THE ROLE OF PRIMIGRAVIDAE, LYMPHATIC VESSEL ENDOTHELIAL RECEPTOR-1 AND PODOPLANIN IN HIV ASSOCIATED EARLY AND LATE ONSET PRE-ECLAMPSIA

by

ONANKOY ATSHAKALA ONYANGUNGA

submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the

Optics and Imaging Centre

College of Health Sciences

University of KwaZulu-Natal

Durban

2017

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 and Imaging Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professors Thajasvarie Naicker and Jagidesa Moodley.

Dr Onankoy A. Onyangunga

(212557379)

Prof Thajasvarie Naicker

(Supervisor)

Prof Jagidesa Moodley

(Co-Supervisor)

DECLARATION

I, Onankoy A. Onyangunga declare that:

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

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

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

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

a) their words have been re-written but the general information attributed to them has been referenced;

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

(v) Where I have reproduced a journal publication of which I am an author, I have indicated in detail which part of the publication was actually written by myself alone and not by other authors, editors or others.

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

Signed: _____ Date: 15-03-2017

DEDICATION

I wish to dedicate this research project to God almighty for the strength given to me. Also, I dedicate it to my late father, Mr Paul Onankoy and beloved mother, Mrs Marie Walo Dolongo.

PEER REVIEWED PUBLICATIONS, ABSTRACT AND CONFERENCE PRESENTATIONS

DOHET APPROVED PEER REVIEWED INTERNATIONAL PUBLICATIONS

1. **Onankoy A. Onyangunga,** Jagidesa Moodley, Vesselina Merhar, David A. Ofusori and Thajasvarie Naicker (2016). Lymphatic vascular endothelial hyaluronan receptor-1 immunoexpression in placenta of HIV infected pre-eclamptic women. *Journal of Reproductive Immunology*, 117, 81-88.

2. J. Moodley, **O. A. Onyangunga** and N. R. Maharaj (2016). Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis. *Hypertension in Pregnancy*. http://dx.doi.org/10.1080/10641955.2016.1193190.

3. **Onyangunga, OA,** Naicker T, Moodley J (2016). Maternal and perinatal outcomes in early and late onset preeclampsia with caesarean delivery in a HIV infected South African population. (Submitted for peer-review to *Nigerian Journal of Clinical Practice*; Manuscript ID no: njcp_364_16).

4. **Onankoy A. Onyangunga,** Jagidesa Moodley, Frederick Odun-Ayo and Thajasvarie Naicker (2016). Immunohistochemical localization of podoplanin in the placenta of HIV infected pre-eclampsia. (Submitted for peer-review to *Journal of Reproductive Immunology*; Manuscript ID no: JRI_2016_180).

ABSTRACTS IN DOHET APPROVED PEER REVIEWED JOURNAL

1. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley, and Thajasvarie Naicker (2015). Analysis of Lymphatic vessel endothelial hyaluronan receptor-1 in the placental bed of HIV associated pre-eclampsia. *Placenta* 36(9), A56.

2. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley, and Thajasvarie Naicker (2015). Vascular endothelial growth factor receptor-3 in placenta of HIV associated normotensive and pre-eclamptic pregnancies. *Placenta* 36(9), A57.

3. **Onankoy Atshakala Onyangunga,** Jagidesa Moodley, and Thajasvarie Naicker (2015). Placental lymphatic vascular endothelial hyaluronan receptor-1 in HIV associated pre-eclampsia. *Placenta* 36(9), A57.

Above Abstracts Were Presented at the International Federation of Placenta Association Conference in Brisbane Australia, 2015

NATIONAL CONFERENCE PRESENTATIONS

1. **O.A. Onyangunga,** D.A. Ofusori, J. Moodley and T. Naicker (2014). Lymphatic vascular endothelial hyaluronan receptor-1 in placenta of normotensive and pre-eclamptic pregnanacies. *Microscopy Society of Southern Africa-Proceedings*, Vol. 44, 46.

2. **O.A. Onyangunga,** D.A. Ofusori, J. Moodley and T. Naicker (2014). Lymphatic vessel endothelial hyaluronan receptor-1 in the placental bed of normotensive and pre-eclamptic pregnanacies. *Microscopy Society of Southern Africa-Proceedings*, Vol. 44, 47.

3. **O.A. Onyangunga,** J. Moodley, D.A. Ofusori, D.A. Margolis and T. Naicker (2014). Immunolocalization of vascular endothelial growth factor receptor-3 in placenta of normotensive and pre-eclamptic pregnancies. *Microscopy Society of Southern Africa-Proceedings*, Vol. 44, 48.

INTERNATIONAL AWARDS

1. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley and Thajasvarie Naicker (2015). Placental lymphatic vascular endothelial hyaluronan receptor-1 in HIV associated pre-eclampsia. *Placenta* 36(9), A57. (Nominated top five in the world, International Federation of Placenta Associations).

2. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley and Thajasvarie Naicker (2015). Analysis of Lymphatic vessel endothelial hyaluronan receptor-1 in the placental bed of HIV associated pre-eclampsia. *Placenta* 36(9), A56. (Received the Elsevier Charlie Loke Travel Award).

FUNDING

This research was funded by:

- The College of Health Sciences,
- Medical Education Partnership Initiative of University of KwaZulu-Natal and
- The South African National Research Foundation.

ACKNOWLEDGEMENTS

A special thank you to:

- Professors T Naicker and J Moodley for allowing me the opportunity to work on this research topic and for accepting me to the Placental Research Group. For their invaluable academic input, continued support, encouragement and generosity with their time and funds making the research process an achievable one.
- Professor Thajasvarie Naicker, my mentor for your guidance in my academic progression and your expertise in the field of Optics and Imaging. Your endless support and encouragement is well appreciated. It has been a great pleasure working with you.
- Professor Jagidesa Moodley for your relentless effort right from the start of this project to ensure the successful completion of my PhD programme.
- Professor J M Foidart, University of Liege, Belgium for your encouragement and support to commence this project. I appreciate the opportunity and exposure you availed me in the course of my invitation to your laboratory in Belgium.
- The staff of Optics and Imaging Centre where this study was conducted.
- My colleagues in Optics and Imaging Centre and Placental Research Group: Dr David Ofusori, Dr Bola Komolafe, Dr Kaminee Maduray, Dr Frederick Odun-Ayo, Vino Dorsamy, and Mrs D Margolis; I am thankful for your help, assistance, patience and continued interest with words of encouragement throughout my study.
- All the masters' students (2016 session) in the Optics and Imaging Centre.
- Vanessa Tombe, Centre for the AIDS Programme of Research in South Africa (CAPRISA) for your assistance during the course of this project.
- Dr N.R Maharaj, Dr B.J Assumani and Miss Nondumiso Nhlumayo (Prince Mshiyeni Memorial Hospital).
- Miss Mavis Nene for your assistance.
- My children; Tony, Carine and Jean Rene Onyangungas- I love you all.
- Finally, my beloved wife, Antoinette Nkoka Onyangunga who has been with me through thick and thin. Thank you for your love, patience and understanding all the way through.

TABLE OF CONTENTS

PREFACE i

DECLARATION ii

DEDICATION iii

PEER REVIEWED PUBLICATIONS, ABSTRACT AND CONFERENCE PRESENTATIONS iv

FUNDING vii

ACKNOWLEDGEMENTS viii

TABLE OF CONTENTS ix

LIST OF ABBREVIATIONS xi

LIST OF FIGURES xiii

LIST OF TABLES xv

ABSTRACT xvi

CHAPTER 1 1

INTRODUCTION 2

1.1 Pre-eclampsia (PE) 2

1.2 Epidemiology 3

1.3 Risk factors, prevention and prediction for pre-eclampsia 4

1.4 Pathogenesis of pre-eclampsia 5

1.5 Genetics of Pre-eclampsia 8

1.5.1 Tumor necrosis Factor (TNF SF13B) 9

1.5.2 CYP11B2 9

1.5.3 HLA-G gene polymorphism 9

1.6 Immunological aspects of Pre-eclampsia 9

1.7 HIV and Pre-eclampsia 10

1.7.1 HIV Infection and Placenta 11

1.8 Vascular Endothelial Growth Factors 11

1.9 Lymphangiogenesis 12

1.9.1 Lymphangiogenic markers 13

1.9.1.1 Lymphatic vascular endothelial receptor-1 (LYVE-1) 13

1.9.1.2 Podoplanin (PDPN) 15

1.10 Rationale for the study 17

1.11 Hypothesis 18

1.12 Aims and Objectives 18

CHAPTER 2: Manuscript 1 20

CHAPTER 3: Manuscript 2 29

CHAPTER 4: Manuscript 3 50

CHAPTER 5: Manuscript 4 60

CHAPTER 6: Abstract 97

CHAPTER 7: Proceedings 103

CHAPTER 8 109

Synthesis 110

Manuscript 1: 110

Manuscript 2: 111

Manuscript 3: 112

Manuscript 4: 113

Conclusion 113

CHAPTER 9 114

REFERENCES 115

CHAPTER 10 127

APPENDICES 128

LIST OF ABBREVIATIONS

AIDS	-	acquired immunodeficiency syndrome
ARV	-	anti-retroviral
BC	-	before Christ era
CCL	-	chemokines ligands
CD4	-	cluster of differentiation 4
CLEC-2	-	C-type lectin-like receptor-2
CNS	-	central nervous system
CT	-	Cytotrophoblast
CYP11B2	-	Cytochrome P450 family II subfamily number 2
EC	-	endothelial cell
ECM	-	extracellular matrix
ERM	-	ezrin/radixin/moesin
EOPE	-	early onset pre-eclampsia
FRC	-	fiboblastic reticular cell
GAL	-	galectin
HA	-	hyaluronic acid
HRE	-	hypoxia responsive element
HELLP	-	hemolysis elevated liver enzymes low platelets
HIF-1	-	hypoxia- inducible factor 1
HIV	-	human immunodeficiency virus
HLA	-	human leukocyte antigen
HMW-HA	-	high molecule weight hyaluronic acid
HRE	-	hypoxia responsive element

IFN-Y	-	gamma interferon
IL	-	interleukin
ISSHP	-	International Society for the Study of Hypertension in Pregnancy
kDa	-	kilo Daltons
KDR	-	Kinase insert domain receptor
LMW-HA	-	low molecule weight hyaluronic acid
LOPE	-	late onset pre-eclampsia
LYVE-1	-	lymphatic vascular endothelial-1 receptor
MTCT	-	mother to child transmission
PDPN	-	podoplanin
PE	-	pre-eclampsia
PP-13	-	Placental protein 13
RHAMM	-	receptor for hyaluronan-mediated motility
sEng	-	soluble endoglin
SF	-	steroidogenic factor-1 site
sFlt-1	-	soluble fms-like tyrosine kinase receptor
TGF-β	-	transforming growth factor beta
Th	-	T-helper
TNF-α	-	tumor necrosis factor alpha
TTR	-	transthyrectin
VEGF	-	vascular endothelial growth factor
VEGFR	-	vascular endothelial growth factor receptor
WHO	-	World Health Organization

LIST OF FIGURES

Figure	Legend
	Chapter 1
Fig 1	Schematic diagram of pathogenesis of pre-eclampsia
Fig 2	sflt1 and sEng cause endothelial dysfunction by antagonizing VEGF and TGF-β signaling
Fig 3	Ligands of vascular endothelial growth factor and its receptor
Fig 4	Binding of HA residues showing LYVE-1 and CD44 interaction
Fig 5	The interaction of podoplanin with a variety of intracellular and Transmembrane proteins to mediate effects on cell migration and adhesion
	Chapter 2
Fig 1	Flow diagram of the study population
	Chapter 3
Fig 1	Flow diagram shows the subgroups of pre-eclampsia and their HIV status
	Chapter 4
Fig 1	LYVE-1 segmentation within (A) conducting and (B) exchange villi of N-group
Fig 2	LYVE-1 immunostaining within placental conducting villi
Fig 3	LYVE-1 immunostaining within placental exchange villi
Fig 4	LYVE-1 expression in (A) conducting and (B) exchange villi across all study groups
Fig 5	LYVE-1 immunoexpression according to HIV status (N-, EOPE-, LOPE-; n=90 and N+, EOPE+, LOPE+; n=90) within (A) conducting and (B) exchange villi
Fig 6	LYVE-1 expression within conducting (n=180) and exchange (n=180) villi irrespective of study groups

Chapter 5

Fig 1 Podolanin immunostained conducting villi across study groups

Fig 2 Podoplanin immunostained exchange villi across study groups

Fig 3 Podoplanin immunoexpression in conducting (A) and exchange villi (B) across all study groups

Fig 4 Podoplanin immunoexpression according to HIV status (N-, EOPE-, LOPE-; n=90 and N+, EOPE+, LOPE+; n=90) within conducting (A) and exchange (B) villi

Fig 5 Podoplanin immunoexpression based on the pregnancy type (N, EOPE and LOPE) within conducting and exchange villi

Fig 6 Podoplanin immunoexpression within conducting (n=180) and exchange (n=180) villi across of study group

Fig S1 Composite image of podoplanin immunostained placental villi of normotensive positive stem (SV), intermediate (IV) and terminal (TV) villi, artery (arrow)

Chapter 6

Fig 1 Exchange villi in HIV pre-eclamptic placenta illustrating LYVE-1 expression (brown) within fetal endothelial cells

Fig 2 Conducting villi in HIV pre-eclamptic placenta showing LYVE-1 expression (brown) within fetal endothelial cells

Fig 1 LYVE-1 expression within decidua from HIV associated early onset pre-eclampsia

Fig 2 LYVE-1 expression within myometrial spiral arteries in HIV associated early onset pre-eclampsia

Fig 1-3 Extravillous trophoblast (1), synctiotrophoblast (2) and cytotrophoblast (2) cell populations.

LIST OF TABLES

Table	Legend
	Chapter 1
Table 1	Principal risk factors in pre-eclampsia development
	Chapter 2
Table 1	Clinical characteristics of all primigravidae with hypertensive disorders of pregnancy
Table 2	Caesarean deliveries in hypertensive primigravidae
Table 3	Perinatal outcome in hypertensive groups
Table 4	Maternal complications in hypertensive primigravidae
	Chapter 3
Table 1	Maternal age and parity in early and late onset pre-eclampsia
Table 2	Clinical data of early and late onset pre-eclampsia
Table 3	Indications for caesarean delivery in early and late onset pre-eclampsia (HIV positive and negative)
Table 4	Maternal complications in early and late pre-eclampsia (HIV positive and negative)
Table 5	Relevant perinatal data in early and late pre-eclampsia (HIV positive and negative mothers)
	Chapter 4
Table 1	Patient demographics across study groups
Table 2	LYVE-1 immuno-reactivity across study groups

ABSTRACT

In South Africa, HIV/AIDS contributes to 40.5% of maternal deaths. The province of KwaZulu-Natal (23.1%) is the epicenter of the HIV global pandemic. It is a serious obstetric dilemma that young women of reproductive age are found to be at risk of HIV infection. Pre-eclampsia is predominantly a condition associated with primigravidae with an incidence of 12% within KwaZulu-Natal. Importantly, HIV infection superimposed upon induced hypertensive pregnancies occur frequently in this province hence, presents a unique paradigm to study these two co-morbidities.

Aim 1: To describe the incidence of obstetric and perinatal outcomes in primigravid Black South Africans with hypertensive disorders in pregnancy.

Method: We examined 5860 primigravidae deliveries at a Regional Hospital in Durban, South Africa. Written informed consent was obtained from all the participants. Maternal demographics and clinical factors for pre-eclampsia, blood laboratory tests, obstetrical findings and outcomes were recorded. Perinatal outcomes were listed on a structured data sheet. Incidence rate of hypertensive disorders was calculated.

All results were presented as frequencies, means, ranges and percentages. Student t-test was used for continuous variables and chi-square was test for categorical variables.

Result: Of the 5860 primigravidae delivered during the study period, 731 had an hypertensive disorders in pregnancy giving an incidence of 12.5%. The diagnosis of gestational hypertension was made in 6.7% of all primigravidae but was the commonest hypertensive disorders in pregnancy subcategory (n= 394/731; 53.9%). On the other hand, mild to moderate pre-eclampsia or non-severe pre-eclampsia, severe pre-eclampsia and eclampsia occurred in 222, 84 and 31 of the 5860 primigravidae respectively. Therefore, pre-eclampsia-eclampsia syndrome occurred in 337/5860 (5.75%) of the study population. Severe pre-eclampsia occurred in 84/5860 (1.43%) of the primigravidae. The rates of caesarean deliveries in women with pre-eclampsia were approximately 50%. There were no perinatal deaths in the gestational hypertension group, but the overall perinatal mortality rate in all pre-eclamptics was 5.9% in comparison to 2.2% in all primigravidae.

Aim 2: To determine the maternal and perinatal outcomes in HIV negative and positive pregnant women with early and late onset pre-eclampsia who had scheduled caesarean section.

Methods: This prospective study was conducted at a regional hospital in Durban, South Africa within a period of 14 months from September 2012 to November 2013. A total of 14304 deliveries were registered. Out of the 1759 pre-eclamptic, 351(19.9%) were early onset pre-eclampsia (EOPE) and 1408(80.1%) were late onset pre-eclampsia (LOPE). Patients with pre-eclampsia (n=120) were divided into 2 groups according to gestational age namely EOPE (n=60) and LOPE (n=60). Each pre-eclamptic category was then further stratified into HIV positive (n=30) and HIV negative (n=30). Demographics and clinical factors for pre-eclampsia, blood laboratory tests, obstetrical findings and outcomes were collated.

Parametric data were analysed using t-test and chi-square tests and Mann-Whitney U test for non-parametric data.

Results: The women with early onset pre-eclampsia were older compared to those with late onset pre-eclampsia (p=0.0001). Also the HIV positive women were older compared to the HIV negative in both categories (p=0.03). However, multiparity and primiparity were more representative in early and late onset pre-eclampsia respectively (p= 0.00 and p=0.00). The severity of the hypertension and the HIV status did not differentiate the 2 groups. However, both maternal complications (eclampsia, persistent postpartum hypertension, HELLP syndrome, maternal death) and poor fetal outcomes occurred predominately in the early onset pre-eclampsia group.

Aim 3 and 4: The aim of this study was to examine lymphatic vessel endothelial receptor-1 (LYVE-1) and podoplanin expressions in the placenta of HIV infected normotensive and early- and/ late onset pre-eclampsia.

Methods: Placental tissue was obtained from normotensive and pre-eclamptic women stratified according to their HIV status. The pre-eclamptic group was divided into early (< 34 weeks) and late (> 34 weeks) onset. Immunohistochemistry utilized monoclonal mouse anti-human LYVE-1 and podoplanin antibodies and was morphometrically evaluated.

Results: There was a significant difference in maternal age and birth weight in the EOPE group based on the HIV status ($p \leq 0.001$; p=0.05 respectively). LYVE-1 immunostaining was localized within endothelium of the arterial supply and venous

drainage of both conducting and exchange villi as well as within medial cells of arteries. LYVE-1 immunostained macrophages were observed within the fetal and maternal circulation. LYVE-1 immunoexpression within the exchange villi was higher in HIV positive compared to HIV negative cohorts ($p \leq 0.0001$). Irrespective of HIV status, LYVE-1 immunoexpression was higher in normotensive and LOPE groups compared to EOPE for both conducting and exchange villi, respectively ($p \leq 0.0001$ and $p=0.006$).

Podoplanin was immunolocalized in a reticular-like stroma complex within the conducting and exchange villi. Its immunoexpression was significantly up-regulated in the exchange versus conducting villi ($p= 0.0001$) irrespective of the pregnancy type and HIV status. Podoplanin was down-regulated in the early onset pre-eclampsia compared to late onset pre-eclampsia group in the exchange villi ($p= 0.05$). Also, podoplanin was up-regulated in HIV positive vs HIV negative groups regardless of pregnancy and villi type. Based on the HIV status, podoplanin immunoexpression within conducting villi was higher in the HIV negative ($8.19 \pm 3.37\%$) versus HIV positive ($7.67 \pm 3.23\%$) group albeit non-significantly ($p = 0.306$). Conversely regardless of pregnancy type, podoplanin immunoexpression within exchange villi was different ($p= 0.008$) between the HIV positive and HIV negative groups.

Overall Conclusion: This study demonstrates that hypertensive disorders in pregnancy developed in 12.5% of primigravidae. We outline that gestational hypertension comprised the commonest sub-category of the hypertensive disorders in pregnancy and were not associated with perinatal deaths. Additionally, this study confirms the heterogeneity of pre-eclampsia and shows that the timing of disease onset is allied with disease severity. Further HIV status influences maternal and neonatal outcomes.

This study reports an absence of lymphatic vessels in the placenta. It provides a novel insight into the elevated distribution of LYVE-1 in the placenta of HIV-infected pregnancy. We propose that placental macrophages provide an innate response against pathogens thereby maintaining maternal-fetal immunity. Using the more specific, podoplanin marker, this novel study demonstrated that placental fluid homeostasis is maintained by a podoplanin reticular-like complex within conducting and exchange villi, being up-regulated in HIV positive pregnancies. However, podoplanin immunoexpression was down-regulated in the exchange villi of EOPE reflecting the distribution deficient trophoblast invasion.

CHAPTER 1

INTRODUCTION

1.1 Pre-eclampsia (PE)

Pre-eclampsia is defined as a new onset of hypertension (arterial blood pressure ≥ 140/90 mmHg on at least 2 occasions and 4 hours apart) with proteinuria at least 1+ on dipstick on 2 occasions at least 6 hours apart but no more than one week, or 300mg/24 hours' urine collection after 20 weeks' gestation (Tranquilli et al., 2014). Pre-eclampsia is a pregnancy specific hypertensive disorder unique to humans (Jeyabalan et al., 2013). The condition "toxaemia of pregnancy" which referred to the classical signs of pre-eclampsia was first reported in ancient Greece as early as 4th-5th BC (Bell, 2010).

Pre-eclampsia may further be classified into mild, moderate and severe according to blood pressure measurement (Tranquilli et al., 2014). The International Society for the Study of Hypertension in Pregnancy (ISSHP) indicates pre-eclampsia in a patient with a blood pressure at ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic is defined as severe (Tranquilli et al., 2014). Factors determining the severity of pre-eclampsia and indication to expedite delivery include difficulty in controlling blood pressure and deteriorating clinical conditions such as the HELLP syndrome, impeding eclampsia, worsening thrombocytopenia and worsening fetal growth restriction. There is no clear consensus on the amount of proteinuria to be considered "severe", although the value between 3 - 5 g/l is acceptable, thus the ISSHP considers that proteinuria should not be criteria of severity in PE (Tranquilli et al., 2013).

Alternatively, pre-eclampsia may be classified into two categories based on the gestational period and onset of symptoms defining the syndrome (Tranquilli et al., 2014)

- Early onset pre-eclampsia (EOPE): presentation of signs and symptoms < 33 weeks + 6 days
- Late onset pre-eclampsia (LOPE): presentation of signs and symptoms >34 weeks. This second category has since been divided into 2 subcategories;
- Preterm pre-eclampsia (starting from 34 weeks + 1 day to 37 weeks + 0 day) and
- Term pre-eclampsia (occurring after 37 weeks +1 day).

The EOPE and LOPE is widely considered as 2 different entities sharing common presentations and some pathogenic roots (Raymond et al., 2011; Soto et al., 2012; Van der Merve et al., 2010; Valensise et al., 2013; Redman, 2014a). Also both maternal and perinatal outcomes are generally poor in EOPE compared to those observed in LOPE (Lisonkwa et al., 2013; Mitsui et al., 2015; Madzali et al., 2014).

In 2000, the International Society for the Study of Hypertension in Pregnancy constituted a Task team to look for universal classification of HDP which would assist countries without their own guidelines (Tranquilli et al., 2014). In 2013, they revised the classification of hypertensive diseases in pregnancy as follow:

- Chronic Hypertension
- Gestational Hypertension
- Pre-eclampsia *de novo* or superimposed on chronic hypertension.
- White Coat Hypertension

1.2 Epidemiology

1.2.1 Pre-eclampsia

Pre-eclampsia affects 5-12% of all pregnancies worldwide (Redman et al., 2012; Jeyabalan et al., 2013). In developing countries, the high incidence of PE remains a serious threat to the health of pregnant women. Maternal deaths related to PE are particularly high in limited resources (15%) compared to industrialized countries (1.8%; Ghulmiyyah and Sibai, 2012). Globally, resource poor countries account for 99% of maternal deaths with 85% occurring in Sub-Saharan Africa and Southern Asia (Rueda-Clausen et al., 2011). The estimated number of maternal death caused by pre-eclampsia is 60 000 annually (Ghulmiyyah and Sibai, 2012). The World Health Organization (WHO) reports that pre-eclampsia (PE) accounts for approximately 16% of maternal deaths in developed countries, 9% in Africa and Asia, and as many as 26% in Latin America and the Caribbean (Khan et al., 2006; WHO Report Maternal Mortality, 2011).

