

**GENE POLYMORPHISMS OF URIC ACID RELATED PROTEINS AND THE  
ANGIOTENSIN RECEPTOR IV (AT4) IN PRE-ECLAMPSIA**

By

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in the

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Doris Duke Medical Research Institute  
College of Health Sciences  
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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 and The National Institute of Health, United States of America under the supervision of Professor T. Naicker and Professor J Moodley.



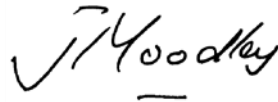
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**(Co-supervisor)**

## DECLARATION

I, Olive Pearl Khaliq declare that:

- (i) The research reported in this dissertation original, except where otherwise indicated.
- (ii) This dissertation has not been submitted for any degree or examination at any other university.
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Signed: \_\_\_\_\_

Date: 14/11/2019

## **DEDICATION**

To God Almighty

*For his guidance, protection and blessings*

My Family

*I would like to dedicate this thesis to the Khaliq and McPherson families for believing in me, supporting me and encouraging me to be where I am today. You are the source of my success and I thank you all from the bottom of my heart.*

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## PUBLICATIONS

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## CONTENTS

PREFACE .....	ii
DECLARATION .....	iii
DEDICATION .....	iv
FUNDING.....	v
PUBLICATIONS .....	vi
ACKNOWLEDGMENTS.....	vii
CONTENTS .....	viii
TABLE OF FIGURES .....	x
ABBREVIATIONS.....	xi
ABSTRACT.....	xiii
ABSTRACT-ISIZULU .....	xiv
THESIS LAYOUT.....	xv
CHAPTER 1.....	1
BACKGROUND AND LITERATURE REVIEW .....	2
1.1 INTRODUCTION .....	2
1.2 PRE-ECLAMPSIA .....	2
1.3 CLASSIFICATION OF PRE-ECLAMPSIA.....	3
1.3.1 Superimposed pre-eclampsia.....	3
1.3.2 Pre-eclampsia .....	3
1.3.3 Sub-classification of pre-eclampsia.....	3
1.3.4 Eclampsia .....	4
1.4 PREVALENCE OF PRE-ECLAMPSIA .....	4
1.5 RISK FACTORS OF PRE-ECLAMPSIA .....	4
1.6 PATHOGENESIS OF PRE-ECLAMPSIA .....	5
1.7 THE ROLE OF GENETICS IN THE PATHOPHYSIOLOGY OF PRE-ECLAMPSIA....	7
1.8 URIC ACID AND PRE-ECLAMPSIA .....	8
1.8.1 Uric Acid Levels in Normal Pregnancies.....	8
1.8.2 Uric Acid Levels in Pre-eclampsia.....	8
1.8.3 Uric Acid Levels and Fetal Outcome .....	10
1.9 GENE POLYMORPHISM OF URATE TRANSPORTING PROTEINS .....	11



1.10 THE RENIN ANGIOTENSIN SYSTEM AND ANGIOTENSIN RECEPTOR IV (AT4) IN PE .....	12
1.11 PROBLEM STATEMENT AND SIGNIFICANCE .....	14
1.12 AIMS AND OBJECTIVES OF THE THESIS .....	15
1.12.1 Aims .....	15
1.12.2 Objectives.....	15
CHAPTER 2.....	2
Review Article: The Role of Uric Acid in Pre-eclampsia: Is Uric Acid a Causative Factor or a Sign of Pre-eclampsia.....	3
CHAPTER 3.....	14
Research Article: Gene Polymorphisms of Uric Acid are associated with Pre-eclampsia in South Africans of African Ancestry .....	15
CHAPTER 4.....	47
Research Article: Soluble Angiotensin IV Receptor Levels in Pre-eclampsia: Is there a Variation?48	
CHAPTER 5.....	70
Research Article: The Role of LNPEP and ANPEP Gene Polymorphisms in the Pathogenesis of Pre-eclampsia .....	71
CHAPTER 6.....	93
6.1 SYNTHESIS, CONCLUSIONS AND FUTURE RESEARCH.....	94
6.2 CONCLUSIONS .....	98
6.3 FUTURE RESEARCH.....	99
CHAPTER 7.....	101
REFERENCES.....	102
APPENDICES.....	114

## TABLE OF FIGURES

Figure 1: Thesis Layout.....	xv
Figure 2: Diagrammatic representation of the stages of pre-eclampsia .....	6
Figure 3: Levels of Uric Acid in Normal pregnancies compared to Pre-eclampsia.....	9
Figure 4: Schematic diagram of the RAAS System .....	13

## ABBREVIATIONS

ABCG2	ATP-Binding cassette super-family G member 2
ACE	Angiotensin Converting Enzyme
AGT	Angiotensinogen
AGTR1	Angiotensinogen receptor 1
ANG 1	Angiotensin 1
ANG II	Angiotensin II
ANG IV	Angiotensin IV
AT1R	Angiotensin 1 receptor
AT2R	Angiotensin 2 receptor
ELISA	Enzyme-Linked Immune Sorbent Assay
EOPE	Early onset Pre-eclampsia
GFR	Glomerular Filtration Rate
HAART	Highly active antiretroviral therapy
HELLP	Hemolysis, elevated liver enzymes, low platelet count
HDP	Hypertensive Disorders of Pregnancy
HIV	Human Immune Deficiency Virus
IUD	Intrauterine Death
IUGR	Intrauterine Growth Restriction
LOPE	Late onset Pre-eclampsia
NaCl	Sodium Chloride
PDKZ1	Postsynaptic density protein
PE	Pre-eclampsia
RAAS	Renin Angiotensin Aldosterone System
ROS	Reactive oxygen species
RUPP	Reduced utero-placental perfusion pressure
sAT-4	Soluble Angiotensin 4
SFI <sub>t</sub> -1	Soluble fms-like tyrosine kinase-1
SGA	Small for Gestational Age
SNP	Single Nucleotide Polymorphism
UA	Uric Acid

URAT 1	Urate Transporter 1
VEGF	Vascular Endothelial Growth Factor
XO	Xanthine Oxidase

## ABSTRACT

**Background:** Hypertensive disorders of pregnancy remain one of the major contributions to maternal and fetal morbidity and mortality around the globe. Pre-eclampsia is a hypertensive disorder of pregnancy which complicates 3-10% of pregnancies worldwide. It is a multi-organ disorder affecting the maternal system, thereby creating a major setback in terms of targeting the aetiology. One of the main organs disrupted is the kidneys and a dysregulation of uric acid levels and the renin angiotensin system have been implicated in pre-eclampsia. Therefore, the aim of this study is to investigate the gene polymorphisms of uric acid, aminopeptidase-N, the angiotensin receptor IV, and plasma levels of the receptor in pre-eclampsia compared to normotensive pregnancies.

**Materials and Methods:** This was a retrospective study consisting of 637 blood samples of which 280 were normotensives and 357 pre-eclamptic. Pre-eclampsia was subdivided into early (n=187) and late onset pre-eclampsia (n=170). DNA was isolated from blood samples using the Thermo Scientific GeneJet whole blood Genomic DNA purification mini Kit. Single nucleotide polymorphisms of uric acid (rs505802, rs1014290, rs12129861, rs2231142), the angiotensin receptor IV (rs18059) and aminopeptidase-N (rs6496603) were amplified using the TaqMan genotyping assay. Plasma levels of angiotensin receptor IV were also measured using the ELISA in pre-eclampsia and compared to normotensives.

**Results:** We found that rs505802 was higher in late onset pre-eclampsia compared to early onset pre-eclampsia and the normotensive group. We also observed a significant elevation of rs1014290 in early onset pre-eclampsia compared to late onset pre-eclampsia and the normotensive group. Gene polymorphisms of the angiotensin IV receptor (rs18059) and aminopeptidase-N (rs6496603) showed no significant association with pre-eclampsia. However, plasma levels of angiotensin IV receptor were significantly lower in pre-eclampsia than in normotensives. Furthermore, we found that the levels decreased with the severity of pre-eclampsia.

**Conclusion:** The single nucleotide polymorphisms of uric acid (rs505802, rs1014290) are associated with the pathogenesis of pre-eclampsia. Furthermore, plasma levels of angiotensin IV are decreased in pre-eclampsia, indicating a dysregulation of the renin angiotensin system in pre-eclampsia.

## ABSTRACT-ISIZULU

**Amathuluzi asetshenziwe:** Lolucwaningo lubizwa nge-retrospective lusebenzise amagazi awu 637 aphuma kwabanomfutho osesimweni sempilo (normotensives) abangu 280 Kanye nabanomfutho ophakeme wegazi kubakhulelwe (pre-eclamptic) abangu 357. Abanomfutho ophakeme wegazi baphinde bahlukaniswa izigaba ezimbili, ezibizwa nge early (n=187) ne late onset (n=170). Ufuzo egazini lutholakale ngokusebenzisa I Thermo Scientific GeneJet whole blood Genomic DNA purification mini Kit. Ama Single nucleotide polymorphisms e uric acid (rs505802, rs1014290, rs12129861, rs2231142), angiotensin receptor IV (rs18059) nawe aminopeptidase-N (rs6496603) acubulungwe ngokusebenzisa i- TaqMan genotyping assay. Izinga le-angiotensin receptor IV egazini likalwe ngokusebenzisa i-ELISA kwabanomfutho wamandla ophakeme kwabakhulelwe (pre-eclamptics) makuqhathaniswa nabanomfutho osesimweni sempilo (normotensive).

**Imiphumela:** Sithole ukuthi amazinga e rs505802 abephezulu ku late onset pre-eclampsia makuqhathaniswa ne early onset pre-eclampsia ne normotensive. Siphinde sabona ukukhuphuka kwenani le rs1014290 ku-early onset pre-eclampsia makuqhathaniswa ne late onset pre-eclampsia Kanye ne normotensive group. Ama gene polymorphisms we angiotensin IV receptor (rs18059) Kanye ne aminopeptidase-N (rs6496603) awakhombisanga mahluko ekuhlobaneni ne pre-eclampsia. Kodwa, inani le angiotensin IV receptor egazini abehlile kulabo abanomfutho wamadla ophakeme egazini (pre-eclampsia) makuqhathaniswa nalabo abanomfutho osesimweni sempilo (normotensive). Siphinde sathola ukuthi lokwehla kuhambisana nokubhebhetheka kwesifo somfutho wamadla ophakeme egazini.

**Ukuvala:** I single nucleotide polymorphisms ye uric acid (rs505802, rs1014290) ihlobene nokuqala kwesifo somfutho wamadla ophakeme egazini kwabakhulelwe (pre-eclampsia). Futhi, izinga le angiotensin IV egazini lehlile kulabo abanomfutho wamadla ophakeme egazini kwabakhululwe (pre-eclampsia), okukhombisa ukungalawuleki kwe renin angiotensin system kulabo abanomfutho wamadla ophakeme egazini (pre-eclampsia).

## THESIS LAYOUT

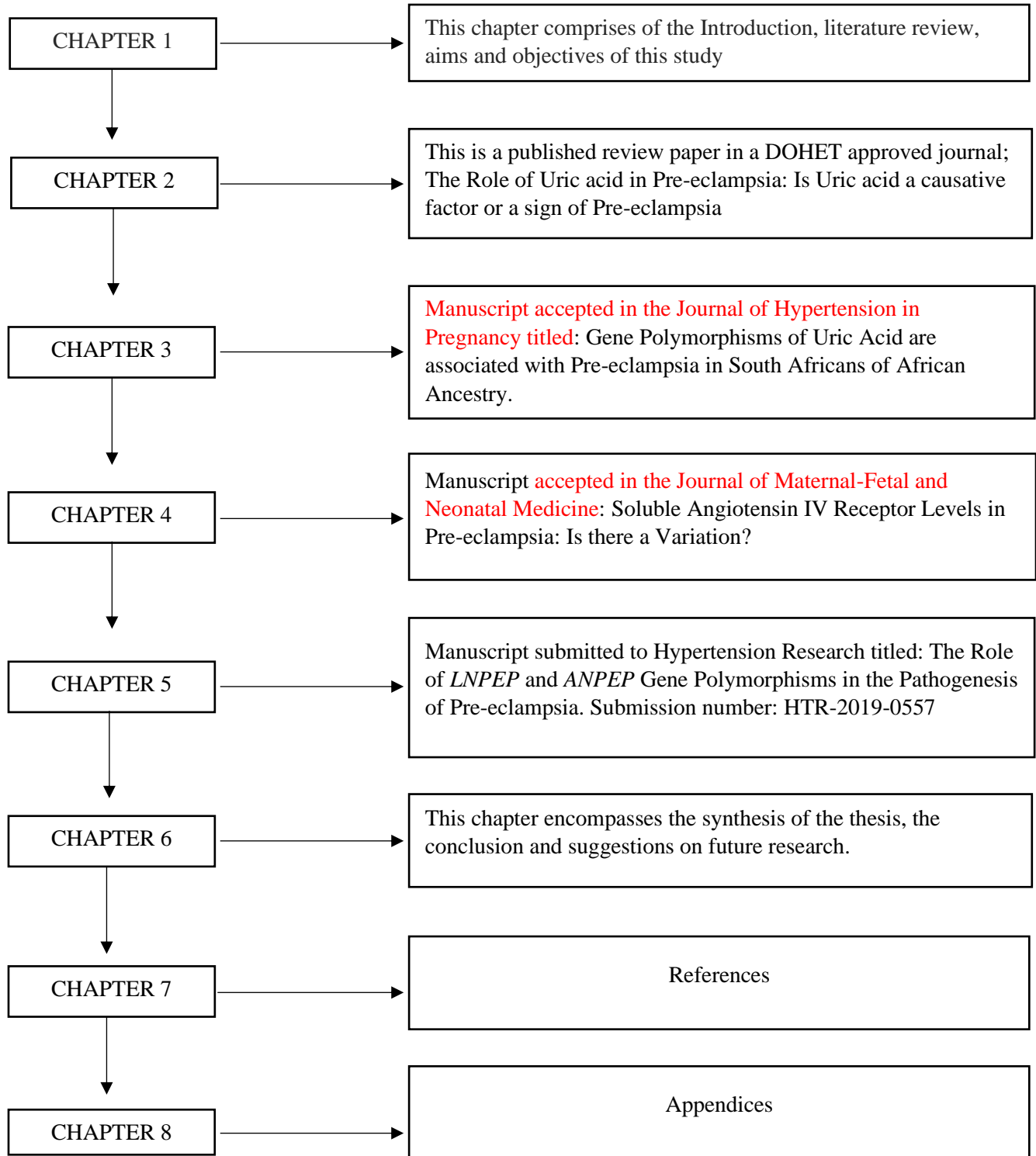


Figure 1: Schematic diagram showing layout of thesis

## **CHAPTER 1**



## **BACKGROUND AND LITERATURE REVIEW**

### **1.1 INTRODUCTION**

Hypertensive disorders of Pregnancy (HDP) are a major cause of maternal and neonatal morbidity and mortality worldwide (Brown *et al.*, 2018). In South Africa, HDP are the commonest direct cause of maternal deaths and account for approximately 18% of all deaths (Saving Mothers' Annual Report, 2017). Most of these deaths are due to severe pre-eclampsia and eclampsia and are predominantly ascribed to intracranial hemorrhage.

The International Society for the Study of Hypertension in Pregnancy classification of HDP has evolved through the years and is now categorized as follows:

- chronic hypertension occurring prior to pregnancy or for the first time at less than 20 weeks of gestation;
- white-coat hypertension, known as high blood pressure (> 140 systolic and or >90 diastolic mm Hg) that occurs at a workplace or a clinic but normal (130/85 mm Hg) at home;
- masked hypertension, which is defined as complicated and difficult to diagnose but occurs occasionally and usually detected by a 24-hour ambulatory monitoring device and
- gestational hypertension which is elevated blood pressure after 20 weeks of gestation without proteinuria.

It is accepted that if gestational hypertension occurs after 34 weeks of gestation, it may progress to pre-eclampsia (Brown *et al.*, 2018).

### **1.2 PRE-ECLAMPSIA**

Pre-eclampsia (PE) is a disorder unique to human pregnancies and is defined as new onset hypertension that occurs after 20 weeks of gestation, characterized by an aberrant increase in blood pressure (systolic blood pressure level of  $\geq 140$  mmHg and or a diastolic blood pressure level of  $\geq 90$  mmHg) and the presence of proteinuria above 0.3g in a 24 hour sample (Gathiram and Moodley, 2016). Pre-eclampsia also affects systemic organs such as the lungs, brain (eclampsia), kidney and the liver (HELLP syndrome) (Gathiram and Moodley, 2016). In some cases hypertension may occur without proteinuria but include complications such as thrombocytopenia, acute kidney dysfunction and fetal restriction (Brown *et al.*, 2018). Furthermore, it is a marker for long term complications

such as cardiovascular diseases and diabetes for both the mother and the baby (Brown *et al.*,2018). Currently delivery of the baby is the only cure as PE resolves with delivery of the placenta.

Pre-eclampsia is associated with hypo-perfusion of the placenta which results in placental ischemia and the release of placenta-derived factors into maternal circulation (Burton *et al.*, 2019). These factors trigger a systemic inflammatory response and vascular endothelial damage. Placenta ischemia also triggers uric acid production (Mador *et al.*,2013). Hyperuricemia precedes proteinuria and hypertension, possibly indicating uric acid involvement in PE pathogenesis (Nahar *et al.*,2020). However, it is unclear whether uric acid may be used as a marker for PE diagnosis (Zangana *et al.*,2018). Nonetheless, a correlation between increased uric acid (hyperuricemia) and maternal and fetal morbidity implicating its diagnostic value in predicting PE development has been reported (Zangana *et al.*,2018).

### **1.3 CLASSIFICATION OF PRE-ECLAMPSIA**

Pre-eclampsia is classified into superimposed pre-eclampsia, pre-eclampsia and eclampsia.

#### ***1.3.1 Superimposed pre-eclampsia***

Superimposed pre-eclampsia commonly occurs in women with chronic hypertension, resulting in maternal and fetal complications (Magee *et al.*, 2014). Abnormality of the kidneys may manifest in superimposed pre-eclampsia before 20 weeks of gestation and is characterized by increased blood pressure and proteinuria at 20<sup>th</sup> weeks of gestation (Watanabe *et al.*, 2013).

#### ***1.3.2 Pre-eclampsia***

Women with PE are at a risk of developing severe complications such as high levels of hypertension, renal diseases, cardiac diseases, and diabetes mellitus (Barrett *et al.*, 2014). The pathogenesis of PE involves deficient trophoblast migration emanating from increased trophoblast cell apoptosis (Naicker *et al.*, 2019). This defective trophoblast invasion leads to a lack of physiological conversion of spiral arteries within the myometrium (Labarrere *et al.*,2017). This leads to reduced utero-placental perfusion pressure (RUPP) and placental hypoxia (Natale *et al.*,2018).

#### ***1.3.3 Sub-classification of pre-eclampsia***

Pre-eclampsia may be sub-categorized according to gestational age and severity. Based on the onset of PE, it may be classified into:

- i) Early onset pre-eclampsia -  $\leq 33$  weeks 6 days of pregnancy.
- ii) Late onset pre-eclampsia-  $\geq 34$  weeks of pregnancy (Lisonkova and Joseph, 2013).

Based on its severity, this complication of pregnancy may be classified into mild and severe PE

- i) Mild pre-eclampsia is characterized by a blood pressure  $> 140/90 < 160/110$  mmHg, and proteinuria  $< 300$  mg but not more than  $2.0$  g in a 24-hour urine sample (++) on a dipstick (Watanabe *et al.*, 2013).
- ii) Severe pre-eclampsia is characterized by a blood pressure of greater than  $160/110$  mmHg and urinary protein concentration  $> 2.0$  g in a 24-hour urine sample (+++) on a dipstick (Watanabe *et al.*, 2013).

#### **1.3.4 Eclampsia**

This is a condition characterized by seizures associated with PE (Watanabe *et al.*, 2013). Factors such as cerebral edema and cerebral vasospasm are accountable for this condition (Soggiu-Duta *et al.*, 2019). Most cases of eclampsia occur during the antepartum period but may also occur during the intrapartum and postpartum periods (Mersha *et al.*, 2019).

### **1.4 PREVALENCE OF PRE-ECLAMPSIA**

Pre-eclampsia complicates 10% of pregnancies worldwide (Berhe *et al.*, 2018). More than 500 000 fetal and 70 000 neonatal deaths occur each year due to PE (Brown *et al.*, 2018). In Africa, 270 000 maternal deaths occur every year with approximately 76 000 attributed to PE development (Vata *et al.*, 2015). The incidence of PE and eclampsia is 5.8%, which is remarkably high considering the frequency is only recorded from Durban, South Africa and that the total frequency of HDP in South Africa is 12% (Moodley *et al.*, 2016).

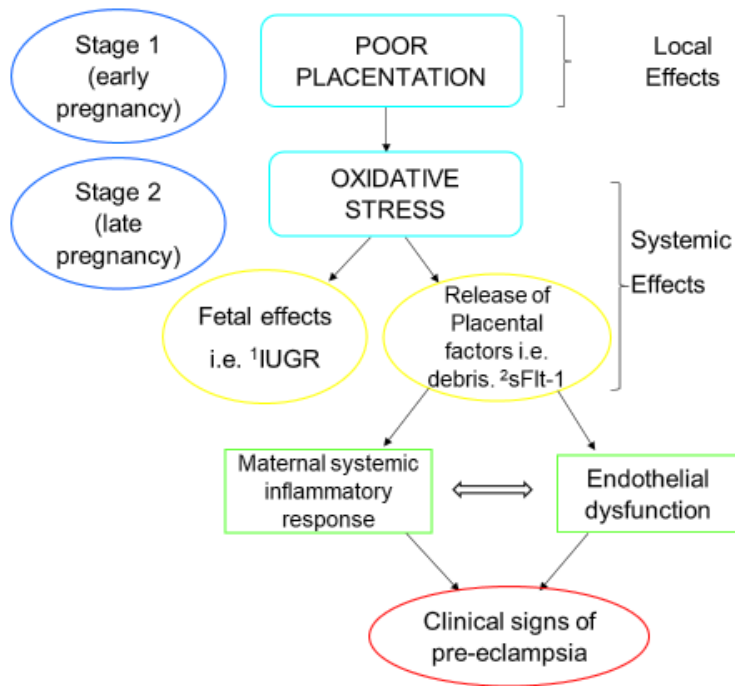
### **1.5 RISK FACTORS OF PRE-ECLAMPSIA**

The pathogenesis of PE is associated with pre-existing factors that include renal diseases, chronic hypertension, type 2 diabetes, autoimmune disorders and thrombophilia syndromes (Brown *et al.*, 2018). Other factors that increase the risk of PE development include: metabolic syndrome, maternal age (Grieger *et al.*, 2018), nulliparity (North *et al.*, 2011), insulin resistance (Hauth *et al.*,

2011), immune factors, nutrient deficiency (calcium, antioxidants, vitamins) and genetic disposition (Sánchez-Aranguren *et al.*, 2014).

## **1.6 PATHOGENESIS OF PRE-ECLAMPSIA**

Pre-eclampsia is a two-stage model condition (Figure 2). The first stage is associated with defective placentation and a lack of spiral artery remodeling that leads to a decrease in blood supply that meets the oxygen and nutrient needs of the developing fetus. This diminished oxygen supply, has a domino effect in that it causes placental hypoxia and ischemia, that lead to the second stage where, placental-derived particles and/or factors are released into maternal circulation (Naljayan and Karumanchi, 2013). These placental-derived particles contribute to the pathogenesis of PE as they have the capacity to initiate an exaggerated inflammatory response (Redman and Sargent, 2001), which leads to the widespread endothelial dysfunction that characterizes PE (Tannetta and Sargent, 2013).



**Figure 2: Diagrammatic representation of the stages of pre-eclampsia:**

Adapted from (Borzychowski et al.,2006). Poor placentation leads to abnormal spiral artery remodeling which promotes the release of placental factors or particles. These factors initiate a systemic inflammatory response with subsequent dysfunction of maternal vascular endothelium that manifests as the clinical signs of PE.

<sup>1</sup>Interuterine Growth Restriction; <sup>2</sup>Soluble fms-like tyrosine kinase-1.

