of placental vascular endothelial cells [25]. It is plausible that the elevated PTX 3 in PE as observed in our study may explain the elevated apoptosis; moreover, severe PE is characterised by vascular foam cells within athetotic lesions [30]. Xiong et al (2020) suggests that PTX 3 may be a novel marker for EOPE.

We also report that HIV infection did not influence PTX 3 concentration, irrespective of pregnancy type (p = 0.371). It is well established that ART reconstitutes immune response thereby influencing TNF-α and IL-1 which inhibits PTX 3 activation. Previous studies have shown that HIV-infected patients have a lower concentration of PTX 3 than those who are ART naïve [31, 32]. However, Minami et al (2020) found that low plasma PTX 3 concentrations correlated with the duration of ART, however, HIV-related variables such as CD4+ T cell counts, HIV-RNA load before ART, and the type of ART did not correlate with plasma PTX 3 levels [33]. Nonetheless, PTX 3 specifically binds to virus-infected cells, rather than uninfected cells, implicating a selective impact during viral infection [34].

C-reactive protein

During normal pregnancy, CRP levels vary across trimesters (2.39 mg/l, 2.44 mg/l, 4.12 mg/l, respectively) [35]. CRP levels in PE compared to normotensive pregnancies are variable, being both up-regulated [23, 36-38] and down-regulated [39] in PE. In our study, we observed a borderline, non-significant difference in CRP concentration between PE and normotensive pregnancies ($p < 0.055$). This difference is supported by the aforementioned studies; however, it may also reflect the sample size, disease severity and gestational age. C-reactive protein levels are associated with lower birth weight and hence would be compromised in EOPE [40]. Moreover, in EOPE the low levels of IL-6, a pre-requisite for CRP synthesis may pre-empt a dysregulation of CRP. [41]. Nonetheless, CRP is an inflammatory marker and was expected to be elevated in PE, a hyperinflammatory condition. It has been reported that the absence of variation in CRP levels during EOPE may

indicate that the maternal inflammatory response in the second trimester of pregnancy is not heightened before the appearance of clinical signs of PE [38].

Our study also demonstrates that HIV infection, regardless of pregnancy type does not affect CRP concentration ($p = 0.371$) with CRP levels being higher in HIV infection than HIV naïve groups. It is controversial that this acute phase protein is elevated in HIV infection compared to HIV naïve patients [42, 43]. Previous studies have reported that the high apoptosis, as occurs in HIV infection may promote the production of CRP, a scavenger for chromatin released from apoptotic cells [44, 45].

It is plausible that ART usage may have influenced CRP concentration in the HIV cohort since ART is a standard of care for HIV infection in South Africa. A higher CRP concentration has been associated with AIDS progression, independent of Highly active antiretroviral treatment usage however, lower levels of CRP are believed to predict longer survival rates of HIV-infected patients [46]. Switching antiretrovirals may have a substantial effect on inflammation [47]. Antiretroviral therapy reduces systemic inflammation. However, levels do not eliminate the inflammatory response. Integrase inhibitors reduce inflammation to a greater extent than NNRTIs [48]

Silva et al (2013) reported that patients who switched from enfuvirtide to raltegravir had significant decreases in CRP, D-dimer, and IL-6 levels [49]. The same was observed when patients switched from a ritonavir-boosted PI or NNRTI treatment to raltegravir [50]. In contrast, Hileman and Funderburg (2017) reported elevated CRP levels in patients receiving efavirenz.

PIs such as lopinavir, darunavir and NNRTIs like nevirapine have been known to cause hepatitis, with NNRTIs having the most severe reactions [51].

In the synergy of PE co-morbid with HIV infection, hepatic dysfunction may exist. HIV is known to cause non-alcoholic fatty liver disease [52], whilst EOPE is associated with elevated liver enzymes and/or liver damage [26, 39]. Since CRP is produced in the liver [9], liver dysfunction may be responsible for the elevated CRP concentrations in the EOPE positive compared to the normotensive positive and negative groups in our study.

Conclusion

The novel study demonstrates a significant up-regulation of PTX 3 concentrations in PE versus normotensive pregnancies, regardless of HIV status. This elevation may originate from the oxidative stress micro-environment which predisposes vascular foam cells and IL1-6 release within the hyperinflammatory condition of PE. CRP concentrations were similar by pregnancy type, HIV status and across study groups, possibly attributed to hepatic dysfunction, IL-6 production, sample size, gestational age and/or disease severity. In the synergy of HIV infection and PE, PTX 3 and CRP concentrations were not significantly different across groups and may be attributable to highly active antiretroviral treatment and switching of ART regimens during pregnancy (fig 3).