The WHO reports that approximately 800 pregnant women die daily due to pre-eclampsia and 50% of those women are from Sub Saharan Africa (WHO, 2011). This high incidence is attributed to limited resources, poor health care infrastructure and poor access to health care facilities. Furthermore, unavailability of certain medications and lack of management protocols for PE exacerbate maternal deaths. Also, more than half of women affected by PE have poor access to health care; hence they are not diagnosed early enough to receive good management (Moodley, 2007). Despite the reported prevalence of early onset pre-eclampsia (EOPE) and late onset pre-eclampsia (LOPE) as 0.3-0.38% and 2.7-3% respectively (Lisonkwa et al., 2013; Fank et al., 2009), caution is advised in accepting this prevalence due to non-uniform diagnostic criteria adopted in these retrospective studies.

1.2.2 HIV

In Sub-Saharan Africa, HIV/AIDS is a major threat to human health. Approximately 30% pregnant women are infected with HIV in South Africa. In South Africa, the three leading causes of maternal deaths are non-pregnancy related infections (HIV infection complicated by tuberculosis and pneumonia) (35.8%), haemorrhage (15.8%) and hypertensive disorders in pregnancy (14.8%; Saving Mothers, 2015). The Saving Mother's surveillance shows an elevation in maternal deaths concurrent with an increase in HIV infection. Moreover, the province of KwaZulu-Natal is considered the epicenter of the HIV pandemic accounting for the highest maternal deaths (22%) with 42.5% emanating from HIV infection and 12% to pre-eclampsia development (Saving Mothers, 2015).

1.3 Risk factors, prevention and prediction for pre-eclampsia

Pre-eclampsia is a multifactorial syndrome; however epidemiological studies have documented numerous risk factors with different degrees of expression (Valenzuela et al., 2011; Leung et al., 2015; English et al., 2015; Lisonkwa et al., 2014). A summary of these risk factors is shown in Table 1.1. In South Africa, maternal deaths due to PE are more common among younger than older women (Saving Mothers, 2015). Also, PE is a condition that mostly affects women of first time pregnancy, teenagers and young women are usually in that category hence the higher incidence (Panday et al., 2004).

Low dose aspirin and calcium have been recommended by the WHO for the prevention of PE (WHO Guidelines Approved by the Guidelines Review Committee, 2011; Holfmeyer et al., 2014). A number of biomarkers have been put identified as predictor test indicators for the early (before 12 weeks) diagnosis of PE development. These include pregnancy associated plasma protein-A (PAPP-A), placenta growth factor (PIGF) and soluble fms-like tyrosine kinase-1 (sFlt-1). Notably the ratio of sFlt-1/ an over production of sFlt-1 and/or decrease of PIGF will have predictive value for the onset of PE by/after 20 weeks of gestation (Akolekar et al., 2011; Kane et al., 2013; Poon et al., 2014; Seely et al., 2016). However, these tests need to be improved and applicable worldwide before becoming routine.

Table 1.1 Principal risk factors in Pre-eclampsia development (Dekker and Sibai, 2001)

Risk factor	Mean RR (95% CI)
Antiphospholipid syndrome	9.72 (4.34–21.75)
Relative risk of preeclampsia	7.19 (5.85–8.83)
Previous preeclampsia	7.19 (5.85–8.83)
Insulin-dependent diabetes	3.56 (2.54–4.99)
Multiple pregnancy	2.93 (2.04–4.21)
Nulliparity	2.91 (1.28–6.61)
Family history of preeclampsia	2.90 (1.70–4.93)
Obesity	2.47 (1.66–3.67)
Age >40 years	1.96 (1.34–2.87)
Preexisting hypertension	1.38 (1.01–1.87)

CI, confidence interval; RR, relative risk

1.4 Pathogenesis of pre-eclampsia

Many enigmatic theories with regards to the origin of PE have been put forward (Bell, 2010). Despite extensive research, the pathogenesis of the disease is still not well understood. Hence, there is a call for new universal definitions, classifications and management. Nevertheless, it is widely agreed that the placenta is the source of the pathogenesis, since delivery or removal of the placenta is followed by the resolution of symptoms of the syndrome (Roberts and Escudero, 2012). The presence of the fetus in the uterus is not mandatory as demonstrated in case of molar pregnancy (Roberts and Escudero, 2012).

Pre-eclampsia is associated with impaired trophoblast invasion in the myometrium (Young et al., 2010; Redman et al., 2011; Powe et al., 2011; Naljayan and Karumanchi, 2013; Staff et al., 2013; Warrington et al., 2013). It is widely accepted that cytotrophoblastic invasion is limited to the decidua of the placental bed with resultant non-physiological remodeling of the spiral arteries. This leads to insufficient blood flow which causes hypoxic conditions responsible of ischemia, apoptosis and excess liberation of cytokines and anti-angiogenic factors (Cerdeira and Karumanchi, 2012; Staff et al., 2013). The consequence of this imbalance between pro- and anti-angiogenic factors results in the clinical manifestation of the syndrome (Figure 1). Pre-eclampsia is also considered a multifactorial condition which involves the renin-aldosterone-angiotensin system, oxidative stress, immune maladaptation and genetic susceptibility possibly modulated by environmental influence (Steegers et al., 2010; Sibai, 2011; Karumanchi et al., 2012).

Placental related pathogenic factors for pre-eclampsia include transforming growth factor beta family (TGF-ß) and its receptor soluble endoglin (Govender et al., 2014); trophoblast invasion related factors such as vascular endothelial growth factors (VEGF) and placenta growth factors (PIGF) (Karumanchi et al., 2016; Chen and Zhang, 2012; Freitag et al., 2013). There is mounting evidence that VEGF and TGF-β1 are required to maintain endothelial health in several tissues, including the kidney and placenta. During normal pregnancy, vascular homeostasis is maintained by physiological levels of VEGF and TGF-β_1 signalling. In PE, excess placental secretion of sFlt1 and sEng (two endogenous circulating anti-angiogenic proteins) inhibit VEGF and TGF-β1 signalling respectively (Figure 2). This results in endothelial cell dysfunction including decreased prostacyclin, nitric oxide production and release of pro-coagulant proteins (Wang et al., 2009).

Other factors include maternal inflammatory factors Th1/Th2 α-tumoural necrosis factor (TNF-α), gamma-interferon (IFN-gamma) and interleukin-2 (IL-2) (EL-Dayem et al., 2016) whilst metabolic related factors include pregnancy-associated plasma protein-A (PAPP-A), insulin-like growth factor and transthyretin (TTR) (Kalkunte et al., 2013; Zhu et al., 2014).

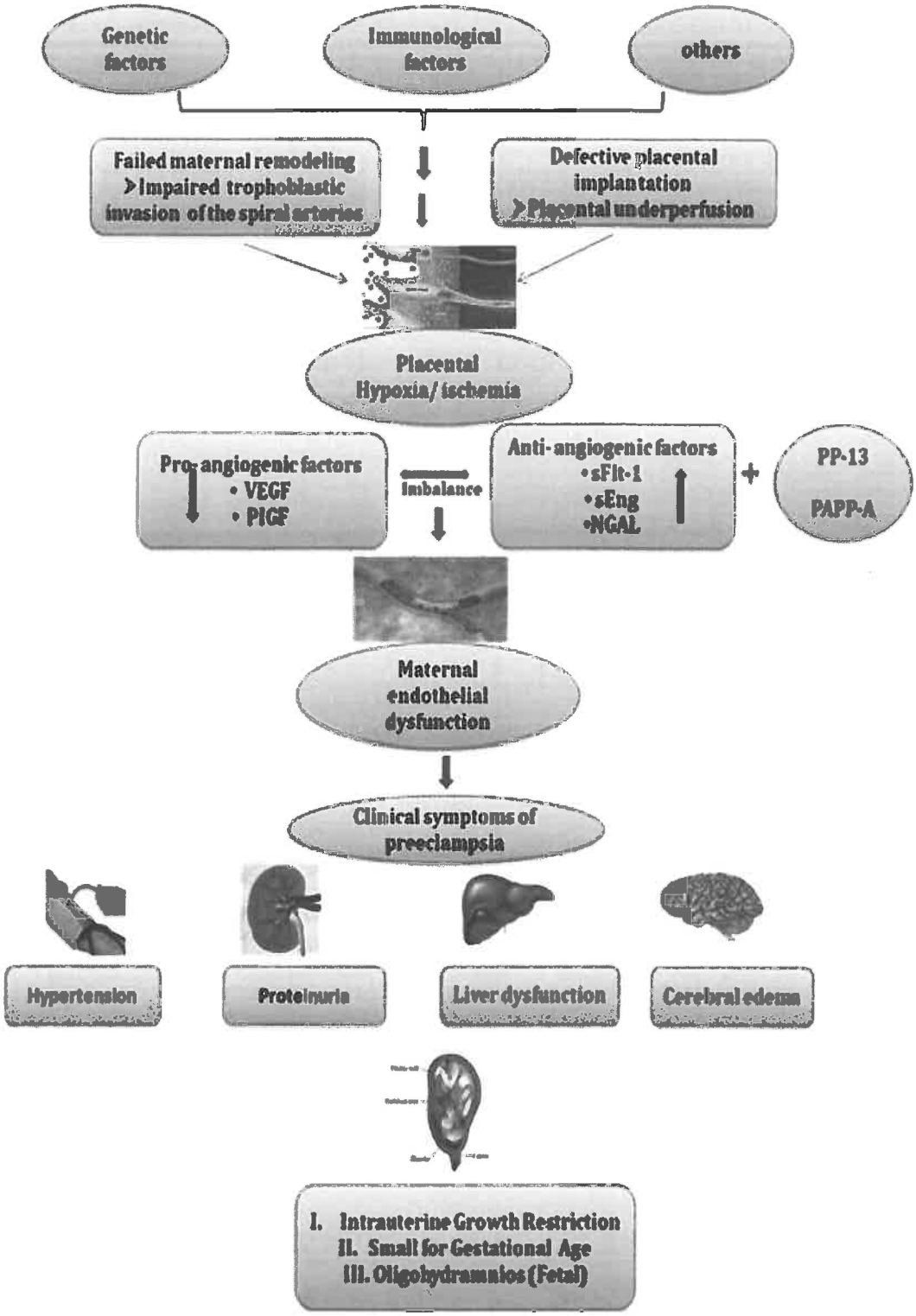


Figure 1. Schematic diagram of pathogenesis of pre-eclampsia (Petla et al., 2013)

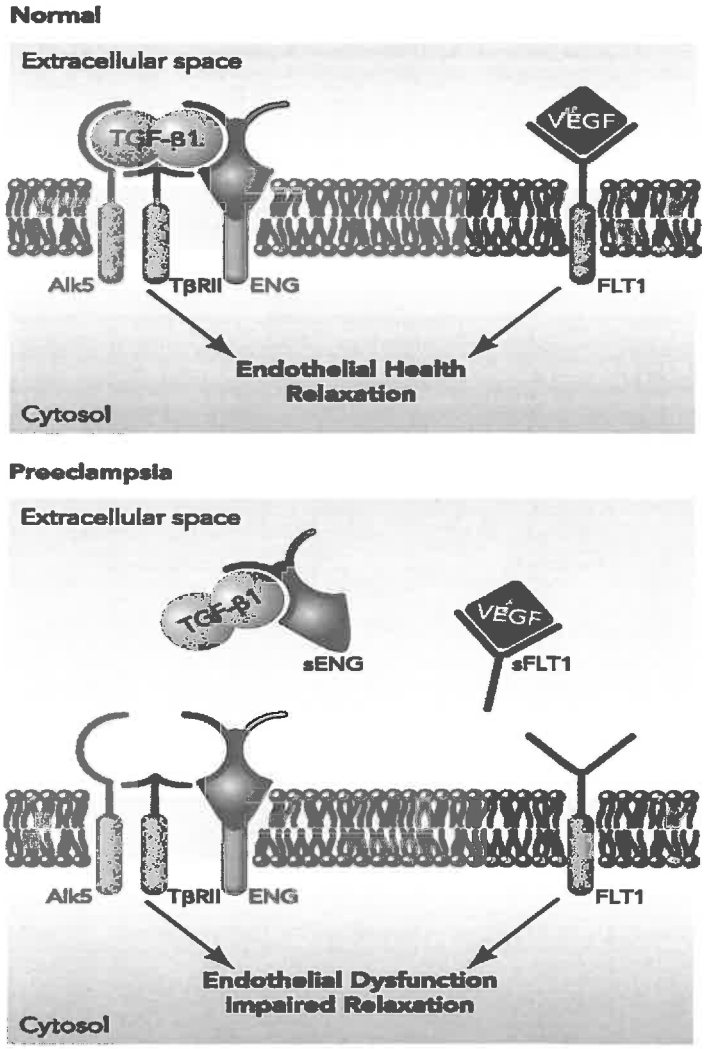


Figure 2. sFlt1 and sEng cause endothelial dysfunction by antagonizing VEGF and TGF-β signalling (Wang et al., 2009).

1.5 Genetics of Pre-eclampsia

PE is a disease with strong familial predisposition, which also varies according to geographical socio-economic and ethnicity (Valenzuela et al., 2011). Women with first-degree relatives that have had PE are five times more at risk of developing PE, whilst those with second-degree relatives have their risk doubled. The genomic imprinting results in the participation of paternal genes in the control of invasion and placental growth, supported by studies showing the increased risk of PE in women with pregnancies of men who have previously involved in pregnancies complicated with PE. Edwards et al (2011) reported n775SNPs in 190 genes has defected six genes with a significance maternal-fetal genotype interaction. These observations propose variable factors of genetic inheritance leading to the

disease progression (Valenzuela et al., 2011). Furthermore, other genes have been found to be related to the categories and severity of PE (Junus et al., 2012; Andraweera et al., 2014; Haram et al., 2014; Zhu et al., 2015).

1.5.1 Tumor necrosis Factor (TNF SF13B)

Tumor necrosis Factor (TNF SF13B) is implicated in the regulation of immune response to infections, autoimmune disease and inflammation in the development of fetal immune system (William and Pipkin, 2011). It is a molecule of the tumor necrosis factor family of ligands and is localized in chromosome region 13932-934. During the third trimester, the human placenta expresses TNF SF13B and its receptors in villous cytotrophoblast cells (CT) and mesenchyme cells where it has an auto-apoptotic role. Recently, a genetic variation of TNF SF13B has been connected with the susceptibility of PE (Williams and Pipkin, 2011; Valenzuela et al., 2011).

1.5.2 CYP11B2

Steroid 11/18-βeta-hydroxylase is encoded by the CYP11B2 gene that is located in the 8924.3. The -344C/T polymorphism within 5' regulatory region of CYP11B2 disrupts a putative steroidogenic factor-1 site, and the homozygosity for SF-IT/T variant has been reported as a protective factor against the risk of PE development ref, in contrast, the heterozygous state is not protective (Valenzuela et al., 2011). Moreover, -344 C/T polymorphism has strong linkage disequilibrium in CYP11B2 and contributes to hypertension in subjects with a varied aldosterone: renin ratio (Valenzuela et al., 2011).

1.5.3 HLA-G gene polymorphism

Trophoblast cells express an unusual repertoire of histocompatibility antigens (HLA-G, HLA-E) of which only HLA-C displays marked polymorphism (William and Pipkin, 2011). HLA-G member of HLA class I is localized in chromosome 6p 21.3. It has an immunosuppressive property and inhibiting role during pregnancy (Valenzuela et al, 2011). The HLA-G displaying a limited polymorphism with PE has been found also in the placenta. It was identified as HLA-G allele G0106 (William and Pipkin, 2011).

1.6 Immunological aspects of Pre-eclampsia

The mechanisms pathologic of the immune system in PE is as a result of complex interactions between placental debris or particles, oxygen-sensing mechanism, endothelial cells, miRNAs,

leucocytes, natural killer cells (NK), dendritic cells (DC), T lymphocyte subsets, B lymphocytes and antibodies (Laresgoiti-Servitje, 2013). These pathologic stages cause placental hypoxia or local changes in oxygen tension or oxygen-sensing mechanism thus result in overexpression of HIF-1α and immunological alterations in early placentation (Roberts and Gammill, 2005). Although, hypoxia alone is not enough to explain the LOPE, most studies have shown predominately normal placentae with normal baby weights in LOPE (Munaut et al., 2008). Maternal inflammatory response, the release of necrotic and/or apoptotic syncytiotrophoblast cells into the maternal circulation contributes to the immunological pathologic interactions (Sargent et al., 2006).

Normal placentation and placental growth require maternal adaptation or tolerance to the fetal allograft (Redman and Sargent, 2010). In PE, specific maternal immune responses to fetal allogenes have been implicated specifically to paternal antigens (Dekker, 2002; Robillard and Dekker, 2006). This adaptation includes a pre-conception stage which considers the number of partners, coitus, sperm and semen experienced by the mother before pregnancy. The pre-conception stage corresponds to stage one of the disease (Redman, 2014b).

1.7 HIV and Pre-eclampsia

HIV causes an immune-dysfunction that has affected millions of people worldwide. This aggressive pandemic, discovered in 1981, was divided into 2 subtypes viz., HIV-1 and HIV-2 (Appay and Sauce, 2008). HIV-1 infection is associated with depletion of CD4+ T cells. Failure of the T helper cells to activate B cells makes the body vulnerable to opportunistic diseases such as tuberculosis (TB) and eczema.

Both HIV infection and PE are common conditions in developing countries. However, literature regarding the association between HIV and PE is conflicting. Studies have shown an increased (Suy et al., 2006), decreased (Mattar et al., 2004) and no difference (Wimalasundera et al., 2002) in the occurrence of PE in HIV infected patients. More recently, a systematic review has found no association between PE development and HIV infection (Conde-Agudelo et al., 2008). Kalumba et al., (2013) and Hall et al., (2014) report an association of PE development in HIV negative pregnant women, in that a major proliferative aseptic inflammation cannot develop enough in an immunocompromised background. Nevertheless, recent studies have shown no difference in the prevalence of pre-eclampsia in HIV negative and HIV positive patients receiving anti-retroviral therapy (ARV's) (Kalumba et al., 2013; Machado et al., 2014).

1.7.1 HIV Infection and Placenta

The placenta is a haemo-chorial organ rich in blood cells and vessel networks, and others cells: macrophages and dendritic cells. Notably, in 14% of cases, the virus will cross the placental barrier and affect the fetus in the absence of highly active anti-retro viral therapy (Derrien et al., 2005). Placental lesions due to HIV infection are not specific and are similar to those of other inflammatory conditions. Mother to child transmission (MTCT) of the virus *in utero* has been reduced to less than 2% with the administration of prenatal antiretroviral therapy. However, their safety for both the baby and mother remains a concern. Indeed, ARVs cause cardiovascular complication with underlying endothelium dysfunction (blood and lymphatic), mitochondrial dysfunction and genotoxicity (Gibellini et al., 2012; Gupta et al., 2012; Elias, et al., 2013; Torriani et al., 2006; Cerrato et al., 2014; Jao et al., 2014).

1.8 Vascular Endothelial Growth Factors

The vascular endothelial growth factor (VEGF) family has 7 isoforms viz., VEGF-A, VEGF-B, VEGF–C and VEGF-D, VEGF-E, VEGF-F and PlGF (Andraweera et al., 2012). It is a signal protein that stimulates vasculogenesis and angiogenesis and is expressed throughout gestation where its main function is during embryonic development (Ferrara, 2003). They work via tyrosine kinase receptors, VEGFR1-R3. As shown in Figure 3, the VEGF-A works by binding to two homologous VEGF receptors expressed on vascular endothelial cells viz., VEGF receptor-1 (Ftl-1) and VEGF receptor-2 (Flk-1/KDR); a third receptor, viz., VEGF receptor-3 (Ftl-4) is involved in VEGF-C and VEGF-D facilitated lymphangiogenesis (Olsson et al., 2006; Sato, 2008). VEGFR-3 is expressed in the lymphatic endothelial cells and participates in mitosis, migration, differentiation and survival of cells. Several polymorphisms and down regulation of VEGF have been implicated in the risk of PE development (Valenzuela et al., 2011).

The most important molecule in the VEGF family that controls lymphangiogenesis is VEGF-C and VEGF-D. VEGFR3 is the major mediator of VEGF-C and VEGF-D driven responses in lymphatic ECs (Jussila and Alitalo, 2002) However, VEGF-A may stimulate VEGFR2 on lymphatic ECs and induce lymphangiogenesis directly. The induction of VEGF-A is important for the initiation of angiogenesis (Shibuya, 2011 and 2014). The principal trigger of angiogenesis is hypoxia, which induces the expression VEGF-A in various cell types. This induction in hypoxia is mediated by a transcription factor known as hypoxia-inducible factor 1 (HIF-1), a heterodimeric complex of HIF-1α and HIF-1β subunits, which binds to hypoxia responsive element (HRE) in the promoter of VEGF-A gene (Liang et al., 2008). Angiogenesis is normally associated with, or followed by, lymphangiogenesis, because

defective lymphangiogenesis causes tissue edema. The angiogenic growth of blood vessels and lymphatic vessels coordinates several biological processes like cell proliferation, migration, differentiation and cell to cell communication (Adams and Alitalo, 2007). However, little is known about the triggering factors of lymphangiogenesis.

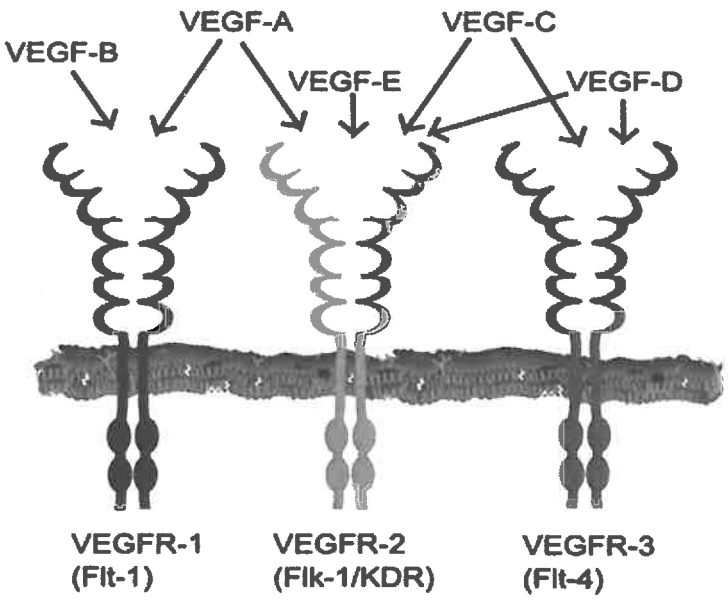


Figure 3. Ligands of vascular endothelial growth factor and its receptors (Sato, 2008)

1.9 Lymphangiogenesis

Lymphangiogenesis is defined as the development of new lymphatic vessels from pre-existing lymphatic vessels (Alitalo et al., 2005). Lymphatic vessels develop shortly after blood vessel formation; hence they have similar origin (Dvorak, 2003). The process of lymphangiogenesis is similar to the process of angiogenesis which is referred to as blood vessel development. Because the lymphatic vessels lack platelets and erythrocytes, it has low blood flow, a lower pressure system and is much less coaguable when compared to blood (Choi et al., 2012). Lymphatic vessels also send out fewer sprouts and are organized in a less complex network as opposed to the blood vessels (Abramson and Dobrin, 2012).

Normally, fluid and proteins leak from the vascular system during blood circulation. These extravasated proteins, fluids, and cells are drained into lymphatic vessels (lymph) and transported back into the venous circulation, thus they have a crucial role in fluid and protein homeostasis (Wang and Oliver, 2010). Lymphatic vessels also transport white blood cells

(such as dendritic cells and macrophages) which patrol from lymph node to lymph node in order to patrol the body for antigens or any foreign particles, thus by doing so, the lymph vessels have an immune-surveillance role. Data on lymphangiogenesis within the placenta and placental bed of HIV infected PE are limited and conflicting (Bellini et al., 2012; Liu et al., 2015). However, the lymphatic system dysfunction may be directly linked to AIDS pathogenesis (Tenner-Rácz et al., 1988).

1.9.1 Lymphangiogenic markers

Circulating anti- (IFN-, TGF-β) and pro- (VEGFs, PIGF) lymphangiogenic factors are implicated in the pathogenesis of pre-eclampsia (Powe et al., 2011; Munaut et al., 2012; Lely et al., 2013). The presence of these markers promotes the growth of lymphatic vessels, hence lymphangiogenesis in metastatic cancer (Stacker et al., 2014).

There is a paucity of data of uterine lymphatic circulation in normal and PE pregnancies (Red-horse et al., 2006). In pregnancy, lymphatic vessels play a vital role in regulating fluid homeostasis, maintaining maternal-fetal immunity and establishing maternal-fetal tolerance. There is a dire lack of information of the presence and function of uterine lymphatics in PE. Recent data has shown that the maternal decidua contains numerous lymphatic vessels with extensive lymphangiogenesis occurring at the maternal-fetal interface (Red-Horse, 2008). Cytotrophoblasts express several lymphangiogenic molecules *in vitro* and *in vivo*, implicating their involvement in inducing lymphatic growth within the decidua (Red-horse et al., 2006).

The identification of specific lymphatic vessel markers viz., lymphatic vascular endothelial receptor 1 (LYVE-1) and podoplanin has provided the tools required to accurately characterize lymphatic distribution (Rodgers et al., 2008).