## 1.7 THE ROLE OF GENETICS IN THE PATHOPHYSIOLOGY OF PRE-ECLAMPSIA

An alteration of genes at distinct loci may contribute to the predisposition of PE development. Furthermore, one gene may not be liable for all PE cases but a number of genes may contribute to subdivisions of the disease (Cox *et al.*,2011). To support this statement, a study was conducted to determine the subcategories of PE based on plasma membrane protein expression and three groups were identified: angiogenesis, mitogen-activated protein kinase signaling, and hormone biosynthesis and metabolism (Cox *et al.*, 2011). In addition, environmental factors may also contribute to the pathogenesis of PE, these include: physiological stress (Vianna *et al.*, 2011) and vitamin D deficiency (Naidoo *et al.*, 2019)

Maternal and fetal genetic functions have been reported to be in conflict during pregnancy. An increased maternal immune response to trophoblast invasion leads to reduced decidualization and placentation. Interestingly, fetal genes function to improve fetal development by increasing nutrient supply to the baby, whereas maternal genes oppose this to secure maternal health; this conflict between fetal and maternal genes exacerbate complications of PE (Varas *et al.*,2018). In addition, fetal genes elevate maternal blood pressure in order to improve utero-placental blood flow but maternal genes resist this and may lead to endothelial dysfunction (Varas *et al.*,2018). The pathway affected in this regard is the vascular endothelial growth factor (VEGF) signaling pathway. This pathway is responsible for angiogenesis and the regulation of endothelial cell function (Ali *et al.*,2019; Echeverria *et al.*,2020; Kaczynski *et al.*,2020).

The circulating and placental soluble form of VEGF receptor: fms related tyrosine kinase (sFlt-1) is elevated in PE, this decreases free circulating VEGF, thus inhibiting their role to activate angiogenesis endothelial integrity management (Echeverria *et al.*,2020). Inactive free VEGF in the kidneys promotes endotheliosis and proteinuria (Echeverria *et al.*,2020). Furthermore, poor trophoblast invasion and reduced uteroplacental blood flow lead to placental ischemia (Echeverria *et al.*,2020), hypoxia and an increase reactive oxygen species (ROS), which in turn, leads to increased oxidative stress (Sánchez-Aranguren *et al.*, 2014).

Pre-eclamptic patients have high levels of oxidative stress and low level of antioxidants. In low- and middle- income countries, pregnant women with a deficiency of antioxidants and an increase in oxidative stress develop PE (Auoache *et al.*,2018; Rana *et al.*,2019). Antioxidants aid in precluding

the process of oxidation, which produces free radicals that can induce chain reactions resulting in cell destruction when activated ( Sánchez-Aranguren *et al.*,2014; Rana *et al.*,2019). Free radicals have also been proposed to initiate endothelial dysfunction (Chaudhary *et al.*,2020). Poor trophoblast invasion, hypoxia, placental ischemia, oxidative stress and inflammation trigger endothelial dysfunction. Endothelial dysfunction and maternal systemic inflammatory response manifest as the clinical signs of PE. The severity of endothelial dysfunction and inflammatory response presents as the different classes of PE.

## **1.8 URIC ACID AND PRE-ECLAMPSIA**

Uric acid (UA) dysregulation has been indicated as one of the clinical signs of PE (Chen *et al.*, 2016). Reports have shown a dramatic increase in uric acid levels in women with PE compared to normal pregnancies (Wu *et al.*, 2012). Uric acid is found in purine derivative foods such as fructose, fatty meat, seafood fruits, alcohol and is also synthesized naturally by the body through purine metabolism (El Din *et al.*, 2017). This biological pathway is initiated by the activation of xanthine oxidase (XO). Xanthine Oxidase converts adenosine triphosphate into adenosine xanthine, then this is further converted into uric acid (Osungbade and Ige, 2011).

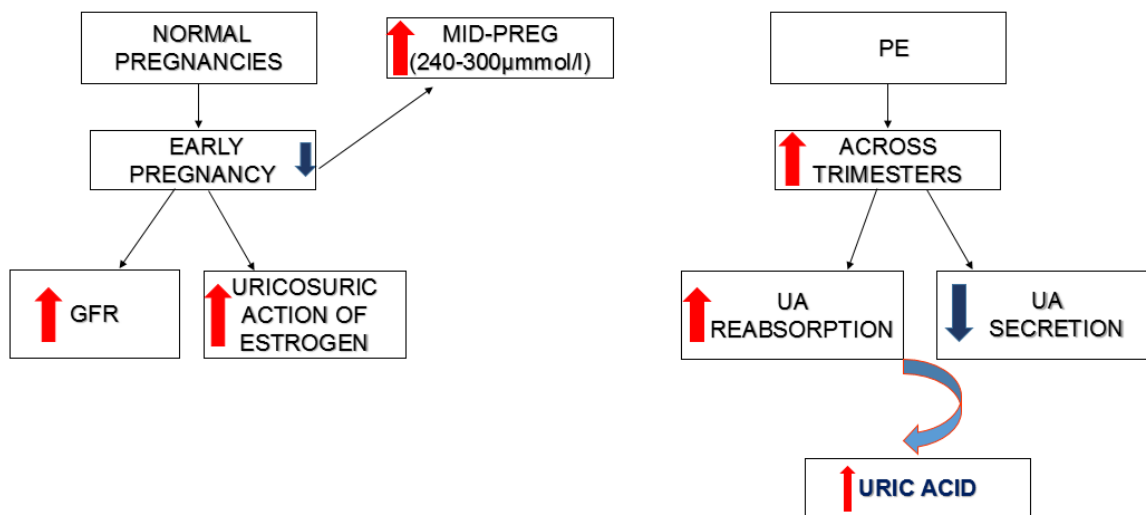
### ***1.8.1 Uric Acid Levels in Normal Pregnancies***

In the first and second trimester of pregnancy, UA levels drop by 20-25% lower than normal ranges of non-pregnant individuals (0.3-6.0 mg/dl) (Martin and Brown, 2010). This is due to an increase in glomerular filtration, an increase in uricosuric acid, and a decrease in proximal tubular reabsorption (Parrish *et al.*, 2010). In the third trimester, levels begin to rise gradually as a result of high fetal development and low uric acid clearance, thereby reaching levels equivalent to non-pregnant individuals (Sultana *et al.*, 2013) (Figure 3).

### ***1.8.2 Uric Acid Levels in Pre-eclampsia***

Levels of uric acid in PE differ from those in normal pregnancies. In Pe, uric acid levels rise throughout pregnancy (Wolak *et al.*,2015; Nair and Savitha, 2017). Pre-eclampsia is displayed with renal dysfunction, oxidative stress and placental ischemia. All these manifestations contribute to the elevated levels of uric acid in PE. It is well documented that angiotensin II is a vasoconstrictor and therefore ascribes to reduced renal blood flow, low urate secretion and low glomerular filtration rate, which in turn lead to reduced uric acid clearance (Sultana *et al.*, 2013; Wolak *et al.*,2015). Reduced

uric acid clearance results in hyperuricemia which activates sympathetic activity and subsequently hypertension, thus aggravating PE (Mumford *et al.*, 2013;Khurshid *et al.*, 2016). (Figure 3).



**Figure 3: Levels of Uric Acid in Normal pregnancies compared to Pre-eclampsia:**

Adapted from (Many *et al.*,1996). Uric acid (UA) is involved in the pathogenesis of pre-eclampsia. In early stages of normal pregnancy, UA levels drop due to an increase in glomerular filtration rate (GFR) and an increase in uricosuric action of estrogen. However, in mid pregnancy serum uric acid levels rise gradually to reach 240-300µmol/l at term-equivalent to levels in non-pregnant women. This may result from hypovolemia that causes an increase in uric acid reabsorption and a decrease in UA secretion. However, in PE UA levels rise throughout the pregnancy and result in placental hypoxia and ischemia in pre-eclampsia.

Uric acid has been classified as a pathogenic factor in PE (Bainbridge and Roberts, 2008). An elevation in uric acid levels at 10 weeks of gestation has been noted in women who develop PE at a later stage in pregnancy (Bainbridge and Roberts, 2008). Uric acid potentiates PE by stimulating inflammation, oxidative stress, and endothelial dysfunction (Bainbridge and Roberts, 2008). In addition, Powers *et al.*, (2006) suggested that hyperuricemia results in hypertension and proteinuria, which are clinical signs commonly used to diagnose PE (Powers *et al.*, 2006).

Uric acid not only promotes hypertension in PE, but also contributes to poor trophoblast invasion (Kang *et al.*, 2004). During normal placentation, cytotrophoblast cells invade the spiral arteries for proper vascular remodeling, and this expands or vasodilates the smooth muscles of the vessel walls



for sufficient flow of blood to the developing fetus. However, in PE, there is poor trophoblast invasion, improper spiral artery remodeling and consequently vasoconstriction of smooth muscles. This leads to reduced blood supply, and hypoxia (Burton *et al.*,2019). In a hyperuricemic environment, nitric oxide (NO) is decreased in endothelial cells and this contributes to poor trophoblast invasion (Zhen *et al.*,2017). Nitric oxide is a potent vasodilator which aids in relaxation of endothelial cells and trophoblast cell migration into the spiral arteries. This evidence suggests the role of uric acid in the pathogenesis of PE, particularly in women who experience elevated circulating uric acid levels at 10 weeks of pregnancy (Bainbridge and Roberts, 2008). Therefore, hyperuricemia is reported to be an early predictor of PE.

The use of UA as a predictor of PE is proposed differently by some studies (Bainbridge *et al.*,2008; Ryu *et al.*,2019). These studies suggest that PE occurs prior to hyperuricemia which occurs as a result of renal injury in PE (Kondareddy and Prathap, 2016). Moreover, it has been mentioned that placental ischemia activates purine metabolism, resulting in hyperuricemia in PE (Kang *et al.*, 2004). Hypoxia is one of the resultant causes of poor placentation in PE. The relationship between hypoxia and uric acid has been established (Many *et al.*, 1996). In a hypoxic environment, adenosine triphosphate (ATP), is broken down into adenosine monophosphate, which is further broken down to adenosine, hypoxanthine then into the end product, which is uric acid (López-Cruz *et al.*, 2016) causing hyperuricemia in PE.

### **1.8.3 Uric Acid Levels and Fetal Outcome**

Hyperuricemia has been implicated as a predictor of fetal outcome in PE. Studies have found that women with hyperuricemia occurring before 35 weeks of gestation have babies with adverse fetal outcome (Williams and Galerneau, 2002). About 56% of women with hyperuricemia had intrauterine growth restriction (IUGR) and intrauterine death (IUD) (Priya *et al.*, 2016). Hyperuricemia was also reported to have a negative impact on fetal birth weight (Sahijwani *et al.*, 2012). Hussain *et al.*, (2011) mentioned that 72% of babies born from mothers with hyperuricemia had low birth weights, while 62% of babies from woman with normal uric acid levels had normal birth weights (Hussain *et al.*, 2011). Another study reported on fetal outcome from pregnancies complicated with both hyperuricemia and pre-eclampsia. About 23.3% of these pregnancies delivered small-for-gestational-age (SGA) babies, while pre-eclamptic women without hyperuricemia only had 9.3% of SGA babies (Khaleeq, 2015).

## 1.9 GENE POLYMORPHISM OF URATE TRANSPORTING PROTEINS

Hyperuricemia is also influenced by genetic determinants, as well as heritability (Nath *et al.*, 2007; Sun *et al.*, 2014). Target organ destruction in patients with hypertension are associated with genetic factors (Li *et al.*, 2016a). Therefore, genome-wide association studies have identified genes that regulate serum uric acid concentration. Gene polymorphisms of uric acid such as *SLC*, *ABCG2* were initially reported to be involved with chronic renal injury (Bhatnagar *et al.*, 2016). Furthermore, gene polymorphisms in Chinese populations affect genetic variations on the regulation of serum uric acid levels in humans (Sun *et al.*, 2014).

Sun *et al.*, (2014) studied the association between gene polymorphisms of 11 loci (*PDZK1*, *GCKR*, *LRP2*, *SLC2A9*, *ABCG2*, *LRRIC16A*, *SLC17A1*, *SLC17A3*, *SLC22A11*, *SLC22A12* and *SF1*) and serum uric acid concentrations in a male and female Chinese population. They reported that *SLC2A9* and *GCKR* variants regulate uric acid concentration in Chinese males while *SF1* and *SLC2A9* were associated with uric acid levels irrespective of sex. *SLC22A12* polymorphism correlates with uric acid concentration in females (Sun *et al.*, 2014a). However, due to the existence of variation in allelic frequencies and effect sizes across different ethnicities, replication studies are required to clarify this effect (Nath *et al.*, 2007).

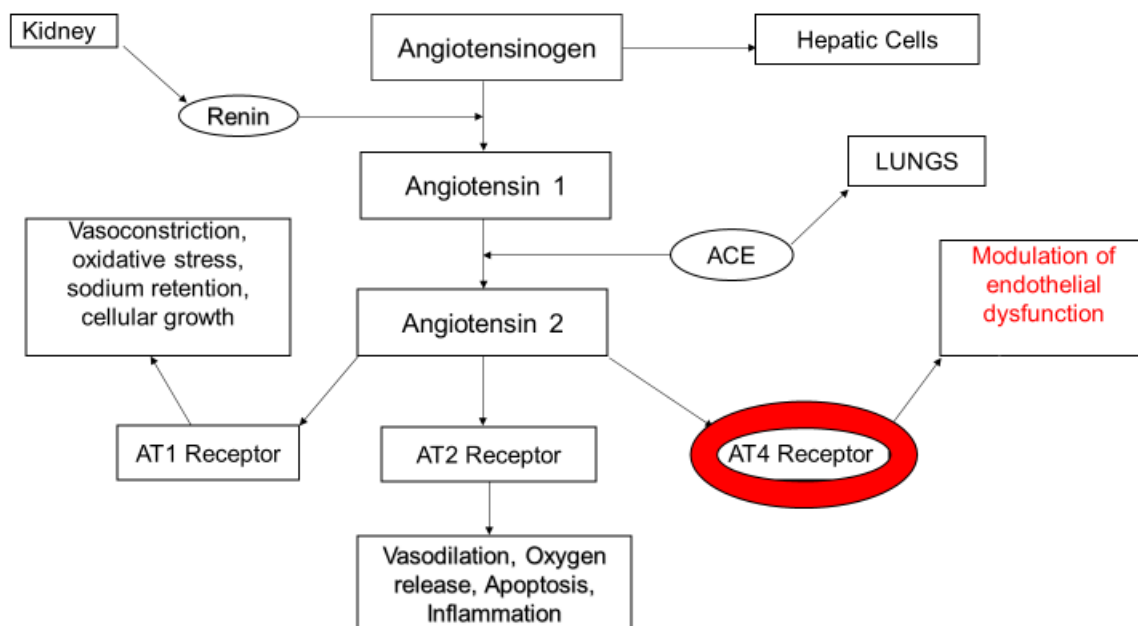
The SNPs in this study were randomly selected dependent on their association with hyperuricemia identified in gout (Sun *et al.*, 2014). It should also be noted that hyperuricemia is a feature of pre-eclampsia (Le *et al.*, 2019). Therefore, the urate transporter gene polymorphisms [(Glucose 9 transporter, *SLC2A0LGLUT9*): *rs1014290*, *SCL22A12* (Urate 1 transporter, *URAT1*): *rs505802*, (Postsynaptic density protein, *PDZK1*, *CD160*): *rs12129861*, (ATP-binding cassette super-family G member 2, *ABC G2*: *rs2231142*)] will be evaluated in a homogenous group of Black African pregnant women of Zulu ethnicity. These genes play an important role in uric acid regulation in the kidneys. The kidneys are accountable for the daily excretion of 70% of uric acid (Maesaka and Fishbane, 1998; El Din *et al.*, 2017; Nair and Savitha, 2017). Although uric acid levels may be elevated by diet and life style factors, the aim of this study was to investigate the genetic association of urate transporters genes. Due to the retrospective nature of this study, uric acid levels were not available. The medications used to manage high blood pressure in pregnancy have not been reported to affect the levels of uric acid in pregnancy (Brown *et al.*, 2018).

## **1.10 THE RENIN ANGIOTENSIN SYSTEM AND ANGIOTENSIN RECEPTOR IV (AT4) IN PE**

The renin-angiotensin-aldosterone (RAAS) system comprises a peptide cascade that plays a role in blood pressure regulation (Konoshita *et al.*, 2014). The key components of the RAAS system are renin, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensin I (ANG I), angiotensin II (ANG II), angiotensin receptor type I (AT1R) and angiotensin receptor type 2 (AT2R) (Yang *et al.*, 2013). Disruptions of the cascade may contribute to the pathogenesis of pre-eclampsia (Aung *et al.*, 2017). In PE, the RAAS system is the chief enhancer of low trophoblast invasion and the impaired remodeling of spiral arteries (Yang *et al.*, 2013).

A disruption of the RAAS system in pre-eclampsia leads to major changes in the RAAS homeostasis including renal impairment, body capillary constriction and a dysregulation in salt and water balance (Yang *et al.*, 2013). Primary components of the RAAS system have been detected in the placenta (Li *et al.*, 1998), therefore, synthesis of certain components of the RAAS system occur within the uteroplacental unit (Shah, 2006).

In a normal pregnancy, the RAAS system undergoes changes to compensate for the demanding fetus. Plasma volume and ANG II levels are elevated compared to non-pregnant individuals. The elevation in ANG II is caused by the decrease in the AT1 receptor activation during pregnancy. Furthermore, normal pregnancies are less reactive to ANG II, therefore, an increase in ANG II does not compromise the pregnancy (Pirani *et al.*, 1973; Yang *et al.*, 2013). However, pre-eclamptic women show decreased levels of ANG II and salt and water retention (Scaife *et al.*, 2017). Figure 4 outlines the RAAS system.



**Figure 4: Schematic diagram of the RAAS System:**

Adapted from (Atlas, 2007). The kidneys secrete renin in response to reduced NACL, ECF volume, and arterial blood pressure. Renin activates Angiotensinogen which is produced by the liver into ANG 1. ANG1 is converted into ANG 2 by the angiotensin converting enzyme (ACE) produced in the lungs. ANG 2 acts as a binding site for AT1R, which is responsible for vasoconstriction, oxidative stress, sodium retention and cellular growth. AT2R is involved in vasodilation, oxygen release, apoptosis and inflammation. Lastly, AT4R plays a role in modulation of endothelial function.

The association of gene polymorphisms of AGT, renin, AT1R, and AT2R have been investigated in pre-eclampsia. In pre-eclampsia, the distribution of the T allele and TT genotype of AGT is significantly increased compared to normotensive pregnancies (Aung *et al.*, 2017). Furthermore, the distribution of the genotypes of renin, AT1R, and AT2R are identical in both pre-eclamptic and normotensive groups, thus indicating that these genotypes are not involved in the pathogenesis of PE (Aung *et al.*, 2017). Another study, performed by Ji *et al.*, (2013) on 41 tag single nucleotide polymorphisms (tag SNPs) in RAAS in a Chinese Han population reported that AGT rs3789678 rs2493132, ACE 4305, and AGTR1 rs275645 are associated with non- pregnant hypertension (Ji *et al.*, 2013).

The AT4 receptor is a component of the RAAS system discovered in the early 2000s by Albiston *et al.*, (2001). It is a binding site for angiotensin IV (Vanderheyden, 2009). Angiotensin IV is responsible for memory, cognition, and vasodilation (Borghetti *et al.*, 2015). Therefore, the AT4 receptor is associated with the regulation of blood flow, memory and vasodilation (Borghetti *et al.*, 2015).

## **1.11 PROBLEM STATEMENT AND SIGNIFICANCE**

Pre-eclampsia (PE) is one of the major categories of the hypertensive disorders of pregnancy worldwide (Rana *et al.*, 2019). Maternal and fetal health are compromised greatly by PE which leads to premature deliveries and sometimes death of the mother and the fetus (Khan *et al.*, 2019). Pre-eclampsia occurs most frequently in sub-Saharan countries compared to those in high-income countries. A global estimate from data of nearly 39 million pregnancies suggests an incidence of 4.6% (Abalos *et al.*, 2013). However, there is great regional variations in incidence rates ranging from 0.4% in Vietnam to almost 12% in primigravidae of African ancestry in Kwa Zulu Natal province, South Africa (Abalos *et al.*, 2013; Moodley *et al.*, 2016).

The commonest direct cause of maternal deaths in South Africa are the hypertensive disorders of pregnancy in particular PE accounting for 18% of all deaths (Ngene and Moodley, 2019). It has been suggested that the high rates of PE and its associated complications in sub-Saharan African countries is due to inaccessibility to maternity care (Burton *et al.*, 2019). It has also been speculated that the human immune deficiency virus (HIV) may contribute to the high prevalence since the treatment (HAART therapy) is reported to result in immune reconstitution and thus PE susceptibility (Phoswa *et al.*, 2019). Furthermore, 50% of PE cases have been reported as genetic and include factors such as race, environment, and social life (Hanson, 2019). This may be the case in the African population; however, justification is required since PE itself is still an enigma. Intensive research has been conducted through the years to resolve this. As a result, several questions remain unanswered.

Pre-eclampsia is very complex since it is a multisystemic disorder (Gathiram and Moodley, 2016). The pathophysiology involves renal injury, hypertension, endothelial dysfunction, inflammation, placental hypoxia and ischemia, but the pathogenesis is unclear (Phipps *et al.*, 2019). There is a need for further investigations in relation to the pathogenesis of PE as well as the genetic aspects in low-

and middle-income countries. The genes associated with the pathogenesis require investigation in order to target the source of the disease and to formulate methods that would prevent its occurrence for healthier pregnancy outcomes.

## **1.12 AIMS AND OBJECTIVES OF THE THESIS**

### ***1.12.1 Aims***

To establish the concentration and the prevalence of genetic variants of urate transporter genes [(*SLC2A0LGLUT9*): rs1014290, *SCL22A12 (URATI)*: rs505802, *PDZK1 (CD160)*: rs12129861] related proteins, aminopeptidase-N (*ANPEP*-rs6496603) angiotensin IV receptor (AT4) (*LNPEP*-rs18059), a new component of the renin angiotensin system in pre-eclampsia compared to normotensive pregnant controls.

### ***1.12.2 Objectives***

1. To compare variant gene polymorphisms of the urate transporter related genes [rs1014290 (*SLC2AL; GLUT9*): rs505802 (*SCL22A12; URATI*): rs12129864 (*PDZK1; CD160*)] in pre-eclampsia versus normotensive controls, to elucidate the role of uric acid in the pathogenesis of pre-eclampsia using the TaqMan genotyping analysis.
2. To assess the plasma concentration of the receptor (sAT-4), in pre-eclampsia versus normotensive controls using an enzyme-linked immune sorbent assay (ELISA).
3. To compare variant gene polymorphisms of *LNPEP* (AT4R) (rs18059) and *ANPEP* (rs6496603) with pre-eclampsia development by performing the TaqMan genotyping assay.



## **CHAPTER 2**



**Review Article: The Role of Uric Acid in Pre-eclampsia: Is Uric Acid a Causative Factor or a Sign of Pre-eclampsia**

This is a review article discussing the role of uric acid in pre-eclampsia. It has been published in current hypertension reports:

Khaliq, O.P., Konoshita, T., Moodley, J. and Naicker, T., 2018. The Role of Uric Acid in Pre-eclampsia: Is Uric Acid a Causative Factor or a Sign of Pre-eclampsia? *Current Hypertension Reports*, 20(9):80.



## The Role of Uric Acid in Preeclampsia: Is Uric Acid a Causative Factor or a Sign of Preeclampsia?

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### Abstract

**Purpose of Review** Because of the significant discrepancies on this topic, this review will focus on the role of uric acid in PE, uric acid as a predictor of preeclampsia and fetal growth retardation. We considered eligible review and original articles relevant to the research question.

**Recent Findings** Hypertensive disorders of pregnancy such as preeclampsia (PE) are a major cause of both maternal and fetal morbidity and mortality worldwide. Uric acid has been reported as a key factor contributing to the pathogenesis of PE. Some studies have indicated that serum uric acid levels increase with the severity of PE, while several studies have shown contradictory results. Some studies suggested high uric acid levels lead to PE, while others state that PE causes an increase in uric acid levels.

**Summary** Despite the strong association of uric acid in the pathogenesis of preeclampsia, current data is still contradictory hence genetic and high-end laboratory investigations may clarify this enigma.

**Keywords** Hypertensive disorders of pregnancy · Preeclampsia · Uric acid · Hyperuricemia

### Introduction

Hypertensive disorders of pregnancy (HDP) are a major cause of maternal and perinatal morbidity and mortality [1]. They affect 2–10% of all pregnancies worldwide [2]. In developing countries, 40,000 women die yearly due to HDP. In South Africa alone, about 20.7% of maternal deaths are caused by

HDP. Hypertensive disorders of pregnancy are divided into different classes, and of these classes, preeclampsia is the most common disorder, with a prevalence of 40 to 60% in developing countries [3–5]. HDP are divided into different classes, which are summarized below according to severity (Table 1).