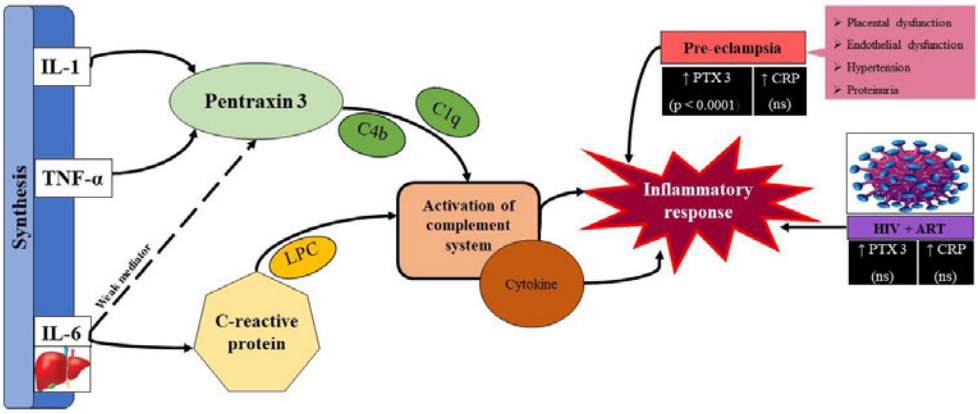

Figure 3 *Graphical abstract*

ART = antiretroviral therapy; HIV= human immunodeficiency virus; LPC= lysophosphatidylcholine ; 1L-1= interleukin -1; IL-6= interleukin -6; TNF-α= tumor necrosis factor α

Declarations

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Ethics approval

Ethical approval was obtained from the institutional Biomedical Research Ethics Committee.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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CHAPTER 4

4. SYNTHESIS

Despite a global reduction in the maternal mortality ratio since 2000 deaths (World Health Organization, 2023b), South Africa had a maternal mortality rate of 148.1 maternal deaths per 100 000 live births, in 2021 (Department of Health South Africa, 2021a). Hypertensive disorders are the main direct cause of maternal deaths in South Africa, with pre-eclampsia accounting for the majority of deaths emanating from hypertensive disorders (Department of Health South Africa, 2021a).

South Africa is the epicentre of the global HIV pandemic (World Health Organization, 2022). Moreover, it is a dilemma that 40.5% of pregnant women are HIV-positive (Department of Health South Africa, 2021a). Anti-retroviral therapy reconstitutes immune response (Fahme *et al.*, 2018). Furthermore, the effect of ART on inflammatory markers in the co-morbidity of HV infection and PE remains unknown.

Since both PE and HIV are known inflammatory conditions, it is important to investigate Women living with HIV who also have PE. This study therefore examined two inflammatory markers: CRP and PTX 3 in the synergy in both these conditions.

4.1 Pentraxin 3 and c-reactive protein in the co-morbidity of HIV infection and pre-eclampsia

C-reactive protein and PTX 3, are both acute inflammatory markers belonging to the pentraxin family (Yamasaki *et al.*, 2009). C-reactive protein is produced in the liver, induced by various cytokines, namely interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α), which are primarily secreted by macrophages and monocytes in inflammatory locations (Kristensen *et al.*, 2009). It primarily contributes to inflammation by activating the C1q molecule in the complement pathway, resulting in the opsonization of pathogens (Sproston and Ashworth, 2018). Pentraxin 3 is produced at the site of infection in response to pro-inflammatory conditions by macrophages, monocytes; epithelial cells; polymorphonuclear cells; endothelial cells; dendritic cells; and fibroblasts. Similar to CRP, PTX 3 is also responsible for the activation of the classical complement system. Specifically, PTX 3 forms a bond with C1q, the initial component of the classical complement system, triggering the activation of the pathway and the deposition of C3 and C4 fragments on target cells (Bally *et al.*, 2019). Additionally, PTX 3 hinders the lytic activity of C1q, limiting its binding to antibody-sensitized red blood cells. resulting in the inhibition of the classical complement pathway (Kunes *et al.*, 2012).