1.9.1.1 Lymphatic vascular endothelial receptor-1 (LYVE-1)

Lymphatic vascular endothelial receptor (LYVE) is a major receptor for hyaluronan on lymphatic endothelial cells (Banerji et al., 1999). Hyaluronic acid (HA) known as hyaluronan was discovered in 1934 by Karl Meyer (Necas et al., 2008). Hyaluronic acid is a mucopolyssacaride primarily found in the extracellular matrix (ECM) and peri-cellular matrix (Necas et al., 2008; Jiang et al., 2007; Wu et al., 2014). Hyaluronic acid, an important and abundant component of ECM has a structure made with non-sulfate, negatively charged linear polymer of repeated disaccharide units of ß-(1, 3)-D, glucuronic acid-ß (1, 3) N-acetyl-D-glucosamine (Wu et al., 2014).

Hyaluronan has different properties which allow it to interact with different molecules through its receptors. It is an important hydrophilic molecule for tissue hydration control (water transport) that maintains the elasto-viscosity of liquid connective tissue (Necas et al., 2008). Hyaluronan regulates tissue morphogenesis such as cell detachment, mitosis, tumors development and metastasis. In addition, HA modulates the expression of inflammatory factors in mesenchymal stem cells (Solis et al., 2011; Wu et al., 2014).

There are two forms of HA: high molecule weight HA (HMW-HA) and low molecule weight HA (LMW-HA). Low molecule weight HA has pro-inflammatory activities, induces angiogenesis, immune response at chemokines, cytokines, growth factors, transcriptome factors with genes interactions while HMW-HA inhibits inflammation, immune response and angiogenesis (Tammi et al., 2002; Evanko et al., 2007; Turley et al., 2002; Stern et al., 2006; Jiang et al.,2001). The LMW-HA can be synthetized *de novo* or generated by either hyaluronidase mediated degradation or hydrolysis of native HA under pathological conditions (Jiang et al., 2007).

Hyaluronic acid interacts with the cell surface viz; at least two ways. First, it binds cell surface receptors, hyal-adherines such as CD44, LYVE-1 and RHAMM. Secondly, it activates other proteins (Solis et al., 2011; Wu et al., 2014). Hyaluronic acid is abundant in the placenta but its role in the lymphangiogenesis is unclear. However, an important data covered its contribution in the function of normal and pathologic placenta (Ponting and Kumar,1995; Matejevic et al., 2001; Zhu et al., 2013; Böckle et al., 2008; Vallet et al., 2010) using different receptors notably LYVE-1.

In 1999, Banerji et al (1999) described for the first time lymphatic endothelial receptor-1 (LYVE-1) as a new homologue of CD44 glycoprotein, lymph-specific receptor for HA. Lymphatic vascular endothelial receptor-1 has been identified as a strong marker for lymphatic endothelium. It has 322 amino acid type 1 integral membrane glycoproteins which is 41% similar to CD44 hyaluronan receptors. LYVE-1 expression has been noted during the uptake and degradation of HA (Johnson et al., 2007) and in the lymphatic and blood endothelial cells (Wrröbel et al., 2005; Gordon et al., 2008; Choi et al., 2013).

LYVE-1 co-localizes with CD44 on lymphatic vessels of placenta. However, at the maternal-fetal interface, LYVE-1 rather than CD44 is the functional HA receptor (Figure 4). LYVE-1 is expressed in both trophoblast and villous fetal vessel endothelial cells (Wang et al., 2011). Although, the precise function and signalling regulation in HA- LYVE-1 pathway in the placenta are not known, the enhanced LYVE-1 protein expression in the syncytiotrophoblast layer of the preterm placenta indicates a unique physiological significance of LYVE-1 during placental and fetal development (Gu et al., 2006). There is no data available on LYVE-1

expression in the placenta and placental bed of HIV associated normotensive and PE pregnancies.

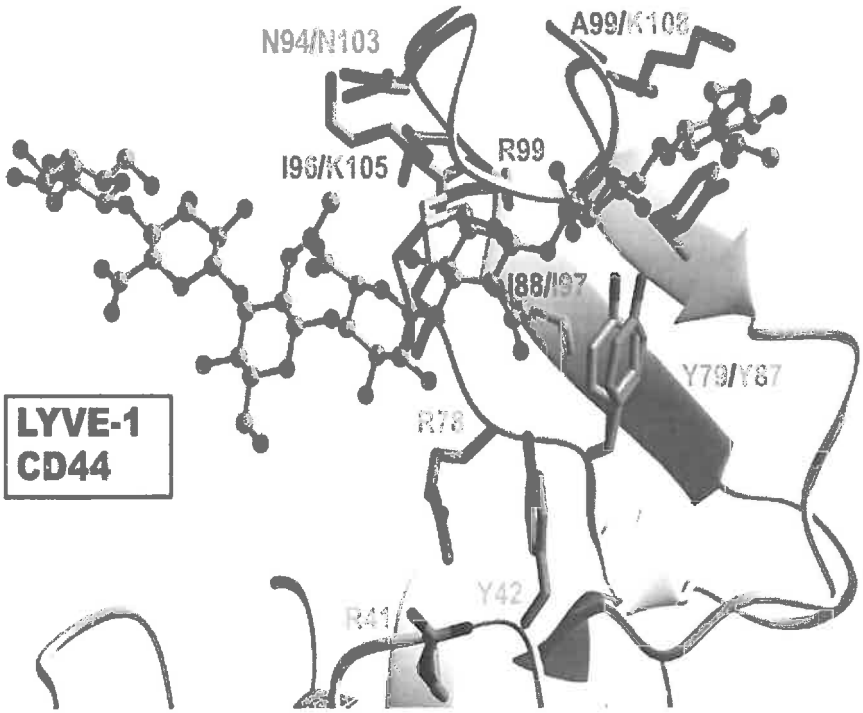


Figure 4. Binding of HA residues showing LYVE-1 and CD44 interaction (Banerji et al., 2010).

1.9.1.2 Podoplanin (PDPN)

Podoplanin is a 36- 43 kDa mucin-type transmembrane protein with a wide variety of functions including regulation of organ development, cell motility, tumorigenesis and metastasis (Suzuki-Inoue et al., 2011). PDPN was first described on lymphatic ECs (Wetterwald et al., 1996), fibroblastic reticular cells (FRCs) of lymphoid organs and thymic epithelial cells (Farr et al., 1992). Fu et al. (2008) reported PDPN expression on ECs is responsible for a blood-lymphatic misconnection. The continued expression of PDPN is required to maintain proper vascular architecture, as an inducible deletion of T-synthase, a major glycosyl-transferase required for O-glycan synthesis while normal levels of PDPN expression show similar blood-lymph mixing (Fu et al., 2008). Given that PDPN is a specific marker of lymphatic vessels, and that increased lymphangiogenesis it is often correlated with poor prognosis in cancer patients, the numbers of PDPN vessels in tumors is often used as a diagnostic marker (Breiteneder-Geleffetal., 1997; Ji, 2006; Swartz and Lund, 2012).

Interactions between PDPN and CLEC-2 also may play a role in tumor progression and metastasis due to platelets interacting with tumor cells. This Interaction is stabilized by multiple regions within human PDPN including three platelet aggregation-stimulating domains (a region between amino acids 80 and 103 that contains potential O glycosylation sites and a region between amino acids 103 to 128) (Kato et al., 2008). Interaction between CLEC-2 in platelets and PDPN on lymphatic ECs induces platelet aggregation and prevents blood from flowing into new lymphatic vessels budding from the cardinal vein. This interaction, in turn, activates downstream signaling in platelets and triggers platelet aggregation. Platelet aggregation is required for a complete separation of the budding lymphatic vessels from the developing blood vessels (Chaipan et al., 2010).

Additionally, recent studies demonstrate following initial separation of blood and lymphatic vessels, the O glycan-dependent PDPN-CLEC-2 interaction maintains functional separation and prevents retrograde flow of blood into lymphatics by sustaining lymphovenous valve integrity (Hess et al., 2014). Therefore, PDPN-CLEC-2 interaction plays an essential role in lymphangiogenesis during development and maintenance of lymphatic vessel integrity after birth. PDPN is crucial for the development and maintenance of the lymphatic vascular system and lymphoid organ homeostasis. However, the exact mechanism of PDPN action is still unclear (Lowe et al., 2012).

Prox1 is expressed in the developing central nervous system (CNS), pancreas, eye lens, liver, heart and skeletal muscles. Mice with tamoxifen induced deficiency of Prox1 (inducible Prox1) during the embryonic, postnatal, or adult stages show reduced expression of lymphatic ECs markers (VEGFR-3, PDPN and LYVE-1) (Pan and Xia, 2015). These indicate that Prox1 regulates PDPN expression at the transcriptional level in the lymphatic vascular system. Thus, PDPN potentially serves as a major downstream target gene of Prox1 in the pathway essential for differentiation and maintenance of lymphatic identity. A more detailed understanding of transcriptional regulation of PDPN in various cell types and their respective downstream signaling pathways is needed for elucidating the biological functions of PDPN (Pan and Xia, 2015).

The binding of PDPN to CD44 or ERMs results in increased cell migration and rearrangement of the actin cytoskeleton to generate actin-rich protrusions of the membrane. Interactions between PDPN and CD9 affect platelet aggregation (Figure 5). The engagement of PDPN by CLEC-2 causes increased motility in DCs and aggregation and activation of platelets. PDPN binds with high affinity to the chemokine CCL21 and while the consequences of this effect have not been examined, it may play a role in facilitating

leukocyte migration. PDPN binding to galectin-8 may modulate adhesion of lymphatic ECs (Astarita et al., 2012).

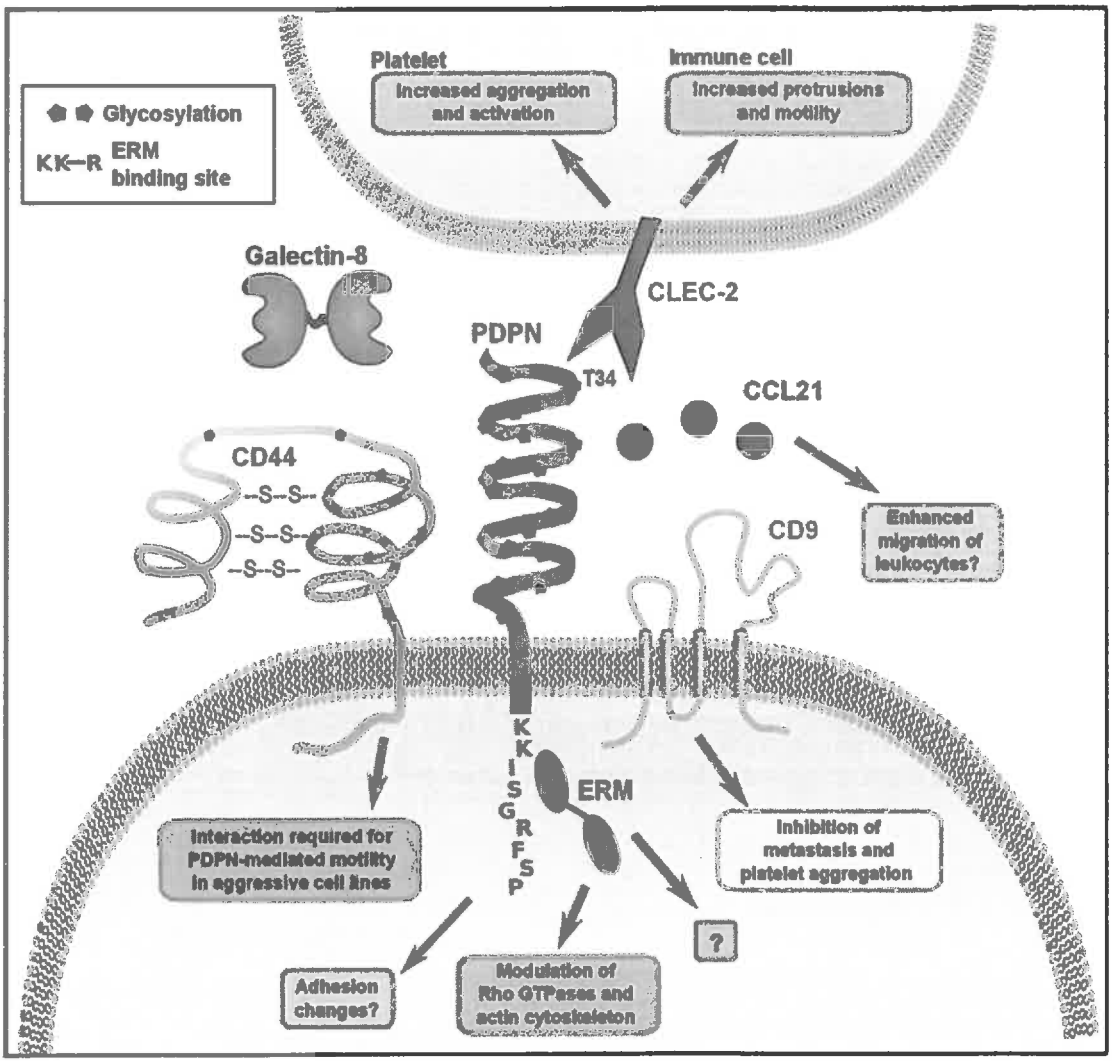


Figure 5. The interaction of podoplanin with a variety of intracellular and transmembrane proteins to mediate effects on cell migration and adhesion. The binding of PDPN to CD44 or ERMs results in increased cell migration and rearrangement of the actin cytoskeleton to generate actin-rich protrusions of the membrane. The three amino acids coloured in pink (K, K, R) are the basic residues requires for ERM protein binding (Astarita et al., 2012).

1.10 Rationale for the study

Pre-eclampsia is a common complication of pregnancy with high morbidity and mortality for both mother and fetus. It is known as well that PE mothers have long term cardiovascular complications. Prevalence rates vary worldwide. It affects mainly young primigravidae. In

South Africa, primigravidae account for more than one third of the total deliveries, however, recent data are limited. Therefore, the rationale of the first study was to establish the prevalence of hypertensive disorders in pregnancy in primigravid Black South African women.

The prevalence of HIV infection is >39% in pregnant women in KwaZulu-Natal, South Africa and in the Sub-Saharan region where it is considered the epicenter of the global HIV pandemic. The co-morbidity of HIV infection and PE remains a serious concern for public health and socio-familial and traditional groups. The second rationale of this study was to determine the maternal and perinatal outcomes in HIV negative and positive pregnant women who had scheduled caesarean section with early and late onset pre-eclampsia.

Although the pathogenesis of PE is not fully understood, it is agreed that the placenta is the origin of the syndrome. The lymphatic system plays a crucial role in tissue fluid homeostasis, immune function, fat metabolism, transport of proteins and macromolecules, and affords protection against viral entry. Off note, lymphatic vessels would play a role in maternal-fetal immunity by boosting survey of the maternal-fetal interface to combat viral infections. It is plausible to assume that the inflammation caused by HIV infection, may be a trigger to enhance lymphangiogenesis in the placenta. The third and fourth rationale of this study was to morphometrically analyse lymphangiogenesis in HIV associated pre-eclampsia using LYVE-1 and PDPN as respective markers in the placenta of HIV-associated normotensive and pre-eclamptic women.

1.11 Hypothesis

The lymphatic system within the placenta will remain unchanged irrespective of HIV status and type of pregnancy (normotensive and pre-eclamptic).

1.12 Aims and Objectives

1. To describe the incidence and obstetric and perinatal outcomes in primigravid Black South Africans with hypertensive disorders in pregnancy.
2. To analyze and compare the maternal and perinatal outcomes of HIV negative and positive pregnant women who are scheduled caesarian deliveries for early and late onset pre-eclampsia.
3. By performing immunocytochemistry utilizing specific markers of lymphatic vessels (LYVE-1; PDPN) and by interfacing this technology with sophisticated

morphometric image analysis, this study examines lymphangiogenesis in the placenta of HIV positive and HIV negative normotensive pregnant women

- To examine whether lymphatic vessels occur in the placenta of normotensive pregnancy
- To compare lymphangiogenesis (LYVE-1; PDPN) in the placenta of HIV positive and HIV negative pregnant women.
- To compare lymphangiogenesis (LYVE-1; PDPN) in the placenta of normotensive pregnant and pre-eclamptic women.
- To compare lymphangiogenesis (LYVE-1; PDPN) in the placenta across all study groups.

CHAPTER 2

Taylor & Francis
Taylor & Francis Group

Hypertension in Pregnancy

ISSN: 1064-1955 (Print) 1525-6065 (Online) Journal homepage: http://www.tandfonline.com/loi/ihip20

Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis

J. Moodley, O. A. Onyangunga & N. R. Maharaj

To cite this article: J. Moodley, O. A. Onyangunga & N. R. Maharaj (2016): Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis, Hypertension in Pregnancy, DOI: 10.1080/10641955.2016.1193190

To link to this article: http://dx.doi.org/10.1080/10641955.2016.1193190

Published online: 08 Jul 2016.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=ihip20

Download by: [La Trobe University] Date: 09 July 2016, At: 17:17

HYPERTENSION IN PREGNANCY
http://dx.doi.org/10.1080/10641955.2016.1193190

Taylor & Francis
Taylor & Francis Group

Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis

J. Moodley, O. A. Onyangunga, and N. R. Maharaj

Department of Obstetrics and Gynaecology and Women's Health and HIV Research Group, University of KwaZulu-Natal, Durban, South Africa

ABSTRACT

Objective: To describe the incidence and obstetric and perinatal outcomes in primigravid Black South Africans with hypertensive disorders of pregnancy (HDP). *Method*: All primigravidae who booked for antenatal care were followed up until hospital discharge. Relevant clinical and demographic data were collected in structured data forms. *Results*: A total of 5860 primigravidae delivered during the study period. Of these, 731 had an HDP, giving an incidence of 12.5%. The diagnosis of gestational hypertension was made in 6.7% of all primigravidae but was the commonest HDP subcategory (n = 394/731; 53.9%). On the other hand, mild to moderate preeclampsia or non-severe preeclampsia, severe preeclampsia, and eclampsia occurred in 222, 84, and 31 of the 5860 primigravidae, respectively. Therefore, preeclampsia–eclampsia syndrome occurred in 337/5860 or 5.75% of the study population. Severe preeclampsia occurred in 1.43% (84/5860) of the primigravidae. The rates of caesarean deliveries in women with preeclampsia were approximately 50%. There were no perinatal deaths in the gestational hypertension group, but the overall perinatal mortality rate in all preeclamptics was 5.9%, in comparison to 2.2% in all primigravidae. *Conclusion*: Hypertensive disorders of pregnancy developed in 12.5% (n = 731/5860) of primigravidae seen over a one-year period. Gestational hypertension comprised the commonest subcategory of the HDP and there were no perinatal deaths in this group.

ARTICLE HISTORY
Received 26 January 2016
Revised 17 May 2016
Accepted 18 May 2016

KEYWORDS
Hypertensive disorders of pregnancy; obstetric outcomes; primigravidae

Downloaded by [La Trobe University] at 17:17 09 July 2016

Introduction

Hypertensive disorders of pregnancy (HDP) (gestational hypertension, mild to moderate preeclampsia, severe eclampsia, eclampsia, HELLP syndrome, and chronic hypertension) are important because they are a leading cause of maternal, fetal, and neonatal morbidity and mortality worldwide [1,2]. Recent reports suggest that preeclampsia is a two-stage disorder [3]. The first stage is one of defective spiral artery remodeling, leading to placental cellular hypoxia, resulting in an imbalance between antiangiogenic and proangiogenic factors. The increase in soluble antiangiogenic factors such as soluble film like tyrosine kinase leads to widespread endothelial dysfunction and the clinical signs of hypertension, proteinuria, and intrauterine fetal growth restriction [3]. The exact etiology is unknown and therefore treatment of this condition is still empirical [1,2]. Identifying

CONTACT J. Moodley jmog@ukzn.ac.za Women's Health and HIV Research Group, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella 4013, South Africa.

© 2016 Taylor & Francis

patients at risk of HDP and instituting emergency care if necessary and timely delivery remain the mainstay of clinical management [1,2].

The frequency of preeclampsia varies globally and occurs between 3% and 8% of all pregnancies [1–3]. In South Africa, a community-based study found a 12% incidence of HDP while a tertiary facility-based study reported a prevalence of 18% [4,5].

First pregnancies have been identified as a risk factor for preeclampsia [6]. There is however a paucity of data on the incidence of preeclampsia in Black African primigravidae in our setting. According to the Saving Mothers Report 2010–2013, primigravidae contributed a significant proportion of maternal deaths due to HDP in South Africa [7]. The aim of our study was to perform a descriptive analysis of all primigravidae admitted with a diagnosis of HDP at a regional hospital facility over a one-year period to establish the prevalence and maternal and neonatal outcomes.

Downloaded by [La Trobe University] at 17:17 09 July 2016

Methods

Regulatory permissions, viz. ethical and health facility, were obtained and all participants provided written informed consent. The study site was a regional hospital in the south of Durban, which caters for a largely Black African population of 1.9 million and had 31 primary healthcare clinics under its supervision. These clinics provided antenatal care and some performed normal vaginal deliveries. All primigravidae with HDP who delivered in the study period (April 2012 to March 2013) were identified at the time of admission and followed till discharge from hospital. Relevant demographic and clinical data was recorded in a purpose-designed form. Preeclampsia was defined as new-onset hypertension (BP ≥ 140/90 mmHg) and proteinuria (≥1+ on urine dipstick) after the 20th week of pregnancy. Gestational hypertension was defined as new-onset hypertension with a negative dipstick for proteinuria or absence of proteinuria in a 24-h urine specimen [1]. Both blood pressure and dipstick proteinuria values were checked 2 h later to confirm the diagnosis of an HDP. Mild to moderate preeclampsia or non-severe preeclampsia was defined as women with a blood pressure value of 140–159/90–109 mmHg and + to ++ of proteinuria on dipstick testing [1]. Severe preeclampsia was defined as a blood pressure of ≥160/110 mmHg and ≥ +++ proteinuria. Patients who had symptoms of persistent headache and increased patella reflexes were considered to have imminent eclampsia. Eclampsia was defined as seizures associated with hypertension. Data on all patients were obtained from the institution's computerized database, but in addition all primigravidae with HDP were followed daily by a research midwife till hospital discharge.

The clinical management of HDP at the study site followed that described in the National Maternity Guidelines of South Africa [8]. Blood pressure measurements were taken in the sitting position to avoid compression of the inferior vena cava by the gravid uterus. The Mindray iMEC12 automated device was used to measure blood pressure levels.

Descriptive statistics were utilized and the results are presented as frequencies, means, range, and percentages. Student's *t*-test was used for continuous variables and chi-square test for categorical variables. A p-value <0.05 was considered statistically significant.

Results

There were 12,973 deliveries during the study period. A total of 5860 (45.20%) were primigravidae and 7,113 (54.8%) were multiparous. The incidence was slightly higher ($\chi^2 = 5.81$; $p < 0.05$) in primigravidae (12.5%) than in multiparous (11.1%). A total of 1520 were diagnosed with HDP, giving an overall incidence of 11.7%. The incidence of HDP in primigravidae was 12.5%; n = 731 (Figure 1). Mild to moderate preeclampsia (non-severe) [1], severe preeclampsia, and eclampsia occurred in 220, 84, and 31 primigravidae, respectively. Of the 5960 primigravidae, 337 (222+84+31) had the preeclampsia–eclampsia syndrome. The incidence of preeclampsia–eclampsia syndrome amongst primigravidae was 5.75% (337/5860). Severe preeclampsia occurred in 1.43% (84/5860) of the primigravidae.

Gestational hypertension occurred in 394 or 53.9% of all hypertensive primigravidae and together with mild to moderate preeclampsia accounted for 84.3% of all cases of HDP.

The number of women ≤24 years (n = 544) formed the majority of cases of HDP; there was a significant difference between women aged ≤24 years compared to those aged ≥24 years (544 vs. 187; $p < 0.001$). In particular, eclampsia occurred commonly in those ≤24 years. There was a significant difference among the hypertensive groups regarding maternal age and blood pressure ($p < 0.001$). The clinical characteristics of all primigravidae with HDP are shown in Table 1.

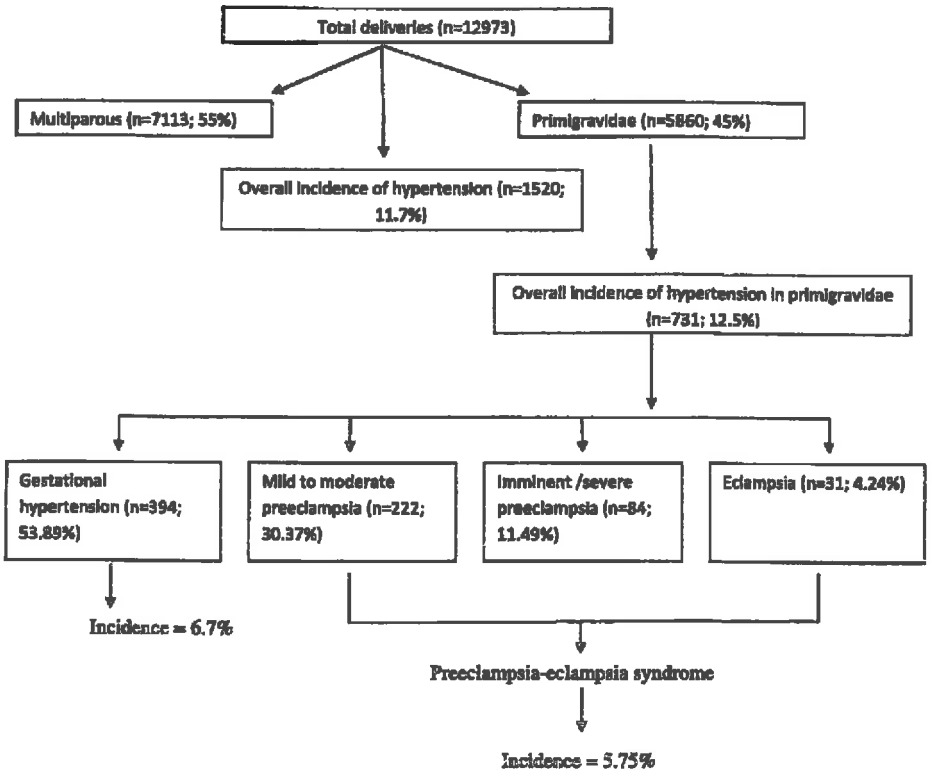


Figure 1. Flow diagram of the study population.