Preeclampsia is the most prevalent hypertensive disorder of pregnancy affecting 3–10% of all pregnancies globally [7–9]. Worldwide, it accounts for 50,000–60,000 deaths each year [10]. The prevalence of this disorder is higher in developing countries (ranging from: 4–18%) [11–13]. Fetal outcomes include premature births, intrauterine growth restriction, and death [14]. Preeclampsia is defined as new onset hypertension, i.e., blood pressure (> 140/90 mmHg) and the presence of protein in the urine (> 0.3 g/24 h or  $\geq$  + 1 dipstick) that occurs after 20 weeks of gestation [10].

Risk factors associated with this disorder include a previous history of preeclampsia, medical history of chronic hypertension, renal diseases, cardiovascular diseases, gestational diabetes, gestational hypertension, and obesity [15, 16]. Factors that predispose pregnant women to preeclampsia (PE) include nulliparity, a new partner, age  $\geq$  35 years, a non-singleton pregnancy [17, 18], and environmental aspects, such as high altitude [19]. PE mostly affects first pregnancies with a rate of 6% worldwide [1, 20]. In South Africa, Moodley et al., (2016) found that 12% of women in their first pregnancies developed PE.

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**Table 1** Classification of hypertensive disorders of pregnancy

Classification	Risk factors	Gestation	Characteristics	References
Superimposed preeclampsia	Chronic hypertension, kidney injury	<20 weeks	Increased blood pressure and proteinuria	[3]
Gestational hypertension	Chronic hypertension	≥ 20 weeks	Increased blood pressure Mild (< 140/90 < 160/110 mmHg) Severe (> 160/110 mmHg)	[6]
Preeclampsia	Diabetes mellitus, renal and cardiac diseases	≥ 20 weeks	Increased blood pressure and proteinuria Mild (< 140/90 < 160/110 mmHg) Severe (> 160/110 mmHg)	[3]
Eclampsia	Cerebral edema, cerebral vasospasm	> 20 weeks	Increased blood pressure and seizures	[3]

The only known cure for PE is delivery of the fetus and placenta together with close monitoring of signs of complications for up to 72 h in the immediate postpartum as a significant proportion of women develop worsening disease in this period. In addition, PE can cause complications later in life for both the mother and the baby [15]. The mother may develop conditions, such as chronic hypertension, immature cardiovascular disease, stroke, and heart disease later in life [21]. The baby may also suffer from the effects of growth restriction and also develop coronary heart disease, metabolic diseases, such as diabetes and stroke later in life [22, 23].

PE is known as a multifactorial disorder, which results in damage to some organs, such as the liver and the kidneys [10]. It has been reported that renal injury may lead to an increase in serum uric acid levels [24], which contributes to the pathogenesis of PE [25]. However, other reports state otherwise: Bainbridge and Roberts, (2008) have reported that uric acid levels rise as early as 10 weeks in women who later develop PE. It is also a known fact that PE occurs after 20 weeks of gestation; therefore, it is speculated that increased uric acid levels may be one of the causes of PE [26••]. In addition, uric acid can also be used as an early predictor for the disease [14, 26••].

Therefore, the goal of this review is to discuss the role of uric acid as a symptom or as a cause of PE by assessing findings of different journal articles based on the production of uric acid in normal pregnancies versus preeclamptic pregnancies, the difference in serum uric acid levels in normal pregnancies compared to preeclamptics as well as symptoms of preeclamptic women with hyperuricemia and fetal outcome.

## The Pathogenesis of Preeclampsia

In a healthy pregnancy, the cytotrophoblast penetrates the inner third of the myometrium causing physiological transformation of the spiral arteries [15]. This adaptation occurs for the placenta to be able to supply sufficient nutrients and oxygen to the developing fetus. In PE, however, the cytotrophoblast fails to invade the inner third of the myometrium. Therefore, the spiral

arteries remain thick-walled and responsive to vasoconstriction. This impaired remodeling of spiral arteries leads to placental ischemia, subsequently resulting in oxidative stress [15]. Placental ischemia is a life-threatening condition for the fetus, since it may result in intrauterine growth restriction and death [15]. Oxidative stress induces the release of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ), oxidized lipid, and free radicals [15]. These conditions occur in the first stage of PE, which is thought to be a two-stage disease [27].

The second stage includes the release of angiogenic factors from the placenta into the maternal circulation [28]. Soluble fms like tyrosine 1 (sFlt-1), also known as the soluble vascular endothelial growth factor receptor 1 (VEGF-1) is released [29].

Angiogenic factors such as the vascular endothelial growth factor (VEGF) promote the formation of nitric oxide, which is responsible for the counterbalance of reactive oxygen species and vasoconstrictor signaling [28]. sFlt-1 is overexpressed in the placentae of preeclamptic women [28, 30–33]. Experimental studies also report that an increase of sFlt-1 in sera of preeclamptic women correlates with a significant decrease in VEGF and placental growth factor (PlGF) [28, 32, 34]. sFlt-1 binds to PlGF, thus inhibiting it from binding to cell surface receptors on endothelial cells resulting in their dysfunction [28].

Research has shown that soluble endoglin (sEng) and sFlt-1 are elevated in the syncytial knots in the placenta and released into maternal circulation as microparticles [35]. Soluble endoglin binds to circulating transforming growth factor beta (TGF- $\beta$ ), thus preventing it from binding to its receptor. The inhibition of the TGF- $\beta$  signaling results in the disability of endothelium-mediated vasodilation. Both sEng and sFlt-1 are upregulated in PE and eclampsia, suggesting that these two conditions have identical pathophysiology [36, 37].

Both circulating sEng and sFlt-1 augment endothelial dysfunction in PE [38]. Poor placentation generates hypoperfusion, hence producing hypoxia, oxidative stress and ischemia, thus promoting endothelial dysfunction [38]. Endothelial dysfunction induces vasoconstriction in all systemic organs, including the kidneys, which are responsible for the long-term management of blood pressure. It also causes complications,

such as the HELLP (hemolysis, elevated liver enzymes and low platelet count) syndrome, cerebral edema, glomerular endotheliosis, and eclampsia [39].

Placental hypoxia and ischemia have also been implicated in the induction of uric acid production in PE [40•]. The relationship between uric acid and PE has been reported by various researchers [29, 40–43]. Uric acid is elevated in PE and may contribute to the pathogenesis of the disease [14, 26••].

### Uric Acid Production

Uric acid is produced in the liver from purine-derived nutritional sources, such as seafood, fatty meat, organ meat, fructose from fruits, added sugars, and alcohol consumption [44, 45]. Uric acid production is triggered by the activation of the xanthine oxidase (XO) enzyme [46]. Moreover, in PE, oxidative stress and cytokines emanating from placental ischemia induces the activity of XO [29, 46]. It has also been reported that preeclamptic placentas contain elevated expression levels XO and serum adenosine [29, 47, 48]. XO converts xanthine into uric acid by producing oxidants, such as the superoxide anion [29]. Figure 1 is a flow diagram depicting uric acid production.

### Serum Uric Acid Levels in Nonpregnant Women, Normal Pregnant Women, and Preeclamptic Women

#### Uric Acid in Nonpregnant Women vs. Normal Pregnancy

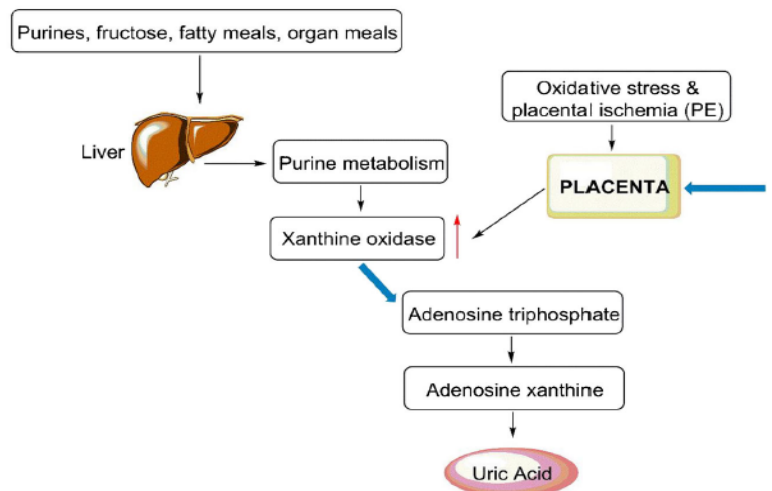
Uric acid is derived from the oxidation of purine nucleotides [46]. In nonpregnant individuals, it ranges typically

between 0.3–6.0 mg/dl. Uric acid levels are lower in women than men because of the uricosuric action of estrogen [49]. In pregnant women, the placenta and the developing fetus contribute to purine metabolism, but surprisingly, the levels of uric acid are 20–25% lower than nonpregnant women until the 20th week of gestation [50]. This decrease in uric acid levels during the first trimester has been attributed to hemodilution caused by an increase in blood level with an associated increase in glomerular filtration and a decrease in proximal tubular reabsorption (Martin and Brown, 2010). In the second trimester, uric acid levels decrease as a consequence of increased uricosuric activity. However, in the third trimester, uric acid levels increase gradually and reach the normal range of nonpregnant individuals at term [49] due to high fetal development with concomitant reduced uric acid clearance [51••, 52].

#### Uric Acid in Preeclampsia

Uric acid levels in PE have been extensively explored from as early as 1917. It is well documented that an elevation occurs in PE compared to normal pregnancies [52, 53]. Several reasons account for elevated uric acid levels in PE. Firstly, vasoconstrictors such as angiotensin II attribute to a decreased renal urate secretion, a decrease in renal blood flow and a decrease in glomerular filtration [53–55]. This is subsequently followed by a decrease in uric acid clearance. Secondly, PE is characterized by poor trophoblast invasion, which aggravates hypoxia, reactive oxygen species (ROS), and oxidative stress [56]. Hypoxia stimulates lactic acid production, which obstructs uric acid secretion [53]. Furthermore, the increased retention of uric acid enhances a sympathetic activity, thereby decreasing angiotensin system action and exacerbating the existing hypertension in PE [57, 58].

**Fig. 1** Schematic representation of uric acid production: Adapted from (Many et al., 2006; Usama et al., 2017). Uric acid is produced in the liver by the metabolism of purines and nutritional sources. In preeclampsia, placental ischemia and oxidative stress trigger or activate the release of xanthine oxidase enzyme. Xanthine oxidase cleaves adenosine triphosphate into adenosine xanthine and it converts it into uric acid, definition: A combination of Chem Draw Drawing software and Microsoft word 13 shapes



In PE, oxidative stress leads to fetal DNA metabolism of uric acid by XO enzyme. Xanthine oxidoreductase has two isoforms: xanthine oxidase (XO) and xanthine dehydrogenase (XDH) [59, 60]. XO is the toxic isoform, since it is accountable for the formation of ROS, while XDH is the active form. It has been proposed that the development of PE is associated with uric acid production via XO, while those without PE produce uric acid via XDH [61]. Increase uric acid levels have been implicated as a predictor of PE development [29, 62].

### Uric Acid and the Pathogenesis of Preeclampsia

PE women present high uric acid levels as early as 10 weeks of pregnancy [40•]. Various studies have reported that an increase in serum uric acid in women with PE indicates the severity of the disease process [40•, 63, 64].

Bainbridge and Roberts (2008) reported an experimental study by Mazzali et al. (2001), which confirmed that increased uric acid levels promote systemic hypertension. Hyperuricemia was induced in rats, with uricase inhibitor (oxonic acid) and the animal subsequently developed hypertension, kidney impairment, and vascular disease. The rats were then treated with a xanthine inhibiting agent (Allopurinol), which lowered uric acid levels and the rats did not develop any complications [40•].

Hyperuricemia has been reported to play a role in abnormal placentation in PE [51•]. Uric acid induces inflammation, oxidative stress, and endothelial dysfunction [51•]. Moreover, increased uric acid levels in circulation activate the release of pro-inflammatory cytokines, such as the TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 [51•]. Increased concentration levels of TNF- $\alpha$  correlate with increased serum uric acid levels in PE [51•, 65].

During pregnancy, trophoblast invasion and migration are critical for fetal development, since it allows sufficient oxygen and nutrient supply from maternal circulation to the fetus. Nitric oxide is essential for trophoblast invasion and migration [66, 67]. However, hyperuricemia diminishes nitric oxide production in endothelial cells, contributing to the dysregulation of trophoblast invasion and resultant lack of physiological transformation of spiral arteries [40•].

The effect of uric acid on nitric oxide production not only compromises placental vasculature but it also affects the maternal vasculature [40•]. Reduced nitric oxide production may also affect endothelial cell function to maternal vasculature by causing vasoconstriction due to lack of nitric oxide. Nitric oxide is essential in facilitating vascular tone [40•]. Moreover, uric acid has been documented to hinder endothelial proliferation by inhibiting the activation of VEGF [68, 69]. Furthermore, preeclamptic women also present with increased levels of vasopressors, such as endothelin in circulation, exacerbating a decrease of vascular tone [40•, 70, 71].

Uric acid has been reported to enhance endothelial cell repair by influencing the mobilization of endothelial progenitor cells, which rejuvenate endothelial lining of blood vessels [72]. Nonetheless, this only occurs during normal levels of uric acid in circulation. Hyperuricemia may attribute to the decrease in endothelial progenitor cells in PE, since there is increased cellular injury that may result in overworking of the progenitor cells, thus its depletion [40•].

There are also reports suggesting that serum uric acid may however not be associated with the pathogenesis of PE. Several studies have reported that renal injury occurs in PE and may lead to increased uric acid levels, since renal injury is characterized by glomerular endotheliosis that results in a decrease in glomerular filtration rate and a decrease in renal blood flow, hence decreasing uric acid clearance [73•, 74–78]. The uric acid level correlates with glomerular endotheliosis [73•, 79].

The level of uric acid correlates with gestational age in PE development. Reports have suggested that serum uric acid levels are highly expressed in the first trimester of pregnant women who later developed PE [73•, 80], while others reported increased serum uric acid levels in the second trimester [26•]. Interestingly, a large prospective study ( $n = 1541$ ) investigated serum uric acid levels in pregnant individuals and found that it was elevated in the first trimester of women who later developed clinical signs of PE [81], while in contrast, another study with a larger sample size ( $n = 5556$ ) showed that serum uric acid levels were not increased in the first trimester (11–13 weeks) and in the second trimester (13–1 weeks) of pregnancy [73•]. Contrasting data shows a reduction in serum uric acid levels in the second trimester, with an observable increase at the same time as the onset of PE (20 weeks) [73•]. These findings support other studies which state that hyperuricemia may be a symptom of PE [40•].

The uncertainty of whether hyperuricemia was the causative agent or a symptom of PE has been investigated [73•]. Using retrospective data and prospective data, Chen et al. (2015) concluded that increased serum uric acid at the onset of PE ( $\geq 20$  weeks of gestation) could be a maternal response and not a promoter of the disease. In a subsequent study, they concluded that serum uric acid might not be a good predictor of an early diagnosis of PE [73•].

### Uric Acid as Biomarker for Preeclampsia

Uric acid is a known biomarker for placental ischemia, oxidative stress, and renal damage [52, 82]. Notably, these are characteristics of PE, which activate XO, which in turn, activates uric acid production, thus resulting in hyperuricemia [46, 82]. Uric acid as a biomarker is corroborated by a recent study that demonstrates 90% sensitivity and 85% specificity of uric acid, and has also noted an increase in serum uric acid levels in PE

compared to normal pregnancies. Therefore, recommends serum uric acid levels as a risk indicator for PE development [83]. Similarly, Kondareddy and Prathap (2016) have recorded serum uric acid levels of PE vs. normotensive women as  $6.2 \pm 1.4$  mg/dl (preeclampsics) and  $4.3 \pm 0.8$  mg/dl (non-preeclampsics) respectively [82]. Their findings support the use of uric acid as a predictor for PE. However, the use of uric acid as a predictor for the development of PE seems to have contradictory results. Some reports have demonstrated no significant difference in serum uric acid levels in PE compared to normal pregnancies [84].

Although, Kondareddy et al. (2016) reported a significant increase in serum uric acid levels in severe PE compared to normal pregnancies, they observed no significant differences between the mild preeclamptic patients versus the normal group. They also reported that preeclamptic women with uric acid levels of  $> 6.0$  mg/dl correlated with premature births (71.9%) in contrast to uric acid levels  $< 6.0$  mg/dl [82].

Serum uric acid levels correlate with the severity of PE [61]. Moreover, uric acid levels of  $> 5.5$  mg/dl were detected in women with PE and (HELLP) syndrome and postpartum hemorrhage (PPH), whereas uric acid levels of  $> 7.8$  mg/dl strongly correlate with maternal deaths [85].

Serum uric acid displayed high diagnostic power for PE development compared to creatinine and urea [86]. No significant differences were found between either creatinine and urea levels in PE compared to normal pregnancies [86]. Most studies conclude that uric acid is a useful predictor of hypertensive disorders of pregnancy [87–89].

Despite the questionable role of serum uric acid as a biomarker of PE, its correlation with maternal and fetal complications has been investigated [90–92]. Sahijuani et al. (2012) observed that out of 50 women with hyperuricemia, only 10 had maternal complications, 6 developed the HELLP syndrome, 2 developed ascites, 13 developed eclampsia, and 4 developed placental abruption. In another study, 76% of women with hyperuricemia had cesarean section deliveries [91]. In another study, the accuracy of serum uric acid in predicting complications of PE was conducted on 60 women; 42 had hyperuricemia, 1 developed the HELLP syndrome, 2 developed ascites, 3 developed eclampsia, and 5 developed placental abruption, 50 had cesarean deliveries and only 4 had maternal complications [90].

Uric acid has a low molecular weight and can therefore cross the placenta into the fetus and is known to impede nephron development in the fetus [93]. Priya et al. (2016) demonstrated that serum uric acid is a good predictor of maternal and fetal outcome, and reported 56% of the fetuses had intrauterine growth restriction (IUGR) and intrauterine death (IUD). Of these, 79% had IUGR, 14% had IUD, and 5% women had both IUGR and IUD [90]. Also, Dhaka et al., (2011) noted significant differences in fetal birth weight of women with hyperuricemia vs. those with normal serum uric acid levels.

Seventy-two percent of babies born from hyperuricemic women had low birth weights and only 20% had normal birth weights. In another study, 62% of babies born to mothers with normal uric acid levels had normal birth weights, while 9.38% were born with low birth weights [93].

Furthermore, hyperuricemia was shown as a predictor of fetal growth restriction (Khaleeq et al., 2015). The results revealed that 23.3% of preeclamptic mothers with hyperuricemia ( $n = 150$ ) had small-for-gestational-age (SGA) babies, while only 9.3% of those with normal serum uric acid levels ( $n = 150$ ) delivered SGA (Khaleeq et al., 2015). This data is in agreement with a study by Kumar et al., (2017), who provided evidence that hyperuricemia can be used a measure of the severity of hypertension in pregnant women and as a predictor test for fetal outcome. In the study, the most extreme fetal outcomes were noted in women with eclampsia, followed by women with PE and lastly in those with gestational hypertension [1].

Studies on uric acid levels and their association with PE have also been done in Africa. In Nigeria, a study was conducted on serum lipid profiles and serum uric acid levels in PE [94]. The results indicated higher levels of serum uric acid in severe PE compared to normal pregnancies. In addition, serum uric acid levels were reported to have an association with advanced maternal age, BMI and multiparity, probably due to the involvement of age and body mass index (BMI) in hypertension, as well as renal injury that could be a susceptibility to PE [94].

In South Africa, similar results were obtained with serum uric acid levels showing a 50% increase in preeclamptic patients compared to normal cases, and this difference was suggested to be due to damaged renal excretion and disintegrating placental tissue, which forms urate in PE [95]. Evidence to this was a decrease in urate levels after delivery of the fetus in PE [95]. Another study in a South African cohort also confirmed that uric acid levels were increased in PE compared to normal pregnancies. Furthermore, a correlation between hyperuricemia and intrauterine growth restriction (IUGR) was also observed. Thirty eight percent of preeclamptic births had (IUGR), but none was noted between hyperuricemia and perinatal deaths [96].

Table 2 is a summary of all the articles that were used in this review on serum uric acid levels as a predictor of preeclampsia. These articles are arranged in order of the years in which they were published. The main purpose of this is to note the differences in data that was collected during the years. In this table, the study sites are also included to note where this form of research has been done, while revealing where such research is lacking in the world. This will help to improve the field of research in other parts of the world as well as to broadcast awareness of the uric acid as a diagnostic tool of preeclampsia.

**Table 2** A summary of the findings used in this review on serum uric acid as a predictor of preeclampsia

Author	Country	Design	Sample size	Study population	Prevalence	Conclusion
(Ondial and Pienaar, 1997)	South Africa	Prospective Analytical study	229 patients	Preeclamptic group and normal group	Not stated	No evidence was found to confirm the relationship between perinatal deaths and hyperuricemia in preeclampsia.
(Berman et al., 2001)	South Africa	Prospective study	160 patients	Preeclamptic group and Normotensive group	Not stated	Hyperuricemia in preeclampsia is caused by the inability of the kidney to increase urate secretion. Also, urate can be used to diagnose preeclampsia, without taking gestational age into consideration
(Williams and Galemeau, 2002)	New Haven USA	Cohort study	459 patients	Normotensives, gestational hypertensives, Preeclampsics and eclampsics	10% of pregnancies	Serum uric acid levels are not sufficiently elevated in women with preeclampsia or pregnancy-induced hypertension to be good prognostic indicators of maternal and fetal complications
(Enaruna et al., 2014)	Nigeria	Prospective study	120 patients	Preeclampsia group and Normal group	Not stated	Serum uric acid levels are augmented in preeclampsia. Therefore, uric acid can serve as a biomarker for the early detection of preeclampsia
(Livingston et al., 2013)	Australia	Prospective study	2514 patients	Hypertension and proteinuria, Hypertension and hyperuricemia HELLP syndrome, and Superimposed preeclampsia	13% of pregnancies	Serum uric acid corrected for gestational age via a Z score was shown to be useful in predicting adverse perinatal outcomes in women admitted to hospital with preeclampsia.
(Tejal and Astha, 2014)	India	Cohort study	100 patients	Hyperuricemic preeclamptic group and Normouricemic preeclamptic group	7–10% of all pregnancies	Estimation of serum uric acid level in pregnancies complicated by both pre-existing hypertension and preeclampsia help to assess the severity of illness, and to identify those fetuses that are likely to have LUGR and high perinatal mortality and morbidity
(Khaleeq et al., 2015)	Pakistan	Cohort study	300 patients	Preeclamptic Normouricemic and pre-eclamptic Hyperuricemic	12–22% of pregnancies	Serum uric acid level measurements are a useful and inexpensive marker for predicting preeclampsia and fetal growth retardation
(Andrew and Patel, 2016)	India	Cohort study	158 patients	PIH group Normal group	7–10% of all pregnancies	Serum uric acid level could be used as a biochemical indicator of preeclampsia/eclampsia and its complications
(Chen et al., 2016)	China	Prospective study	5556 patients	Preeclamptic group Normal group	3–5% of all pregnancies	Serum levels of uric acid were only increased after the presentation of clinical symptoms of preeclampsia. Therefore, it is likely that uric acid is not involved in the development of preeclampsia and cannot be an early prediction biomarker of this disease
(Kondareddy and Prathap, 2016)	India	Prospective observational case control study	180 patients	Normotensives, Mild preeclampsics, severe preeclampsics and eclampsics	5–15% of pregnancies	Uric acid was very significantly higher in severe preeclampsia ( $P < 0.01$ ) and eclampsia ( $P < 0.01$ ) than that in normal healthy pregnant controls. The uric acid in mild preeclampsia was not significantly higher than the healthy pregnant control.
(Priya et al., 2016)	India	Prospective study	60 Patients	Preeclamptic women	2–10% of all pregnancies	Serum uric acid is statistically significant predictor ( $p$ value 0.01) of fetal complications of preeclampsia even though not of maternal complications ( $P$ value 0.42).
(Jeevitha, 2017)	India	Prospective observational study	120 patients	Antenatal normotensives	15–37% of the study population	Serum uric acid level can be used as a predictor of preeclampsia to manage expectant women for a good maternal outcome
(Kumar et al., 2017)	India	Prospective comparative study	110 patients	Gestational hypertension, preeclampsia, eclampsia.	2–10% of all pregnancies	Routine estimation of serum uric acid levels may be useful as a predictor of severity of disease in women with HDP and overall fetal outcome.
(Niraula et al., 2017)	Nepal	Comparative cross-sectional study	90 patients	PIH group Control group	7–10% of all pregnancies	serum UA is significantly raised in PIH compared to the control group. Serum uric acid can still be used as a prevalent marker for risk assessment in PIH.