During normal placental development, the extravillous trophoblast cells invade maternal spiral arteries, gradually replacing decidual endothelial cells, resulting in the remodelling of spiral arteries (Sato *et al.*, 2012). However, during PE deficient trophoblast cell invasion, and non-physiological conversion of myometrial spiral arteries (Hong *et al.*, 2021). This causes hypoxia resulting in widespread endothelial dysfunction and an increased inflammatory response (Agostinis *et al.*, 2021). The inflammatory

response, during PE, is largely influenced by the complement system; this is evident by the increased levels of C1q, C3a, C5a, and the C5b-9 complex (Agostinis *et al.*, 2021).

Although the link between PE and HIV infection is still uncertain, studies have shown that during HIV infection the presence of C5a, which is linked to higher levels of pro-inflammatory cytokines such as TNF-α and IL-6, may amplify HIV-1 infection (David and Naicker, 2023). The inhibition of C5a receptor may reverse this enhancement of HIV infection. Therefore, excessive complement activation may worsen the chronic inflammatory state seen in the duality of HIV infection and PE (Pillay *et al.*, 2019).

4.2 The role of C-reactive protein and Pentraxin 3 in HIV-associated pre-eclampsia.

This study focused on the evaluation of CRP and PTX 3 concentrations in normotensive pregnant and PE women stratified by HIV status in women of South African ancestry.

Furthermore, to ensure the homogeneity of the PE group all participants in this study were diagnosed with EOPE. In contrast to LOPE, the aetiology of EOPE involves defective placentation and is associated with adverse maternal and perinatal outcomes (Guo *et al.*, 2021; Jehangir *et al.*, 2023). Early onset pre-eclampsia is linked to an increased risk of multi-organ dysfunction, including the liver, haematological systems, and kidneys.

4.3 Pentraxin 3 and c-reactive protein concentration by pregnancy type

The main findings of this study were a significant up-regulation of plasma PTX 3 concentration in PE compared to normotensive pregnancy [p = <0.0001], irrespective of HIV status. This elevation of PTX 3 in the hypoxic microenvironment of PE is corroborated by other studies (Turkmen *et al.*, 2015; Colmenares-Mejia *et al.*, 2020; Xiong *et al.*, 2020). Pentraxin 3 is known as an early predictor for PE, with levels higher in women with PE compared to normotensive pregnancies within the first trimester (Garg *et al.*, 2018). Endothelial dysfunction triggers an inflammatory response, releasing cytokines and growth factors, into the maternal circulatory system (Colmenares-Mejia *et al.*, 2020).

Activation of the inflammatory system in PE results in an up-regulation in plasma levels of inflammatory cytokines, such as TNF-α and IL-6 (Suzuki *et al.*, 2008). Pentraxin 3, plays a crucial role in the initial stages of inflammation and is synthesized in response to primary inflammatory signals such as IL-1 and TNF-α, thereafter triggering the classical complement pathway (Kunes *et al.*, 2012).

We report a weak non-significant difference in CRP based on pregnancy type [$p = 0.055$], irrespective of HIV status. This is an unexpected finding as PE is regarded as a hyperinflammatory condition

(Homera *et al.*, 2008). This weak, non-significance ($p < 0.055$) may be attributed to our small sample size. Previous studies have reported an elevation of plasma CRP in PE compared to those with normotensive pregnancies (Suliman *et al.*, 2022).

However, PE is associated with pathology of the liver and the kidney (Agostinis *et al.*, 2021). Since CRP is synthesised in the liver, damage to this organ may significantly impair its production. Patients with liver dysfunction exhibited a downregulation of CRP concentrations compared to patients without liver dysfunction (Mackenzie and Woodhouse, 2006).

4.4 Pentraxin 3 and c-reactive protein concentration by HIV-infection and ART effect

This study also reports an absence of a significant difference for both plasma CRP; and PTX 3 concentrations between HIV-positive and HIV-negative groups, regardless of pregnancy type.; Of-note all HIV-infected women in our study received ART, a standard of care management in South Africa. Despite immune restoration with ART, the duration of ART (pre- or intra-pregnancy) was unknown. The extended use of ART is believed to enable lipopolysaccharides to induce endothelial dysfunction (Fahme *et al.*, 2018). When HIV-infected monocytes and macrophages are exposed to lipopolysaccharides, they bind to CD14 to form an LPS/CD14 complex. This complex activates TLR4, which in turn promotes the production of pro-inflammatory cytokines. The lymphoid tissue is impacted by chronic immune activation and inflammation because it increases the production of TGF-β, which in turn stimulates the production of collagen. This process replaces the fibroblastic reticular network and results in a progressive loss of naïve T cells. (Zicari *et al.*, 2019; Teer *et al.*, 2021).