Downloaded by [La Trobe University] at 17:17 09 July 2016

Table 1. Clinical characteristics of all primigravidae with hypertensive disorders of pregnancy (n = 731).

Variable	Gestational hypertension (n = 394)	Preeclampsia mild to moderate (n = 222)	Severe preeclampsia and imminent eclampsia (n = 84)	Eclampsia (n = 31)	*p*-Value
Age* (mean ± SD)	21.5 ± 3.4	25.3 ± 5.7	20.3 ± 3.7	19.7 ± 3.3	0.01
Age groups					
≤24 years	265 (67.3)	170 (76.6)	78 (92.9)	31 (100)	0.001
≥25 years	129 (32.7)	52 (23.4)	6 (7.1)	0 (0)	0.01
Gestational age @ delivery (weeks, range)	37 (36–39)	36 (35–39)	35 (35–38)	35 (34–36)	0.3
Blood pressure					
Systolic	148 .7 ± 11.2	158.3 ± 15.0	156.5 ± 13.6	163.6 ± 14.7	.001
Diastolic	85.0 ±11.2	97.4 ± 15	101.1 ± 11.5	104.8 ± 14.5	0.001
Maternal weight	73.0 ± 14.7	75.2 ± 15.3	78 ± 16.6	74.8 ± 21.0	0.2

*in years.

The total number of caesarean deliveries in all primigravidae was 1645 (28%) while that in hypertensive primigravidae was 325 (45%) (Table 2).

There were 130 stillbirths (2.2%; n = 5860) in all primigravidae, while the number of stillbirths in those with HDP was 32 (Table 3); 21 of these were in women with severe preeclampsia and eclampsia. There were 11 early neonatal deaths. The overall perinatal mortality rate was 5.9% of all hypertensive primigravidae. The birthweights of babies born to the gestational hypertension group were slightly heavier than those in the other subcategories of the HDP.

There were two maternal deaths, viz. severe preeclampsia (n = 1) and eclampsia (n = 1). The rest of the complications are shown in Table 4. Most complications occurred in severe preeclampsia and eclampsia subcategories. There were no maternal complications associated with gestational hypertension.

The mean hospital stay was 6 days (range: 2–38), and 4 (0.6%) and 48 (6.6%) of the hypertensive primigravidae required admission to the intensive care unit (ICU) and high care, respectively.

Table 2. Caesarean deliveries in hypertensive primigravidae.

Hypertensive group	Total	Emergency	Elective	*p*-Value
Gestational hypertension	87 (22.08%)	70 (90.5%)	17 (19.5%)	0.04
Mild to moderate preeclampsia	141 (63.5%)	29 (20.6%)	112 (79.4%)	0.001
Severe preeclampsia/imminent eclampsia	66 (78.6%)	38 (57.6%)	28 (42.4%)	0.01
Eclampsia	31 (100%)	31 (100%)	0 (0%)	0.001
Total	325	115	210	

Table 3. Perinatal outcome in hypertensive groups.

Variable	Gestational hypertension	Preeclampsia mild to moderate	Severe preeclampsia/imminent eclampsia	Eclampsia
Alive	394*	211	78	23
Baby weight (g)	2902 ± 342**	2540 ± 234	2396 ± 216	2150 ± 198
Early neonatal deaths	0	2	4	5
Stillbirths	2	9	15	6
Admission to ICU	15	25	39	11

*Four twin pregnancies; ** $p < 0.01$.

Downloaded by [La Trobe University] at 17:17 09 July 2016

Table 4. Maternal complications in hypertensive primigravidae.

Complications	Gestational hypertension	Mild to moderate preeclampsia	Severe preeclampsia/ Imminent eclampsia	Eclampsia
Maternal death (n = 2; 0.3%)	0	0	1	1
Postpartum hemorrhage (n = 8; 1.1%)	0	2	5	1
Stroke (n = 1; 0.1%)	0	0	1	0
Abruption placentae (n = 8; 1.1%)	0	1	4	3
HELLP (n = 1; 0.1%)	0	0	1	0

HELLP, hemolysis, elevated liver enzymes, and low platelets.

The overall HIV infection rate among all primigravidae was 29% (1580/5860). Of the 731 hypertensive primigravidae, 19.3% (141 of 731) were HIV positive versus 590 (90.7%) who were HIV negative. There was significant difference between the groups ($p < 0.05$).

Downloaded by [La Trobe University] at 17:17 09 July 2016

Discussion

The rates of HDP are high in South Africa. In our study the overall incidence of HDP was 11.7% (n = 1520: Figure 1) and that in primigravidae was 12.5% (n = 731). These figures are at the upper range of figures for HDP quoted in the world literature [1–3]. Figures for the incidence of gestational hypertension, preeclampsia–eclampsia syndrome are difficult to obtain in low- and middle-income countries. Our findings are in keeping with a previous study of the overall incidence of hypertension, taking into account a regional hospital and its clinics in the Durban area by Panday et al. [4]. Therefore we can reliably state that the frequency of HDP in the Durban area is approximately 12%. The incidence of preeclampsia–eclampsia syndrome was 5.8% while severe preeclampsia occurred in 1.43%. These figures are important for researchers working in the field of HDP of pregnancy in South Africa and for all health professionals providing maternity care. Furthermore they indicate contemporary estimates from a regional hospital that conducts approximately 12,000 deliveries annually.

An earlier study (1999) conducted in a tertiary teaching hospital in the Eastern Cape found that 760 (4.6%) of all the deliveries had hypertensive disorders of pregnancy, of which 502 (66%) had proteinuric hypertension [9]. These differences in the rates of HDP in the same country are probably due to the differing referral patterns as demonstrated by finding of a high number of proteinuric hypertensives in the Eastern Cape.

Our study found that gestational hypertension was the commonest hypertensive subcategory and that this group also had good obstetric outcomes. This may indicate that once the diagnosis of gestational hypertension is made, clinical protocols are followed and timely delivery is being carried out. Two other studies on pregnancy outcomes in primigravidae in our geographical area have shown similar favorable outcomes and greater birthweights than normotensive and proteinuric hypertensive counterparts [10,11]. It is plausible that the slightly higher blood pressures might cause an increased placental blood flow to the placenta, resulting in greater birthweights. Furthermore, none of the primigravidae in the gestational hypertension group had severe hypertension because it is known that severe gestational hypertension developing before 35 weeks gestational age is associated with high perinatal and maternal morbidity rates [12]. The maternity care guidelines which are being followed at the study site recommends delivery at the 38th week of gestation in women with

gestational hypertension [8]. It is surprising that the prevalence of HELLP is low in the population study. The standard practice in the hospital for all patients with hypertension in pregnancy is to do a complete blood count including platelet count, urea, uric acid, 24 h urine estimation for protein and creatinine clearance, and liver function tests (including lactate dehydrogenase). Furthermore, all patients with platelet counts $<50 \times 10^9$/L have a peripheral blood smear, a coagulation screen, and a thromboelastogram. It therefore is unlikely that differences of prevalence of HELLP in other countries [12] are due to lack of testing for the requisite biochemical studies, but will require further investigation. Similar to the low prevalence of HELLP, we had no cases of postpartum preeclampsia. This may be due to the fact that at the study site, normotensive patients are discharged from the hospital after 24 h of observation. If they develop hypertension or seizures following delivery, they are usually admitted to an internal medicine ward. This may be a limitation of our study in that the patients were not followed up post delivery. However, in a recent study of over 50 consecutive cases of eclampsia conducted in a rural area of South Africa, not a single case of atypical eclampsia was found [13]. This also needs further investigation.

The prevalence of HIV in the South African population is high. At the time of study it was approximately 39% [14]. We did not investigate the role of HIV on the frequency of preeclampsia because the great majority of patients had HIV testing for the first time on the initial antenatal visit. They were therefore only started on HAART therapy at this time. Also, it was difficult to obtain information on the duration of HAART therapy. It was not our intention to study the role of HIV on preeclampsia. However, our group has done a case control study on the prevalence of preeclampsia in HIV-affected pregnancies and found that the rate of HIV/AIDS was significantly lower in women with preeclampsia than in normotensive healthy pregnant women [15].

Another limitation of our study is that we did not evaluate clinical decision making and avoidable factors. This might have been useful given the overall large number of stillbirths in our study. Furthermore, preeclampsia is known to be a placental disorder and it is possible that in certain cases the fetus is predominantly affected and that fetal growth restriction is not detected clinically. The high rates of stillbirths need in-depth investigation.

The high incidence of the preeclampsia–eclampsia syndrome in primigravidae in our environment is of obvious concern and supports the National Committee on Confidential Enquiries into Maternal Deaths in South Africa of primigravidae aged ≤24 years, being at risk of pregnancy complications [7]. In our study, 74.4% of the hypertensive primigravidae were 24 years and less and 25.6% were ≥25 years (Table 1). Significantly, all the eclamptics were aged ≤24 years.

We did not analyze the data on the use of contraception because of missing information in a number of the records. However, many young women do not use contraceptives and those that do, do not use them consistently [7].

There were two maternal deaths; one occurred in an unbooked patient with eclampsia admitted with a Glasgow Coma Scale of 3. The other occurred in a severe hypertensive who had a vaginal delivery following induction of labor. She was prematurely discharged within a period of 24 h of delivery, to a community clinic, with what seems like labile hypertension. Blood pressure levels are very labile in severe preeclampsia and warrant high care management by multidisciplinary specialist teams. It goes without saying that

Downloaded by [La Trobe University] at 17:17 09 July 2016

high blood pressure levels must be stabilized for at least 48–72 h in severe HDP despite shortages in staffing levels and bed space [5,6].

Conclusion

The incidence of HDP among Black South African primigravidae was 12.5%. This high figure contributes a significant workload in the maternity unit of the regional/level 2 hospital in which this study was performed. Patients ≤19 years of age in particular must be regarded at being at high risk of severe hypertension and guidelines on antenatal care of this age group needs reconsideration.

Since most primigravidae are young as shown in our study, more attention needs to be given to the provision of contraceptive services to this age group in the local population.

References

1. Magee LA, Abalos E, von Dadelszen P, Sibai B, Easterling T Walkinshaw S. How to manage hypertension in pregnancy effectively. Br J Clin Pharmacol 2011;72:3:394–401
2. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJ, Hofmeyr GJ. Pre-eclampsia 2015. Lancet http://dx.doi.org/10.1016/S0140-6736(15)00070-7 accessed on 22 December 2015.
3. Govender N, Naicker T, Moodley J. Endoglin in HIV-associated preeclamptic placentae. Hypertens Pregnancy 2015;34(3):342–54. doi: 10.3109/10641955.2015.1046603.
4. Panday M, Mantel G D, Moodley J. Audit of severe acute morbidity in hypertensive pregnancies in a developing country. J Obstet Gynecol 2004;24(4):387–91.
5. Moodley J, Kalane G. A review of the management of eclampsia: Practical issues. J Hypertens Pregnancy 2006;25:47–62.
6. Committee Opinion. First trimester risk assessment for early onset preeclampsia. Obstets Gynecol 2015;126;3:e25–e27
7. National Committee on Confidential Enquiries into Maternal Deaths. Saving Mothers: Fourth Report on Confidential Enquiries into Maternal deaths in South Africa 2005–2007. Pretoria: Department of Health. 2010.
8. Guidelines for maternity care in South Africa. National Department of Health, Pretoria, 2007.
9. Buga GA, Lumu SB. Hypertensive disorders of pregnancy at Umtata General Hospital: Perinatal and maternal outcomes. East Afr Med J 1999;76(4):217–222
10. Rankhethoa NM, Moodley J, Gouws E. Perinatal outcomes of babies born to Black South African women with hypertension. East Afr Med J 1998;75(7):388–91
11. Moodley J, Mphatsoe M, Gouws E. Pregnancy outcome in primigravidae with late onset hypertensive disease. East Afr Med J 1999;76(9):490–4
12. Sibai BM Diagnosis and management of gestational hypertension and preeclampsia. Obstet Gynecol 2003;102(1):181–92
13. Makhanya V, Moodley J, Govender L. Eclampsia, still a major problem in a regional health facility in rural KwaZulu-Natal, Durban, South Africa. S Afr J Obstet Gynaecol (accepted for publication).
14. The 2012 National Antenatal Sentinel HIV and herpes simplex type 2 prevalence survey in South Africa. Pretoria: National Department of Health,2014.
15. Kalumba VMS, Moodley J, Naidoo TD. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. Cardiovas J Afr 2013;24(2):24–27. doi: 10.5830/CVJA-2012-078.

Downloaded by [La Trobe University] at 17:17 09 July 2016

CHAPTER 3

Covering Letter

The Editor

Nigerian Journal of Clinical Practice

29th August 2016

Dear Sir

Sub: Submission of Manuscript for publication

We intend to publish an article entitled "Maternal and Perinatal outcomes in early and late onset preeclampsia in a HIV infected South African Population" in your esteemed journal as an original article to the Editor.

On behalf of all the contributors I will act as a guarantor and will correspond with the journal from this point onward.

Prior publication: None

Support: Medical Education Partnership Initiative and College of Health Sciences of University of KwaZulu-Natal, South Africa

Conflicts of interest: None

Permissions: None

We hereby transfer, assign, or otherwise convey all copyright ownership, including any and all rights incidental thereto, exclusively to the journal, in the event that such work is published by the journal.

We would like to suggest following referees for the article.

1.

Thanking you.

Yours sincerely,

Signature

Corresponding contributor: Dr Onankoy Atshakala Onyangunga

Optics and Imaging Centre, College of Health Sciences
University of KwaZulu-Natal, South Africa
E-mail onyangunga51@gmail.com ; jmog@ukzn.ac.za

Encl : Three copies of the manuscript
Three copies of the photograph
Floppy
Contributor's form signed by all the contributors
Checklist

Manuscript type: Original article

Manuscript title: Maternal and perinatal outcomes after caesarean delivery in HIV infected early and late onset pre-eclampsia in South African population

[1*]Onyangunga OA, [1]Naicker T, [2]Moodley J

[1]Optics and Imaging Centre, Doris Duke Medical Research Institute and [2]Women's Health and HIV Research Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

***Corresponding author:** Dr Onankoy Atshakala Onyangunga; [1]Optics and Imaging Centre, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South Africa, **4001.**

Emails: Onyangunga (onyangunga51@gmail.com); Naicker (naickera@ukzn.ac.za); Moodley (jmog@ukzn.ac.za).

Running title: maternal and perinatal outcomes in early and late onset preeclampsia in HIV infected women

Acknowledgement

The authors appreciate The College of Health Sciences, The Medical Education Partnership Initiative (MEPI), University of KwaZulu Natal and The National Research Foundation (South Africa) for their financial supports. We thank Dr K Maduray and Dr F Odun-Ayo for their contributions.

Maternal and perinatal outcomes after caesarean delivery in HIV infected early and late onset pre-eclampsia in South African Population

[1]Onyangunga OA, [1]Naicker T, [2]Moodley J

[1]Optics and Imaging Centre, School of Laboratory Medicine and Medical Sciences and [2]Women's Health and HIV Research Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

Abstract

Objectives: We analysed the maternal and perinatal outcomes of HIV negative and positive pregnant women who had scheduled caesarean deliveries for early and late onset pre-eclampsia. The study looked at maternal and fetal outcomes in the two clinical entities. **Methods:** This prospective study was conducted at a regional hospital in Durban, South Africa within a period of 14 months from September 2012 to November 2013. A total of 14304 deliveries were registered. Out of the 1759 preeclamptic, 351(19.9%) were EOPE and 1408(80.1%) were LOPE. Patients with the former and latter scheduled for caesarean delivery were selected based on exclusion criteria and written informed consent. Patients with preeclampsia (n=120) were divided into 2 groups according to gestational age namely EOPE (n=60) and LOPE (n=60). Each preeclamptic category was then further stratified into HIV positive (n=30) and HIV negative (n=30). Maternal demographics, clinical details for pre-eclampsia, blood laboratory tests, maternal and perinatal outcomes were recorded.

Results: Women with early onset preeclampsia were older compared to those with late onset preeclampsia (p=0.0001). Also the HIV positive women were older compared to the HIV negative in both early- and late-onset preeclampsia (p=0.03). However multiparous and primiparous were more representative in early and late onset preeclampsia respectively (p= 0.00 and p=0.00). The severity of hypertension and the HIV status did not differentiate the 2 groups. Overall, maternal complications (eclampsia, persistent postpartum hypertension, HELLP syndrome, maternal death) and poor fetal outcomes occurred predominately in early onset preeclampsia. **Conclusion:** This study confirms the heterogeneity of preeclampsia and shows that the timing of onset of this pregnancy disorder is important to disease severity. Further HIV status seems to influence maternal and neonatal outcomes.

Key words: preeclampsia, early and late onset preeclampsia, HELLP syndrome, HIV-1 infection

Running title: maternal and perinatal outcomes in early and late onset preeclampsia in HIV infected women

Introduction

Hypertension disorders of pregnancy (HDP) are leading cause of pregnancy-related morbidity and mortality in South Africa. [1] Recently, the International Society for the Study of Hypertension in Pregnancy (ISSHP) classified HDP into chronic hypertension, gestational hypertension and preeclampsia (PE) - *de novo* or superimposed on chronic hypertension and white coat hypertension. Preeclampsia was also classified into mild and moderate sub-categories (non–severe hypertension), severe preeclampsia and the HELLP syndrome. The ISSHP further suggested that for clinical purposes, preeclampsia can be divided into early- and late-onset preeclampsia. Early-onset preeclampsia (EOPE) manifests before 33 weeks + 6 days gestation while late-onset preeclampsia (LOPE) presents at or after 34 weeks of gestation. [2, 3] Early- and late-onset preeclampsia shares some pathogenic roots, but risk factors vary and often lead to different outcomes. Therefore, the two preeclampsia categories should be treated as distinct entities from an etiologic, clinical and prognostic standpoint. [4-8]

HIV infection is a major public health concern worldwide [9, 10] and has a significant cause of maternal death in South Africa. [1] Preeclampsia and HIV infection are immune related thus might have significant interaction with each other. Literature has reported conflicting results on the state that HIV influences the incidence of PE [11-13] hence, this study aims to analyse the maternal and perinatal outcomes of HIV negative and positive pregnant women who had scheduled caesarean deliveries for early and late onset preeclampsia in South African women

Material and methods

This prospective study was conducted at a regional hospital in Durban, South Africa within a period of 14 months from September 2012 to November 2013. A total of 14304 deliveries were registered. Out of the 1759 preeclamptic, 351(19.9%) were EOPE and 1408(80.1%) were LOPE. Patients with the former and latter scheduled for caesarean delivery (CD) were selected based on exclusion criteria and written informed consent. Planned and emergency caesarean deliveries [14] before the onset of labor were also enrolled for the study. Patients with preeclampsia (n=120; BP ≥ 140/90 mmHg and proteinuria ≥ 1+ on dipstick or 24 hours proteinuria of 300mg) were divided into 2 groups according to gestational age namely EOPE (n=60; <33 weeks + 6 days) and LOPE (n=60; ≥34 weeks at the time of diagnosis). Each preeclamptic category was then further stratified into HIV positive (n=30) and HIV negative (n=30) as shown in Fig 1.

Severe hypertension was defined as systolic blood pressure ≥ 160 mmHg and or diastolic blood pressure ≥ 110 mmHg; mild to moderate preeclampsia was defined when systolic blood pressure < 160 mmHg and diastolic blood pressure < 110 mmHg. Early persistent postpartum hypertension was defined as blood pressure ≥ 140/90 mmHg 48 hours post-delivery. In the antepartum period, blood pressures were initially monitored half hourly until the high blood pressure was "stabilized" followed by four hourly intervals measurement. After CD, the blood pressures were checked at an hour interval for 24 hours. Records were taken at every 4 hours up to 48 hours after delivery. Women's weight at the initiation of antenatal care and at the time of delivery was recorded. The difference between the 2 measurements was considered as the weight gain during pregnancy. We used the ratio between the weight gain and gestational age at delivery to evaluate the impact of weight gain on early and late PE.

All HIV infected women received HAART (Highly Active Antiretroviral Therapy). Women with diabetes, previous history of hypertension, auto immune disease, history of seizures, chorioamnionitis, intrauterine death and multiple pregnancies were excluded. Demographic, clinical and obstetrical data of all patients were recorded in a structured format. Institutional ethical clearance was obtained from the Biomedical Research and Ethics Committee, University of KwaZulu-Natal (BE: 040/12).

Statistical analysis

Data were analysed using SPSS version 23 (IBM, USA). Results are expressed as mean ± standard deviation (SD). Parametric data was analysed using the Independent t-test, Pearson Chi-Square test, whilst non-parametric data was analysed using the Mann-Whitney U test. A p value of $p < 0.05$ was considered as statistically significant.

Results

Maternal demographic characteristics in early and late onset preeclampsia

The demographic features of the EOPE and LOPE are presented in Table 1. Irrespective of the HIV status, the mean maternal age was higher in the EOPE (27.9 ± 7.0 years) vs LOPE (25.1 ± 6.03 years) groups. The parity in EOPE and LOPE (2.1 ± 1.2 and 1.8 ± 0.9) respectively were similar (p=0.1). However, in LOPE and EOPE groups, primiparous and multiparous were more frequent respectively (p=0.001). The age mean in the HIV positive group was higher compared to the HIV negative group (p=0.0001). The parity in the two LOPE categories were statistically different (2.2 ± 0.5 and 1.6 ± 1.0) (p=0.03).

Clinical and obstetrical parameters studied in early and late preeclampsia

The PE was stratified into mild/moderate and severe (Fig 1). The systolic and diastolic blood pressure were not different in both EOPE and LOPE categories (p=0.9) (Table 2). Among the 53 cases of severe preeclampsia, 32(60.37%) were from the EOPE group. Out of the 32 women, 20(62.2%) were HIV negative and 12(37.8%) were HIV positive (p=0.0001). Furthermore severe forms of PE were predominately found in LOPE negative 17(80.9%) cases compared to LOPE positive 4(19.1%) cases (p=0.000). The mean proteinuria by dipstick was (++) in EOPE and (+) in LOPE. The difference was significant (p=0.02). The gestational age at delivery differed in EOPE (33.1 ± 9 weeks) compared to LOPE groups (36.8 ± 1.7 weeks) (p=0.001). We observed a lower gestational age (32.9 ± 2.60 weeks) in EOPE negative compared to (33 ± 1.7 weeks) in EOPE positive (p=0.05). The LOPE subgroups did not show any difference (p= 0.4).

Irrespective of HIV status, maternal weight in EOPE (75.6 ± 18.3 kg) was higher than the LOPE group (72.1 ± 18.1g) (p=0.0001). The maternal weight of 8(13.3%) women was above the mean in EOPE group. The weight gain was 7.2 ± 5.5 kg and 7.6 ± 5.2 kg for EOPE and LOPE respectively. In the HIV positive, the weight gain was lower in both EOPE (6.4 ± 5.4 kg) and LOPE (6.3 ± 4.4 kg) compared to the HIV negative; EOPE (7.9 ± 5.6 kg and LOPE (8.6 ± 5.5 kg) (p=0.001). The weight gain/gestational age ratios in EOPE (3:20) was lower compared to LOPE (5:20) (p=0.05). The ratio was low particularly in the HIV positive subgroups.

Laboratory findings early and late onset pre-eclampsia

Hemoglobins level did not differ in both EOPE and LOPE but were overall <11 g/dl. The level of urea, creatinine, uric acid, alkaline phosphatase, alanine transaminase and aspartate transaminase were higher in EOPE compared to the LOPE group except for lactate

dehydrogenase. There was a lower CD4 count in the EOPE (367.71 ± 149.04) compared to the LOPE (462.44 ± 311.1) groups (p=0.032).

Indications for caesarean delivery in early and late preeclampsia

Without considering the HIV status of the women, caesarean delivery for severe preeclampsia as indication were more common in LOPE (n=21) than in EOPE (n=3) (p=0.001). Based on HIV status, the number of caesarean deliveries was equal between EOPE and LOPE and their subgroups (Table 3). All 22 cases of CD for severe and preterm PE occurred in EOPE group. Furthermore, elective CD for oligohydramnios (n=4, 80%) in EOPE positive (p=0.001) and for previous CD (n=13, 65%) in LOPE positive (p=0.02) were more frequent than the EOPE HIV negative group.