## Summary

1. Hyperuricemia occurs early in PE. Since uric acid levels are increased as early as 10 weeks of pregnancy, it is plausible that glomerular endotheliosis may account for the decreased uric acid clearance. Notably, the elevated uric acid exacerbates the abnormal trophoblast invasion in PE.

2. Also, uric acid is not a prime source of the disease. However, hyperuricemia correlates with the severity of PE.

3. Although hyperuricemia plays a role in the progression of the disease, it is a useful predictor index for maternal and fetal outcome. Studies conducted on hyperuricemia and PE in South Africa have used very small sample sizes compared to other countries, and is warranted on a larger population to confirm the results that were obtained by the smaller groups. Also more research needs to be done in African countries, since Table 2 in this review indicates that most investigations were done in Asian populations.

## Conclusion

Despite the strong association of uric acid in the pathogenesis of preeclampsia, current data is still contradictory; hence, genetic and high-end laboratory investigations may clarify this enigma.

## Compliance with Ethics Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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## **CHAPTER 3**

**Research Article: Gene Polymorphisms of Uric Acid are associated with Pre-eclampsia in South Africans of African Ancestry**

This chapter consists of a research article examining the association of gene polymorphisms of uric acid in pre-eclamptic women of African ancestry.

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**Gene Polymorphisms of Uric Acid are associated with Pre-eclampsia in South Africans of African Ancestry**

**Running Head: Gene Polymorphisms in Pre-eclampsia**

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## Abstract

**Objectives:** To investigate the association of uric acid gene polymorphisms and Pre-eclampsia.

**Methods:** 637 women of African ancestry [280 controls, 357 pre-eclampsia (early-onset=187, late-onset=170)] retrospectively. The rs505802, rs1212986 and rs1014290 SNPs were genotyped from purified DNA using real-time PCR.

**Results:** CT genotype (rs505802) was higher in pre-eclampsia [Adjusted  $p=0.028^*$ : OR (95% CI) = 1.73 (1.258 - 2.442) and late-onset pre-eclampsia [Adjusted  $p=0.027^*$ : OR (95 % CI) =1.75 (1.165 - 2.2628) than controls. CT genotype (rs1014290) was higher in early-onset pre-eclampsia [Adjusted  $p$  value= $0.040^*$ : OR (95% CI) = 1.60 (1.102 - 2.325)] than controls.

**Conclusion:** The genotyped rs505802 and rs1014290 are significantly associated with pre-eclampsia

**Keywords** Pre-eclampsia, Single nucleotide polymorphisms, Uric acid, Hyperuricemia



## Introduction

Pre-eclampsia (PE) is a common cause of maternal and fetal complications worldwide, accountable for > 500 000 fetal deaths and > 70 000 maternal deaths [1]. Pre-eclampsia has a global prevalence of 5-8% [2]. Despite extensive research, the exact etiology of PE remains obscure, with the only known cure being the early delivery of the fetus and placenta [3, 4]. The pathogenesis of PE is influenced by environmental and genetic factors [5]. To date, the etiology of PE is unclear but studies suggest that it involves deficient trophoblast invasion and inadequate spiral artery transformation with resultant ischemia, oxidative stress, and hyperuricemia [2, 5].

Uric acid is a result of purine metabolism arising from purine nutritional products such as seafood, fruit-derived fructose, alcohol and organ meat [6]. The biological synthesis of uric acid is activated by the xanthine oxidase (XO) enzyme. [6]. The production of uric acid in PE is exaggerated compared to that of normal pregnancy [7]. Increased uric acid levels may arise from renal impairment noted in PE, or in an ischemic placenta [8]. It is reported that placental ischemia promotes the activation of XO, further activating uric acid production [9].

Despite several studies highlighting the role of uric acid in the etiology of PE, the data remains controversial [10-13]. Nonetheless, the uric acid elevation is reported to occur as early as 10 weeks of gestation thereby increasing the risk of PE development [5]. On the other hand, PE may manifest clinical features prior to hyperuricemia and cause renal impairment with resultant elevation in serum uric acid levels [12].

An association of gene polymorphisms with serum uric acid elevation has been previously demonstrated [14-16]. Single nucleotide polymorphisms SNPs of *PDZK1*, *GCKR*, *LRP2*, *SLC2A9*, *ABCG2*, *LRRRC16A*, *SLC17A1*, *SLC17A3*, *SLC22A11*, *SLC22A12* and *SF1* were mapped for serum uric acid association in both males and females in a Chinese population. The results revealed that *SLC2A9* rs11722228, *SF1* rs606458, and *GCKR* rs780094 SNPs altered serum uric acid concentration in males, whilst *SF1* rs606458 and *SLC2A9* rs3775948 altered uric acid concentration across both sexes [15]. Moreover, the gene *SLC2A9* (*GLUT9*) was found to be associated with hyperuricemia in both Caucasians and Asians [16]. Also, Zhang et al., [14] genotyped the SNP rs2231142 of the *ABCG2* gene and found an association with hyperuricemia in an American population consisting of European Americans, African Americans, Mexican Americans and Indian Americans [14]. Analogous reports also link the urate transporter 1 (*URATI*) gene with hyperuricemia [17], this gene is an anion transporter that functions mainly to control uric acid reabsorption within renal proximal

tubules. Nonetheless, in a Korean population, SNPs *viz.*, rs7929627, rs75786299, rs3825017 and rs121907892 of the *URATI* gene are strongly associated with increased serum uric acid levels in individuals with gout [18]. Thus, the latter studies highlight the role of polymorphisms of the uric acid polymorphisms in various disease types, albeit most strongly in gout [14-16, 18]. It is well known that the kidney is affected in PE compared to normal pregnancies with a consequential elevation of serum uric acid [19-22]. All previous studies on uric acid polymorphisms have investigated them in relation to gout, as far as we are aware, there are no studies investigated in pregnant populations. Therefore, the four SNPs selected in this study were randomly chosen based on their association with hyperuricemia detected in individuals with gout [16, 17, 23]. The aim of this study was to determine the association and the prevalence of genetic variants of uric acid [(*SLC2ALGLUT9*): rs1014290, *SCL22A12 (URATI)*:rs505802, *PDZK1 (CD160)*: rs12129861: (*ABCG2*): rs2231142] in South African women of African ancestry with PE.

## **Materials and Methods**

### **Study Population and Design**

Stored samples of pregnant women (n=637) recruited from an urban hospital in Durban, South Africa were used for the purposes of this study. Informed consent for the storage and future studies was obtained following institutional ethical approval (BCA 338/17). Study groups included healthy normotensive pregnant women and women with a diagnosis of PE. The PE group was sub-divided into early (occurring <33 weeks plus 6 days of gestation; EOPE) and late-onset PE (occurring >34 weeks of gestation; LOPE). Pre-eclampsia was defined as new-onset hypertension (systolic blood pressure  $\geq 140$ mmHg and or diastolic blood pressure of  $\geq 90$ mmHg) with or without proteinuria (300mg in a 24-hour quantitative urine test or at least 1+ on a urinary dipstick test). All blood pressure and proteinuria measurements were recorded at the time of admission to hospital or recorded at the time of recruitment for the controls.

## **DNA Isolation**

DNA was isolated from 500 µl of whole blood using the Thermo Scientific GeneJet whole blood Genomic DNA Purification Mini Kit (Thermo Scientific). Samples were stored at -20°C for genotyping analysis.

## **TaqMan Genotyping of Uric Acid Gene Polymorphisms**

Four SNP probes were amplified to detect specific polymorphisms from purified DNA samples using TaqMan genotyping master mix (Applied Biosystems), according to manufacturer's protocol. Genotyping of SNPs was performed using the QuantStudio 7 real-time Flex PCR (Life Technologies, California, USA). TaqMan reagents to run the polymerase chain reaction included: TaqMan universal master mix (x2), No Amp Erase UNG (12.5 µl), 20 X working stock of SNP Genotyping Assay (1.25 µl), DNase-free water (5.25 µl), and 50 ng/µl of DNA (1 µl), with a total volume of 20 µl per well. Following PCR amplification, allelic discrimination results were analyzed using the QuantStudio 7 Flex v1.3 software. To confirm our results, the experiments were repeated in two independent laboratories with the same cohort (University of KwaZulu-Natal, University of Fukui).

## **Sample Size**

The sample size was determined by an institutional biostatistician. The Cohens effect was used to calculate the sample size. To assess a two-fold difference in the specified genes between study groups with 80% power and the probability of 95% assuming 50% controls, 274 participants were required with 137 participants per subgroup. A total number of 600 participants was expected, of which 400 would be PE and 200 controls. The PE group was subdivided into early (n=200) and late (n=200) onset PE.

## **Statistical Analysis**

The Hardy-Weinberg equilibrium (HWE) test was used to check for conformance to observed frequencies of the genotypes [24]. Frequency and percentage were used to describe the presence of the genotypes. Subgroups were compared using the Chi-squared test or Fisher's exact test as appropriate. The strength of association was reported as odds ratios (OR) and 95% confidence interval (CI) for categorical data and Wilcoxon rank sum tests for numeric data. A *p*-value <0.05 was considered statistically significant. For table demographics analysis, one-way ANOVA test was performed, with the Graph pad prism 5 software (Graph Pad Software, San Diego, CA, USA). Also, the Bonferroni correction test was conducted for multiple comparisons.

## Results

This study consisted of 637 participants. The two main groups included the PE group (n=357; 56%) and the control group (n=280;44%). The PE group was subdivided into early (EOPE: n=187; 29%) and late pre-eclampsia (LOPE: n=170; 27%).

### Genotyping

The association of gene polymorphisms of uric acid (rs505802, rs1014290, rs12129861, rs2231142) in PE is tabulated below. An allelic (Table 2) and genotypic comparison of frequencies were calculated and four genetic models tested; *i.e.* the codominant (equal effect of two alleles from a gene pair), dominant (alleles with the same phenotype irrespective of whether the paired allele is identical or not) [25], recessive (creates a phenotype only when the paired alleles are identical) and overdominant model (heterozygote has a greater effect compared to the homozygote) [26]. These four genetic models were tested for associations with PE for each of the three variants examined. (Table 3, 4 and 5). The SNP rs2231142 of the *ABCG2* gene showed a very low frequency of < 1% in our study population and is therefore not included in the results on table 2 and below.

### Allelic association of rs505802 (*URAT 1*) in Pre-eclampsia

#### *Pre-eclampsia vs Controls*

The frequency of the C allele (529 cases and 438 controls) and T allele (185 cases and 126 controls) showed no significant association with PE (C vs T: Adjusted  $p=0.480$ ). Similarly, the frequencies of CC (199 cases and 173 controls), CT (131 cases and 88 controls), and TT (27 cases and 19 controls) showed no significant association with PE compared to controls [ (CC vs CT + TT: Adjusted  $p=1.000$ ); (TT vs CC+CT: Adjusted  $p=0.360$ ); (Table 2)].

#### *Early-onset pre-eclampsia vs Controls*

Early-onset pre-eclampsia was compared to the control group. The frequency of the C allele (292 cases and 438 controls) and the T allele (82 cases and 129 controls) showed no statistical significance (C vs T: Adjusted  $p=1.000$ ). Likewise, the frequencies of CC (117 cases and 173 controls), CT (58 cases and 88 controls), and TT (12 cases and 19 controls) showed no significant difference between EOPE and the control group [(CC vs CT +TT: Adjusted  $p=1.000$ ); (TT vs CC+CT: Adjusted  $p=1.000$ ); (Table 2)].

### ***Late-onset pre-eclampsia vs Controls***

The C allele (237 cases and 434 controls) and the T allele (103 cases and 126 controls) frequencies were higher in the controls compared to LOPE. There was a significant difference between LOPE and controls [C vs T: Adjusted  $p=0.028^*$ : OR (95%CI) =1.49 (1.104 - 2.030)]. With regards to the frequencies of CC (82 cases and 173 controls), CT (73 cases and 88 controls), and TT (15 cases and 19 controls), a significant difference was noted between TT and CC + CT in LOPE compared to the controls [(TT vs CC +CT: Adjusted  $p=0.015^*$ : OR (95%CI) =1.74 (1.180 - 2.551)]. However, there was no significant difference between (CC vs CT+ TT: Adjusted  $p=1.000$ ); (Table 2).

### ***Early-onset pre-eclampsia vs Late-onset pre-eclampsia***

The allele frequencies of C (292 EOPE and 237 LOPE) and T (82 EOPE and 103 LOPE) were significantly different between EOPE and LOPE [C vs T: Adjusted  $p=0.032^*$ : OR (95%CI) = 1.55 (1.105 - 2.168)]. Also, the frequencies of CC (117 EOPE and 82 LOPE), CT (58 EOPE and 73 LOPE), and TT (12 EOPE and 15 LOPE) showed a significant difference between EOPE and LOPE (TT vs CC+CT: Adjusted  $p=0.019^*$ : OR (95%CI) =1.79 (1.176 - 2.736)]. In contrast, CC vs CT +TT showed no significant difference between EOPE and LOPE (Adjusted  $p=1.000$ ). The C and the T alleles were higher in controls compared to the EOPE and LOPE groups; (Table 2).

### **Allelic Association of rs12129861(PDZK1-CD160) in Pre-eclampsia**

#### ***Pre-eclampsia vs Controls***

The allelic frequency of C (409 cases and 329 controls) and T (305 cases and 231 controls) of rs12129861(PDZK1-CD160) were lower in the controls compared to PE. However, no significant association was observed between PE and the controls (C vs T: Adjusted  $p=1.000$ ). The frequencies of CC (122 cases and 101 controls), CT (165 cases and 127 controls) and TT (70 cases and 52 controls) also showed no significant association with PE. [(CC vs CT+TT: Adjusted  $p=1.000$ ); (TT vs CT + CC: Adjusted  $p=1.000$ ); (Table 2)].

#### ***Early-onset pre-eclampsia vs Controls***

The frequency of the C allele (207 cases and 329 controls) and the T allele (167 cases and 231 controls) of rs12129861 showed no significant association with EOPE compared to controls (C vs T: Adjusted  $p=0.900$ ). The C and T allele frequencies were lower in EOPE than in controls. Equally, frequencies of CC (58 cases and 101 controls), CT (91 cases and 127 controls) and TT (52 cases and

38 controls) were not significantly different in EOPE compared to controls [(CC vs CT+TT: Adjusted  $p=1.000$ ); (TT vs CC + CT: Adjusted  $p=0.780$ ); (Table 2)].

#### ***Late-onset pre-eclampsia vs Controls***

The frequencies of the C allele (202 cases and 329 controls) and the T allele (138 cases and 231 controls) showed no significant difference in LOPE compared to controls (C vs T: Adjusted  $p=1.000$ ). The C and T alleles were lower in LOPE compared to controls. Similarly, the frequencies of CC (64 cases and 101 controls), CT (74 cases and 127 controls) and TT (32 cases and 38 controls) showed no significant association with LOPE (CC vs CT+TT: Adjusted  $p=1.000$ ), (TT vs CC+CT: Adjusted  $p=1.000$ ); (Table 2).

#### ***Early-onset pre-eclampsia vs Late-onset pre-eclampsia***

The allelic frequencies of C (207 EOPE and 202 LOPE) and T (167 EOPE and 138 LOPE) were not significantly different between EOPE and LOPE (C vs T: Adjusted  $p=0.810$ ). No significant difference was noted with the frequencies of CC (58 EOPE and 64 LOPE), CT (91 EOPE and 74 LOPE) and TT (52 EOPE and 32 LOPE) between EOPE and LOPE [(CC vs CT+TT: Adjusted  $p=1.000$ ); (TT vs CC+CT: Adjusted  $p=0.570$ ); (Table 2)].

#### **Allelic Association of rs1014290 (*GLUT 9*) with Pre-eclampsia**

##### ***Controls vs Pre-eclampsia***

The frequency of the C allele (209 cases and 144 controls) and the T allele (503 cases and 414 controls) of rs1014290 showed no significant association with PE compared to controls (C vs T: Adjusted  $p=0.480$ ). Equivalently, the CC (25 cases and 20 controls), CT (159 cases and 104 controls) and TT (172 cases and 155 controls) were not significantly different in PE compared to controls [(CC vs CT+TT: Adjusted  $p=1.000$ ); (TT vs CC+CT: Adjusted  $p=1.000$ ); (Table 2)].

##### ***Early-onset pre-eclampsia vs Controls***

The allelic frequency of C (122 cases and 144 controls) and T (82 cases and 155 controls) of rs1014290 were lower in EOPE compared to the controls. [C vs T: Adjusted  $p=0.072$ ]. Correspondingly, the frequencies of CC (17 cases and 20 controls), CT (88 cases and 104 controls) and TT (82 cases and 155 controls) were significantly different between EOPE and controls (CC vs

CT+TT: [Adjusted  $p=0.040^*$ : OR (95% CI) =1.60 (1.102 - 2.325)]. No significant association was found between EOPE and the control group with TT vs CC+CT: Adjusted  $p=1.000$ ); (Table 2).

#### ***Late-onset pre-eclampsia vs Controls***

The frequency of the C allele (87 cases and 144 controls) and the T allele (251 cases and 414 controls) of rs1014290 SNP showed no significant association with LOPE (C vs T: Adjusted  $p=1.000$ ). The C and T alleles were lower in LOPE compared to the control group. Similarly, the frequencies of CC (8 cases and 20 controls), CT (71 cases and 104 controls) and TT (90 cases and 155 controls) showed no significant difference between LOPE and the controls [(CC vs CT+TT: Adjusted  $p=1.000$ ); (TT vs CC+CT: Adjusted  $p=1.000$  )]; (Table 2)].

#### ***Early-onset pre-eclampsia vs Late-onset pre-eclampsia***

The frequency of the C allele (122 EOPE and 87 LOPE) and the T allele (82 EOPE and 251 LOPE) did not show any significant difference between the EOPE and the LOPE group [C vs T: Adjusted  $p=0.132$ ]. In the same way, the frequencies of CC (17 EOPE and 8 LOPE), CT (88 cases and 71 LOPE) were not significantly different between EOPE and LOPE [CC+CT vs TT: Adjusted  $p=0.073$ ]. No significant difference was noted with CC vs CT+TT: Adjusted  $p=0.300$ ); (Table 2).

### **Genotype Association of *URAT1* (rs505802) with Pre-eclampsia**

#### ***Pre-eclampsia vs Controls***

The genotype frequencies CC (173 controls and 199 cases), CT (88 controls and 131 cases) and TT (19 controls and 27 cases) of rs505802 showed no significant association with PE in the codominant genetic model [(CC vs TT: Adjusted  $p=1.000$  CC vs CT: Adjusted  $p=1.000$  CT vs TT: Adjusted  $p=1.000$ )]. The dominant model showed no significant difference between the control group and PE (CC vs CT+TT: Adjusted  $p=1.000$ ). Similarly, the genotype frequencies in the recessive model were not statistically significant between the controls and PE (CC+CT vs TT: Adjusted  $p=0.498$ ). However, the overdominant genetic model showed a significant difference between the controls and PE, [TT+CC vs CT: Adjusted  $p=0.004^{**}$ : OR (95% CI) = 1.73 (1.258 - 2.442)] with the CT genotype having an association with PE; (Table 3).

### ***Early-onset pre-eclampsia vs Controls***

The genotype frequencies of CC (117 cases and 173 controls), CT (58 cases and 88 controls) and TT (12 cases and 19 controls) of rs505802 showed no significant association with PE in the codominant model [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=1.000$ ); (CT vs TT: Adjusted  $p=1.000$ )]. The dominant genetic model showed no significant association with PE (CC vs CT+TT, Adjusted  $p=1.000$ ). Similarly, the recessive genetic model (CC + CT vs TT: Adjusted  $p=1.000$ ) and the overdominant genetic model were not statistically significant (TT+CC vs CT: Adjusted  $p=1.000$ ); (Table 3).

### ***Late-onset pre-eclampsia vs Controls***

The genotype frequencies were CC (82 cases and 173 controls), CT (73 cases and 88 controls) and TT (15 cases and 19 controls). The codominant model, CC vs CT, showed significant association with LOPE [CC vs CT: Adjusted  $p=0.027^*$ : OR (95 % CI) =1.75 (1.165 - 2.628)]. There was no significant association noted with CC vs TT: Adjusted  $p=0.661$ ) and CT vs TT: Adjusted  $p=1.000$ ). The dominant genetic model showed a statistically significant association of rs505802 with LOPE [CC vs CT+TT: Adjusted  $p=0.020^*$ : OR (95% CI) = 1.74 (1.180-2.551)]. The CT genotype showed a strong association of rs505802 (*URAT1*) with LOPE. No statistical significance was found with the recessive genetic model in LOPE compared to controls (CC+CT vs TT: Adjusted  $p=1.000$ ). The overdominant model displayed no association with LOPE compared to controls [CC+TT vs CT: Adjusted  $p=0.054$ ]; (Table 3).

### ***Early-onset pre-eclampsia vs Late-onset pre-eclampsia***

The genotype frequencies of CC (117 EOPE and 82 LOPE), CT (58 EOPE and 73 LOPE) and TT (12 EOPE and 15 LOPE) in the codominant genetic model showed no significant difference: (CC vs CT: Adjusted  $p=0.630$ ) and (CT vs TT: Adjusted  $p=1.000$ ). Nevertheless, a significant association was noted between EOPE and LOPE with; [CC vs CT: Adjusted  $p=0.039^*$ : OR (95% CI) =1.79 (1.150-2.804)]. Similarly, the dominant genetic model was significantly different between EOPE and LOPE [CC vs CT+TT: Adjusted  $p=0.026^*$ : OR (95% CI) = 1.79 (1.176-2.736)]. The recessive model was not significantly different between EOPE and LOPE (CC+ CT vs TT: Adjusted  $p=1.000$ ). No significant association with LOPE was noted with the overdominant genetic model; [CT+TT vs CC: Adjusted  $p=0.078$ ]; (Table 3).



## **Genotype Association of PDZK1 CD160 (rs12129861) with Pre-eclampsia**

### ***Pre-eclampsia vs Controls***

The genotypic frequencies of CC (122 cases and 101 controls), CT (165 cases and 127 controls) and TT (70 cases and 52 controls) in the codominant genetic model showed no significant association between the controls and PE, [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=1.000$ ); (CT vs TT: Adjusted  $p=1.000$ )]. The dominant genetic model was not significantly different between PE and the control group (CC vs CT+TT: Adjusted  $p=1.000$ ). No significant difference was found between PE and controls in the recessive genetic model (CC+CT vs TT: Adjusted  $p=1.000$ ) and the overdominant genetic model (TT+CC vs CT: Adjusted  $p=1.000$ ); (Table 4).

### ***Early onset pre-eclampsia vs Controls***

The frequencies of CC (58 cases and 101 controls), CT (91 cases and 127 controls) and TT (70 cases and 52 controls) in the codominant genetic model showed no significant difference: [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=1.000$ ); (CT vs TT: Adjusted  $p=1.000$ )]. Similarly, no significant association found with the dominant genetic model (CC vs CT +TT: Adjusted  $p=1.000$ ), the recessive genetic model (CC+CT vs TT: Adjusted  $p=1.000$ ) and the overdominant genetic model (TT+ CC vs CT, Adjusted  $p=1.000$ ); (Table 4).

### ***Late-onset pre-eclampsia vs Controls***

The genotype frequencies of CC (64 cases and 101 controls), CT (74 cases and 127 controls) and TT (32 cases and 52 controls) in the codominant model were not significantly different between the controls and LOPE: [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=1.000$ ) and (CT vs TT: Adjusted  $p=1.000$ )]. Similarly, the dominant genetic model (CC vs CT + TT: Adjusted  $p=1.000$ ), the recessive genetic model (CC+CT vs TT: Adjusted  $p=1.000$ ) and the overdominant genetic model were not significantly associated with LOPE (TT + CC vs CT: Adjusted  $p=1.000$ ); (Table 4).

### ***Early-onset pre-eclampsia vs Late-onset pre-eclampsia***

The frequencies of CC (58 EOPE and 64 LOPE), CT (91 EOPE and 74 LOPE) and TT (38 EOPE and 32 LOPE) of the codominant genetic model showed no statistical significance between EOPE and LOPE: [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=0.808$ ); (CT vs TT: Adjusted  $p=1.000$ )]. The dominant genetic model displayed no significant difference between EOPE and LOPE (CC vs CT+TT: Adjusted  $p=0.748$ ). Likewise, the recessive genetic model (CC+CT vs TT: Adjusted  $p=1.000$ ) and the overdominant genetic model were not significant association with EOPE and LOPE (TT+CC vs CT: Adjusted  $p=1.000$ ); (Table 4).