Additionally, there is a high occurrence of metabolic risk factors and non-alcoholic fatty liver disease in patients living with HIV infection (Michel *et al.*, 2023). This may suggest that even though CRP is an effective inflammation marker, it may not be the most reliable indicator when looking at EOPE patients with HIV. However, additional research should be conducted to investigate this further.

4.5 Pentraxin 3 and c-reactive protein concentration in the co-morbidity of PE and HIV infection

We report a significant difference in PTX 3 concentration across the study population (the synergy of pregnancy type and HIV status) ($p = 0.0002$). More specifically PTX 3 concentrations were significantly different between normotensive HIV-negative versus PE HIV-negative groups ($p = 0.0001$); and normotensive HIV-negative versus PE HIV-positive groups ($p = 0.0049$). In contrast, we also report that CRP concentration did not differ across the study population ($p = 0.230$). These unexpected results may be attributed to immune reconstitution induced by ART. Systemic inflammation is down-regulated by ART, however, does not stop the inflammatory reaction. In contrast to NNRTIs, integrase inhibitors reduce inflammation more effectively (Hileman and Funderburg, 2017).

Moreover, the long-term usage of ART significantly reduces systemic inflammation and immune activation (Hileman and Funderburg, 2017), which may result in the non-significant difference of both CRP and PTX 3 as noted in our study.

4.6 Limitations

This study utilized plasma obtained at term, therefore gestational age may be a limitation. Furthermore, the small sample size and unknown duration of ART may have confounded results.

4.7 Conclusions

This novel study demonstrated elevated pentraxin 3 concentrations in pre-eclampsia compared to normotensive pregnancies, irrespective of HIV status. This up-regulation emanates from oxidative stress and inflammation within the hypoxic micro-environment of pre-eclampsia. Conversely, c-reactive protein levels were lower, albeit not significantly different in pre-eclampsia compared to normotensive pregnancies, regardless of HIV status. This decline may be attributed to hepatic dysfunction, gestational age, disease severity, interleukin-6 production, and sample size. We also report no similar pentraxin levels by HIV status, probably due to the immune restorative effects of antiretroviral therapy. The intricate interplay between these inflammatory markers provides a comprehensive insight into the complex pathophysiology of HIV-associated pre-eclampsia, laying the groundwork for potential therapeutic interventions.

4.8 Recommendations for future research

Future research should be conducted on a larger sample size, with a comparison of data across the different trimesters of pregnancy. Additionally, other members of the pentraxin family should be investigated in the synergy of HIV comorbid PE, in order to understand their inter-relationships and downstream effect on signalling pathways.

CHAPTER 5

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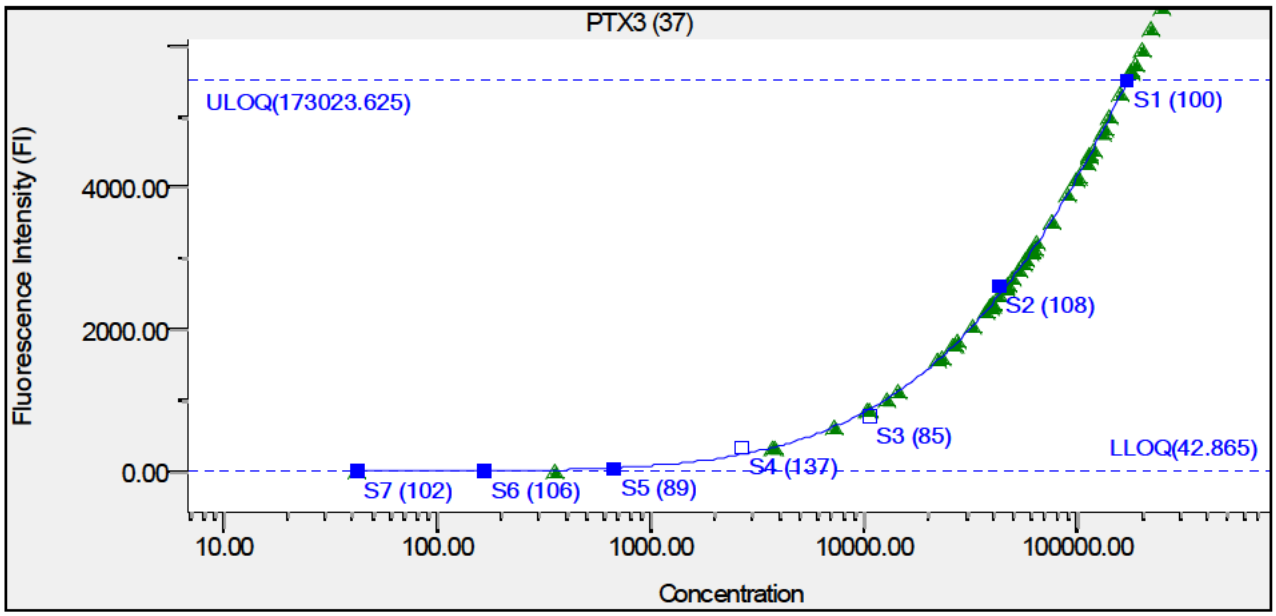
CHAPTER 6