Maternal mortality and mortality in early and late onset preeclampsia

Only one case of post-partum eclampsia was recorded in LOPE group within 24 hours after delivery. Imminent eclampsia, HELLP syndrome and early post-partum persistent hypertension were the major adverse events that occurred predominantly in the EOPE group. These complications were associated with severe renal and liver impairment (p=0.001). The EOPE group had a longer in-hospital stay compared to the LOPE group (4.7 ± 3.5 versus 3.6 ± 1.1 days; p=0.05) respectively.

Perinatal outcomes in early and late preeclampsia

Table 5 shows the perinatal outcomes and main complications. A highly significant difference between the birth weight in EOPE (2148 ± 739g) and LOPE (2900 ± 536g) was observed in our study. The placental weight in the EOPE group was less than that of the LOPE group (p=0.05). Intra-uterine growth restriction (IUGR) or small for gestational age and fetal distress were more frequent in EOPE than the LOPE group. The Apgar score at first minute (7.2 ± 1.3 versus 7.9 ± 0.5) and fifth minute (8.5 ± 1.0 versus 8.9 ± 0.3) minute were

significantly lower in EOPE group (p=0.002 and p=0.001 respectively). These neonates stayed longer in hospital compared to those of the LOPE group (5.17 ± 2.47 versus 1.06 ± 0.86 days; p=0.0001)

DISCUSSION

In our study, the maternal age was significantly higher in the EOPE group compared to the LOPE group. These results were similar to previous studies. [15, 16] Advanced maternal age has been reported to be associated with EOPE. [16,17] Fang *et al.* [16] and Leung *et al.* [17] showed statistically significant differences in maternal age and incidence of PE development. Leung *et al.* [17] studied two populations (Australian and British) and showed an odds ratio (OR) of 2.06(95% CI) for the age group <25 years and OR of 1.12 (95% CI) for age group 36-40 years in Australia and a lower OR in the UK with but with the same higher trend. Other reports [18, 19] found no difference in maternal age between early and late onset pre-eclampsia and suggest that this may be due to the age of first maternity in the population studied. Indeed in high-income countries, PE (late onset) are today more frequent in older age group. An interesting finding in our study was the higher age in EOPE positive group compare to the negative subgroup. We can only hypothesize the possibility of gene interactions in different age groups, regulating the development of preeclampsia in the HIV infected mother. [21, 22] A literature review implicates a possible maladaptation to pregnancy changes in advanced aged women; loss of vascular compliance, decline vascular responsiveness to endothelium-dependent vasodilators, down-regulation of gene expression of angiogenesis as co-responsible factors for the early and late occurrence of preeclampsia. [23, 24] Maternal age also has effects on myometrium and vasculature gene expression which impair on placental implantation and development. [23] This finding however needs further investigation.

Primiparity has been cited as a higher risk factor for preeclampsia development. [15-20] The pathogenic mechanism of preeclampsia in primiparity is unclear. Primipaternity and long interval between coitus among the primparous women has been postulated as possible causal factors. [25] A number of studies [16-19] have found primiparity more frequent in late rather than early onset preeclampsia, a similar observation was noted in our study. In contrast Madzali *et al* [26] in a retrospective study reported 56.0% nulliparous in EOPE and 53.9% in LOPE group. Whilst Gulec *et al.* [27] found no differences in the frequency of primiparity in both EOPE and LOPE, suggesting further investigation to clarify these controversies.

Early onset preeclampsia is associated with preterm delivery. [14, 18, 20, 26, 27] In our study, the gestational age at delivery for EOPE was 33 ± 1 weeks versus 36.4 ± 1.7 weeks for LOPE. In a cohort study, Li *et al.* [20] report the gestational age of 32^{+1} weeks for EOPE and 37^{+2} weeks for LOPE respectively. Similarly, Aksornphusitaphong *et al.* [28] reported lower gestational age means, 30.5 + 3.3 weeks for EOPE and 36.8 + 2.2 weeks for LOPE at delivery, however, their results are confounded by the inclusion of diabetic patients into the cohort. Clinicians are cautious and have different views on the expectant or interventionist management of severe EOPE. Churchill *et al.* [29] has shown no statistical differences for maternal outcomes than the caesarean sections. The differences on timing for delivery in EOPE could be related to the severity of the disease, maternal or fetal affectation, protocols and institutional facilities.

Few studies [15, 25] which included vaginal and CD report a higher mean systolic and/or diastolic blood pressure in the EOPE compared to the LOPE group. Our study was limited to caesarean deliveries and no difference in blood pressure was observed. However, severe hypertension was more frequent in the EOPE compared to the LOPE group. Moreover we noted a predominance of severe hypertension in the HIV negative subgroups suggesting the "protective role" of HIV infection for the development of preeclampsia. [12, 13]

Pre-gestational weight, body mass weight and gestational weight gain have been used to evaluate the risk of preeclampsia. [17, 18, 30,] An excessive maternal weight or weight gain is associated with the development of preeclampsia. In our study, irrespective of HIV status, the maternal weight at booking was higher in the EOPE group compared to the LOPE group. We also report a lower gestational weight gain in HIV positive patients and these findings are supported by Floridia *et al.* [31]

The high prevalence of complications particularly the HELLP syndrome in EOPE expounds the severity and the heterogeneity of the 2 forms of preeclampsia. [24,32] Haram *et al.* [33] linked polymorphism of chromosomes 2q, 5q and 13q to preeclampsia development. Also polymorphisms of genes STOX, ERAP1, syncytin envelop gene, -670 Fas receptor as well as ACVR2A gene on chromosome 2q22, the toll like receptor -4(TLR-4) and factor V Leiden mutation have all been implicated in the development of preeclampsia and HELLP syndrome Haram *et al* [33].

The poor fetal outcome in EOPE is probably the result of severe hypoxia of the placental bed and placental microenvironment. [4, 34] Placental lesions emanating from under perfusion have also reported similar perinatal outcomes in LOPE. [4] In our study, we report a correlation between the severity of hypertension and adverse fetal outcome. Prematurity, neonatal deaths and cost effective issues are a concern in the management and outcome of women with EOPE's. In our study, 20(33.3%) of EOPE women in our study delivered after 36 weeks. It is hence important, that the management of each patient should be individualized in view of life threatening conditions.

A limitation of our study is the sample size; a larger cohort of preeclamptic women undergoing caesarean delivery is warranted. Nevertheless, it has the merit of giving the impact of preeclampsia and HIV infection co-morbidity on mothers who had caesarean

delivery. Indeed our study has confirmed findings such as those of Leung *et al* [17] that demographic information and clinical findings are important tools in the management of preeclampsia particularly in resource limited settings. As preeclampsia is no longer considered solely an obstetrical disease, a multidisciplinary follow-up in early and late postpartum period is recommended.

Conclusion

Our study confirms the heterogeneity of preeclampsia and shows that the timing of disease onset is an important indicator of disease severity and possibly of disease etiology. Furthermore, HIV status influences perinatal and maternal outcomes. Maternal age and parity are risk factors for both EOPE and LOPE development. Although HIV infection is related to poor outcome, it has a possible protective role in the occurrence of severe preeclampsia.

Acknowledgement

The authors appreciate The College of Health Sciences, The Medical Education Partnership Initiative (MEPI), University of KwaZulu Natal and The National Research Foundation (South Africa) for their financial supports. We thank Dr K Maduray and Dr F Odun-Ayo for their contributions.

Conflict of Interest

None.

References

1. Saving Mothers 2011-2013: 2015. Sixth Report on the Confidential Enquiries into Maternal Deaths in South Africa. 1-78. http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf
2. Tranquilli AL, Dekker G , Magee L, Roberts J, Sibai BM, Steyn W, Zeeman GG, Brown MA. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertension 2014; 4 (2): 97-104.
3. Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. 2013; 3(1):44-47.
4. Soto E, Romero, R, Kusanovic JP, Ogge G, Hussein Y, Yeo L, Hassan SS Jai Kim C, Chaiworapongsa T. Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal under-perfusion. J Matern Fetal Neonatal Med. 2012; 25(5): 498–507.
5. Raymond D, Peterson E. A Critical Review of Early-Onset and Late- Onset Preeclampsia.2011; 66(8) :497-506.
6. van der Merwe JL, Hall DR, Wright C, Schubert P, Grové D. Are early and late preeclampsia distinct subclasses of the disease--what does the placenta reveal? Hypertens Pregnancy. 2010;29(4):457-467.
7. Valensise H, Novelli GP, Vasapollo B. Pre-Eclampsia: One Name, Two Condition- The Case For Early And Late Disease Being Different. Fetal and Maternal Medicine Review 2013; 24: 32-37.

8. Redman CW. Pre-eclampsia: A complex and variable disease. Pregnancy Hypertension: An International Journal of Women' Cardiovascular Health. 2014, 4(3); 241-242.

9. Maternal Health, HIV and AIDS: Examining Research through a Programmatic Lens. Meeting, 2013- Maternal and Mortality and HIV: An Overview.

10. Say L, Chou D, Gemmil A, Tunçalp O, Moller AB, Daniels J *et al.* Global causes of maternal death: A WHO systematic analysis. Lancet Glob Health 2014; 2: e323-333.

11. Kalumba VM, Moodley J, Naidoo TD. 2013 Is the prevalence of PE affected by HIV/AIDS? A retrospective case- control study. Cardiovasc J Afr 2, 24-27

12. Hall D, Gebhart S, Theron G, Grové D. Pre-eclampsia and gestational hypertension are less common in HIV infected women. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 2014,4;91-96.

13. Landi B, Bezzeccheri V, Guerra B, Piemontese M, Cervi F, Cecchi L *et al.* HIV Infection in Pregnancy and the Risk of Gestational Hypertension and Preeclampsia. *World Journal of Cardiovascular Diseases* 2014, 4: 257-267.

14. NICE Guidelines [CG132], 2012.

15. Lisonkwa S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obst Gynecol, 2013;209: 544e1-544e12.

16. Fang R, Dawson A, Lohsoonthorn V, Williams MA Risk Factors of Early and Late Preeclampsia among Thai women. Asian Biomed (Res Rev News). 2009; 1(3) : 477-486.

17. Leung C, Saaid R, Pedersen L, Park F, Poon L, Hyett J. Demographic factors that can be used to predict early-onset pre-eclampsia. J Matern Fetal Neonatal Med. 2015 Mar; 28(5):535-539.

18. Stubert J, Ullmann S, Dieterich M, Diedrich D, Reimer T Clinical differences between early- and late-onset severe preeclampsia and analysis of predictors for perinatal outcome. J Perinat Med. 2014 Sep; 42(5):617-627.

19. Mitsui T., Masuyama H., Eto E., Nobumoto E., Hayata K, Hiramatsu Y. Different Fetal and Neonatal Growth between Early- and Late-Onset Preeclampsia. Open Journal of Obstetrics and Gynecology 2015; 5: 516-521.

20. Li XL, Guo PL, Xue Y, Gou WL, Tong M, Chen Q. An analysis of differences between early and late preeclampsia with severe hypertension. Pregnancy Hypertension: An International Journal of Women' Cardiovascular Health.2016; 6:47-52.nnn

21. Miranda J, Romero R, Korzeniewski SJ, Schawartz AG, Chaemsaithong P, Stampalija T *et al.* The anti-aging factor α-klotho during human pregnancy and its expression in pregnancies complicated by small-for- gestational neonate and /or preeclampsia. J Matern Fetal Neonatal Med,2014; 27(5): 449-457

22. Richardson K, Weinberg A. Dynamics of Regulatory T-cells during pregnancy: Effect of HIV infection and correlations with others immune parameters. PloS One 2011,6(11) e28172.doi:10.137/1jo urnal.pone.0028172

23. Yazdani M, Amirshahi E, Shakeri E, Amirshahi R, Malekmakan L. Prenatal and Maternal Outcomes in Advanced Maternal Age, a Comparative Study. Women' Health Bull.2015; 2(2): e 23092 e1-5.

24. Junus K, Centlow M, Wilkström A-K, Larsson I, Hansso SR, Olovson M. Gene expression profiling of placentae from women with early- and late-onset preeclampsia down-regulation of angiogenesis-related genes ACVRL1 and EGFL7 in early-onset disease Molecular Human Reproduction 2012; 18(3): 146-155.

25. Deen ME, Ruurda LGC, Wang J, Dekker GA. Risk factors for preeclampsia in multiparous women: Primiparity versus the birth interval hypothesis .J Matern Fetal Neonatal Med 2006, 19(2):79-84

26. Madzali R, Yuksel MA, Imamoglu M, Tuten A, Oncul M, Aydin B, Demirayak G. Comparison of clinical and perinatal outcomes in early- and late-onset preeclampsia. Arch Gynecol Obstet.2014; 290 (1):53-57

27. Gulec UK, Ozgunen FT, Buyukkurt S, Guzel AB, Urunsak IF, Demir SC, Evruke IC. Comparison of clinical and laboratory findings in early- and late-onset preeclampsia. J Matern Fetal Neonatal Med. 2013; 26(12):1228-33.

28. Aksornphusitaphong A, Phupong V Risk factors of early and late onset pre-eclampsia. J Obstet Gynaecol Res. 2013; Mar; 39 (3):627-631.

29. Churchill D, Duley L, Thornton JG, Jones L. Interventionist versus expectant care for severe preeclampsia before term. Pregnancy and Childbirth Group's Trial. Cochrane Library, 2013.

30. Macdonald-Wallis C, Tilling K, Frazer A,Nelson SM, Lawlor DA. Gestational weight gain as a risk factor for hypertensive disorders of pregnancy. Am J Obstet Gynecol, 2013; 209 (4): 327.e1-327.e17.

31. Floridia M, Ravizza M, Masuelli G, Dalzero S, Pinetti C, Cetin I *et al.* Body mass index and weight gain in HIV: a national study in Italy. Clin Infect Dis 2013 Apr,56(8): 1190-119332'

32. Zhu L, Lu R, Kong L, Cheng H, Lan F, Li X. Genome –Wide Mapping of 5mC and 5hmC. Identified Differentially Modified Genomic Regions in Late-Onset Severe Pre-eclampsia: A Pilot Study. PloS One 2015; 10(7): e 0134119 doi: 10.1371/journal.pone33.

33. Haram K, Mortensen JH, Nagy B. Genetic aspects of preeclampsia and the HELLP syndrome. Journal Pregnancy: 2014 (2014):dx.doi.org/10.1155/2014/910751

34. Andraweera PH, Dekker GA, Thompson SD, Dissanayake VH, Jayasekara RW, Roberts CT. Hypoxia-inducible factor-1α gene polymorphisms in early and late onset preeclampsia in Sinhalese women. Placenta. 2014 Jul;35(7):491-495.

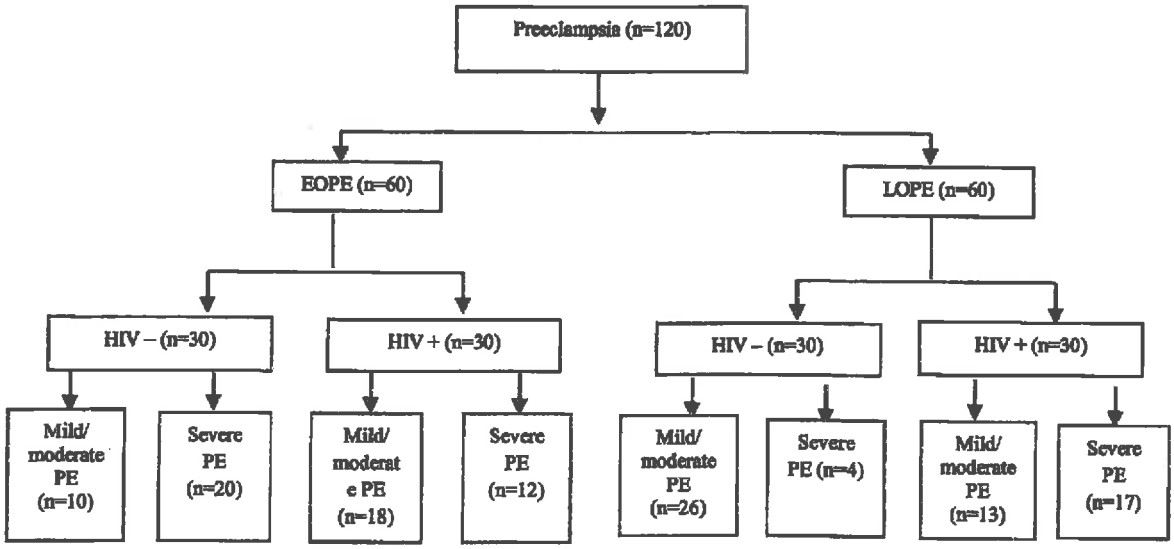


Fig 1: Flow diagram illustrating the subcategories of preeclampsia and their HIV status.

*HIV- =HIV negative; HIV+ = HIV infected; PE = preeclampsia

Table 1: Maternal age and parity in early and late onset preeclampsia

Variable	EOPE (n = 60)		LOPE (n = 60)		Significance
	HIV-	HIV+	HIV-	HIV+	p-value
Age (years)	25.2 ± 6.5	31.8 ± 5.9*	24.2 ± 6.4	26.7 ±4.6†	0.02
Age groups <20 20-34 ≥ 35	 4 22 4	 0 12 8	 9 24 3	 1 10 3	 0.04 - 0.04
Parity Primiparous Multiparous	2.0 ± 1.2 16 14	2.4 ± 1.1 8 22	1.6 ±1.0 17 13	2.2 ± 0.5 ‡ 18 12	0.1 0.001 0.001

*p=0. 0001; p value comparing age mean between EOPE- and EOPE+; †p=0.001; p value comparing age mean between LOPE- and LOPE+; ‡p=0.03; p value comparing parity mean between LOPE- and LOPE+

Table 2: Clinical Data of Early and Late Onset Preeclampsia

Variable	EOPE (n=60)	LOPE (n=60)	p value
Blood Pressure (mmHg) Systolic blood pressure Diastolic blood pressure	 152,3 ±17.5 97.5 ± 12.0	 152.7± 20 97.9 ± 13.0	 0.9 0.9
Severe blood pressure n (%)	32 (62.3%)	21(37.7%)	0.0001
HIV Status (%) HIV negative HIV positive	 20 (62.2%) * 12 (37.8%)	 17 (80.9%) † 4 (19.1%)	 0.3 0.001
Proteinuria	2+ (1+ - 3+)	1+(1+ - 3+)	0.02
Gestational age (weeks) At diagnosis At delivery	 27.7 ± 3.9 33.3 ± 3.9	 36.5 ± 3.3 36.8 ± 2.2	 0.0001 0.0001
Maternal weight (kg)	75.6 ± 18.3	72,1 ± 1.8	0.000
Maternal weight gain (kg) HIV negative HIV positive	7.6 ± 5.2 7.9 ± 5.6‡ 6.4 ± 5.4	7.2 ± 5.5 8.6 ± 5.5§ 6.3 ± 4.4	0.9 0.001 0.1

*p=0.001; Comparing severe preeclampsia in EOPE- and in EOPE+; †p=0.0001; Comparing severe preeclampsia in LOPE- and in LOPE+; ‡p=0.05; Comparing gestational at delivery in EOPE- and in EOPE+; §p=0.01; Comparing weight gain in EOPE and in LOPE categories

Table 3: Indications for caesarean delivery in early and late onset preeclampsia (HIV positive and negative)

Indications	EOPE (n=60)		LOPE (n=60)		p value
	HIV – (n=30)	HIV + (n=30)	HIV – (n=30)	HIV + (n=30)	
Previous CS	9 (60)	6 (40)	7 (35)	13 (65)	0.3
Severe preeclampsia	0 (0)	3 (100)	12 (57.1)	9 (42.9)	0.001
Oligohydramnios	1 (20)	4 (80)	1(50)	1 (50)	0.2
Severe PE + preterm	13 (59)	9 (41)	-	-	-
CTG abnormalities	6 (46.2)	7 (53.8)	7 (58.3)	5 (41.7)	0.8
Imminent eclampsia	1 (50)	1 (50)	1 (50)	1 (50)	
Big baby*	-	-	2 (66.7)	1 (33.3)	-
Total (n=120)	60 (50%)		60 (50%)		-

CTG= cardiotocograph *Big baby: ultrasound weight > 4000grams

Table 4: Maternal complications in early and late preeclampsia (HIV positive and negative)

Complication	Total	EOPE (n=60)	LOPE (n=60)	p value
Postpartum eclampsia	1	0 (0)	1 (100)	0.000
Imminent eclampsia	11	7 (63.6%)	4 (36.4)	0.02
HELLP syndrome	4	4 (100)	0 (0)	-
Imminent eclampsia/ HELLP	1	1 (100)	0 (0)	-
Persistent postpartum hypertension	32	20 (62.5%)	12 (37.5%)	0.02
Oligohydramnios	11	8(72.7%)	3(27.3%)	0.04
Postpartum hemorrhage	0	0 (0)	0 (0)	-
Maternal death		1 (100)	0 (0)	0.000
Hospital stay(days)	1	4.7 ± 3.5	3.6 ± 1.1	0.05

Table 5: Relevant Perinatal Data in Early and Late Preeclampsia (HIV positive and negative mothers)

Variable	EOPE (n=60)	LOPE (n=60)	p value
Sex			
Male	26 (43.3%)	35 (58.3%) 0.1	0.1
Female	34 (56.7%)	25 (41.7%) 0.1	0.1
Birth weight (g)	2148 ± 739	2900 ± 536	0.015
HIV negative	2187 ± 799	3078 ± 457	0.001
HIV positive	2083 ± 852	2962 ± 277	0.001
Birth weight groups			
< 1500g	19 (31.7)	0 (0)	0.001
1500 - 2499 g	23 (38.3)	6 (10)	0.001
2500 - 4499 g	18 (30.0)	54 (90)	0.001
Oligohydramnios	8 (13.3)	3 (5)	0.04
Intrauterine growth restriction (IUGR)	5 (8.3)	0 (0)	0.001
Early neonatal death	5 (8.3)	0 (0)	0.001
Fetal distress	13 (21.7)	12 (20)	0.8
Placenta weight (g)	463 ± 135	514 ± 107	0.05
Apgar score:			
1 minute	7.2 ± 1.3	7.9 ± 0.5	0.002
5 minute	8.5 ± 1.0	8.9 ± 0.3	0.001
Hospital stay (days)	5.17 ± 2.47	1.06 ± 0.86	0.0001

CHAPTER 4

Journal of Reproductive Immunology 117 (2016) 81–88

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Lymphatic vascular endothelial hyaluronan receptor-1 immunoexpression in placenta of HIV infected pre-eclamptic women

Onankoy A. Onyangunga[a,*], Jagidesa Moodley[b], Vesselina Merhar[a], David A. Ofusori[a], Thajasvarie Naicker[a]

[a] Optics and Imaging Centre, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

[b] Women's Health and HIV Research Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 30 May 2016
Accepted 30 June 2016

Keywords:
LYVE-1
HIV infection
Pre-eclampsia
Placenta
Macrophage-like cells

ABSTRACT

Introduction: Lymphangiogenesis is the formation of new vessels from pre-existing lymphatic vessels. Data on lymphangiogenesis in the placenta of HIV-infected pre-eclamptics are sparse and the findings are conflicting. The aim of this novel study was to evaluate LYVE-1 immunoexpression in the placenta of HIV infected normotensive versus pre-eclamptic women.

Methods: Placental tissue was obtained from normotensive and pre-eclamptic women stratified according to their HIV status. The pre-eclamptic group was divided into early (<34 weeks) and late (>34 weeks) onset. Immunohistochemistry utilized mouse anti-human LYVE-1 antibody and was morphometrically evaluated.

Results: LYVE-1 immunostaining was localized within endothelium of the arterial supply and venous drainage of both conducting and exchange villi as well as within medial cells of arteries. LYVE-1 immunostained macrophage-like cells were observed within the fetal and maternal circulation. LYVE-1 immunoexpression was higher ($p = 0.0001$) in HIV positive cohort, regardless of pregnancy and villous type. Irrespective of HIV status and pregnancy type, LYVE-1 immunoexpression was significantly elevated in the conducting compared to the exchange villi ($p = 0.01$). LYVE-1 immunoexpression was higher in N and LOPE compared to EOPE groups for both conducting and exchange villi types respectively ($p = 0.0001$ and $p = 0.006$). There is a decrease of LYVE-1 expression in EOPE+ (conducting villi) and EOPE− (exchange villi) compared to N and LOPE subgroups.

Conclusion: This study provides a novel insight into an up-regulation of LYVE-1 expression in the fetal circulation of conducting and exchange villi of HIV-infected pre-eclamptics.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Pre-eclampsia,unique to human pregnancy, is a serious complication of pregnancy characterized by new onset hypertension (≥140/90 mmHg) and proteinuria (1+ or 300 mg/24 h urine) after the 20th week of gestation (Steegers et al., 2010; Tranquilli et al., 2013; Redman, 2014). A number of studies suggest that the frequency of pre-eclampsia may be affected by immunosuppressive conditions such as HIV/AIDS (Moodley, 2013; Kalumba et al., 2013; Landi et al., 2014).