## **Genotype Association of *SLC2ALGLUT9* (rs1014290) with Pre-eclampsia**

### ***Pre-eclampsia vs Controls***

The frequencies of CC (25 cases and 20 controls), CT (159 cases and 105 controls) and TT (172 cases and 155 controls) of the codominant genetic model showed no significant association with PE: [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=1.000$ ); (CT vs TT: Adjusted  $p=0.224$ )]. The dominant genetic model was not statistically different between PE and the controls (CC vs CT+TT: Adjusted  $p=1.000$ ). Likewise, the recessive genetic model (CC+CT vs TT: Adjusted  $p=0.280$ ) and the overdominant genetic model indicated no significant difference between PE and the control group (TT+CC vs CT: Adjusted  $p=0.243$ ); (Table 5).

### ***Early onset pre-eclampsia vs Controls***

The frequencies of CC (17 cases and 20 controls), CT (88 cases and 104 controls) and TT (82 cases and 155 controls) in the codominant model were not significantly associated with EOPE [CT vs TT: Adjusted  $p=0.072$ ]. No significant association was noted with: CC vs TT: Adjusted  $p=0.726$ ) and CC vs CT: Adjusted  $p=1.000$ ). The dominant genetic model demonstrated no significant association with EOPE (CC vs CT +TT: Adjusted  $p=1.000$ ). Likewise, the recessive genetic model [CC+CT vs TT: Adjusted  $p=0.053$ ] and the overdominant genetic model (TT+CC vs CT) showed no association of rs1014290 with EOPE [TT+ CC vs CT: Adjusted  $p=0.142$ ];(Table 5).

### ***Late-onset pre-eclampsia vs Controls***

The genotype frequencies of CC (8 cases and 20 controls), CT (71 cases and 104 controls) and TT (90 cases and 155 controls) in the codominant genetic model were not significantly associated with LOPE: [(CC vs TT: Adjusted  $p=1.000$ ); (CC vs CT: Adjusted  $p=0.906$ ) and (CT vs TT: Adjusted  $p=1.000$ )]. The dominant model showed no significant difference between LOPE and the controls (CC vs CT+TT: Adjusted  $p=1.000$ ). Similarly, the recessive model :(CC+CT vs TT: Adjusted  $p=1.000$ ) and the overdominant model was not significantly associated with LOPE (TT + CC vs CT: Adjusted  $p=1.000$ ); (Table 5).

### ***Early-onset pre-eclampsia vs Late-onset pre-eclampsia***

The frequencies of CC (17 EOPE and 8 LOPE), CT (88 EOPE and 71 LOPE) and TT (82 EOPE and 90 LOPE) of rs1014290 showed no significant association with EOPE and LOPE in the codominant genetic model: [(CC vs TT: Adjusted  $p=0.230$ ); (CC vs CT: Adjusted  $p=0.938$ ) and (CT vs TT: Adjusted  $p=0.652$ )]. The dominant model showed no significant association of rs1014290 with EOPE and LOPE (CC vs CT+TT: Adjusted  $p=0.432$ ). In the same way, the recessive genetic model (CC+CT vs TT: Adjusted  $p=0.305$ ) and

the overdominant genetic model were not significantly associated with EOPE and LOPE (TT+CC vs CT: Adjusted  $p=1.000$ ); (Table 5).

## Discussion

The key findings in this study revealed that of the four SNPs selected, rs2231142 (*ABCG2*) has a frequency of <1% in our population and rs12129861 (*PDZK1 CD160*) showed no significant association with PE. However, rs505802 (*URAT 1*) and rs1014290 (*GLUT 9*) denote a significant association with PE.

### *URAT 1 SLC22A12* (rs505802)

The study demonstrates a strong association of the human urate transporter 1 (*URAT 1 SLC22A12*; rs502802) genetic variant with PE, specifically LOPE compared to the control group [Adjusted  $p=0.028^*$ : OR (95% CI) = 1.73 (1.258 - 2.442)] (Table 3). Late-onset pre-eclampsia occurs after 34 weeks of gestation and affects 75-80% of all pregnancy cases [27]. This condition is mainly associated with maternal health disorders which include metabolic syndrome, obesity, dyslipidemia, and insulin resistance [28]. Our findings are the first to demonstrate uric acid polymorphisms in pregnancy, however, they are similar to a previous study that reported *URAT 1* gene association with increased inflammatory response in non-pregnant patients with metabolic syndrome, obesity, and hypertension [29].

Notably, the human urate transporter gene (*URAT 1*) is responsible for uric acid reabsorption in the proximal tubule of the nephron [30]. A study investigating whether *URAT 1* was a candidate gene for hyperuricemia or hypouricemia in a non-pregnant Korean population demonstrated a strong significance with hyperuricemia [OR=32.07,  $p=1.20 \times 10^{-8}$ ] [18]. Although the current study has no clinical data on serum uric acid levels, we speculate that rs505802 may be involved with hyperuricemia found in PE, as well as the clinical manifestations are seen in the participants with LOPE. Moreover, the impairment of renal function may also lead to the disruption of uric acid regulation with resultant hyperuricemia[30]. Previous studies in a Chinese cohort with cardiovascular diseases have demonstrated that hyperuricemia is associated with elevated blood pressure, diabetes, obesity, and insulin resistance[31]. These symptoms are also risk factors for PE development [32]. Sultana et al., [33] also reported that increased serum uric acid levels stimulate inflammation, oxidative stress and endothelial dysfunction: all of which are characteristics of PE [33]. In addition, it was reported that the *URAT 1* gene is found in adipocytes where it activates oxidative stress and inflammation in response to hyperuricemia [34]. Moreover, it has been mentioned that uric acid mediates inflammation and can, therefore, promote the production of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and the TNF- $\alpha$  through monocytes [35]. Increased concentration levels of TNF-

$\alpha$  has been noted in PE individuals with a significant correlation with increased serum uric acid levels [35]. In addition, a previous study by Redman et al., [36] reported that pro-inflammatory cytokines are exaggerated in PE compared to healthy controls [36].

Noteworthy, in our study, pre-eclamptic individuals had a higher heterozygote genotype (CT) associated with PE; a heterozygous disadvantage since the heterozygous genotype (CT) increases the risk of PE during pregnancy. The CT genotype was more prevalent in PE (37%) [TT+CC vs CT: Adjusted  $p=0.004^{**}$ : OR (95% CI) = 1.73 (1.258 - 2.442)] and in LOPE (42%) [CC vs CT: Adjusted  $p=0.027^*$ : OR (95 % CI) =1.75 (1.165 - 2.2628)] than the control group (31%) and EOPE (31%); (Table 3). Because it is very rare to find the association of a disease with a heterozygous genotype, these findings are similar to a study by Liu et al., [26] that found a heterozygous genotype to be protective against Tuberculosis [26]. Currently, there is no literature on heterozygous genotypes associated with pregnancy complications. However, in our case, individuals with the CT genotype may have a predisposition to PE development.

#### ***PDZK1 CD160* (rs12129861)**

*PDZK1 CD160* is a scaffolding protein, necessary for uric acid transporter activity [37]. In our study, there was no significant association of rs12129861 with PE (Table 4). Therefore, our results demonstrate that rs1212986 is not associated with the pathogenesis of pre-eclampsia or hyperuricemia in our study population. However, several studies have been conducted on Asian and European descent and these studies have reported the involvement of the *PDZK1 CD160* gene, specifically the rs12129861 SNP, with a uric acid level in gout [15, 31, 37-39].

#### ***SLC2ALGLUT9* (rs1014290)**

The *GLUT 9* gene functions as a transporter for fructose, glucose and uric acid. It is located within kidney and liver cells [23]. In the present study, significant association was noted with rs1014290 in controls compared to EOPE [Adjusted p value= $0.040^*$ : OR (95% CI) = 1.60(1.102 - 2.325)] (Table 2). The CT genotype was more prevalent within the EOPE group (47%) compared to controls (37%), confirming that individuals with the CT genotype are at a 1.6 risk fold of PE development (Table 2). Our study indicates that this gene is associated with the pathogenesis of EOPE. Early-onset pre-eclampsia contributes to 5-20% of all cases of PE and is probably the greatest contributor to maternal

and fetal morbidity and mortality associated with this condition [27]. Early-onset pre-eclampsia has been reported to be more detrimental to the fetus than it is for the mother as it involves poor placentation that leads to intrauterine growth restriction and subsequently neonatal death [27]. More importantly, a study by Kristensen et al.,[40] confirmed that kidney glomerular and proteinuria diseases are associated with EOPE. Hyperuricemia is a result of renal insufficiency since a decrease in the glomerular filtration causes an increase in tubular reabsorption and a decrease in uric acid secretion [41]. In contrast, *GLUT 9* mutations result in hypouricemia, indicating an increase in urate excretion [42]. However, it has been mentioned that uric acid triggers endothelial dysfunction, advances hypertension, vascular and renal diseases [33]. An increase in maternal vascular resistance has been suggested to account for poor placentation in EOPE [27]. Similarly, a study published approximately 53 years ago showed that hyperuricemia was associated with hypertension [43]. Nonetheless, the *GLUT 9* gene has been reported to directly lower blood pressure level in an Amish population, implicating that a mutation in this gene triggers high blood pressure [44].

The current study has shown a strong association of the *GLUT 9* gene with EOPE. These results are similar to that of a previous study that reports an inactivation of the gene coding for GLUT 9 in the liver of mice promoting severe hyperuricemia [45]. The latter findings suggest that damage to hepatic cells may lead to an inactivation of *GLUT 9* and result in hyperuricemia. Interestingly, EOPE has been shown to have an aggressive hematological, arterial, renal, hepatic adverse maternal and fetal outcome in comparison to LOPE [27].

Several studies have reported the association of *GLUT 9* with uric acid [44-51]. Interestingly, Wang et al., [52], reported that hyperuricemia was strongly associated with hypertension in both males ((SUA)> 420  $\mu\text{mol/l}$ ) and females ((SUA)> 360  $\mu\text{mol/l}$ ), thus pregnant individuals with hyperuricemia may be susceptible to PE development [52]. Vitart *et al.*, (2008) also showed that the C>T alleles of this SNP were associated with hyperuricemia in patients with gout from the northern and western Europe [53]. Moreover, these alleles are also associated with hyperuricemia in a Minnesota Chinese ethnic Hmong population [39]. Nonetheless, our findings may provide an explanation as to why women of African ancestry seem to have the more clinically aggressive form of PE, which is EOPE and have high prevalence rates of renal impairment and end-stage renal disorder [54]. Early-onset PE is more aggressive since it occurs due to poor placentation, which could cause intrauterine growth restriction followed by death of the fetus [27].

### ***ABCG2 (rs2231142)***

We found a frequency of < 1% in our study population and this is in keeping with a study by Zhang et al., [14] which showed a significant association of rs2231142 with hyperuricemia in four American population groups [European Americans ( $p = 2.37 \times 10^{-67}$ ), Mexican Americans ( $p = 6.97 \times 10^{-9}$ ), African Americans ( $p = 3.98 \times 10^{-5}$ ), and Indigenous Americans ( $p = 5.33 \times 10^{-4}$ ) with gout. However, significance was strongly noted in males and menopausal women compared to women in their reproductive phase of life. No data was reported on pregnant women; nevertheless, it was concluded that rs2231142 may be regulated by sex hormones [14].

### **Strengths and Limitations of the Study**

To the best of our knowledge, this is the first study to examine uric acid polymorphisms in a South African population of African ancestry. In South Africa, PE has the highest prevalence in the province of KwaZulu-Natal (12%), occurring predominantly in primigravidae. The SNPs examined in this study do not represent all the SNPs available in uric acid, however, the four SNPs were selected based on their association with hyperuricemia in gout. Furthermore, this study utilizes retrospectively collected samples and therefore the number of samples was limited, and the levels of uric acid in pre-eclamptic individuals were not available.

### **Summary**

In this study, we hypothesized that gene variants of uric acid (rs505802-*URAT 1*, rs12129681-*PDZK1*, rs1014290-*GLUT 9*) associated with hyperuricemia in gout are also associated with pre-eclampsia. We demonstrate a prevalence of these variants within the study population and showed a strong association with PE. Therefore, this study provides new evidence linking these genetics variations associated with hyperuricemia in gout to PE. Furthermore, this study suggests that women of African ancestry are a risk factor for PE, particularly the EOPE subtype since this type of PE is known to be clinically associated with renal insufficiency.

## **Conclusion and Future Recommendations**

Two (*URAT 1*, rs505802; *GLUT 9*, rs1014290) of the three gene polymorphisms of uric acid showed significant association with PE. Therefore, these gene variants are involved in the pathogenesis of PE. However, further studies need to be conducted in an independent cohort with a larger sample size to confirm these results. In addition, the SNPs investigated in the current study may also be used to genetically screen patients for the risk of pre-eclampsia.

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## **Declaration of Interest**

There are no conflicts of interest in this study.



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## Appendices

**TABLE 1: Patient Demographics and Clinical Data**

	<b>Control (n=280)</b>	<b>EOPE (n=187)</b>	<b>LOPE (n=170)</b>	<b>p-value</b>
<b>Mean &amp; SD</b>				
Maternal Age (years)	26.78±6.5	29.76±6.5	27.29±6.8	
Control vs EOPE				<b>&lt;0.0001</b> <sup>***</sup>
Control vs LOPE				0.9701 <sup>ns</sup>
EOPE vs LOPE				<b>&lt;0.0001</b> <sup>***</sup>
Systolic BP (mmHg)	109.8±12.3	158.9±13.7	151.4±12.1	
Control vs EOPE				<b>&lt;0.0001</b> <sup>***</sup>
Control vs LOPE				<b>&lt;0.0001</b> <sup>***</sup>
EOPE vs LOPE				<b>0.0026</b> <sup>**</sup>
Diastolic BP (mmHg)	67.65±8.4	102.0±9.3	98.23±6.6	
Control vs EOPE				<b>&lt;0.0001</b> <sup>***</sup>
Control vs LOPE				<b>&lt;0.0001</b> <sup>***</sup>
EOPE vs LOPE				<b>0.0300</b> <sup>*</sup>
Gestational Age (weeks)	38.24±1.7	28.51±4.2	37.06±1.9	
Control vs EOPE				<b>&lt;0.0001</b> <sup>***</sup>
Controls vs LOPE				<b>&lt;0.0001</b> <sup>***</sup>
EOPE vs LOPE				<b>&lt;0.0001</b> <sup>***</sup>
Maternal Weight (Kg)	72.79±16.8	81.96±18.5	73.48±18.2	
Control vs EOPE				<b>0.0120</b> <sup>*</sup>
Control vs LOPE				0.2253 <sup>ns</sup>
EOPE vs LOPE				0.3585 <sup>ns</sup>
Proteinuria	0.00±0.06	1.51±0.74	1.44±0.69	
Control vs EOPE				<b>&lt;0.0001</b> <sup>***</sup>
Control vs LOPE				<b>&lt;0.0001</b> <sup>***</sup>
LOPE vs EOPE				0.4970 <sup>ns</sup>

A *p-value* of <0.05 was considered statistically significant, \*\*\* = highly significant with a *p-value* of *p*<0.001. *ns*= not significant. SD (Standard deviation), EOPE (Early onset pre-eclampsia), LOPE (late onset pre-eclampsia).

**Table 2: Allelic Association of Rs505802, Rs12129861, Rs1014290 in Pre-eclampsia and Control groups**

ALLELES	Rs505802 OR (95%CI) <i>p</i> -value	Rs12129861 OR (95%CI) <i>p</i> -value	Rs1014290 OR (95%CI) <i>p</i> -value
<b>CONTROL vs PE</b>			
FREQUENCIES CC, CT, TT	<b>Controls</b> -(173;27%), (88;14%), (19;3%). <b><sup>1</sup>PE</b> -(199;31%), (131;21%), (27;4.2%) <b><sup>2</sup>EOPE</b> - (117;18%), (58;9%), (12;1.8%). <b><sup>3</sup>LOPE</b> -(82;13%), (73;11%), (15;2.4%)	<b>Controls</b> -(101;16%), (127;20%), (52;8%). <b>PE</b> -(122;19%), (165;26%), (70;11%). <b>EOPE</b> - (58;9%), (91;14%), (38;6%). <b>LOPE</b> -(64;10%), (74;12%), (32;5%)	<b>Controls</b> -(20;3%), (104;16%), (155;24%). <b>PE</b> -(25;4%), (156;25%), (172;27%). <b>EOPE</b> -(17;3%), (88;14%), (82;13%). <b>LOPE</b> -(8;1%), (71;11%), (90;14)
C vs T OR (95% CI) P VALUE	1.20 (0.9292 -1.562)  Adjusted <i>p</i> value= <i>0.480</i> (False)	1.06 (0.8487 - 1.329)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.19 (0.9313 - 1.532)  Adjusted <i>p</i> value= <i>0.480</i> (False)
CC vs CT/TT OR (95% CI) P VALUE	1.12 (0.6112 - 2.067)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.07 (0.7177 - 1.594)  Adjusted <i>p</i> value= <i>1.000</i> (False)	0.97(0.5313 - 1.800)  Adjusted <i>p</i> value = <i>1.000</i> (False)
CC/CT vs TT OR (95% CI) P VALUE	1.28 (0.9331 - 1.766)  Adjusted <i>p</i> value= <i>0.360</i> (False)	1.09 (0.7832 - 1.508)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.07 (0.7888 - 1.451)  Adjusted <i>p</i> value = <i>1.000</i> (False)
<b>CONTROL vs EOPE</b>			
C vs T OR (95% CI) P VALUE	1.03 (0.7543 - 1.417)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.15 (0.8821 - 1.497)  Adjusted <i>p</i> value= <i>0.900</i> (False)	1.39 (1.044 - 1.856)  Adjusted <i>p</i> value= <i>0.072</i> (False)
CC vs CT/TT OR (95% CI) P VALUE	0.94 (0.4459 - 1.990)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.12 (0.7013 - 1.783)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.29 (0.6593 - 2.544)  Adjusted <i>p</i> value = <i>1.000</i> (False)
CC/CT vs TT OR (95% CI) P VALUE	0.96 (0.6602 - 1.417)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.26 (0.8459 - 1.862)  Adjusted <i>p</i> value= <i>0.780</i> (False)	1.60(1.102 - 2.325)  Adjusted <i>p</i> value = <b><i>0.040*</i></b> ( <b>True</b> )
<b>CONTROL vs LOPE</b>			
C vs T OR (95% CI) P VALUE	1.49 (1.104 - 2.030)  Adjusted <i>p</i> value= <b><i>0.028*</i></b> ( <b>True</b> )	1.03 (0.7813 - 1.352)  Adjusted <i>p</i> value= <i>1.000</i> (False)	1.00 (0.7368 - 1.367)  Adjusted <i>p</i> value = <i>1.000</i> (False)
CC vs CT/TT	1.33 (0.6564 - 2.692)	1.02 (0.6237 - 1.657)	1.55 (0.6686 -3.612)

OR (95% CI) P VALUE	Adjusted p value= <i>1.000</i> (False)	Adjusted p value= <i>1.000</i> (False)	Adjusted p value = <i>1.000</i> (False)
CC/CT vs TT OR (95% CI) P VALUE	1.74 (1.180 - 2.551)  Adjusted p value= <b><i>0.015*</i></b> ( <b>True</b> )	1.07 (0.7210 to 1.588)  Adjusted p value = <i>1.000</i> (False)	1.10 (0.7477 - 1.610)  Adjusted p value = <i>1.000</i> (False)
<b>EOPE vs LOPE</b>			
C vs T OR (95% CI) P VALUE	1.55 (1.105 - 2.168)  Adjusted p value= <b><i>0.032*</i></b> ( <b>True</b> )	1.18 (0.8771 - 1.590)  Adjusted p value= <i>0.810</i> (False)	1.40 (1.008 - 1.935)  Adjusted p value = <i>0.132</i> (False)
CC vs CT/TT OR (95% CI) P VALUE	1.41 (0.6408 - 3.108)  Adjusted p value= <i>1.000</i> (False)	1.10 (0.6511 - 1.858)  Adjusted p value = <i>1.000</i> (False)	2.01 (0.8450 - 4.793)  Adjusted p value = <i>0.300</i> (False)
CC/TT vs TT OR (95% CI) P VALUE	1.79 (1.176 - 2.736)  Adjusted p value= <b><i>0.019*</i></b> ( <b>True</b> )	1.34 (0.8661 - 2.082)  Adjusted p value = <i>0.570</i> (False)	1.55 (1.058 - 2.281)  Adjusted p value = <i>0.073</i> (False)

<sup>1</sup>Pre-eclampsia,<sup>2</sup>Early onset Pre-eclampsia,<sup>3</sup>Late onset Pre-eclampsia. A *p*-value of <0.05 was considered statistically significant. \*= denotes significance, \*\* = *p*<0.01 and \*\*\* = *p*<0.001. Bonferroni corrected test was used for multiple comparisons.