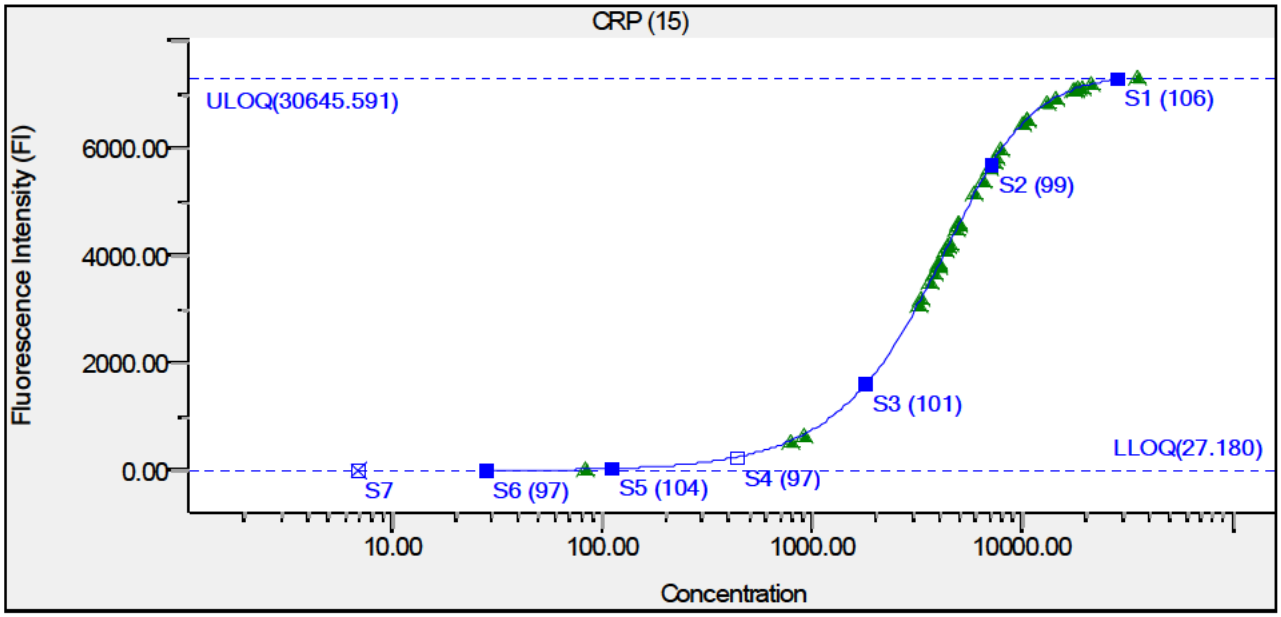
6.0 ADDENDUM

6.1 Standard curves

6.1.1 Pentraxin 3



6.1.2 C-reactive protein



6.2 Ethical approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

12 June 2023

Miss Thalia Moodley (217002747)
School of Lab Med & Medical Sc
Westville

Dear Miss Moodley

Protocol reference number: BREC/00005735/2023
Project title: C a€. reactive protein and pentraxin 3 in the co-morbidity of HIV infected pre-eclampsia
Degree Purposes: MMedSc

EXPEDITED APPLICATION: APPROVAL LETTER
(sub-study of BCA338/17)

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 as a sub-study of BCA338/17 and may begin as from 12 June 2023. 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 12 June 2023. 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.

[redacted]