The exact cause of pre-eclampsia is not fully understood. Our current understanding of the mechanism causing the pathologic changes observed in pre-eclampsia is complex and inconclusive. Recent findings suggest that pre-eclampsia is a two-stage disorder (Redman, 2014; Roberts, 2014). The first stage is thought to be due to failure of the myometrial spiral arteries to undergo vascular remodeling that converts them into flaccid wide-bore channels. This vascular maladaptation results in a marked reduction in blood flow to the placenta with consequential ischaemia and onset of the second stage and maternal symptoms. This reduction in blood flow stimulates cellular hypoxia and the release of a variety of anti-angiogenic circulating factors such as soluble *fms*-like tyrosine kinase -1 and soluble endoglin (Govender et al.,

Abbreviations: LYVE-1, lymphatic vascular endothelial hyaluronan receptor-1; HIV−, human immunodeficiency virus negative; HIV+, human immunodeficiency virus positive; N−, normotensive HIV negative; N+, normotensive HIV positive; EOPE−, early onset pre-eclamptic HIV negative; EOPE+, early onset pre-eclamptic HIV positive; LOPE−, late onset pre-eclamptic HIV negative; LOPE+, late onset pre-eclamptic HIV positive.

* Corresponding author.
E-mail address: felyonya@yahoo.com (O.A. Onyangunga).

http://dx.doi.org/10.1016/j.jri.2016.06.010
0165-0378/© 2016 Elsevier Ireland Ltd. All rights reserved.

2014). It has been postulated that excessive anti-angiogenic factors bind with pro-angiogenic factors (vascular endothelial and placental growth factors), inhibiting their biological activities and subsequently resulting in widespread endothelial damage and subsequent new onset of hypertension and proteinuria, known as pre-eclampsia (Alladin and Harrison, 2012; Sircar et al., 2015). Recently, work by our group and others have identified an imbalance of pro-angiogenic and anti-angiogenic proteins as a key factor in the pathogenesis of the pre-eclampsia (Govender et al., 2014; Govender et al., 2013; Govender et al., 2015). Current research suggests that the placenta is the key organ in the pathogenesis of pre-eclampsia as its delivery resolves the symptoms of the disorder (Lambert et al., 2014).

The common pathway of transmission of HIV at mucosal site is sexual transmission. Once the virus is in the mucosa, it may utilize lymphatic endothelial channels to disseminate infected cells to the draining lymph nodes (Zhang et al., 2012). The latter study demonstrated that HIV-1 gp120 induces hyper permeability of lymphatic cells monolayer and modulates the expression of fibronectin, slit 2 and robo 4. Although endothelial cells do not express CD4 receptor, they express HIV co-receptors CXCR4 and CCR5 (Zhang et al., 2012). A number of studies have shown the effects of HIV infection and antiretroviral therapy on vasculature (Dubé et al., 2008; Kristoffersen et al., 2009; Mata-Marín et al., 2013; Sinxadi et al., 2013).

Lymphangiogenesis is the formation of new vessels from pre-existing lymphatic vessels (Zheng et al., 2014). The lymphatic vascular system provides an exclusive microenvironment not only for maintaining homeostasis but also for playing a critical role in immune responses. Literature on lymphangiogenesis in the placenta of HIV infected pre-eclamptics are limited and conflicting (Gu et al., 2006; Volchek et al., 2010; Castro et al., 2011; Bellini et al., 2012; Liu et al., 2015). Hyaluronan (HA) is an extracellular glycosaminoglycan involved in cell adhesion and migration (Wu et al., 2014). Lymphatic vascular endothelial receptor (LYVE-) is a major receptor for HA on lymphatic endothelial cells (Banerji et al., 1999). LYVE-1 co-localizes with CD44 on lymphatic vessels, however, at the maternal-fetal interface LYVE-1, rather than CD44, is the functional HA receptor (Gu et al., 2006).

The aim of this novel study was to evaluate LYVE-1 immunoexpression in the placenta of HIV infected normotensive versus pre-eclamptic women.

2. Material and methods

This prospective study was conducted at a Regional Hospital, Durban, South Africa. Ethical approval was obtained from the Biomedical Research Ethics Committee, University of KwaZulu-Natal (BE: 040/12). Written informed consent was obtained prior to enrollment in the study.

The study population were randomly selected and grouped into normotensive and pre- eclamptic pregnancies and further stratified by their HIV status. The pre-eclamptic group was stratified by gestational age into early (EOPE; <34 weeks) and late (LOPE; >34 weeks) pre-eclampsia. The study groups are outlined below:

a) Normotensive HIV negative (N−): (n = 30);
b) Normotensive HIV positive (N+): (n = 30);
c) Early onset pre-eclamptic HIV negative (EOPE−): (n = 30);
d) Early onset pre-eclamptic HIV positive (EOPE+): (n = 30);
e) Late onset pre-eclamptic HIV negative (LOPE−): (n = 30);
f) Late onset pre-eclamptic HIV positive (LOPE+): (n = 30).

The relevant clinical and demographic data were documented in a structured data form.

2.1. Placenta collection and tissue preparation

After delivery of the baby during elective caesarean section, placental tissue was removed from central area of the placenta and was fixed in 10% buffered formaldehyde and processed into wax blocks using conventional techniques. Three micron thick sections were cut using a rotary microtome (Leica Microsystems, Germany) and mounted onto coated slides (X-tra Adhesive, Leica Microsystems, Germany).

2.2. Immunohistochemistry

Immunohistochemistry was performed using a Dako Envision Flex detection system Kit (Envision + System + HRP, K800021, Dako, Denmark) together with a Dako Autostainer Link machine. Epitope retrieval was performed with a target antigen retrieval (pH 9.0; Envision Flex Target Retrieval solution -high pH; S2368) for 20 min. Endogenous peroxidase block was performed with peroxidase blocking reagent (SM801). A mouse anti-human lymphatic vascular endothelial hyaluronan receptor-1 antibody was incubated for 20 min (1:160, clone#537028; MAB20892, R & D systems, UK & Europe; Dako diluent). This was followed by a peroxidase labeled polymer which was conjugated to secondary goat anti-mouse immunoglobulins and followed by the chromogen. Primary antibody, substituted with non-immune sera of the same IgG class or a buffer served as a method control. Lymph node served as a positive control.

2.3. Morphometric image analysis of LYVE-1 expression

All specimens were viewed with an Axioscope A1 microscope (Carl Zeiss, Germany). Image capturing, processing and analysis were performed using the AxioVision software (Carl Zeiss, Germany; version 4.8.3). At least four fields of view per slide for each type of villi (*i.e.* conducting and exchange) were randomly selected and captured using a 20× objective magnification.

The auto measure mode of analysis was selected. LYVE-1 expression was determined as a percentage of immunostaining (brown) within the villi. Since the conducting and exchange villi differ significantly in histology, a single object analysis of immunostaining within conducting villi was applied. Each conducting villi was framed and the amount of immunostaining was expressed as percentage per frame area (Fig. 1A). The amount of label (green) within the exchange villi (red) was determined by a two phase threshold and expressed as a total percentage of LYVE-1 immunostaining within the frame area (Fig. 1B).

2.4. Statistical analysis

Data were analyzed using SPSS version 23 (IBM, USA). Results are expressed as mean ± standard deviation. Parametric data was used directly whilst non-parametric data was analyzed using the Kruskal-Wallis test and Mann-Whitney *U* tests. A two-way ANOVA was performed to examine the effect of HIV infection (HIV+ *vs* HIV−), pregnancy type (normotensive *vs* pre-eclampsia) and villous type (exchange *vs* conducting) on LYVE-1 immunostaining. A p value of $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Patient demographics

Patient demographics are shown in Table 1. Significant difference in maternal age and birthweight based on HIV status was noted in the EOPE group only ($p = 0.001$; $p < 0.05$ respectively). The

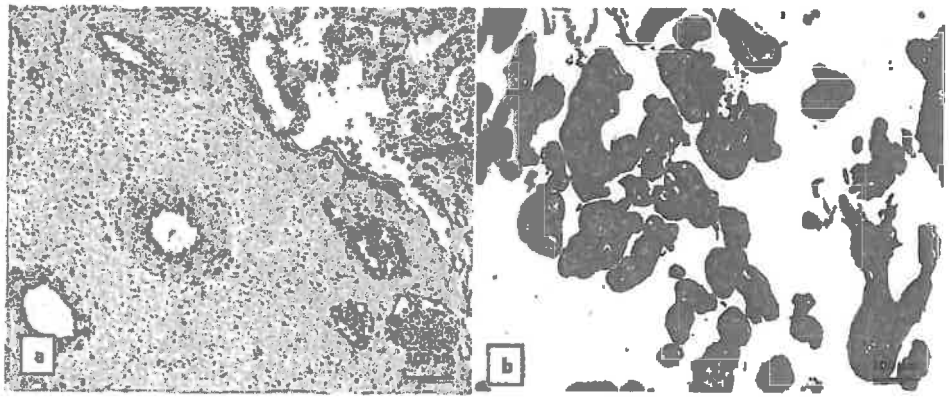


Fig. 1. LYVE-1 segmentation within (A) conducting and (B) exchange villi of N- group. Red = area of villi; Green = LYVE-1 immunoexpression.

Table 1
Patient demographics across study groups.

Parameters	Normotensive		EOPE		LOPE	
	HIV− (n = 30)	HIV+ (n = 30)	HIV− (n = 30)	HIV+ (n = 30)	HIV− (n = 30)	HIV+ (n = 30)
Maternal Age (years)	26.5 ± 6.4	27.7 ± 5.5	25.2 ± 6.5**	31.8 ± 5.9**	24.2 ± 6.4	28.7 ± 4.6
Gestational Age (wks)	38.5 ± 1.1	38.7 ± 0.7	32.9 ± 2.6*	33 ± 3.60*	37.1 ± 6.1	37.4 ± 1.2
Baby Weight (g)	3346 ± 321	3255 ± 396	2187 ± 799*	2083 ± 852*	3078 ± 457*	2962 ± 277*
Baby Sex (M:F)	14:16	14:16	14:16	12:18	15:15	20:10

* $p < 0.05$.
** $p = 0.001$.

mean gestational age at delivery in EOPE was statistically lower compared to both N and LOPE groups ($p < 0.05$).

3.2. Qualitative LYVE-1 expression in placenta

LYVE-1 immunostaining was observed within the conducting and exchange villi across normotensive and pre-eclamptic placentae, irrespective of HIV status (Fig. S1-composite montage). Label was localized to endothelial cells, of both the arterial supply and venous drainage of the conducting (stem; Figs. 2 a–f and S1) villi. Additionally, endothelial cells of capillaries within the exchange villi were immunostained (intermediate and terminal; Figs. 3 a–f and S1). The endothelial localization of LYVE-1 was noted across all study groups, although the level of immunoreactivity within medial cells of arteries was variable, appearing stronger in the LOPE groups (Fig. 2e–f), irrespective of HIV status. LYVE-1 was also observed in the fibroblast-like cells of the mesenchymal stroma of conducting villi (Fig. 2e). LYVE-1 immunostaining was low to absent within trophoblast cell populations (syncytiotrophoblast and cytotrophoblast; Figs. 2 and 3).

Numerous LYVE-1 immunostained macrophage-like cells were observed within veins, arteries and capillaries of the fetal circulation (Figs. 2d and 3b), as well as within the intervillous space of the maternal circulation (Fig. 3b) and this distribution appeared to be qualitatively less in the N−, compared with the rest of the groups. No lymphatic vessels were detected in the placenta. Replacement of the primary antibody with a non-immune serum of the same IgG class produced no immunoreactivity (Fig. S2a). Lymph node was used as a positive control (S2b).

3.3. Morphometric analysis of LYVE-1 immunostaining

3.3.1. Pregnancy type irrespective of HIV status

LYVE-1 immunoexpression was higher in N and LOPE compared to EOPE groups for both conducting and exchange villi types respectively ($p = 0.0001$ and $p = 0.006$). LYVE-1 immunoexpression was statistically different between EOPE (≤34 weeks gestation) and LOPE (>34 weeks gestation) in conducting and exchange villi respectively ($p = 0.0001$; $p = 0.006$). Within conducting villi, LYVE-1 immunoexpression did not differ between N and EOPE ($p = 0.066$); however, it was higher in LOPE compared with the N ($p = 0.0001$). Within exchange villi, LYVE-1 immunoexpression was the lowest in the EOPE group compared to the N ($p = 0.0001$), however there was no significant difference between N and LOPE groups ($p = 0.333$).

3.3.2. Across sub-groups

Percentage LYVE-1 (Mean ± SD) immunoexpression within conducting and exchange villi across study groups are shown in Table 2 and Fig. 4A, B. Within the exchange villi, there was a significant decrease of LYVE-1 immunoexpression in the EOPE−ve group compared with the rest of the groups. A similar trend, was observed within the conducting villi amongst all groups, but a significant LYVE-1 decline was noted between EOPE− and the LOPE−/+ groups only [EOPE− *vs* LOPE− ($p = 0.01$); EOPE− *vs* LOPE+ ($p = 0.0001$); Table 2, Fig. 4A].

In the conducting villi, LYVE-1 immunoexpression did not differ between N− *vs* EOPE− ($p = 0.5$), whilst in the exchange villi a significant difference was noted between these groups ($p = 0.006$). On the other hand, LYVE-1 immunoexpression within both conducting and exchange villi was significantly different between N+ *vs* EOPE+ respectively ($p = 0.026$; $p = 0.008$). Within the conducting villi, LYVE-1 immunoexpression differed between N and LOPE (N− *vs* LOPE−, $p = 0.007$; N+ *vs* LOPE+, $p = 0.002$). In the exchange villi, LYVE-1 immunoexpression did not differ between N and LOPE (N− *vs* LOPE−, $p = 0.7$; N+ *vs* LOPE+, $p = 0.066$).

With regards to early and late presentation of pre-eclampsia, LYVE-1 immunoexpression within conducting villi was significantly different between EOPE and LOPE (EOPE− *vs* LOPE−, $p = 0.006$; EOPE+ *vs* LOPE+, $p = 0.0001$). Whereas, LYVE-1 immunoexpression within exchange villi only differed between EOPE− and LOPE− ($p = 0.0003$).

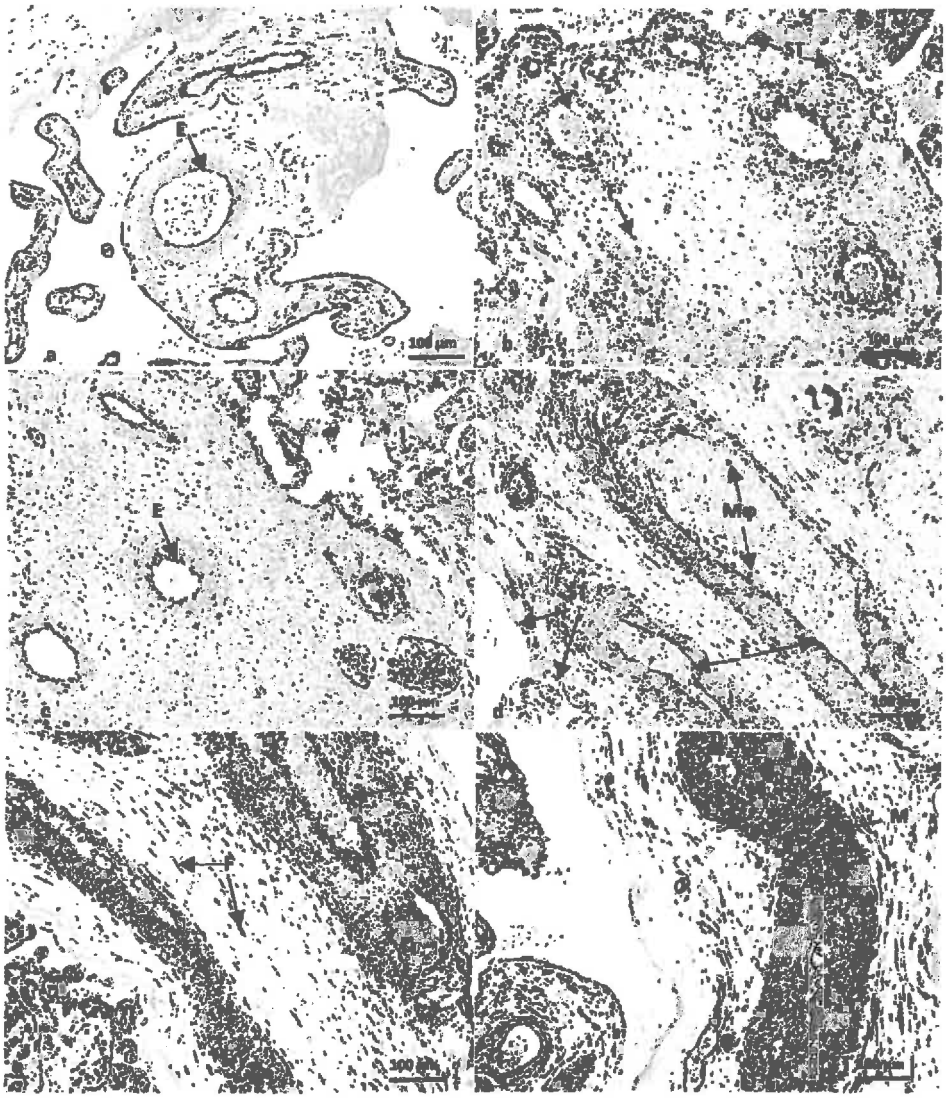


Fig. 2. LYVE-1 immunostaining within placental conducting villi in (a) N−; (b) N+; (c) EOPE−; (d) EOPE+; (e) LOPE− and (f) LOPE+ groups. F = fibroblast-like cells; E = endothelial cells; ST = syncytiotrophoblast; Mφ = macrophage-like cells; M = media.

Table 2
LYVE-1 immuno-reactivity across study groups. Results are expressed as (%) mean ± SD.

Villi Type	Normotensive		EOPE		LOPE	
	HIV−	HIV+	HIV−	HIV+	HIV−	HIV+
Conducting	14.01 ± 4.72	15.88 ± 4.42	13.00 ± 7.21#	13.35 ± 4.61#	17.44 ± 4.64	19.55 ± 3.84
Exchange	13.07 ± 7.13	18.96 ± 7.80	8.84 ± 4.18	13.69 ± 7.51	13.77 ± 7.69	15.64 ± 6.33

N.B. There was a significant difference across the study groups and subgroups except the ones represented with (#) indicating that in EOPE− and EOPE+ were similar in LYVE-1 expression in conducting villi only.

3.3.3. *HIV status*

Considering HIV status across the study population (n = 180) LYVE-1 immunoexpression was higher (p = 0.0001) in HIV positive cohort (n = 90), regardless of pregnancy and villous type. Based on villous type (irrespectively of pregnancy type), there was no significant difference in LYVE-1 immunoexpression within conducting villi (p = 0.1; Fig. 5A) between the HIV positive and negative groups, whereas LYVE-1 immunoexpression within the exchange villi differed (p = 0.0001; Table 2; Fig. 5B).

3.3.4. *Villous types*

Irrespective of HIV status and pregnancy type, LYVE-1 immunoexpression was significantly elevated in the conducting compared to the exchange villi (p = 0.01; Fig. 6).

4. Discussion

Our study shows distinctive LYVE-1 immunoexpression within the fetal circulation of the placenta. It was predominantly local-

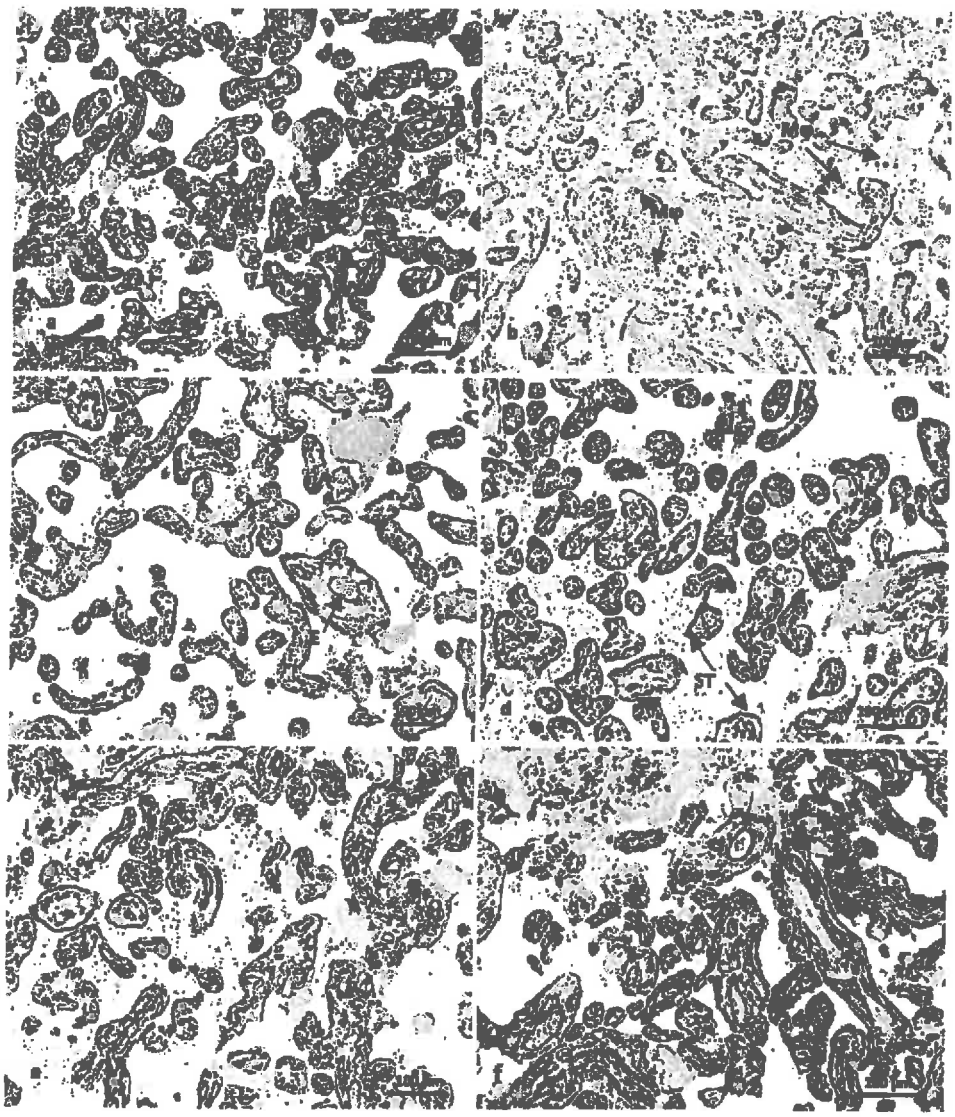


Fig. 3. LYVE-1 immunostaining within placental exchange villi in (a) N−; (b) N+; (c) EOPE−; (d) EOPE+; (e) LOPE− and (f) LOPE+ groups. E = endothelial cells; ST = syncytiotrophoblast; Mφ = macrophage-like cells.

ized to endothelial and medial cells of fetal arteries, veins and mesenchymal fibroblast-like cells of conducting, as well as within the capillaries of exchange villi. These results are supported by the positive gene expressions for lymphatic markers of VEGF-C, Flt-1, LYVE-1, and Prox-1 in villous core vessel endothelium indicating the probability of their lymphatic-lineage phenotype (Gu et al., 2006). In contrast, Böckle et al. found no immunostaining on cryopreserved chorionic villi using a polyclonal LYVE-1 antibody, concluding that the human term placenta has no lymphatic drainage (Böckle et al., 2008). It should be emphasised that we also did not observe any lymphatic vessels within the fetal circulation of wax embedded placenta.

Notably, in human non-pregnant endometrium the presence of lymphatic vessels is controversial (Red-Horse et al., 2006; Tomita and Mah, 2014). Here, lymphatics would ensure protection from autoantigens during menstruation and prevention of antibody production against sperm (Naz and Menge, 1994). In normal pregnancy the placenta triggers the formation of a new lymphatic system within the decidua (Red-Horse, 2008), hence a functioning lymphatic system is necessary to maintain maternal-fetal immunity, maternal tolerance and tissue fluid homeostasis (Lamont, 2003). In the absence of a lymphatic system, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) positive macrophages are reported to accomplish an innate response against pathogens within the placenta (Böckle et al., 2008). In our study we found a LYVE-1 expression in macrophage-like cells in foetal circulation within the placenta. This macrophage-like infiltration was not quantified due to the fact that we do not perform CD68 or DC-SIGN immunostaining. Qualitative observations showed a predominant appearance of these cells in the N+ as well as pre-eclamptic (both HIV+ and HIV−) women compared with the normotensive HIV− controls. Similarly, Evsen et al. found an increase of Hofbauer cells in women with HELLP syndrome (haemolysis, elevated liver enzymes, and low platelets count) compared with normotensive patients, suggesting that the increase in these cells may have an adaptive mechanism at the fetal site of the placenta in patients with HELLP syndrome (Evsen et al., 2013). The occurrence of macrophage-like cells may be responsible for

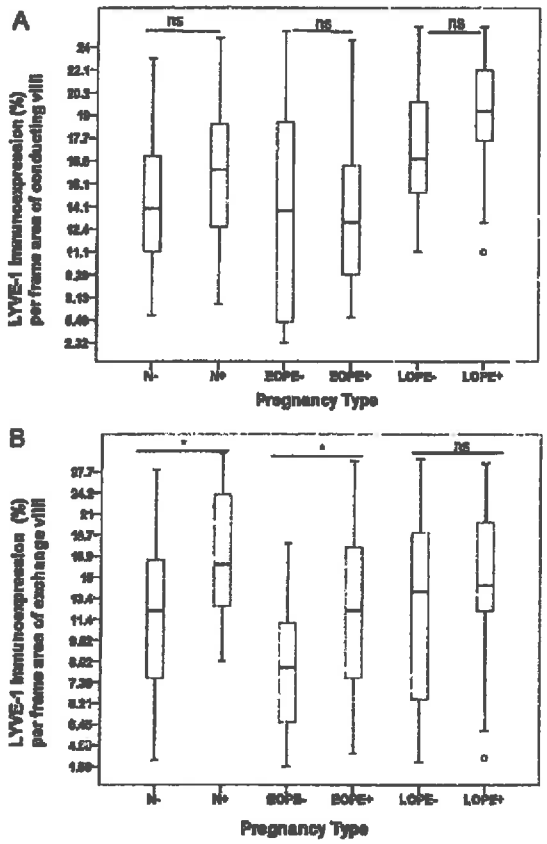


Fig. 4. LYVE-1 expression in (A) conducting and (B) exchange villi across all study groups (n = 30 per subgroup). *The difference between subgroups is statistically significant ($p < 0.05$); ns = non-significant.