**Table 3: Genetic Association of Rs505802 (URAT 1) in Controls vs Pre-Eclampsia (PE) groups**

SNP	GENETIC MODEL	GENOTYPES	CONTROL	CONTROLS vs PE			CONTROLS vs EOPE			CONTROLS vs LOPE			EOPE vs LOPE	
				PE	OR (95% CI)	P-VALUE and Adjusted value	EOPE	OR (95% CI)	P VALUE and Adjusted value	LOPE	OR (95% CI)	P VALUE	OR (95% CI)	P VALUE and Adjusted value
Rs505802	Codominant	CC CC vs TT	<b>173 (27%)</b>	<b>199 (31%)</b>	1.00 1.24 (0.6636 - 2.300)	- 1.000 (False)	<b>117 (18%)</b>	1.00 1.07 (0.5008 - 2.290)	- 1.000 (False)	<b>82 (13%)</b>	1.00 1.67(0.8056 - 3.444)	- 0.661 (False)	1.00 1.78(0.7934 - 4.010)	- 0.630 (False)
		CT CC vs CT	<b>88 (14%)</b>	<b>131 (21%)</b>	1.00 1.29 (0.9226 - 1.815)	- 0.540 (False)	<b>58 (9%)</b>	1.00 1.03 (0.6836 - 1.540)	- 1.000 (False)	<b>73 (11%)</b>	1.00 1.75 (1.165 - 2.628)	- <b>0.027* (True)</b>	1.00 1.79 (1.150 - 2.804)	- <b>0.039 (True)</b>
		TT CT vs TT	<b>19 (3%)</b>	<b>27 (4.2%)</b>	1.00 1.05 (0.5489 - 1.999)	- 1.000 (False)	<b>12 (1.8%)</b>	1.00 1.04 (0.4711 - 2.312)	- 1.000 (False)	<b>15 (2.4%)</b>	1.00 1.05(0.4989 - 2.213)	- 1.000 (False)	1.00 1.01(0.4373 - 2.318)	- 1.000 (False)
	Dominant	CC	<b>173 (27%)</b>	<b>199 (31%)</b>	1.00	-	<b>117 (18%)</b>	1.00	-	<b>82 (13%)</b>	1.00	-	1.00	-
		CT + TT	<b>107 (17%)</b>	<b>158 (25%)</b>	1.12 (0.6112 - 2.067)	1.000 (False)	<b>70 (11%)</b>	1.03 (0.7056 - 1.515)	1.000 (False)	<b>88 (14%)</b>	1.74 (1.180 - 2.551)	<b>0.020* (True)</b>	1.79 (1.176 - 2.736)	<b>0.026* (True)</b>
		Recessive	CC + CT	<b>261 (41%)</b>	<b>330 (52%)</b>	1.28 (0.9331 - 1.766)	0.498 (False)	<b>175 (27%)</b>	1.06 (0.5026 - 2.243)	1.000 (False)	<b>155 (24%)</b>	1.33 (0.6564 - 2.692)	1.000 (False)	1.41 (0.6408 to 3.108)
		TT	<b>19 (3%)</b>	<b>27 (4.2%)</b>	1.00	-	<b>12 (1.8%)</b>	1.00	-	<b>15 (2.4%)</b>	1.00	-	1.00	-
	Overdominant	TT + CC	<b>192 (30%)</b>	<b>226 (35%)</b>	1.75 (1.258 - 2.442)	<b>0.004** (True)</b>	<b>129 (20%)</b>	1.02 (0.6835 to 1.520)	1.000 (False)	<b>97 (15%)</b>	1.64 (1.106 - 2.437)	0.054 (False)	1.67(1.085 - 2.583)	0.078 (False)
		CT	<b>88 (14%)</b>	<b>131 (21%)</b>	1.00	-	<b>58 (9%)</b>	1.00	-	<b>73 (11%)</b>	1.00	-	1.00	-

\*= denotes significance of  $p < 0.05$ , \*\*= $p < 0.01$  and \*\*\* =  $p < 0.001$ . The Bonferroni test was used for multiple comparisons

**Table 4: Genetic Association of Rs12129861 (PDZK1 CD160) in Controls vs Pre-eclampsia (PE) groups**

SNP	GENETIC MODEL	GENOTYPES	CONTROL	CONTROLS vs PE			CONTROLS vs EOPE			CONTROLS vs LOPE			EOPE vs LOPE	
				PE	OR (95% CI)	P-VALUE and Adjusted value	EOPE	OR (95% CI)	P VALUE and Adjusted value	LOPE	OR (95% CI)	P VALUE and Adjusted value	OR (95% CI)	P VALUE and Adjusted value
Rs12129861	Codominant	CC CC vs TT	<b>101 (16%)</b>	<b>122 (19%)</b>	1.00 1.11 (0.7139 - 1.740)	- 1.000 (False)	<b>58 (9%)</b>	1.00 1.27 (0.7501 - 2.159)	- 1.000 (False)	<b>64 (10%)</b>	1.00 1.03(0.5998 - 1.768)	- 1.000 (False)	1.00 1.31(0.72 67 - 2.363)	- 1.000 (False)
		CT CC vs CT	<b>127 (20%)</b>	<b>165 (26%)</b>	1.00 1.08 (0.7573 - 1.528)	- 1.000 (False)	<b>91 (14%)</b>	1.00 1.25 (0.8194 - 1.900)	- 1.000 (False)	<b>74 (12%)</b>	1.00 1.08(0.7112 - 1.663)	- 1.000 (False)	1.00 1.36(0.84 85 - 2.170)	- 0.808 (False)
		TT CT vs TT	<b>52 (8%)</b>	<b>70 (11%)</b>	1.00 1.04 (0.6760 - 1.588)	- 1.000 (False)	<b>38 (6%)</b>	1.00 1.02 (0.6201 - 1.677)	- 1.000 (False)	<b>32 (5%)</b>	1.06(0.6244 - 1.786)	- 1.000 (False)	1.00 1.04(0.59 06 -1.816)	- 1.000 (False)
	Dominant	CC	<b>101 (16%)</b>	<b>122 (19%)</b>	1.00	-	<b>58(9%)</b>	1.00	-	<b>64 (10%)</b>	1.00	-	1.00	-
		CT + TT	<b>179 (28%)</b>	<b>165 (26%)</b>	1.09 (0.7832 - 1.508)	1.000 (False)	<b>129 (20%)</b>	1.25 (0.8459 - 1.862)	1.000 (False)	<b>106 (17%)</b>	1.07(0.7210 - 1.588)	1.000 (False)	1.34(0.86 61 - 2.082)	0.748 (False)
		Recessive	CC + CT	<b>228 (36%)</b>	<b>287 (45%)</b>	1.07 (0.7177 - 1.594)	1.000 (False)	<b>149 (23%)</b>	1.12 (0.7013 - 1.783)	1.000 (False)	<b>138 (22%)</b>	1.02 (0.6237 - 1.657)	1.000 (False)	1.14(0.77 54 - 1.673)
		TT	<b>52 (8%)</b>	<b>70 (11%)</b>	1.00	-	<b>38 (6%)</b>	1.00	-	<b>32 (5%)</b>	1.00	-	1.00	-
	Overdominant	TT + CC	<b>153 (24%)</b>	<b>192 (30%)</b>	1.02 (0.7419 - 1.393)	1.000 (False)	<b>96 (15%)</b>	1.14 (0.7880 - 1.655)	1.000 (False)	<b>96 (15%)</b>	1.08(0.7336 - 1.581)	1.000 (False)	1.23(0.81 01 - 1.867)	1.000 (False)
		CT	<b>127 (20%)</b>	<b>165 (26%)</b>	1.00	-	<b>91 (14%)</b>	1.00	-	<b>74 (12%)</b>	1.00	-	1.00	-

\*= denotes significance of  $p < 0.05$ , \*\*= $p < 0.01$  and \*\*\* =  $p < 0.001$ . The Bonferroni test was used for multiple comparisons

**Table 5: Genetic Association of Rs1014290 (*GLUT 9*) in Controls vs Pre-eclampsia (PE) groups**

SNP	GENETIC MODEL	GENOTYPES	CONTROL	CONTROLS vs PE			CONTROLS vs EOPE			CONTROLS vs LOPE			EOPE vs LOPE	
				PE	OR (95%CI)	P-VALUE and Adjusted value	EOPE	OR (95% CI)	P VALUE and Adjusted value	LOPE	OR (95%CI)	P VALUE and Adjusted value	OR (95%CI)	P VALUE
Rs1014290	Codominant	CC vs TT	<b>20 (3%)</b>	25 (4%)	1.00 1.13(0.6018 - 2.109)	- 1.000 (False)	<b>17 (3%)</b>	1.00 1.61 (0.7979 - 3.235)	- 0.726 (False)	<b>8 (1%)</b>	1.00 1.45(0.6141 - 3.431)	- 1.000 (False)	1.00 2.33(0.9556 - 5.692)	- 0.230 (False)
		CT vs CT	<b>104 (16%)</b>	159 (25%)	1.00 1.22(0.6462 - 2.315)	- 1.000 (False)	<b>88 (14%)</b>	1.00 1.01 (0.4957 - 2.036)	- 1.000 (False)	<b>71 (11%)</b>	1.00 1.71(0.7123 - 4.090)	- 0.906 (False)	1.00 1.71(0.6993 - 4.204)	- 0.939 (False)
		TT vs TT	<b>155 (24%)</b>	172 (27%)	1.00 1.38 (0.9914 - 1.915)	- 0.224 (False)	<b>82 (13%)</b>	1.00 1.60 (1.083 - 2.363)	- 0.072 (False)	<b>90 (14%)</b>	1.00 1.18(0.7895 - 1.751)	- 1.000 (False)	1.00 1.36(0.8824 - 2.097)	- 0.652 (False)
	Dominant	CC	<b>20 (3%)</b>	25 (4%)	1.00	-	<b>17 (3%)</b>	1.00	-	<b>8 (1%)</b>	1.00	-	1.00	-
		CT + TT	<b>259 (41%)</b>	331 (52%)	1.02 (0.5554 - 1.882)	1.000 (False)	<b>170 (27%)</b>	1.29 (0.6593 - 2.544)	1.000 (False)	<b>161 (25%)</b>	1.55(0.6686 - 3.612)	1.000 (False)	2.01(0.8450 - 4.793)	0.432 (False)
		Recessive	CC + CT	<b>124 (19%)</b>	184 (29%)	1.34 (0.9762 - 1.832)	0.280 (False)	<b>105 (16%)</b>	1.60 (1.102 - 2.325)	0.053 (False)	<b>79 (12%)</b>	1.09(0.7477 - 1.610)	1.000 (False)	1.46(0.9603 - 2.216)
		TT	<b>155 (24%)</b>	25 (4%)	1.00	-	<b>82 (13%)</b>	1.00	-	<b>90 (14%)</b>	1.00	-	1.00	-
	Overdominant	TT + CC	<b>175 (27%)</b>	197 (31%)	1.36 (0.9859 - 1.871)	0.243 (False)	<b>99 (15%)</b>	1.50 (1.027 - 2.179)	0.142 (False)	<b>98 (15%)</b>	1.22(0.8252 - 1.801)	1.000 (False)	1.23(0.8067 - 1.866)	1.000 (False)
		CT	<b>104 (16%)</b>	159 (25%)	1.00	-	<b>88 (14%)</b>	1.00	-	<b>71 (11%)</b>	1.00	-	1.00	-

\*= denotes significance of  $p < 0.05$ , \*\*= $p < 0.01$  and \*\*\* =  $p < 0.001$ . The Bonferroni tests was used for multiple comparisons



## **CHAPTER 4**

**Research Article: Soluble Angiotensin IV Receptor Levels in Pre-eclampsia: Is there a Variation?**

The following manuscript investigated plasma levels of soluble AT-4 in pre-eclampsia compared to normotensive pregnancies:

Citation: Khaliq, O.P., Konoshita, T., Moodely, J. and Naicker, T., 2019. Soluble Angiotensin IV Receptor Levels in Pre-eclampsia: Is there a Variation? **Accepted in** The *Journal of Maternal-Fetal & Neonatal Medicine* and will be available at the following link.

<https://doi.org/10.1080/14767058.2020.1743665> .

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 **The Journal of Maternal-Fetal & Neonatal Medicine** <onbehalf@manuscriptcentral.com> Fri, 13 Mar, 21:06 (5 days ago)   

to me ▾  
13-Mar-2020

Dear Dr Khaliq:

Ref: Soluble Angiotensin IV Receptor Levels in Pre-eclampsia: Is there a Variation?

Our referees have now considered your paper and have recommended publication in *The Journal of Maternal-Fetal & Neonatal Medicine*. We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting.

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**Title: Soluble Angiotensin IV Receptor Levels in Pre-eclampsia: Is there a Variation?**

**Running Title: Angiotensin IV Receptor levels in Pre-eclampsia**

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Conflicts of interest: NONE

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**Word Count:** 3575



## ABSTRACT

**Objective:** To measure the concentration of plasma soluble angiotensin IV receptor (sAT-4), a component of the renin angiotensin system in healthy normotensive pregnancies and pre-eclampsia.

**Study Design:** Stored maternal plasma samples obtained at time of diagnosis from pregnant women of African ancestry were stratified into normotensive and pre-eclampsia groups. Pre-eclampsia was subdivided into early onset, late onset, and into and severe pre-eclampsia. Plasma concentrations of sAT-4 were measured at 450nm using the ELISA technique (LNPEP KIT).

**Results:** The systolic and diastolic blood pressure (BP) levels of the normotensive group were statistically lower compared to pre-eclampsia groups ( $p<0.05$ ) and the mean gestational age in early onset pre-eclampsia was lower compared to late onset pre-eclampsia and the normotensive group ( $p<0.05$ ). Plasma sAT-4 levels were significantly elevated ( $p<0.0001$ ) in the normotensive group (median 1.95, range 1.89 – 2.02 ng/ml) compared to the pre-eclampsia group (median 1.55, range 1.42 – 1.74 ng/ml), regardless of gestational age. Soluble AT-4 was decreased in relation to the severity of pre-eclampsia ( $p<0.001$ ), the level in pre-eclampsia without severe features (median 1.57, range 1.42 – 1.74 ng/ml) was significantly higher than in pre-eclampsia with severe features (median 1.51, range 1.42 - 1.55 ng/ml). There was no significant difference in the sAT-4 level between early onset pre-eclampsia ( $1.60\pm 0.13$  ng/ml) and late onset pre-eclampsia ( $1.65\pm 0.29$  ng/ml) groups ( $p=0.59$ ).

**Conclusion:** Plasma circulating levels of sAT-4 in women with severe features of pre-eclampsia had lower levels than normotensives and those with preeclampsia without severe features.

**Keywords:** Pre-eclampsia, soluble angiotensin IV receptor (sAT-4), Renin-Angiotensin-Aldosterone System

## INTRODUCTION

Preeclampsia (PE) is a pregnancy specific multisystemic disorder, characterized by widespread endothelial dysfunction with clinical manifestations being end-organ dysfunction [1]. Pre-eclampsia causes substantial maternal and perinatal morbidity and mortality globally [2], and the exact cause is unknown [3]. More importantly, PE is a placental disorder therefore treatment requires the delivery of the fetus and placenta, however iatrogenic preterm delivery of the fetus and placenta is associated with obstetric and perinatal complications [2]. Despite several theories examining the pathogenesis of PE, molecular mechanisms underlying its precise development remains unclear [4].

During normal pregnancy, modification of cardiovascular function occurs, characterized by vasodilation that mediates increased blood flow to the uterus and kidneys [1]. The renin–angiotensin–aldosterone system (RAAS) serves as a key regulator of electrolyte balance, fluid homeostasis, blood pressure and placentation [5].

Normal placentation occurs in a low oxygen environment with hypoxia inducible factors (HIFs) modulating transcription genes involved in essential physiological processes that promote angiogenesis, cell proliferation, inflammation regulation of vascular tone and protection against oxidative stress [6]. The RAAS is involved in the early stages of placenta development thereby ensuring the development of a healthy pregnancy [7]. More specifically, angiotensin peptides *viz.*, angiotensin II (Ang II) and the hexapeptide angiotensin IV (Ang IV) promote normal placentation [8-10].

Angiotensin II and IV are highly expressed in early pregnancy and function by binding to angiotensin I (AT1R), angiotensin II (AT2R) and angiotensin IV (AT4R) receptors respectively [11]. The AT4 receptor is a class II transmembrane protein often referred to as insulin regulated aminopeptidase (IRAP) or oxytocinase (OTase) [9, 10]. The activation of AT4R improves cell signal transmission, and it has both antioxidant and anti-inflammatory properties [12]. Off note, Ang IV elevates intracellular calcium to boost nitric oxide synthase (NOS) thereby modifying superoxide production [13]. A reduction in AT4R expression in PE placenta [8] and Ang II and Ang IV has been reported to result in deficient trophoblast invasion, non-physiological remodeling of myometrial spiral arteries, a decrease in nitric oxide, which is an effective tissue vasodilator [4].

The imbalance of RAAS components in circulation heralds the clinical signs of PE [1]. Despite the low expression of AT4R in the placenta of PE, circulating plasma levels of AT4R remains unknown in PE. Therefore, the aim of this study was to establish the concentration of plasma soluble Ang IV receptor (sAT-4) in PE vs healthy normotensive pregnancies stratified by gestational age and severity using the enzyme-linked immunosorbent assay (ELISA) technology.

## **METHODS**

### **Study design**

This was a retrospective study consisting of a total of 250 stored maternal plasma samples obtained at the time of diagnosis from pregnant women of African ancestry. Written informed consent was obtained prior to sample collection from women recruited at a large regional hospital, South Africa. The study was approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal, South Africa (BCA338/17). Of the 250 women, 150 were normotensive pregnant (BP  $\leq$ 120/80 mmHg, with no obstetric complications) and 100 were pre-eclamptic (BP  $\geq$ 140/90 mmHg). All participants were between the ages of 18-41 years.

Pre-eclampsia was defined as new onset hypertension (blood pressure of  $\geq 140/90$  mmHg taken on two occasions four hours apart and at least 1+ proteinuria measured by urinary dipstick) commencing at 20 weeks or more of gestation, with or without proteinuria and/or with evidence of liver disease, renal complications, thrombocytopenia, neurological dysfunctions [14]. The PE group was subdivided into early onset pre-eclampsia (EOPE), which refers to hypertension that occurs at  $\leq 33$  weeks + 6 days of pregnancy and may result in the delivery of the fetus at  $< 34$  weeks of gestation (n=50) and late onset pre-eclampsia (LOPE), which is hypertension that occurs at  $\geq 34$  weeks of pregnancy and may result in the delivery of the fetus at  $>34$  weeks of gestation (n=50) [15]. Based on severity, the PE group was divided into the mild pre-eclampsia (n=69) and severe pre-eclampsia (n=31) groups. Pre-eclampsia without severe features (MPE) was defined as blood pressure of  $\geq 140/90$  mmHg and  $\leq 159/109$  mmHg and proteinuria of  $\geq 0.3$  g but  $\leq 2.0$  g obtained from a 24 hour urine test or a 1+ to 2+ on a urine dipstick [16]. Pre-eclampsia with severe features (SPE) was identified as systolic blood pressure  $\geq 160$  mmHg and/or diastolic blood pressure  $\geq 110$  mmHg, with proteinuria  $\geq 2.0$  g in a 24- hour urine test or  $\geq 3+$  on a dipstick [16].

Patients with a previous history of hypertension, renal disease, liver disease or chronic hypertension and gestational hypertension were excluded from the study. Participants were matched according to maternal age and gestational age (weeks).

### **Quantification of plasma circulating sAT-4**

The plasma sAT-4 level was determined using the Human LNPEP Assay Kit (Immuno-Biological Laboratories Co., Ltd Japan) according to the guidelines of the manufacturer. All the samples were analyzed in duplicate. The measurement range for this kit was 0.39 - 25ng/ml. Standard concentrations were plotted against absorbance to obtain a standard curve. The concentration of sAT-4 in the different samples were obtained by extrapolating the absorbance of the test samples on the

standard curve. The measurement of sAT-4 concentration in the standards and test samples were done against a test sample blank at a wavelength of 450nm.

### **Statistical analysis**

Results obtained in this study were analyzed using GraphPad Prism, V 5.03 (Graph-Pad Software Inc., California). Non-parametric method of data analysis was adopted as the sample size in the different experimental groups varied. The Mann-Whitney test was used to compare the results between the normotensive and the experimental groups. The Kruskal-Wallis test was also used to compare quantitative variables across the experimental groups. The patient demographic data are expressed as mean  $\pm$  SD. In this study, a p-value  $< 0.05$  was considered significant.

## **RESULTS**

**Table 1** represents the demographic data of all study groups. No significant differences were noted for maternal weight, maternal height, body mass index (BMI) and maternal age across all groups. However, systolic blood pressure, diastolic blood pressure and gestational age were significantly different across all three groups ( $p < 0.05$ ).

### **sAT-4 Quantification**

The plasma circulating level of sAT-4 in the different experimental groups is graphically illustrated in There was a significantly higher level of circulating sAT-4 ( $p < 0.0001$ ) in the normotensives (median 1.95, range 1.89 – 2.02 ng/ml) compared with the level in the PE (median 1.55, range 1.42 – 1.74 ng/ml) (Figure 1). Plasma sAT-4 level was significantly higher ( $p < 0.0001$ ) in the normotensives (median 1.95, range 1.89 – 2.02 ng/ml) compared with the level in EOPE (median 1.55, range 1.51 – 1.64 ng/ml) and LOPE (median 1.56, range 1.42 – 1.74 ng/ml) respectively. The

level of circulating sAT-4 in the early onset PE group did not statistically differ from that in the late onset PE group ( $p = 0.59$ ) (Figure 2).

In figure 3, circulating level of sAT-4 in the normotensive group (median 1.95, range 1.89 – 2.02 ng/ml) was significantly higher ( $p < 0.0001$ ) compared with the level in PE without severe features (Mild PE;MPE) (median 1.57, range 1.42 – 1.74 ng/ml) and PE with severe features (severe PE;SPE) (median 1.51, range 1.42 - 1.55 ng/ml) respectively. It was also observed that the level of circulating sAT-4 in severe PE group was relatively lower compared to that in the mild PE group ( $p < 0.0005$ ).

## Discussion

Circulating sAT-4 levels were found to be lower in the PE compared to the healthy normotensive group ( $p<0.001$ ). Furthermore, plasma sAT-4 levels were significantly lower in both the EOPE and LOPE subtypes compared to normotensive group ( $p<0.0001$ ) and in PE without severe features and in PE with severe features, compared to healthy normotensive pregnancies ( $p<0.0001$ ). The levels of sAT-4 were also observed to be significantly higher in PE with severe features, compared to PE without severe features respectively ( $p<0.0005$ ). Despite clinical differences, sAT-4 levels were similar between EOPE and LOPE groups ( $p=0.59$ ). Therefore, a variation was noted in the levels of sAT-4 in healthy normotensive groups compared to PE. Moreover, a significant variation was also observed with PE severity.

Notably, circulating RAAS components are elevated in uncomplicated gestations [17]. It is plausible to hypothesize that an increase in AT4R expression in pregnancy serves as a regulatory mechanism to ensure Ang IV binding to AT4R rather than to AT1R [8]. This shift in AT4R and AT1R expression regulates the role of Ang IV in placental development during pregnancy [8]. Furthermore, a decreased expression of AT4R in early pregnancy negatively impacts trophoblast invasion a hallmark of PE development, and it may also cause a downregulation of Ang IV facilitated vasodilatation [8, 18, 19]. It has been reported that Ang IV receptors facilitate the activation of the nitric oxide synthase (NOS) isoform in the endothelial cells which helps in vasorelaxation [20]. This is due to Ang IV stimulating an increase in the release of intracellular calcium which in turn is essential for regulating NOS [21].



Nonetheless, the results of the current study are corroborated by other reports that have also shown a significant decline in ATII and renin levels in PE [1, 5, 22]. Unlike AT1R and AT2R, AT4R is highly expressed throughout gestation [23, 24] highlighting its vital role in the maintenance of a successful pregnancy. Soluble AT4 is found on endothelial cells, smooth muscle cells, on the extravillous trophoblast and is responsible for trophoblast invasion [8]. A deficient extravillous trophoblast invasion leads to an absence of physiological conversion of myometrial spiral arteries in EOPE development [25]. In the current study, levels of sAT-4 were decreased compared to normal pregnancies. This probably explains the decrease noted in EOPE which is characterized by poor trophoblast invasion and abnormal remodeling of spiral arteries [25]. Nonetheless, oxidative stress emanating from poor placental perfusion contributes to the development of PE [26]. However, defective placentation is more pronounced in EOPE compared to LOPE [26, 27]. Early onset pre-eclampsia is pathophysiologically different from LOPE and it is a more severe clinical form of the condition associated with renal diseases, characterized by an earlier gestational age, onset and delivery [28].

This study is highlighted by the fact that it is the first, as far as we are aware, to compare the differences of plasma sAT-4 in healthy normotensives versus PE, as well as mild PE vs severe PE, we observed that 67.7% of the patients with severe features PE were of the EOPE type. Early onset pre-eclampsia is severely burdened with poor neonatal morbidity with prematurity, increased foetal growth restriction as well as small for gestational age babies[29]. Unlike EOPE, LOPE is characterised by a shorter duration of the ailment and it may be efficiently managed in settings with standard antenatal care services and also in cases where delivery of the baby is induced after the 37<sup>th</sup> week of gestation [29]. Markedly poor placental development in severe EOPE stimulates exaggerated inflammatory responses and causes a change in the intrauterine haemodynamic environment [30].

These changes cause placental malperfusion which when coupled with increased “placental oxidative and ER stress” triggers EOPE development and its severity [29].

A key finding in our study is the significant decrease in sAT-4 based on severity of PE *i.e.* in PE with severe features compared to PE without severe features. These results are corroborated by other studies that report a correlation of significantly lower Ang II with an increase in the severity and clinical outcome in PE [1]. This difference of sAT-4 in PE with severe features compared to PE without severe features may be due to the difference in placental pathology of PE without severe features compare to PE with features. The placental pathology in this two sub groups of PE differs, in that PE with severe features is associated with more infarcts and syncytial knots compared to that in PE without severe features [31, 32]. This finding of a significant decrease in the level of sAT-4 in PE with severe features compared to PE without severe features throws more light on the evidence that these two conditions are distinctly different entities [33, 34].

## **Conclusion**

Plasma circulating level of sAT-4 decreased in PE. Women with severe features of PE have lower levels of s-AT4 than those without severe features and normotensives.

## **Acknowledgments**

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Finally, we would also like to acknowledge the Optics and Imaging Center, with special thanks to Miss Wendy Phoswa and Miss Zinhle Mkhize for their support in this project.

### **Conflicts of Interests**

All authors declare that there are no conflicts of interest.

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## List of Figures

**Figure 1.** Plasma sAT-4 level in Normotensives and PE (ng/ml). There was a significantly higher level of circulating sAT-4 ( $p < 0.0001$ ) in the normotensives (median 1.95, range 1.89 – 2.02 ng/ml) compared with the level in the PE (median 1.55, range 1.42 – 1.74 ng/ml).

**Figure 2.** Plasma sAT-4 level in Normotensives, early onset PE and late onset PE (ng/ml). Plasma sAT-4 level was significantly higher ( $p < 0.0001$ ) in the normotensives (median 1.95, range 1.89 – 2.02 ng/ml) compared with the level in EOPE (median 1.55, range 1.51 – 1.64 ng/ml) and LOPE (median 1.56, range 1.42 – 1.74 ng/ml) respectively. The level of circulating sAT-4 in the early onset PE group did not statistically differ from that in the late onset PE group ( $p = 0.59$ ).

**Figure 3.** Plasma sAT-4 level in Normotensives, mild PE and severe PE (ng/ml). Shows the circulating level of sAT-4 in the normotensive group (median 1.95, range 1.89 – 2.02 ng/ml) was significantly higher ( $p < 0.0001$ ) compared with the level in PE without severe features (Mild PE;MPE) (median 1.57, range 1.42 – 1.74 ng/ml) and PE with severe features (severe PE;SPE) (median 1.51, range 1.42 - 1.55 ng/ml) respectively. It was also observed that the level of circulating sAT-4 in severe PE group was relatively lower compared to that in the mild PE group ( $p < 0.0005$ ).

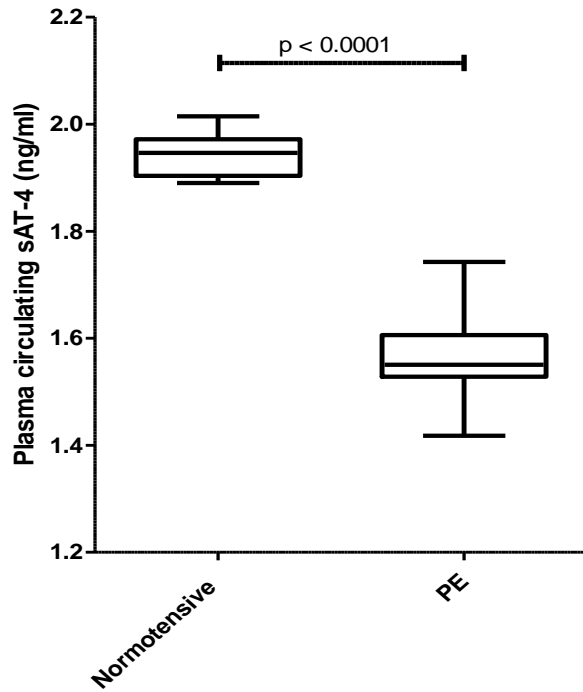
**Table 1: Patient Demographic Data**

Mean & <sup>1</sup> SD	Normotensive pregnant (n=100)	PE (n=357)				p-value
		<sup>2</sup> EOPE (n=50)	<sup>3</sup> LOPE (n=50)	<sup>4</sup> MPE (n=31)	<sup>5</sup> SPE (n=69)	
Age (years) Control vs EOPE Control vs LOPE EOPE vs LOPE Control vs MPE Control vs SPE MPE vs SPE	28.60±5.90	28.19±7.35	28.45±7.13	29.31±7.90	29.07±4.50	p>0.05 p>0.05 p>0.05 p>0.05 p>0.05 p>0.05 p>0.05
BMI (Kg/M <sup>2</sup> ) Control vs EOPE Control vs LOPE EOPE vs LOPE Control vs MPE Control vs SPE MPE vs SPE	32.59±7.301	33.52±9.483	37.20±8.021	34.36±7.10	32.98±8.10	p>0.05 p<0.05 p>0.05 p>0.05 p>0.05 p>0.05
Gestational Age (weeks) Control vs EOPE Control vs LOPE EOPE vs LOPE Control vs MPE Control vs SPE MPE vs SPE	31.88±6.73	24.25±5.77	35.95±1.96	34.46±3.72	31.89±4.60	p<0.05 p<0.05 p<0.05 p>0.05 p>0.05 p>0.05
Systolic BP (mmHg) Control vs EOPE Control vs LOPE EOPE vs LOPE Control vs MPE Control vs SPE MPE vs SPE	108.0±11.25	161.10±4.13	149.51±6.13	146.8±5.55	165.5±4.57	p<0.05 p<0.05 p>0.05 p<0.05 p<0.05 p<0.05
Diastolic BP (mmHg) Control vs EOPE	65.52±9.38	94.36±6.09	93.44±3.40	92.27±4.85	98.45±9.52	p<0.05 p<0.05 p>0.05

Control vs LOPE						p<0.05
EOPE vs LOPE						p<0.05
Control vs MPE						p>0.05
Control vs SPE						
MPE vs SPE						

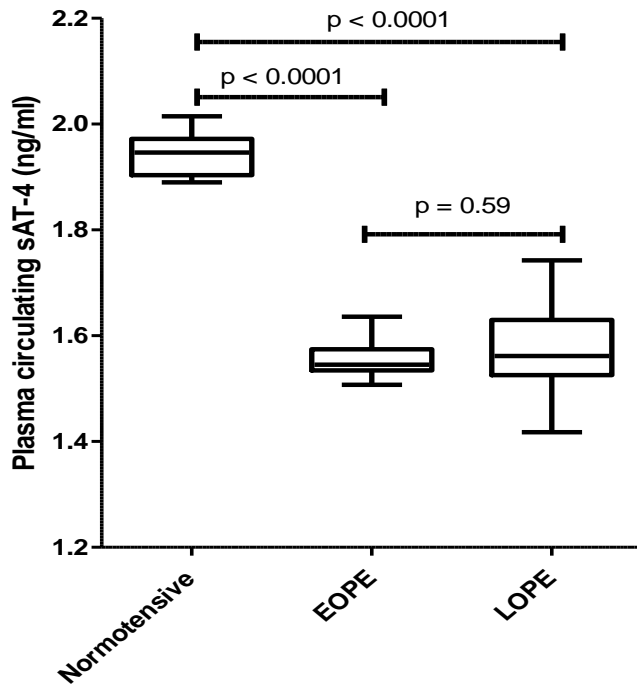
<sup>1</sup>SD-Standard Deviation, <sup>2</sup>EOPE- Early onset Pre-eclampsia, <sup>3</sup>LOPE-Late onset Pre-eclampsia,  
<sup>4</sup>MPEMild  
Pre-eclampsia, <sup>5</sup>SPE- Severe Pre-eclampsia

### Figure Legends

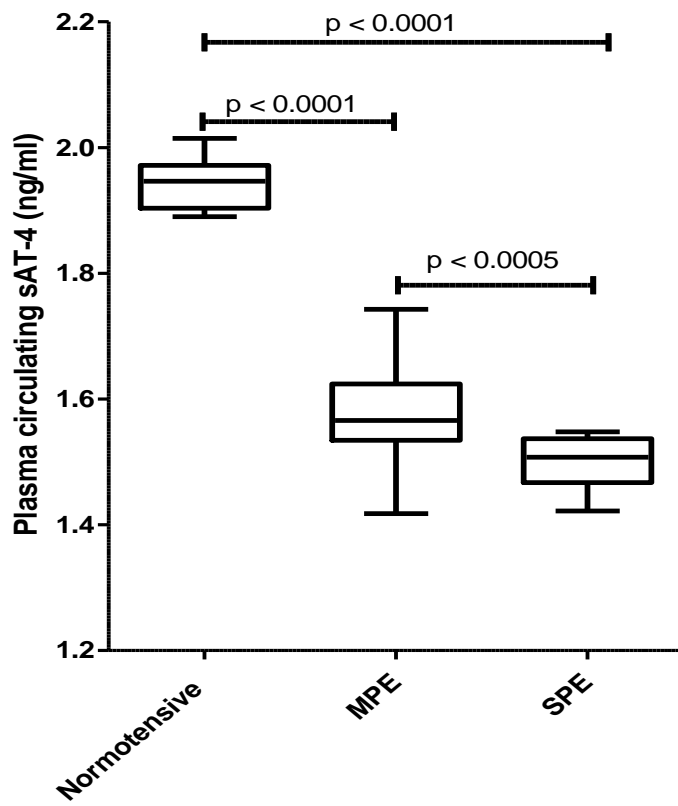


**Figure 1:** Plasma sAT-4 level in Normotensives and PE (ng/ml).





**Figure 2:** Plasma sAT-4 level in Normotensives, early onset PE and late onset PE (ng/ml)



**Figure 3:** Plasma sAT-4 level in Normotensives, mild PE and severe PE (ng/ml).



## **CHAPTER 5**

**Research Article: The Role of LNPEP and ANPEP Gene Polymorphisms in the Pathogenesis of Pre-eclampsia**

This research article focuses on the association of *LNPEP* and *ANPEP* gene polymorphisms in pre-eclampsia:

Citation: Khaliq, O.P., Konoshita, T., Moodley, J. and Naicker, T.,2019. The Role of LNPEP and ANPEP Gene Polymorphisms in the Pathogenesis of Pre-eclampsia. Submitted to *Hypertension Research*, HTR-2019-0557.

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## **The Role of *LNPEP* and *ANPEP* Gene Polymorphisms in the Pathogenesis of Pre-eclampsia**

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## **Abstract**

The role of the renin angiotensin system in the pathogenesis of pre-eclampsia is well established. Pre-eclampsia is a leading cause of maternal and neonatal morbidity and mortality worldwide. Therefore, the aim of this study was to investigate the association of the angiotensin receptor 4 and aminopeptidase-N in the pathogenesis of pre-eclampsia. Stored blood samples of 637 South African women of African ancestry were utilized. The study population were divided into controls (n=280) and pre-eclampsia (n=357). Pre-eclampsia was sub-divided into early (n=187) and late (n=170) onset subtypes. DNA was extracted from whole blood and genotyped. Odds ratio and 95% confidence intervals were used to assess the association. The allele and genotype frequencies of the angiotensin receptor 4 and aminopeptidase-N showed no significant difference between the control versus the pre-eclampsia groups. Similarly, allele and genotype frequencies of the control group versus the subtypes of pre-eclampsia (early onset and late onset pre-eclampsia) showed no significant differences. The single nucleotide polymorphisms of angiotensin receptor 4 (rs18059) and aminopeptidase-N (rs6496603) are not associated with the pathogenesis of pre-eclampsia in women of African ancestry.

**Keywords:** Pre-eclampsia, Angiotensin receptor 4, Aminopeptidase-N, Single nucleotide polymorphisms



## Introduction

Pre-eclampsia (PE) is a pregnancy specific disorder which presents clinically with hypertension and proteinuria [1]. It complicates 1-10% of pregnancies globally and is significantly associated with maternal and neonatal morbidity and mortality [2]. Although the aetiology of PE is poorly understood, it is widely accepted that it is characterised by an inadequate trophoblast invasion and lack of myometrial remodelling spiral arteries, with subsequent endothelial dysfunction [3].

The involvement of the renin-angiotensin system (RAS) in the pathophysiology of PE has also been documented [4]. The chief function of RAS is to modulate blood pressure and electrolyte balance [4, 5]. The RAS is activated primarily by the enzyme renin released by juxtaglomerular cells of the kidney during low blood pressure and salt concentration [6]. The renin enzyme converts angiotensinogen released by the liver into angiotensin 1, which is catalysed by angiotensin-converting enzyme (ACE) to form the potent vasoconstrictor angiotensin II [7]. Angiotensin II (Ang II) binds to angiotensin 1 (ATR1) and angiotensin 2 (ATR2) receptors to perform its physiological function [8]. The key role of ATR1 is to activate vasoconstriction, regulate sympathetic activity, as well as in aldosterone release [8]. In contrast, AT2R is weakly expressed in the kidney, inhibits cell proliferation and stimulates cell death [9].

Angiotensin II may also be cleaved to form other Ang peptides such as the Ang (1-7), Ang III and Ang IV [10-12]. The enzyme facilitating the conversion of Ang III to Ang IV is aminopeptidase N (ANPEP) [13]. This protein is located on the brush border of proximal tubules [14-16]. It is involved in angiogenesis, inflammation, cell invasion and death hence is vital for pregnancy success [17].

Ang IV binds to AT1R and AT2R with low affinity but has a high affinity for the angiotensin receptor 4 (AT4R), also known as the leucyl-N-exopeptidase (LNPEP) [12]. AT4R is found on endothelial and smooth muscle cells where it binds Ang IV to mediate blood flow [18]. Furthermore, Ang IV is also found in the extravillous trophoblast (EVT) cells hence is involved in trophoblast invasion and spiral artery remodelling during the first trimester of pregnancy [19].

The AT4R is highly expressed in the heart, skeletal muscles kidneys, small intestine and the placenta [20]. Interestingly, the AT4R is up-regulated in early pregnancy placentae implicating its role in trophoblast migration [21]. High expression levels of the AT4R aid in Ang IV binding since it has a higher affinity for AT4R rather than AT1R [21]. The AT4R has been reported as

an antagonist of the AT1R. The binding of Ang IV to AT1R may lead to pregnancy complications. [22]. Elevated expression levels of AT1R in placentae of PE pregnancies compared to normotensive pregnancies have been reported [21, 23].

These alterations of RAS components have led to comprehensive investigations on their gene polymorphisms in hypertension, cardiovascular and renal diseases [24]. Siphai *et al.*, (2009) demonstrated that gene polymorphism of the AT1R (A1166C) gene in Turkish patients are not associated with ischemic stroke. Likewise, Aung *et al.*, (2017) demonstrated that polymorphisms of the AT1R (A1166C) and AT2R (C3123A) SNPs were not associated with PE development in Black South African females [25].

Existing data and information on the AT4R in pregnancy are very limited and poorly understood. In order to elucidate why the placental expression of AT4R are down-regulated in PE, this study aims to investigate the association of single nucleotide polymorphisms (SNPs) of AT4R viz., (*LNPEP*; rs18059) and *ANPEP* (rs6496603) in Black preeclamptic women of African ancestry.

## **Materials and Methods**

### **Study population**

The samples utilized in this study were stored blood samples from pregnant women (n=637), obtained from a public sector hospital in Durban, South Africa. Informed consent for the storage and future studies was obtained from the Ethical Biomedical Committee at the University of KwaZulu-Natal (BCA338/17). The study population included normotensive pregnant (n=280) and PE (n=357) women. Pre-eclampsia was defined as high blood pressure ( $\geq 140/90$  mmHg occurring during the second half of pregnancy ( $\geq 20$  weeks of gestation) with proteinuria ( $\geq 300$  mg in a 24-hour quantitative urine test/ +1 dipstick) or without proteinuria but with evidence of one or more of the conditions such as thrombocytopenia, raised liver enzymes, and abnormal renal function tests [26]. All demographic and clinical parameters were recorded at the time of hospital admission for PE patients during pre-natal visits. Controls were matched according to age and gestational age.

### **DNA Isolation**

DNA was extracted from whole blood (500  $\mu$ l) following the protocol from the Thermo Scientific GeneJet whole blood genomic DNA mini Kit (Thermo Scientific). Purified DNA samples were stored at  $-20^{\circ}\text{C}$  for genotyping analysis.

## **Genotyping**

The SNPs (rs18059) of the *LNPEP* and (rs6496603) of *ANPEP* genes were genotyped from purified DNA samples using the TaqMan Universal PCR master mix (Applied Biosystems) following the manufacturer's instructions (Life Technologies LTD, Warrington, UK). These primers (rs18059 and rs6496603) were amplified and allelic discrimination data were analysed using the Real-time Polymerase Chain Reaction (PCR) together with the StepOne v2.2.2 Applied Biosystems software.

## **Sample Size**

The sample size was calculated using the specified gene between cases and controls. A two-fold difference in the level of gene was used based on a 95% probability level assuming 50% were controls. The required sample size was 274 participants (137/group), with 80% power. However, a sample size of 637 participants was obtained respectively, sub-divided into the normotensive (280) group and the PE (357) group.

## **Statistical Analysis**

Frequency and percentages were used to describe the presence of alleles and genotypes. Sub-groups were compared using the Chi-square test or Fisher's exact test as appropriate. The strength of association was reported as odds ratio (OR) and 95% confidence interval (CI) for categorical data and Wilcoxon rank sum tests for numerical data. A *p*-value of <0.05 was considered statistically significant. Demographical data were analysed using the one-way ANOVA test with the Graph pad prism 5 software (Graph pad Software, San Diego, CA, USA).

## **Results**

The study population (n=637;100%) consisted of PE (n=357;56%) and normotensive pregnant (n=280;44%) participants. The PE group was divided into early onset (EOPE) (n=187;29%) and late onset (LOPE) (n=170;27%).

## **Demographics and Analysis**

Table 1 validates demographic and clinical data amongst the three study groups. A significant difference was found for maternal age, gestational age, maternal weight, systolic, and diastolic blood pressure and proteinuria between normotensive pregnant *vs* EOPE and LOPE.

## **Genotyping**

An allelic and genotypic comparison of frequencies between PE and normotensive pregnant groups for SNPs *LNPEP* (rs18059) and *ANPEP* (rs6496603) are outlined in Table 2 and 3 respectively.

### **Allelic Association of *LNPEP* (rs18059) with Pre-eclampsia**

#### ***Pre-eclampsia (PE) vs Controls***

There was no significant association between the C (372 PE vs 317 controls) and T (332 PE vs 241 controls) alleles (Table 2).

#### ***Early onset (EOPE) and late onset (LOPE) Pre-eclampsia vs Controls***

Early onset PE vs controls showed no significant association for C and T alleles (C=189 EOPE vs 317 controls; T=177 EOPE vs 241 controls), and LOPE vs controls for both alleles were not significantly different (C=183 LOPE vs 317 controls; T=155 LOPE vs 241 controls). Similarly, the C and T alleles showed no significant difference between EOPE and LOPE (Table 2).

### **Allelic Association of *ANPEP* (rs6496603) with Pre-eclampsia**

#### ***Pre-eclampsia (PE) vs Controls***

No significant difference was found for C (332 PE and 268 controls) and T (382 PE and 292 controls) alleles in PE compared to the controls (Table 3).

#### ***Early onset (EOPE) and late onset (LOPE) Pre-eclampsia vs Controls***

There was no significant difference between the C and T alleles in EOPE (C=180 EOPE and 268 controls; T=194 EOPE and 292 controls) and LOPE (C=152 EOPE and 268 controls; T=188 EOPE and 292 controls) compared to the control group. No significance was noted between EOPE compared to LOPE (Table 3).

### **Genotype Frequencies of *LNPEP* (rs18059) with Pre-eclampsia**

#### ***Pre-eclampsia (PE) vs Controls***

The genotype frequencies of rs18059 (CC, CT, and TT) showed no significant difference between PE (103, 166, and 83) versus the controls (95, 127 and 57); (Table 4).

### ***Early onset and late onset Pre-eclampsia vs Controls***

A comparison of genotype frequencies for rs18059 (CC, CT, and TT) showed no significant association with EOPE (53,83 and 47) and LOPE (50,83 and 36) and controls (95,127 and 57); (Table 4).

### **Genotype Frequencies of ANPEP (rs6496603) with Pre-eclampsia**

#### ***Pre-eclampsia (PE) vs Controls***

There was no significant difference between the genotypes of rs6496603 (CC, CT, and TT) in PE (72, 188 and 97) versus the controls (63,142 and 75); (Table 5).

#### ***Early onset and late onset Pre-eclampsia vs Controls***

The genotype frequencies of rs6496603 (CC, CT, and TT) was not significantly different between EOPE (41, 98 and 48) and controls (63,142 and 75). Similarly, an absence of significant difference was noted between EOPE versus LOPE (31, 90 and 49) and between LOPE versus controls (Table 5).

## Discussion

The main findings of this study advocate that gene polymorphisms of the AT4R (*LNPEP*, rs18059) and *ANPEP* (rs6496603) genes are not associated with PE development. The results demonstrate no statistical difference in allele and genotype frequencies of *LNPEP* (rs18059) and *ANPEP* (rs6496603) between normotensive and PE pregnancies. Our results are similar to that of Aung *et al.*, (2017) who found no significant associations of the gene polymorphisms of the RAS components; angiotensin-AGT (M235T), renin-REN (C-5312T), AT1R (A1166C), AT2R (C3123A) in PE compared to controls. Furthermore, Aung *et al.*, (2017) found no association of these genes in PE subgroups (EOPE and LOPE) compared to controls [25]. Similarly, no significant differences in allele and genotype frequencies of the *LNPEP* (rs18059) and *ANPEP* (rs6496603) in normotensive pregnancies compared to EOPE and LOPE groups were noted respectively. Likewise, a comparison of alleles and genotype frequencies between EOPE and LOPE presented no significant difference.

In contrast to the latter study, Akbar *et al.*, (2009) observed frequencies of gene polymorphisms of AT1R and AT2R in different populations of women with PE compared to healthy normotensive pregnancies [27]. They found the AT2R (A1675G) polymorphism to be significantly higher in Afro-Caribbean women with PE compared to controls ( $p=0.004$ ) [27]. Furthermore, Akbar *et al.*, (2009) noted that AT1R (A1166C) was not significantly associated with PE in Afro-Caribbean, Caucasian and Asian women [27]. Additionally, Aung *et al.*, (2017) and Roberts *et al.*, (2004) reported similar results in a Zulu population group [28].

Li *et al.*, (2016) demonstrated that the AT1R (AGTR1; 275645) was significantly different between PE and the control group in a Chinese population ( $p=0.021$ ) but found no significance between GH and the controls. However, angiotensinogen (AGT; rs3789678) was significantly associated with GH and not PE compared to the controls ( $p=0.0088$ ) [29].

Also, Zangbar *et al.*, (2018) studied different SNPs of AT1R (+1166A/C; rs5186) and AT2R (+1675A/G; rs5194) and noted no genotype and allele frequency differences between PE and controls. However, a combination of the genotypes of the two SNPs showed a statistical significance between PE and the control group. The AC/AG genotypes were lower in PE compared to controls, while the CC/AA genotypes were higher in PE compared to controls ( $p<0.01$ ). These findings led to the conclusion that these polymorphisms are more likely to cause PE complications in pregnant women [30].

The gene polymorphisms of the RAS were also investigated in patients with essential hypertension and the results obtained were analogous to results obtained from PE patients [31-33]. In a Burkina Faso population with essential hypertension, gene polymorphisms of AGT and AT1R (AGT 235M/T, AT1R 1166A/C) were not associated with essential hypertension [32]. In contrast, Yang *et al.*, (2015) found that the allele and genotype frequencies of ACE and AT1R in a Chinese Yi ethnic group were associated with essential hypertension (ACE G2350A,  $p=0.000, 0.002$ ; AT1R A1166C:  $p=0.008, 0.011$ ) [31].

In relation to AT4R, Pipkin *et al.*, (2007) reported that the angiotensin peptide Ang IV acts via the AT4R on the placenta to stimulate angiogenesis, cell proliferation, inflammation, therefore AT4R contributes crucially to the development and preservation of a healthy pregnancy [34]. In addition, the Ang IV peptide physiologically moderates trophoblast invasion in the maternal endometrium, angiogenesis and endothelial dysfunction. In addition, Ang IV enhances nitric oxide activity, which is a potent vasodilator and contributes positively to maternal blood flow and endothelial dysfunction [21].

Williams *et al.*, (2010) noted a low expression of AT4R in the placenta of PE compared to healthy pregnancies [21]. Pre-eclampsia is characterized by poor trophoblast invasion, inflammation and endothelial dysfunction [35-38], changes which may be attributed to the decrease in the AT4R levels in PE. However, our results show that the rs18059 polymorphism of *LNPEP* is not associated with PE, therefore, other biochemical pathways may be associated with AT4R down-regulation.

The binding of Ang IV to AT4R inhibits the action of the AT1R by boosting blood flow and inhibiting sodium reabsorption by the kidneys, thus regulating blood pressure [10]. Moreover, Mistry *et al.*, (2013) noted that AT1R is highly expressed in pre-eclamptic placentas [23]. Also the binding of Ang II to AT1R promotes vasoconstriction, peripheral vascular resistance, defective angiogenesis, cell proliferation and vascular permeability all factors that are dysregulated in PE [39]. Since AT1R is also a binding site for Ang IV, it may also contribute to the pathology of PE when highly expressed [21]. Many previous studies corroborate our findings and report no association of gene polymorphisms of the RAS with PE development [11, 25, 28, 29].

## **Strengths, Limitations and Future Recommendations**

The study was conducted in a homogenous population of African ancestry in which the prevalence of PE is high. The limitation of this study was that only one single nucleotide polymorphism (SNP) was tested in both the *LNPEP* and the *ANPEP* gene. Other polymorphisms of the same genes need to be investigated in PE to confirm that the genes of the RAS components are not associated with PE.

## **Conclusion**

Despite reports on low AT4 expression levels in placentae of women with PE, the role of RAS genes in PE remains unclear. Several investigations on the gene polymorphisms of RAS components have revealed an absence of association with pre-eclampsia development. Similarly, our results show that the gene polymorphisms of the AT4 receptor (*LNPEP*; rs18059) and aminopeptidase-N (*ANPEP*; rs6496603) are not associated with PE in women of African ancestry. We recommend further investigations to examine additional RAS genes in the pathogenesis of PE.

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## **Conflicts of Interest**

There are no conflicts of interest.