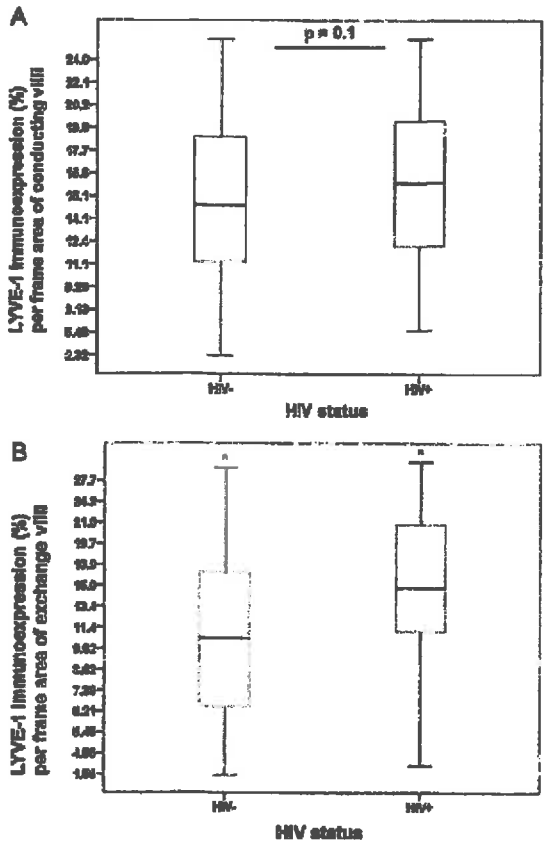


Fig. 5. LYVE-1 immunoexpression according to HIV status (N+, EOPE+, LOPE+; n = 90 and N+, EOPE+, LOPE+; n = 90) within (A) conducting and (B) exchange villi. *$p = 0.0001$.

detecting, engulfing and destroying viral antigens within the placenta thereby maintaining maternal-fetal immunity. Additionally, macrophages are implicated in placental infection like chorioamnionitis (Amara, 2013).

Data on lymphangiogenesis in relation to HIV infection in pregnancy is scarce. To our knowledge, our study is the first report demonstrating a significant increase in placental LYVE-1 immunoexpression in HIV infected compared to uninfected pregnant women ($p = 0.0001$). It is important to note that LYVE-1 morphometry in this study may be confounded by the anti-retroviral management of all HIV infected women within our sample population as it is a standard of care in our environment. Our findings on LYVE-1 immunoexpression might be influenced by other viral infections such as the one observed in Kaposi sarcoma–associated Herpes virus lead to their lymphatic reprogramming; induction of ~70% of the main lymphatic lineage–specific genes, including LYVE-1 and down-regulation of blood vascular genes (Gupta et al., 2012; Hong et al., 2004). It is known that HIV infection affects lymphatic endothelial cells which have co-receptors of HIV specifically gp120 (Hong et al., 2004; Dube et al., 2011). *In vitro* studies advocate a candidate angiogenic and lymph-angiogenic factor encoded by HIV, the matrix protein p17 (Popovic et al., 2005). This protein is found in lymph nodes before initiation and during highly active antiretroviral therapy (Popovic et al., 2005). Recent data implicates a variant form of p17, called S75X, which prompts cell growth by activating MAPK/ERK and PI3K/AKT pathways (Basta et al., 2015).

HLA-G plays a role in immune tolerance during pregnancy by protecting the trophoblast against NK cell-mediated death. HLA-G levels are up-regulated occur during cytomegalovirus and HIV infection (Lozano et al., 2002; Onno et al., 2000). In contrast, hypoxia down-regulates the expression of HLA-G, pre-eclampsia is a hypoxic microenvironment (Kilburn et al., 2000). Hence, the immunosuppressive ability of HLA-G is neutralized in HIV associated pre-eclampsia.

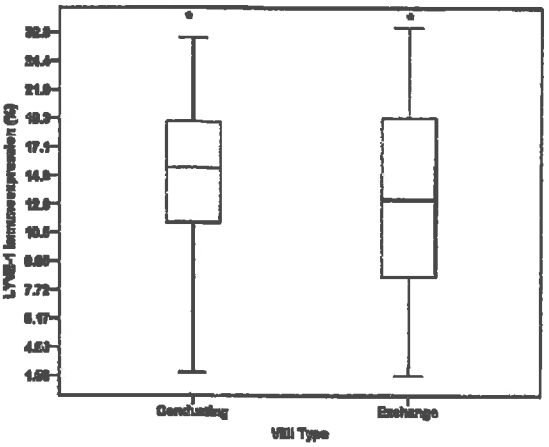


Fig. 6. LYVE-1 expression within conducting (n = 180) and exchange (n = 180) villi irrespective of study group. *The difference between villi is statistically significant ($p = 0.0001$).

With regards to pregnancy type, we found a lower LYVE-1 reactivity in the EOPE compared to the normotensive ($p = 0.0001$) and LOPE groups ($p = 0.006$). It is well known that women with EOPE develop a more severe and complicated form of pre-eclampsia (Valensise et al., 2008); with an exaggerated immune response, hence the lymphatic profile within this group may be affected.

In our study, few immunoreactive sites of LYVE-1 expression was noted within trophoblast cell populations (syncytiotrophoblast and cytotrophoblast). Our results are in accordance with the findings by Gu et al. who showed a higher LYVE-1 staining within the trophoblast cells of pre-term compared to term placentas (Gu et al., 2006). The few immunoreactive sites of LYVE-1 expression within trophoblastic cells in our study, might reflect the endothelial phenotype of these cells. It is well known that trophoblast cells express many endothelial lineage molecules such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and VEGFR-1 (Flt-1) (Govender et al., 2014), hence they may play a role in endothelial phenotype change during placentation. Furthermore, it has been shown that other endothelial markers such as VEGF-C and its receptor (VEGFR3) and angiopoietin-2 (Ang-2) are involved in lymphangiogenesis within embryonic and adult tissues (Karkkainen et al., 2004).

Our results show LYVE-1 immunostaining in fibroblast-like cells within the mesenchymal villous stroma. It should be noted that placental fibroblasts are heterogenic and their lineage may include mesothelial, endothelial epithelial cells, and circulating fibrocytes (Gale et al., 2002). These fibroblast-like cells may also be implicated in secreting various chemokines to recruit inflammatory cells, hence be involved in immune response to viral infection.

To-date there are conflicting results in studies using lymphatic-specific endothelial cell markers (Gu et al., 2006; Volchek et al., 2010; Castro et al., 2011; Bellini et al., 2012; Liu et al., 2015). Recent developments have shown that numerous blood and lymphatic markers are expressed in human placental tissue, and that the expression and localization of these lymphatic markers is compartmentally different (Bellini et al., 2012). Liu et al. described a distinct population of lymphatic vessels in the decidua and not in the human placenta based on immunohistochemistry (Liu et al., 2015). Similarly, other studies also report an absence of lymphatics in the human placenta (Castro et al., 2011). It should be noted that the latter 2 reports examined HIV negative samples, our study is unique in that we compare LYVE-1 expression in HIV infected and non-infected placenta. Additionally, a previous study has shown that human placental implants induce the infiltration of LYVE-1 positive lymphatic endothelial cells that forms vessel (Red-Horse et al., 2006). It is apparent that caution is still required when examining lymphatic vessels within placenta, even when lymphatic endothelial cell markers are used. A recent study, found the expression levels of the angiogenic markers VEGF1, CD34 and CD31 were significantly lower in the placenta of the PE than the control group whilst the lymphangiogenic markers PROX-1 and VEGFR3 were negatively expressed in the placenta (Liu et al., 2015). Unlike the latter study, our pre-eclamptic group was stratified by gestational age into early (EOPE; ≤34 weeks) and late (LOPE; >34 weeks) to maintain homogeneity of the groups.

In conclusion, the present study provides novel insights into the distribution of LYVE-1 expression in the placenta of HIV-infected pre-eclamptics. Based on HIV status, we report an up-regulation of LYVE-1 expression in the fetal circulation of conducting and exchange villi of immunocompromised patients. There is a decrease of LYVE-1 expression in EOPE+ (conducting villi) and EOPE− (exchange villi) compared to N and LOPE subgroups studied. This study has also shown that LYVE-1 is a heterogeneous marker for both lymphatic and arterial and venous endothelial cells. Further work that outlines the framework of the basic histology of the lymphatic system in the placental bed will enable a better understanding of maternal-fetal immunity during HIV infection in pregnancy.

Acknowledgements

The authors wish to thank Mrs. D.A. Margolis for technical assistance, Dr. K. Maduray and Dr. A. Ajith for the statistical analysis. Funders included the College of Health Sciences, UKZN and the Medical Education Programme Initiative.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jri.2016.06.010.

References

Aladin, A.A., Harrison, M., 2012. Pre-eclampsia: systemic endothelial damage leading to increased activation of blood coagulation cascade. J. Biotechnol. Res. 4, 26–43.

Amara, A.B., 2013. Placental macrophages are impaired in chorioamnionitis, an infectious pathology of the placenta. J. Immunol. 191, 5501–5514.

Böckle, B.C., Sölder, E., Kind, S., Romani, N., Sepp, N.T., 2008. DC-sign+ CD163+ macrophages expressing hyaluronan receptor LYVE-1 are located within chorion villi of the placenta. Placenta 2, 187–192.

Banerji, S., Ni, J., Wang, S., Clasper, S., Su, J., Tammi, R., Jones, M., Jackson, D.G., 1999. LYVE-1, a new homologue of CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J. Cell Biol. 144, 789–801.

Basta, D., Latinovic, O., Lafferty, M.K., Sun, L., Bryant, J., Lu, W., Caccuri, F., Caruso, A., Gallo, R., Garzino-Demo, A., 2015. Angiogenic, lymphangiogenic and adipogenic effects of HIV-1 matrix protein. Pathog. Dis., http://dx.doi.org/10.1093/femspd/ftv062.

Bellini, T., Bonioli, E., Pulcheri, E., Bellini, C., Rutigliani, M., Boccardo, F., Campisi, C., 2012. Are there lymphatic vessels in the placenta? Lymphology 45, 34–36.

Castro, E., Tony, P.W., Galambos, C., 2011. Neither normal nor diseased placentas contain lymphatic vessels. Placenta 4, 310–316.

Dubé, M.P., Lipshultz, S.E., Fichtenbaum, C.J., Greenberg, R., Schecter, A.D., Fisher, S.D., 2008. Working group 3: effects of HIV infection and antiretroviral therapy on the heart and vasculature. Circulation 118, e36–40.

Dube, M.P., Gorski, J.C., Shen, C., 2011. Severe impairment of endothelial function with the HIV-1 protease. Indian J. Clin. Biochem. 3, 249–256.

Evsen, M.S., Kalkanli, S., Deveci, E., Sak, M.E., Ozler, A., Baran, O., Erdem, E., Seker, U., 2013. Human placental macrophages (Hofbauer cells) in severe preeclampsia complicated by HELLP syndrome: immunohistochemistry of chorionic villi. Anal. Quant. Cytopathol. Histpathol. 35, 283–288.

Gale, N.W., Thurston, G., Hackett, S.F., Renard, R., Wang, Q., McClain, J., Martin, C., Witte, C., Witte, M.H., Jackson, D., Suri, C., Campochiaro, P.A., Wiegand, S.J., Yancopoulos, G.D., 2002. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by angiopoietin-1. Dev. Cell. 3, 411–423.

Govender, N., Naicker, T., Rajakumar, A., Moodley, J., 2013. Soluble fms-like tyrosine kinase-1 and soluble endoglin in HIV-associated preeclampsia. Eur. J. Obstet. Gynecol. Reprod. Biol. 170, 100–105.

Govender, N., Moodley, J., Gathiram, P., Naicker, T., 2014. Soluble fms-like tyrosine kinase-1 in HIV infected pre-eclamptic south African black women. Placenta 35, 618–624.

Govender, N., Naicker, T., Moodley, J., 2015. Endoglin in HIV-associated preeclamptic placentae. Hypertens. Pregnancy 34, 1–13.

Gu, B., Alexander, J.S., Gu, Y., Zhang, Y., Lewis, D.F., Wang, Y., 2006. Expression of lymphatic vascular endothelial hyaluronan receptor-1 (LYVE-1) in the human placenta. Lymphat. Res. Biol. 1, 11–17.

Gupta, S.K., Shen, C., Moe, S.M., Kamenduliis, L.M., Goldman, M., Dubé, M.P., 2012. Worsening endothelial function with efavirenz compared to protease inhibitors: a 12-month prospective study. PLoS One 9, e45716.

Hong, Y., Foreman, K., Shin, J.W., Hirakawa, S., Curry, C.L., Sage, D.R., Libermann, T., Dezube, B.J., Fingeroth, J.D., Detmar, M., 2004. Lymphatic reprogramming of blood vascular endothelium by Kaposi sarcoma-associated herpesvirus. Nat. Genet. 36, 683–685.

Kalumba, V.M., Moodley, J., Naidoo, T.D., 2013. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. Cardiovasc. J. Afr. 2, 24–27.

Karkkainen, M.J., Haiko, P., Sainio, K., Partanen, J., Taipale, J., Petrova, T.V., Jeltsch, M., Jackson, D.G., Talikka, M., Rauvala, H., Betsholtz, C., Alitalo, K., 2004. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat. Immunol. 5, 74–80.

Kilburn, B.A., Wang, J., Duniec-Dmuchowski, Z.M., Leach, R.E., Romero, R., Armant, D.R., 2000. Extracellular matrix composition and hypoxia regulate the expression of HLA-G and integrins in a human trophoblast cell line. Biol. Reprod. 62, 739–747.

Kristoffersen, U.S., Kofoed, K., Kronborg, G., Giger, A.K., Kjaer, A., Lebech, A.M., 2009. Reduction in circulating markers of endothelial dysfunction in HIV-infected patients during antiretroviral therapy. HIV Med. 2, 79–87.
Lambert, G., Brichant, J.F., Hartstein, G., Bonhomme, V., Dewandre, P.Y., 2014. Pre-eclampsia: an update. Acta Anaesthesiol. Belg. 65, 137–149.
Lamont, R.F., 2003. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. BJOG 20, 71–75.
Landi, B., Bezzeccheri, V., Guerra, B., Piemontese, M., Cervi, F., Cecchi, L., Margarito, B., Giannubilo, S.R., Ciavattini, A., Tranquilli, A.L., 2014. HIV infection in pregnancy and the risk of gestational hypertension and preeclampsia. World J. Cardio Vasc. Dis. 4, 257–267.
Liu, H., Li, Y., Zhang, J., Rao, M., Liang, H., Liu, G., 2015. The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia. Placenta 3, 279–286.
Lozano, J.M., Gonzalez, R., Kindelan, J.M., Rouas-Freiss, N., Caballos, R., Dausset, J., Carosella, E., Pena, J., 2002. Monocytes and T lymphocytes in HIV-1 positive patients express HLA-G molecule. AIDS 16, 347–351.
Mata-Marin, J.A., Méndez-Cruz, R., Arroyo-Anduiza, C.I., Mata-Marin, L.A., Gaytán-Martínez, J., Asbún-Bojalil, J., 2013. Effect of antiretroviral therapy on inflammatory markers of endothelial dysfunction in HIV treatment-naïve infected patients. J. Med. Virol. 8, 1321–1326.
Moodley, J., 2013. Impact of HIV on the incidence of pre-eclampsia. Cardiovasc. J. Afr. 24, 5.
Naz, R.K., Menge, A.C., 1994. Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility. Fertil. Steril. 61, 1001–1013.
Onno, M., Pangault, C., Le Friec, G., Guilloux, V., Andre, P., Fauchet, R., 2000. Modulation of HLA-G antigens expression by human cytomegalovirus: specific induction in activated macrophages harbouring human cytomegalovirus infection. J. Immunol. 164, 6426–6434.
Popovic, M., Tenner-Racz, K., Pelser, C., Stellbrink, H.J., van Lunzen, J., Lewis, G., 2005. Persistence of HIV-1 structural proteins and glycoproteins in lymph nodes of patients under highly active antiretroviral therapy. PNAS 102, 14807–14812.
Red-Horse, K., Rivera, J., Schanz, A., Zhou, Y., Winn, V., Kapidzic, M., Maltepe, E., Okazaki, K., Kochman, R., Vo, K.C., Giudice, L., Erlebacher, A., McCune, J.M., Stoddart, C.A., Fisher, S.J., 2006. Cytotrophoblast induction of arterial apoptosis and lymphangiogenesis in an *in vivo* model of human placentation. J. Clin. Invest. 10, 2643–2652.
Red-Horse, K., 2008. Lymphatic vessel dynamics in the uterine wall. Placenta 29, 55–59.
Redman, C., 2014. The six stages of pre-eclampsia. Pregnancy Hypertens 3, 241–247.
Roberts, J.M., 2014. Pathophysiology of ischemic placental disease. Semin. Perinatol. 3, 139–145.
Sinxadi, P.Z., Dave, J.A., Samuels, D.C., Heckmann, J.M., Maartens, G., Levitt, N.S., Wester, C.W., Haas, D.W., Hulgan, T., 2013. Mitochondrial genomics and antiretroviral therapy-associated metabolic complications in HIV-infected Black South Africans: a pilot study. AIDS Res. Hum. Retroviruses 7, 1031–1039.
Sircar, M., Thadhani, R., Karumanchi, S.A., 2015. Pathogenesis of pre-eclampsia. Curr. Opin. Nephrol. Hypertens. 24, 131–138.
Steegers, E.A., Von Dadelszen, P., Duvekot, J.J., Pijnenborg, R., 2010. Pre-eclampsia. Lancet 41, 631–644.
Tomita, T., Mab, K., 2014. Cyclic changes of lymphatic vessels in human endometrium. Open J. Pathol. 4, 5–12.
Tranquilli, A.L., Brown, M.A., Zeeman, G.G., Dekker, G., Sibai, B.M., 2013. The definition of severe and early-onset preeclampsia: statements from the International Society for the Study of Hypertension in Pregnancy. Pregnancy Hypertens 1, 44–47.
Valensise, H., Vasapollo, B., Gagliardi, G., Novelli, G.P., 2008. Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. Hypertension 52, 873–880.
Volchek, M., Girling, J.E., Lash, G.E., Cann, L., Kumar, B., Robson, S.C., Bulmer, J.N., Rogers, P.A., 2010. Lymphatics in the human endometrium disappear during decidualization. Hum. Reprod. 10, 2455–2464.
Wu, M., Du, Y., Liu, Y., He, Y., Yang, C., Wang, W., Gao, F., 2014. Low molecular weight hyaluronan induces lymphangiogenesis through LYVE-1-mediated signaling pathways. PLoS One 3, e92857.
Zhang, X., Yu, J., Kuzontkoski, P.M., Zhu, W., Li, D.Y., Groopman, J.E., 2012. Slit2/Robo4 signaling modulates HIV-1 gp120-induced lymphatic hyper permeability. PLoS Pathog. 8, 1–13, e1002461.
Zheng, W., Aspelund, A., Alitalo, K., 2014. Lymphangiogenic factors, mechanisms, and applications. J. Clin. Invest. 124, 878–887.

CHAPTER 5

Manuscript Details

Manuscript number	JRI_2016_160
Title	Immunohistochemical localization of podoplanin in the placenta of HIV infected pre-eclampsia
Article type	Full Length Article

Abstract

Introduction: Impaired lymphatic function is associated with pathological conditions. The presence of placental lymphatics is still under debate. The aim of this study was to examine podoplanin (PDPN) placental expression in normotensive and pre-eclampsia (PE) complicated by HIV infection. Methods: Normotensive pregnant and pre-eclamptic women were stratified by gestational age into early (<34 weeks) and late (≥34 weeks) onset PE and further divided by HIV status. Immunostaining was performed using a monoclonal mouse anti-human PDPN antibody prior to morphometric image analysis. Results: Podoplanin was immunolocalized in a reticular-like stroma complex within the conducting and exchange villi. Its immunoexpression was significantly up-regulated in the exchange versus conducting villi ($p = 0.0001$) irrespective of the pregnancy type and HIV status. Podoplanin was down-regulated in the EOPE group compared to LOPE in the exchange villi ($p = 0.05$). Also, PDPN was up-regulated in HIV+ vs HIV- groups regardless of pregnancy and villi type. Based on the HIV status, PDPN immunoexpression within conducting villi was higher in the HIV- (8.19 ± 3.37%) versus HIV+ (7.67 ± 3.23%) group albeit non-significantly ($p = 0.306$). Conversely regardless of pregnancy type, PDPN immunoexpression within exchange villi was different ($p = 0.008$) between the HIV+ and HIV- groups. Conclusion: This novel study demonstrated that placental fluid homeostasis is maintained by a PDPN reticular-like complex within conducting and exchange villi, being up-regulated in HIV positive pregnancies. However, PDPN immunoexpression was down-regulated in the exchange villi of EOPE reflecting the distribution deficient trophoblast invasion.

Keywords	Podoplanin; HIV infection; pre-eclampsia; placenta
Manuscript category	Clinical Studies

Corresponding Author	Onankoy A Onyangunga
Corresponding Author's Institution	Optics and Imaging
Order of Authors	Onankoy A Onyangunga, Jagidesa Moodley, Frederick Odun-Ayo, Thajasvarie Naicker

Suggested reviewers	Nalini Govender, Olugbenga Ayannuga

Submission Files Included in this PDF

File Name [File Type]

JReproimmunol cover letter.pdf [Cover Letter]

Conflict of interest.pdf [Conflict of Interest]

Manuscript.docx [Manuscript File]

Figure 1.pdf [Figure]

Figure 2.pdf [Figure]

Figure 3 .pdf [Figure]

Figure 4.pdf [Figure]

Figure 5.pdf [Figure]

Figure 6.pdf [Figure]

FIGURE CAPTIONS.docx [Figure]

TABLE 1.docx [Figure]

TABLE 2.docx [Figure]

TABLES CAPTION.docx [Figure]

Supplementary image.pdf [Figure]

HIGHLIGHTS.docx [Highlights]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Cover letter

Dear Editor
Journal of Reproductive Immunology

Manuscript Title: Immunohistochemical localization of podoplanin in the placenta of HIV infected Pre-eclampsia

We intend to publish an article entitled-above in your esteemed journal as an original research to the Editor. We declare that we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have our names listed as a contributor. We believe the manuscript represents valid work. Each author confirms they meet the criteria for authorship. Neither is this manuscript been published nor is being considered for publication elsewhere, except as described in the covering letter. We certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately. We attest that, if requested by the editors, we will provide the data/information or will cooperate fully in obtaining and providing the data/information on which the manuscript is based, for examination by the editors or their assignees. Sources of outside support of the project are named in the cover letter.

We give the rights to the corresponding author to make necessary changes as per the request of the journal, do the rest of the correspondence on our behalf and he/she will act as the guarantor for the manuscript on our behalf. We declare that there is no conflict of interest and the final version of the manuscript has been approved for submission to your journal.

All persons who have made substantial contributions to the work reported in the manuscript, but who are not contributors, are named in the Acknowledgment and have given us their written permission to be named. If we do not include an Acknowledgment that means we have not received substantial contributions from non-contributors and no contributor has been omitted.

	Name	Signature	Date signed
1	OA ONYANGUNGA		05.10.2016.
2	J Moodley	J Moodley	5 October 2016
3	F ODUN-AYO		05-10-2016
4	T NAICKER	T Naicker	05.10.2016

Conflict of Interest

Dear Editor

Journal of Reproductive Immunology

05 October 2016

Manuscript Title: Immunohistochemical localization of podoplanin in the placenta of HIV infected Pre-eclampsia

I declare on behalf of all authors that there is no conflict of interest and the final version of the manuscript has been approved for submission to your journal.

Thank you.

Regards

Dr OA Onyangunga

Title: Immunohistochemical localization of podoplanin in the placenta of HIV infected pre-eclampsia

Authors: Onyangunga OA, *Moodley J, Odun-Ayo F and Naicker T

Affiliations: Optics and Imaging Centre, and *HIV and Women's HIV Research Group, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Corresponding author:

Dr OA Onyangunga,

Optics and Imaging Centre,

College of Health Sciences,

University of KwaZulu-Natal, South Africa.