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**TABLE 1: Patient Demographics and Clinical Data**

Mean & <sup>1</sup> SD	Normotensive pregnant (n=280)	PE (n=357)		p-value
		<sup>2</sup> EOPE (n=187)	<sup>3</sup> LOPE (n=170)	
Maternal Age (years) Control vs EOPE Control vs LOPE EOPE vs LOPE	26.78±6.5	29.76±6.5	27.29±6.8	<0.0001 <sup>***</sup> 0.9701 <sup>ns</sup> <0.0001 <sup>***</sup>
Systolic BP (mmHg) Control vs EOPE Control vs LOPE EOPE vs LOPE	109.8±12.3	158.9±13.7	151.4±12.1	<0.0001 <sup>***</sup> <0.0001 <sup>***</sup> 0.0026 <sup>**</sup>
Diastolic BP (mmHg) Control vs EOPE Control vs LOPE EOPE vs LOPE	67.65±8.4	102.0±9.3	98.23±6.6	<0.0001 <sup>***</sup> <0.0001 <sup>***</sup> 0.0300 <sup>*</sup>
Gestational Age (weeks) Control vs EOPE Control vs LOPE EOPE vs LOPE	38.24±1.7	28.51±4.2	37.06±1.9	<0.0001 <sup>***</sup> <0.0001 <sup>***</sup> <0.0001 <sup>***</sup>
Maternal Weight (Kg) Control vs EOPE Control vs LOPE EOPE vs LOPE	72.79±16.8	81.96±18.5	73.48±18.2	0.0120 <sup>*</sup> 0.2253 <sup>ns</sup> 0.3585 <sup>ns</sup>
Proteinuria (mg) Control vs EOPE Control vs LOPE EOPE vs LOPE	0.00±0.06	1.51±0.74	1.44±0.69	<0.0001 <sup>***</sup> <0.0001 <sup>***</sup> 0.4970 <sup>ns</sup>

A *p*-value of <0.05 was considered statistically significant, \*\*\*= highly significant with a *p*-value of <0.001, \*\*= *p*<0.01, *ns*= not significant.

<sup>1</sup>SD= standard deviation; <sup>2</sup>EOPE =early onset preeclampsia; <sup>3</sup>LOPE =late onset preeclampsia

**TABLE 2: Allele Frequencies of LNPEP (rs18059) in Pre-eclampsia vs Controls**

<b>FREQUENCIES CC, CT, TT (rs18059)</b>	<b>ALLELE Rs18059-LNPEP</b>	<b>OR (95%CI) p-value</b>
<b>Controls (n=280;44%)</b>	<b>PE vs CONTROL</b>	
CC=95 (14.9%)	C vs T	1.174 (0.9387 - 1.468) <i>p</i> =0.1596
CT=127 (19.9%)	CC vs CT/TT	1.248 (0.8904 - 1.750) <i>p</i> =0.1979
TT=57 (8.9%)	CC/CT vs TT	1.202 (0.8208 - 1.759) <i>p</i> =0.3443
<b>PE (n=357;56%)</b>	<b>EOPE vs CONTROLS</b>	
CC=103 (16.2%)	C vs T	1.232 (0.9453 - 1.605) <i>p</i> =0.1225
CT=166 (26.1%)	CC vs CT/TT	1.266 (0.8453 - 1.897) <i>p</i> =0.2516
TT=83 (13.0%)	CC/CT vs TT	1.346 (0.8657 - 2.093) <i>p</i> =0.1861
<b>EOPE (n=187;29%)</b>	<b>EOPE vs LOPE</b>	
CC=53 (8.3%)	C vs T	1.106 (0.8221 - 1.487) <i>p</i> =0.5063
CT=83 (13.0%)	CC vs CT/TT	1.031 (0.6509 - 1.632) <i>p</i> =0.8977
TT=47 (7.4%)	CC/CT vs TT	1.277 (0.7778 - 2.096) <i>p</i> =0.3333
<b>LOPE (n=170;27%)</b>	<b>LOPE vs CONTROLS</b>	
CC=50 (7.8%)	C vs T	1.114 (0.8490 - 1.462) <i>p</i> =0.4357
CT=83 (13.0%)	CC vs CT/TT	1.229 (0.8131 - 1.857) <i>p</i> =0.3276
TT=36 (5.7%)	CC/CT vs TT	1.054 (0.6592 - 1.686) <i>p</i> =0.8255

**Table 3: Frequency Alleles of ANPEP (rs6496603) in Pre-eclampsia groups vs Controls**

<b>FREQUENCIES CC, CT, TT (rs6496609)</b>	<b>ALLELE Rs6496603-ANPEP</b>	<b>OR (95%CI) p-value</b>
<b>Controls (n=280;44%)</b>	<b>PE vs CONTROL</b>	
CC=63 (9.9%)	C vs T	1.056 (0.8461 - 1.318) <i>p</i> =0.6297
CT=142 (22.3%)	CC vs CT/TT	1.149 (0.7847 - 1.683) <i>p</i> =0.4747
TT=75 (11.8%)	CC/CT vs TT	1.020 (0.7167 - 1.451) <i>p</i> =0.9135
<b>PE (n=357;56%)</b>	<b>EOPE vs CONTROLS</b>	
CC=72 (11.3%)	C vs T	1.011 (0.7779 - 1.314) <i>p</i> =0.9352
CT=188 (29.5%)	CC vs CT/TT	1.034 (0.6619 - 1.615) <i>p</i> =0.8837
TT= 97 (15.2%)	CC/CT vs TT	1.059 (0.6950 - 1.615) <i>p</i> =0.7882
<b>EOPE (n=187;29%)</b>	<b>EOPE vs LOPE</b>	
CC=41 (6.4%)	C vs T	1.148 (0.8546 - 1.541) <i>p</i> =0.3598
CT=98 (15.4%)	CC vs CT/TT	1.259 (0.7477 - 2.121) <i>p</i> =0.3855
TT=48 (7.5%)	CC/CT vs TT	1.173 (0.7353 - 1.870) <i>p</i> =0.5033
<b>LOPE (n=170;27%)</b>	<b>LOPE vs CONTROLS</b>	
CC=31 (4.9%)	C vs T	1.135 (0.8660 - 1.488) <i>p</i> =0.3582
CT=90 (14.1%)	CC vs CT/TT	1.302 (0.8056 - 2.104) <i>p</i> =0.2806
TT=49 (7.7%)	CC/CT vs TT	1.107 (0.7240 - 1.692) <i>p</i> =0.6390

**TABLE 4: Genotype Frequencies of *LNPEP* (rs18059) in Pre-eclampsia groups vs Controls**

<b>FREQUENCIES CC, CT, TT (rs18059)</b>	<b>GENOTYPE Rs18059-<i>LNPEP</i></b>	<b>OR (95%CI) p-value</b>
<b>Controls (n=280;44%)</b>	<b>PE vs CONTROL</b>	
CC=95 (14.9%)	CC vs TT	1.343 (0.8670 - 2.080) p=0.1860
CT=127 (19.9%)	CC vs CT	1,206(0.8392 - 1.732) p=1.206
TT=57 (8.9%)	CT vs TT	1.114 (0.7402 - 1.677) p=0,6045
<b>PE (n=357;56%)</b>	<b>EOPE vs CONTROLS</b>	
CC=103 (16.2%)	CC vs TT	1.478 (0.8857 - 2.466) p=0.1340
CT=166 (26.1%)	CC vs CT	1.171 (0.7580 - 1.810) p=0,4760
TT=83 (13.0%)	CT vs TT	1.262 (0.7845 - 2.029) p=0.3372
<b>EOPE (n=187;29%)</b>	<b>EOPE vs LOPE</b>	
CC=53 (8.3%)	CC vs TT	1.232 (0.6887 - 2.203) p=0.4820
CT=83 (13.0%)	CC vs CT	1.060 (0.6481 - 1.734) p=0.8164
TT=47 (7.4%)	CT vs TT	1.306 (0.7683 - 2.219) p=0.3237
<b>LOPE (n=170;27%)</b>	<b>LOPE vs CONTROLS</b>	
CC=50 (7.8%)	CC vs TT	1.200 (0.6994 - 2.059) p=0.5078
CT=83 (13.0%)	CC vs CT	1.242 (0.7995 - 1.929) p=0.3348
TT=36 (5.7%)	CT vs TT	1.035 (0.6272 - 1.707) p=0.8935



**Table 5: Genotype Frequencies of ANPEP (rs6496603) in Pre-eclampsia groups vs Controls**

<b>FREQUENCIES CC, CT, TT (rs6496609)</b>	<b>GENOTYPE Rs6496603-ANPEP</b>	<b>OR (95%CI) p-value</b>
<b>Controls n=280;44%)</b>	<b>PE vs CONTROL</b>	
CC=63 (9.9%)	CC vs TT	1.132 (0.7194 - 1.780) p=0.5924
CT=142 (22.3%)	CC vs CT	1.158 (0.7747 - 1.732) p=0.4734
TT=75 (11.8%)	CT vs TT	1.024 (0.7057 - 1.485) p=0.9019
<b>PE (n=357;56%)</b>	<b>EOPE vs CONTROLS</b>	
CC=72 (11.3%)	CC vs TT	1.017 (0.5956 - 1.736) p=0.9511
CT=188 (29.5%)	CC vs CT	1.060 (0.6627 - 1.697) p=0.8066
TT= (15.2%)	CT vs TT	1.078 (0.6914 - 1.682) p=0.7394
<b>EOPE (n=187;29%)</b>	<b>EOPE vs LOPE</b>	
CC=41 (6.4%)	CC vs TT	1.350 (0.7311 - 2.493) p=0.3368
CT=98 (15.4%)	CC vs CT	1.215 (0.7026 - 2.100) p=0.4859
TT=48 (7.5%)	CT vs TT	1.112 (0.6808 - 1.815) p=0.6723
<b>LOPE (n=170;27%)</b>	<b>LOPE vs CONTROLS</b>	
CC=31 (4.9%)	CC vs TT	1.328 (0.7577 - 2.327) p= 0.3213
CT=90 (14.1%)	CC vs CT	1.288 (0.7776 - 2.134) p=0.3249
TT=49 (7.7%)	CT vs TT	1.031 (0.6595 - 1.611) p= 0.8940



## **CHAPTER 6**

## 6.1 SYNTHESIS, CONCLUSIONS AND FUTURE RESEARCH

To-date, the etiology of PE remains a perplexity worldwide. Pre-eclampsia is a multisystem disorder, that requires ongoing research to elucidate its underlying etiology (Gathiram and Moodley, 2016). Globally, 2-10% of pregnancies are affected by PE at  $\geq 20$  weeks of gestation, resulting in maternal and fetal morbidity and mortality (Kamath, 2006). Although the etiology is an enigma, the clinical signs and symptoms are well established. These include hypertension ( $\geq 140$  mmHg systolic BP and  $\geq 90$  mmHg diastolic BP), proteinuria ( $>0.3$  mg) or without proteinuria with one of the following conditions; thrombocytopenia, liver or renal dysfunctions, neurological disorders and fetal growth restriction (Brown *et al.*, 2018). The exact cause of PE is unknown; however, the pathophysiology involves poor trophoblast invasion into the spiral arteries, resulting in irregular spiral artery remodeling, which triggers placental hypoxia and ischemia. These factors activate maternal systemic inflammation, endothelial dysfunction, hypertension, proteinuria and hyperuricemia (Redman and Sargent, 2005).

In South Africa, hypertension in pregnancy accounts for above 14% of all maternal deaths and is a direct contribution to maternal mortality (Saving Mothers 2014-2016, 2017). Moreover, the condition is affected by HIV infection in pregnancy, in fact 30% of parturients in SA are HIV infected (Kalumba *et al.*, 2013). To-date, the etiology of PE remains a perplexity worldwide. Pre-eclampsia is associated with injury to the kidney and it is well documented that uric acid is elevated in PE (Nair and Savita 2017).

The role of hyperuricemia in PE is poorly described, therefore this thesis investigated the role of uric acid in PE as a causative factor or a symptom of the disease. The review indicated discrepancies amongst other studies (Bainbridge and Roberts, 2008; Chen *et al.*, 2016; Kondareddy and Prathap, 2016; Priya *et al.*, 2016; Staff and Redman, 2016). Bainbridge *et al.*, (2008) identified hyperuricemia as a pathogenic factor of PE since women with hyperuricemia at 10 weeks of gestation developed PE later in pregnancy. In contrast, Kondareddy and Prathap, (2016) suggested that hyperuricemia occurred after the clinical signs of PE in some patients, and stated that high levels cannot be a pathogenic factor, a predictor or a biomarker of the disease (Kondareddy and Prathap, 2016). In addition, William and Galerneau, (2002) suggested that serum uric acid levels were not significantly elevated in PE compared to normal pregnancies (Williams and Galerneau, 2002).

The above debates on the role of uric acid in PE led to the first objective of the current study, which was to investigate the association of gene polymorphisms of uric acid levels (*URAT1*; rs505802, *GLUT 9*; rs1014290; *PDZK1 CD160*; rs12129861) in PE. Early in normal gestation, uric acid levels rise, with a gradual decline as the pregnancy progresses reaching an equilibrium to equate that of non-pregnant women at term (Parrish *et al.*, 2010). Moreover, in pre-eclamptic pregnancies, this elevation results in hyperuricemia (Mustaphi *et al.*, 1996).

Hyperuricemia of gout is known to be associated with polymorphisms of various uric acid related genes *viz.*, rs1014290 (*SLC2AL*; *GLUT9*): rs505802 (*SCL22A12*; *URAT1*): rs12129861 (*PDZK1*; *CD160*) (Zhang *et al.*, 2013; Sun *et al.*, 2014; Zhang *et al.*, 2016). We report that of the four SNPs selected in our study, rs2231142 (*ABCG2*) had a frequency of <1% in women of African ancestry. We also show that SNPs rs505802 (*URAT 1*) and rs1014290 (*GLUT 9*) rather than rs12129861 (*PDZK1 CD160*) were significantly associated with PE.

The *URAT 1* transporter gene is located within the proximal tubules where uric acid reabsorption occurs. We found a significantly higher prevalence of the SNP (rs505802) in PE (37%) compared to healthy pregnancies (31%) and EOPE (31%). These results are tabulated in chapter 3 (table 2-5) and include; the frequencies of the alleles and genotypes of all the urate transporter genes as well as their association, in PE vs normotensives, which is reported as odds ratio at 95% confidence interval and a p value of <0.05 was considered significant. Our findings are supported by an association of the *URAT 1* gene with an inflammatory response in non-pregnant women with metabolic syndrome, hypertension and obesity (Shafiu *et al.*, 2012). Similar clinical manifestations have been reported in LOPE, (Madazli *et al.*, 2014) which, according to our findings is influenced by the *URAT 1* SNP (rs505802). Moreover, the association of rs505802 with hyperuricemia has been previously demonstrated (Cho *et al.*, 2015). Using a Chinese cohort with cardiovascular disease, Kolz *et al.*, (2009) reported that patients with hyperuricemia also presented with exaggerated blood pressure, diabetes, insulin resistance and obesity (Kolz *et al.*, 2009), all medical conditions associated with PE development (English *et al.*, 2015).

In the current study, the heterozygous genotype CT of rs505802 demonstrated a higher frequency in PE compared to healthy pregnancies. Therefore, individuals with the CT genotype were twofold more likely to be preeclamptic. These findings are analogous to various other studies that have suggested

that PE is of heterozygous origin with variation by ethnicity (Valenzuela *et al.*, 2012; Rudic *et al.*, 2018).

In contrast the *PDZK1 CDI60* SNP (rs12129681) in our study showed no significant association with PE despite its association with hyperuricemia in gout (Kolz *et al.*, 2009; Wright *et al.*, 2010; Reginato *et al.*, 2012; Sun *et al.*, 2014; Roman *et al.*, 2016).

In our study *GLUT 9* (rs1014290) was significantly associated with EOPE compared to normotensive pregnant women. The CT genotype was more prevalent within the EOPE group (47%) compared to controls (37%), confirming that women of African ancestry with the CT genotype have a 1.5 times risk of PE development. The *GLUT 9* gene is a uric acid transporter within kidney and liver cells (Mobasheri *et al.*, 2002). Our findings are in accordance with studies that show an association of rs1014290 with hyperuricemia in gout (Li *et al.*, 2007; Stark *et al.*, 2008; Preitner *et al.*, 2009; Urano *et al.*, 2010; Parsa *et al.*, 2012; Testa *et al.*, 2014; Meng *et al.*, 2015; Ruiz *et al.*, 2018). Moreover, the association between hyperuricemia and hypertension has been demonstrated across both sexes (Wang *et al.*, 2014). Additionally, Parsa *et al.*, (2012) reported that the consequence of a mutation of the *GLUT 9* gene was hypertension development (Parsa *et al.*, 2012).

In our study, the rs1014290 SNP was associated with EOPE, a sub-type of PE characterized by defective placentation with/without renal injury, revealed by proteinuria. It is widely accepted that uric acid provokes endothelial dysfunction, hypertension, vascular resistance and renal disease (Sultana *et al.*, 2013). Early onset pre-eclampsia is associated with abnormal placentation and a lack of myometrial spiral artery remodeling (Gomathy *et al.*, 2018). Furthermore, EOPE presents with an aggressive hematological, arterial, renal, hepatic adverse maternal and fetal outcome in comparison to LOPE. An inactivation of the gene coding for *GLUT 9* in hepatic cells of mice results in hyperuricemia, therefore it is plausible that a consequence of liver pathology in EOPE may be due to *GLUT 9* inactivation (Gomathy *et al.*, 2018).

The second objective of our study was to assess the plasma concentration of the receptor (sAT-4), in pre-eclampsia. Soluble AT4 receptor is a vital component of RAS (Williams *et al.*, 2010). The activation of AT4 receptor (AT4R) improves cell signal transmission, and it has both anti-oxidant and anti-inflammatory properties (Wright *et al.*, 2015). Soluble AT4R is a binding ligand for angiotensin IV which mediates blood flow and normal placentation by its involvement in extravillous

trophoblast invasion (Williams *et al.*, 2010). Importantly, Ang IV elevates intracellular calcium to boost nitric oxide synthase (NOS), which is a crucial vasodilator (Patel *et al.*, 1998). A reduction in Ang IV results in poor trophoblast invasion, poor placentation, vasoconstriction and subsequently PE development.

In the present study, a significant decrease in sAT-4R was noted in PE, EOPE, LOPE, mild PE (MPE) and severe PE (SPE) compared to normotensive pregnant women of African ethnicity. Moreover, SPE levels were also significantly different to MPE. Our findings are in keeping with other studies that observed a significant decrease in ATII and renin levels in PE (Kurlak *et al.*, 2019a). Furthermore, a significant down-regulation in sAT-4 was reported in PE compared to normotensive placentae (Williams *et al.*, 2010). The AT4R is highly expressed throughout gestation in normal pregnancies, portraying its necessity to maintain a successful pregnancy. A decline in AT4 expression leads to poor extravillous trophoblast invasion resulting in an absence of the physiological conversion of myometrial spiral arteries in EOPE development (Reister *et al.*, 2006). Furthermore, oxidative stress emanating from poor placental perfusion contributes to the development of PE (Redman *et al.*, 2014).

Another primary finding in our study was the significant decrease of sAT-4 aligned with the severity of PE. This finding is corroborated with other studies that observed a significant decrease of Ang II (Leaños-Miranda *et al.*, 2018). Notably in our study, 67.7% of SPE were EOPE women. Early onset pre-eclampsia correlates with neonatal morbidity, premature births, fetal growth restrictions and small for gestational age babies (Staff and Redman, 2018), complications emanating from poor placentation and a hypoxic microenvironment that is detrimental to the fetus (Herzog *et al.*, 2017).

The last objective of our study was to compare variant gene polymorphisms of AT4 (rs18059) and *ANPEP* (rs6496603) with pre-eclampsia development. Since the AT4 receptor levels are altered in PE pregnancies, it is vital that one evaluates the underlying cause for this dysregulation. The RAS is a significant contributor to blood pressure regulation and salt retention and a dysregulation of any component may compromise maternal and fetal health (Shah, 2005). In pregnancy, the different components of RAS function synergistically to maintain a homeostatic environment for fetal development (Chung *et al.*, 1998). These components include Ang II which binds to AT1R and AT2R for activation as well as Ang III and Ang IV. The conversion of Ang III to IV is mediated by *ANPEP*, which plays a fundamental role in angiogenesis, cell invasion, cell death and inflammation, designating its importance in a healthy pregnancy (Poumarat *et al.*, 2002). Ang IV on the other hand

binds to the AT4 receptor (*LNPEP*) for functionality. It increases blood flow, mediates proper extravillous trophoblast invasion and activates nitric oxide for vasodilation of endothelial cells (Williams *et al.*, 2010). A down-regulation of either Ang IV or its receptor may be detrimental to maternal and fetal health.

Our study demonstrates no significant association of *LNPEP* (rs18059) and *ANPEP* (rs6496603) SNPs with PE development in a homogenous Black African population. These findings are corroborated by Aung *et al.*, 2017 who reported no significance in the frequencies of the ATIR SNPs (A1166C), AT2R (C3123A), angiotensin-AGT (M235T), renin-REN (C-5312T) in PE compared to normotensive controls in the same population (Aung *et al.*, 2017). In contrast, Akbar *et al.*, (2009) observed a significant increase in frequencies of AT2R (A1675G) in Caribbean women with PE compared to healthy pregnancies. However, analogous to Aung *et al.*, 2017, Akbar *et al.*, 2009 noted that ATIR (A1166C) was similar amongst Afro-Caribbean, Caucasian and Asian women with PE compared to those with normotensive pregnancies (Akbar *et al.*, 2009).

Our study supports the evidence that PE is of heterozygous origin. Also, we are the first to report on the association of uric acid polymorphisms in PE and our findings depict that uric acid polymorphisms are associated with the pathogenesis of PE. Moreover, the RAS system is dysregulated in PE and this study shows that sAT-4 levels in circulation are lower in PE compared to normotensive pregnant women. However, gene polymorphisms of the RAS show no significant association with PE.

## 6.2 CONCLUSIONS

In conclusion, our review has shown that uric acid levels may be elevated in the first trimester of pregnancy. This elevation may emanate from the glomerular endotheliosis and contribute to the deficient trophoblast invasion and lack physiological spiral artery remodeling. This study demonstrates for the first time, polymorphisms of uric acid genes (*URAT 1*; rs505802 and *GLUT 9*; rs1014290). These two genes were significantly associated with the pathogenesis of PE in women of African ancestry hence may be used to genetically test for the risk of PE development. The increased retention of uric acid enhances sympathetic activity thereby decreasing the angiotensin system action and exacerbating the existing hypertension in PE. Moreover, we show that the AT4 receptor gene (*LNPEP*) and *ANPEP* are not significantly associated with PE development in a homogenous Black African population. However, sAT-4 plasma levels in PE are lower than normal pregnancies. This



decline in sAT-4 levels may negatively impact trophoblast invasion and may also cause a down regulation of angiotensin IV facilitated vasodilatation. Nonetheless, this finding of a downregulation of s-AT4 pre-eclampsia with severe features compared with PE with mild features reflects that these two conditions are distinctly different entities.

### **6.3 FUTURE RESEARCH**

Further investigations are required to confirm the association of uric acid with the pathogenesis of PE in an independent cohort. Furthermore, the involvement of the RAS with PE is poorly understood since concentrations of the RAS components are dysregulated, but their SNPs show no significant association with the disease. There is a need for further research in this regard for the improvement in the management of PE for healthier pregnancy outcomes. It is also important to note that it is challenging to perform studies on genes, therefore case-control studies should be matched correctly in genetic studies. Since it is difficult to implicate a single SNP with a disease, for future studies, a mendelian randomization test would be useful to test the cause-effect of genes in pre-eclampsia.



## **CHAPTER 7**

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## APPENDICES



## **APPENDIX 1**



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19 April 2018

Prof T Naicker  
Discipline of Optics and Imaging  
School of Laboratory Medicine and Medical Sciences  
[naickera@ukzn.ac.za](mailto:naickera@ukzn.ac.za)

Dear Prof Naicker

**Title of Project: Exploring the pathogenesis HIV associate pre-eclampsia syndrome in a homogenous South African population group.**  
**BREC Ref No.: BCA338/17**

We wish to advise you that your response received on 19 April 2018 to BREC letter dated 22 March 2018 has been noted by a sub-committee of the Biomedical Research Ethics Committee.

Your request received on 15 February 2018 to append the study below to the above study has now been approved by a sub-committee of the Biomedical Research Ethics Committee.

**Study details:**

**PI: Olive Khaliq**

**Title: Gene Polymorphism of Uric Acid related Proteins and Angiotensin IV (AT4) in pre-eclampsia**

This approval will be ratified at the next BREC meeting to be held on 08 May 2018.

Yours sincerely

Mrs A Marimuthu  
Senior Administrator: Biomedical Research Ethics