Email: onyangunga51@gmail.com; naickera@ukzn.ac.za

Fax: 031 2604311

Telephone: 031 2604435

Keywords: podoplanin, HIV-1 infection, pre-eclampsia, placenta

Running title: podoplanin expression in placenta of HIV infected pre-eclampsia

Abstract

Introduction: Impaired lymphatic function is associated with pathological conditions. The presence of placental lymphatics is still under debate. The aim of this study was to examine podoplanin (PDPN) placental expression in normotensive and pre-eclampsia (PE) complicated by HIV infection.

Methods: Normotensive pregnant and pre-eclamptic women were stratified by gestational age into early (<34 weeks) and late (≥34 weeks) onset PE and further divided by HIV status. Immunostaining was performed using a monoclonal mouse anti-human PDPN antibody prior to morphometric image analysis.

Results: Podoplanin was immunolocalized in a reticular-like stroma complex within the conducting and exchange villi. Its immunoexpression was significantly up-regulated in the exchange versus conducting villi (p = 0.0001) irrespective of the pregnancy type and HIV status. Podoplanin was down-regulated in the EOPE group compared to LOPE in the exchange villi (p = 0.05). Also, PDPN was up-regulated in HIV+ *vs* HIV- groups regardless of pregnancy and villi type. Based on the HIV status, PDPN immunoexpression within conducting villi was higher in the HIV- (8.19 ± 3.37%) versus HIV+ (7.67 ± 3.23%) group albeit non-significantly (p = 0.306). Conversely regardless of pregnancy type, PDPN immunoexpression within exchange villi was different (p = 0.008) between the HIV+ and HIV- groups.

Conclusion: This novel study demonstrated that placental fluid homeostasis is maintained by a PDPN reticular-like complex within conducting and exchange villi, being up-regulated in HIV positive pregnancies. However, PDPN immunoexpression was down-regulated in the exchange villi of EOPE reflecting the distribution deficient trophoblast invasion.

Keywords: Podoplanin; HIV infection; pre-eclampsia; placenta

Running title: Podoplanin expression in placenta of HIV infected pre-eclamptics

Abbreviations: PDPN, Podoplanin; HIV-, Human Immunodeficiency Virus negative; HIV+, Human Immunodeficiency Virus positive; N-, Normotensive HIV negative; N+, Normotensive HIV positive; EOPE-, Early onset pre-eclamptic HIV negative; EOPE+, Early onset pre-eclamptic HIV positive; LOPE-, Late onset pre-eclamptic HIV negative; LOPE+, Late onset pre-eclamptic HIV positive

1. Introduction

Pre-eclampsia (PE) is a systemic syndrome, unique to human pregnancy affecting 5-12% of all pregnancies (Moodley et al., 2016; Redman et al., 2012). Its pathogenesis is still not fully understood, however the placenta and the placental bed is implicated as the source. Complex interactions at the maternal fetal interface lead to deficient placentation and remodeling of spiral arteries (Wang et al., 2011). Inadequate utero-placental perfusion leads to an ischemic placenta with resultant endothelial dysfunction. The hypoxic microenvironment creates an imbalance of circulating angiogenic [vascular endothelial growth factor (VEGF); placental growth factor (PlGF)] and anti-angiogenic factors, [soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng)] (Govender et al., 2014). In a recent study, we reported the presence of pro and anti-angiogenic factors in HIV associated PE (Govender et al., 2015). Additionally, evidence of an exaggerated expression of inflammatory markers in PE placenta are reported although the exact role of the placenta in the direct dissemination of inflammatory cytokines remains to be determined (Pinheiro et al., 2013).

As early as 1627, Ascillices discovered "milky veins", known today as lymphatic vessels mingled amongst blood capillaries (Alitalo and Carmeliet, 2002). Lymphatic vessels transport lymph-fluid, proteins and other cells that leak into tissues from the bloodstream and return it to the circulatory system (Norrmén et al., 2011). Both blood and lymphatic capillaries are lined by a monolayer of endothelial cells of mesodermal origin. The lymphatic system plays a crucial role in tissue fluid homeostasis, immune function, fat metabolism, transport of proteins and macromolecules, and affords protection against viral entry (Norrmén et al., 2011). Impaired lymphatic function is associated with pathological conditions such as lymphedema and hypertension

(Martel et al., 2013). Recently, a study by our group showed an absence of lymphatic vasculature (LYVE-1) in the chorionic villi at term (Onyangunga et al., 2016).

Podoplanin (PDPN) also known as T1A; GP36; GP40; Gp38; OTS8; T1A2; TI1A; T1A-2; AGGRUS; HT1A-1; PA2.26 is a 40 kDa O-linked sialoglycoprotein molecule that is selectively expressed in lymphatic endothelial cells (LECs) but absent in normal vasculature (Fukunaga, 2005). It is specifically recognized by a novel monoclonal antibody named D2-40, a M_r 40,000 O-linked sialoglycoprotein that reacts with a fixation-resistant epitope on lymphatic endothelium. Podoplanin is regulated by the lymphatic-specific gene Proxy-1, a master gene that controls the development of lymphatic progenitors (Kato et al., 2006). Studies have proven podoplanin/D2-40 to be a useful marker in determining lymphatic invasion (Schmid et al., 2005) and demonstrating lymphatic endothelial differentiation (Ordóñez, 2005).

The presence of placental lymphatics is still under debate (Bellini et al., 2012; Castro et al., 2011; Liu et al., 2015; Onyangunga et al., 2016). Off note, lymphatic vessels would play a role in maternal-fetal immunity by boosting survey of the maternal-fetal interface to combat viral infections. HIV-infected women are at increased risk for placental membrane inflammatory lesions (Al-Husaini, 2009) such as chorioamnionitis, placental membrane inflammation, and decidualitis (Schwartz et al., 2000). It is plausible to assume that the inflammation caused by HIV infection, may be a trigger to enhance lymphangiogenesis in the placenta. Hence the aim of this study was to examine if PDPN is expressed in term human placentae, and to compare its expression in normotensive and PE complicated by HIV infection.

2. Material and Methods

2.1 Study population

The study protocol was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE: 040/12). Post informed consent, the placenta was obtained from 180 pregnant women immediately after delivery at a large urban regional hospital. The study population consisted of normotensive pregnant (N; n=60) and pre-eclamptic (n=120) women. This group was further stratified based on the gestational age into early onset PE (EOPE; <34 weeks of gestation; n=60) and late onset PE (LOPE; >34 weeks of gestation; n=60). Each group was further

stratified by HIV status (n=30 each). Primigravida and multigravida patients with a diagnosis of PE (new onset blood pressure of ≥140/90 mmHg and at least 1+ proteinuria after 20 gestational weeks) formed the study group. All study participants were delivered by elective caesarean section.

Clinical and demographic data were recorded on a structured data form. Exclusion criteria included chronic hypertension, diabetes, intra uterine death, chorioamnionitis, autoimmune diseases, cardiovascular diseases and a history of seizures.

2.2 Immunohistochemistry

Following delivery of the placenta, a central wedge biopsy was fixed in 10% buffered formaldehyde, dehydrated and embedded in paraffin wax. Sections (3 µm) were cut on a rotary microtome (Leica RM 2135, Germany) and mounted onto coated slides (X-tra adhesive; Leica, Germany). This was followed by de-parafinization and rehydration in a descending series of ethanol.

Immunostaining was performed using the Dako Envision Flex detection system Kit (Envision + System + HRP; K800021; Dako, Denmark). Tissue sections were immersed into the preheated target retrieval solution (pH 9; Envision Flex retrieval solution; S2368) and incubated for 20 min at 97°C. The slides were then placed in a LINK Autostainer instrument and immunostained using the Envision™ Flex peroxidase blocking reagent (5 min; SM801); monoclonal mouse anti-human podoplanin antibody [ready-to-use; clone D2-40; 20 min; Dako, Germany]; Dako Flex HRP (goat secondary antibody; 20 min) followed by Envision™ Flex DAP + chromogen. Sections were counterstained with Mayer's haematoxylin. The lymph node served as a positive control. Replacement of the primary antibody with a buffer or with non-immune sera of the same IgG class served as a method control and IgG control respectively. A qualitative evaluation of PDPN immunolocalization was performed.

2.3 Morphometric image analysis of podoplanin expression

Placental villi were viewed with an Axioscope A1 microscope (Carl Zeiss, Germany). At least four fields of view per slide for villi type (i.e. conducting and exchange) were randomly selected and archived at an initial objective magnification of X20. Image capturing, processing and analysis were performed using the AxioVision Image

Analysis software (Version 4.8.3 Carl Zeiss, Germany). The percentage of PDPN immunostaining (brown chromogen) within the villi was calculated using the automeasure mode of analysis (Onyangunga et al., 2016). Specifically, each conducting villi was framed and the amount of immunostaining was expressed as percentage per frame area. However, within the exchange villi (red), the amount of label (green) was determined by a two phase threshold and expressed as a total percentage of PDPN within the frame area.

2.4 Statistical Analysis

IBM SPSS Statistics Version 23 was used to analyze the data. Descriptive statistics for continuous data is presented either by mean ± standard deviation or median (minimum-maximum). To determine the statistical difference between study groups, the Kruskal-Wallis test was used for non-parametric data. Two-way ANOVA test was used to examine the effect of HIV infection (HIV+ *vs* HIV-), pregnancy type (normotensive *vs* pre-eclampsia) and villous type (exchange *vs* conducting) on PDPN immunostaining. A p value of < 0.05 was considered statistically significant.

3. Results

3.1 Patient demographics

Demographics of the study population are shown in Table 1. Based on HIV status, a significant difference in maternal age and birth weight was noted in the EOPE group only ($p = 0.001$ and $p = 0.05$ respectively).

3.2 Podoplanin immunoexpression in placental tissue

Irrespective of the HIV status, villi type and pregnancy type (Normotensive, EOPE and LOPE), PDPN was strongly immunostained in a reticular-like complex that traversed the mesenchymal stroma around arteries within the conducting (stem) and exchange villi. However, it was absent within vascular endothelial cells of both conducting and exchange (intermediate and terminal) villi (Fig. 1 and 2). The syncytiotrophoblast and cytotrophoblast cells population showed no immunostaining (Fig. 2a). Macrophage-like cells or Hofbauer cells were immunostained within the fetal and maternal supply. Positive control displayed PDPN immunostaining whilst buffer and method controls produced no staining.

3.3 Morphometric image analysis of podoplanin immunostaining

3.3.1 Pregnancy types (Normotensive vs EOPE vs LOPE)

Regardless of HIV status and villi type, the immunoexpression of PDPN was higher in the normotensive compared to the pre-eclamptic group. Moreover, the percentage immunoreactivity of PDPN was statistically different between the EOPE *vs* LOPE (15.28 ± 3.46% *vs* 16.93 ± 4.74%; $p = 0.05$).

Within the conducting villi, normotensive negative (N-) presented the highest PDPN immunoreactivity across all the groups. A significant difference in the percentage of PDPN immunoexpression was noted in the N- *vs* EOPE+ ($p = 0.009$) and EOPE+ *vs* LOPE+ ($p = 0.04$) groups only. There was an increase in PDPN immunoexpression in the EOPE+ *vs* LOPE+ (Table 2, Fig 3a).

In the exchange villi, the highest percentage of PDPN immunoreactivity was observed in the normotensive positive (N+) group. There was a significant increase of PDPN immunoexpression in the N- *vs* N+ and EOPE+ *vs* LOPE+ ($p = 0.003$ and $p = 0.0001$ respectively). The PDPN immunoexpression differed significantly amongst all the groups with exception of N- *vs* EOPE- ($p = 0.30$), N- *vs* LOPE- ($p = 0.312$) and N+ *vs* LOPE+ ($p = 0.70$) which also were not significantly different in the conducting villi (Table 2, Fig 3b).

3.3.2 HIV status (HIV- vs HIV+)

Based on the HIV status, PDPN immunoexpression within conducting villi was higher in the HIV- (8.19 ± 3.37%) compared to the HIV+ (7.67 ± 3.23%) group albeit non significantly ($p = 0.306$) (Fig.4a). Conversely regardless of the pregnancy type the PDPN immunoexpression within exchange villi, was significantly different ($p = 0.008$) between the HIV+ and HIV- groups (Fig.4b).

3.3.3 Villi types (exchange vs conducting)

The percentage of PDPN immunexpression within the conducting and exchange villi with regards to the pregnancy type (Normotensive, EOPE and LOPE) and HIV status is shown in Fig. 5. The immunoexpression of PDPN was significantly up-regulated in the exchange compared to conducting villi ($p = 0.0001$) irrespective of the pregnancy type and HIV status (Fig. 6). Based on pregnancy type (normotensive and pre-

eclamptic), PDPN expression was significant in the exchange ($p = 0.032$) and non-significant in the conducting ($p = 0.396$) villi.

Exchange villi: The immunoexpression of PDPN was significantly elevated between the EOPE and LOPE (15.29 ± 4.27% *vs* 16.94 ± 4.74%; $p = 0.05$). Although PDPN was high in the normotensive, it was not significantly different compared to the LOPE group ($p = 0.352$). The PDPN immunoexpression was the lowest within the EOPE group and differed with the normotensive group ($p = 0.007$).

Conducting villi: Across pregnancy types (Normotensive *vs* EOPE *vs* LOPE), PDPN immunoexpression within the conducting villi were not significantly different (N *vs* EOPE-, $p = 0.145$; N *vs* LOPE-, $p = 0.968$; EOPE *vs* LOPE-, $p = 0.186$).

4. Discussion

This study demonstrates an intense reticular-like network of PDPN immunostaining within the mesenchymal stroma of conducting and exchange placental villi. Regardless of HIV status and villi type, the immunoexpression of PDPN was reduced in PE compared to the normotensive group ($p < 0.03$). Our findings are similar to that of Wang et al. (2011) who reported a strong expression of PDPN within villous stroma throughout gestation albeit with a significant reduction in PE compared to normotensive placentae. In contrast, an up-regulation of PDPN was reported in inflammatory conditions; PE is a heightened inflammatory state (Astarita et al., 2015). We propose that the PDPN rich stromal network within the placental villi may serve as a lymphatic conductive network that maintains interstitial fluid balance in pregnancy.

It is plausible that the complex reticular-like network of stromal PDPN as observed in our study may be derived from its phylogenetic and progenitor source. These mesenchymal stromal cells are multipotent cells that may differentiate into neurogenic, chondrogenic, osteogenic, adipogenic, and myogenic lineage (Portmann-Lanz et al., 2006). We observed that PDPN is expressed in stromal fibroblasts and myofibroblasts. This may be associated with lymphangiogenesis especially as PDPN is implicated in the lymphatic spread of cancer cells (Kitano et al., 2010; Navarro-Núñez et al., 2013).

In our study, PDPN was absent in vascular endothelial cells. The ontogeny of lymphatic endothelial cells (LECs) is debatable. In mammals, the lymphatic endothelial system seems to be exclusively derived from the blood endothelial compartment, as the contribution of hematopoietic cells integrating into lymphatic structures is absent (Zumsteg and Christofori, 2012). In contrast, others believe that LECs may have a dual origin emanating either from embryonic veins or lymphangioblasts (Barozzi et al., 2003).

Podoplanin may play a fluid flux with different phenotypes in perivascular cells and stroma of the placenta (Hultgård-Ekwall et al., 2006). We observed strong immunolocalisation of PDPN at the perivascular zone of placenta villi. Our observations are in keeping with the findings of Bellini et al. (2012). Mesenchymal stem cells may also differentiate into perivascular cells, the precursor of capillary endothelial cells. Fetal blood vessels of stem villi are enclosed by perivascular tissue sheaths, which have abundant α-actin (Graf et al., 1995).

In our study, the immunoexpression of PDPN was reduced in PE compared to normotensive pregnancies, irrespective of HIV status and villi type. It is well established that the placenta stimulates the production of Th2 cytokines (Wegmann et al., 1993). In normal pregnancy there is a shift from the Th1 to a Th2 immune response, however this shift is absent in PE (Laresgoiti-Servitje et al., 2010). Podoplanin is expressed on the surface of the pro-inflammatory T cell, Th17 cells (Peters et al., 2011) and participate in pregnancy-related pathologies, such as PE and recurrent spontaneous miscarriage (Lee et al., 2012; Saito, 2010).

In acute inflammation, PDPN has an inhibitory function on T cell response where it controls Th17 response by reducing sensitivity to IL-7 survival signals (Peters et al., 2015). It is plausible that PDPN serves to shut-off Th17 response thereby limiting the pathogenicity of these cells. This action may be mediated via the interaction of PDPN with its 3 ligands viz., CLEC-2, galectin-8 and the chemokine CCL21 (Cueni and Detmar, 2009; Kerjaschki et al., 2004; Suzuki-Inoue et al., 2007). During the acute inflammatory state such as PE, PDPN probably binds to either CLEC-2 or galectin-8 thereby reducing CD4 effector T cells. In the chronic state of HIV infection, PDPN interacts with CCL21, thus may promote chronic inflammation (Peters et al., 2015). In our study, PDPN was up-regulated in HIV+ *vs* HIV- groups regardless of

pregnancy and villi type. This may be due to the fact that the immune insufficiency of HIV infection is inadequate to neutralize the exaggerated immune response in PE. Alternatively, one should take cognizance of the fact that all HIV patients in our study received ART, the duration and level of CD4 T cell count were however, not available.

Podoplanin can be integrated into the HIV-1 envelope thereby influencing HIV-1 infection by interacting with its ligand CLEC-2, an HIV attachment factor on platelets (Chaipan et al., 2010). The interaction of HIV with CLEC-2 on platelets might induce platelet activation and thrombocytopaenia which was found to be associated with HIV infection thus has the potential to control viral expansion (Gramberg et al., 2008; Scaradavou, 2002; Shen and Frenkel, 2004).

Early and late PE is thought to reflect different disease conditions with the former being associated with adverse maternal and neonatal outcomes (Valensise et al., 2008). In our study, regardless of HIV status, PDPN was down-regulated in the EOPE group compared to the LOPE in the exchange villi. This distribution may reflect the deficient trophoblast invasion that occurs in the EOPE group (Valensise et al., 2008). Notably, PDPN triggers epithelial-mesenchymal transition related to the stimulation of RhoA and rise in cell activation, migration and invasiveness (Martín-Villar et al., 2006). The exchange villi constitute the site of most feto-maternal exchanges and are comparable to alveolar t-1 cells in the lungs were PDPN has similar role to the one in the placenta (Ramirez et al., 2003). Exchange villi are directly bathed in maternal blood hence diffusion occurs directly across this barrier affecting interstitial fluid accumulation.

Whilst the presence of placental lymphatics is still under debate, we report an absence of regular lymphatic vessels. The presence of a reticular-lymphatic-like conductive network within the placental stroma suggests that this system may be involved in placental fluid homeostasis. We demonstrated an up-regulation of PDPN in HIV positive pregnancies irrespective of the pregnancy type and villi type. However, PDPN immunoexpression was down-regulated in the exchange villi of EOPE reflecting deficient trophoblast invasion. The dual paradigm of the effect of PDPN on T cell response in HIV infection and pregnancy pathologies differ hence the functional role of PDPN *via* its ligand interaction requires further investigation.

Acknowledgements

The authors wish to thank Dr DA Ofusori and Ms DA Margolis for technical assistance. Financial support was obtained from College of Health Sciences, UKZN; NRF and the Medical Education Programme Initiative.

References

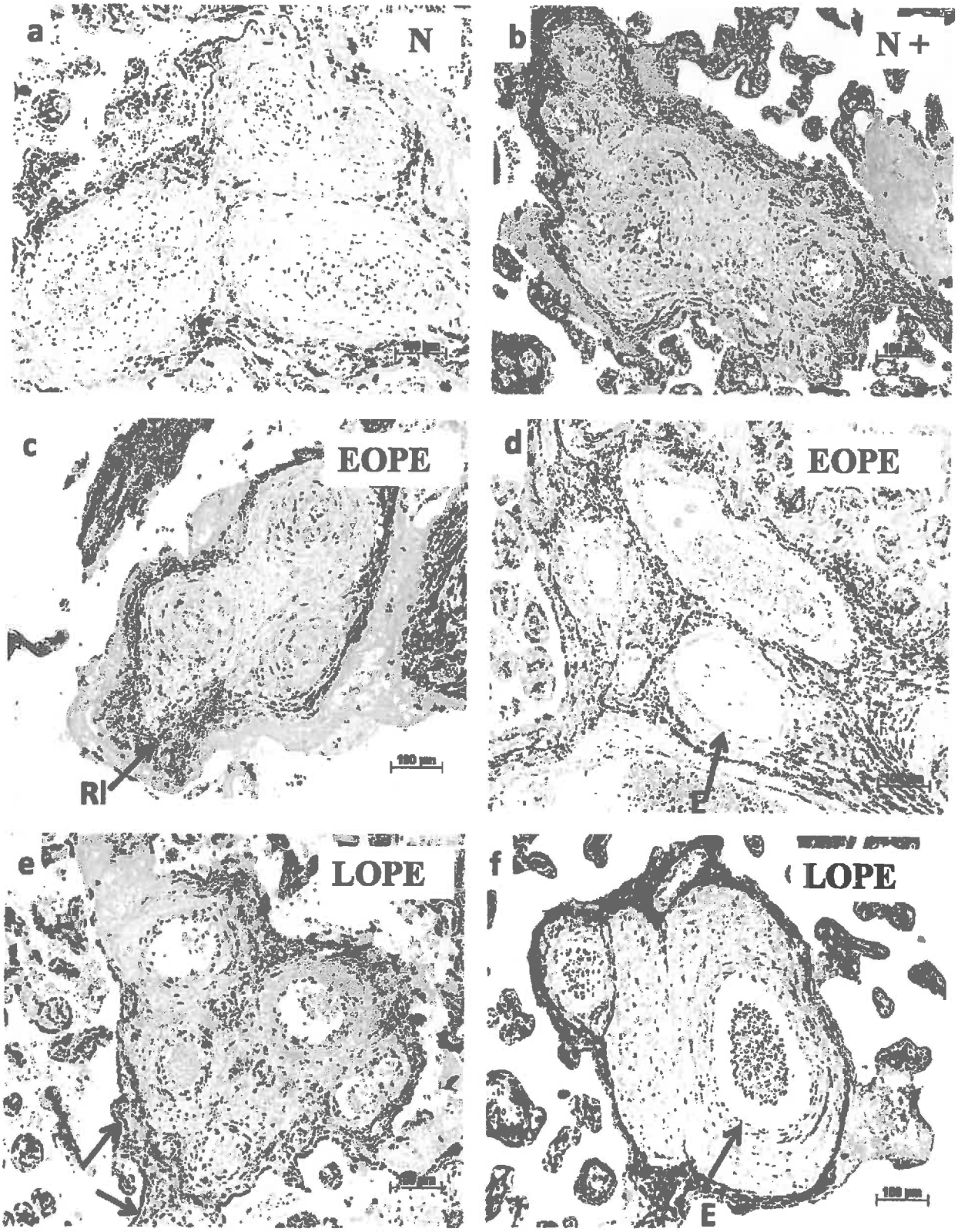
1. Al-Husaini, A., 2009. Role of placenta in the vertical transmission of human immunodeficiency virus. J Perinatol. 29, 331-336.
2. Alitalo, K., Carmeliet, P., 2002. Molecular mechanisms of lymphangiogenesis in health and disease. Cancer Cell, 1, 219-227.
3. Astarita, J.L., Cremasco, V., Fu, J., Darnell, M.C., Peck, J.R., Nieves-Bonilla, J.M., Song, K., Kondo, Y., Woodruff, M.C., Gogineni, A., 2015. The CLEC-2-podoplanin axis controls the contractility of fibroblastic reticular cells and lymph node microarchitecture. Nature immunol. 16, 75-84.
4. Barozzi, P., Luppi, M., Facchetti, F., Mecucci, C., Alù, M., Sarid, R., Rasini, V., Ravazzini, L., Rossi, E., Festa, S., 2003. Post-transplant Kaposi sarcoma originates from the seeding of donor-derived progenitors. Nat. med. 9, 554-561.
5. Bellini, C., Rutigliani, M., Boccardo, F., Campisi, C., Bellini, T., Bonioli, E., Fulcheri, E., 2012. Are there lymphatic vessels in the placenta? Lymphology 45, 34-36.
6. Castro, E., Parks, W.T., Galambos, C., 2011. Neither normal nor diseased placentas contain lymphatic vessels. Placenta, 32, 310-316.
7. Chaipan, C., Steffen, I., Tsegaye, T.S., Bertram, S., Glowacka, I., Kato, Y., Schmökel, J., Münch, J., Simmons, G., Gerardy-Schahn, R., 2010. Incorporation of podoplanin into HIV released from HEK-293T cells, but not PBMC, is required for efficient binding to the attachment factor CLEC-2. Retrovirology, 7, 1.
8. Cueni, L.N., Detmar, M., 2009. Galectin-8 interacts with podoplanin and modulates lymphatic endothelial cell functions. Exp. Cell Res. 315, 1715-1723.
9. Fukunaga, M., 2005. Expression of D2-40 in lymphatic endothelium of normal tissues and in vascular tumours. Histopathol. 46, 396-402.

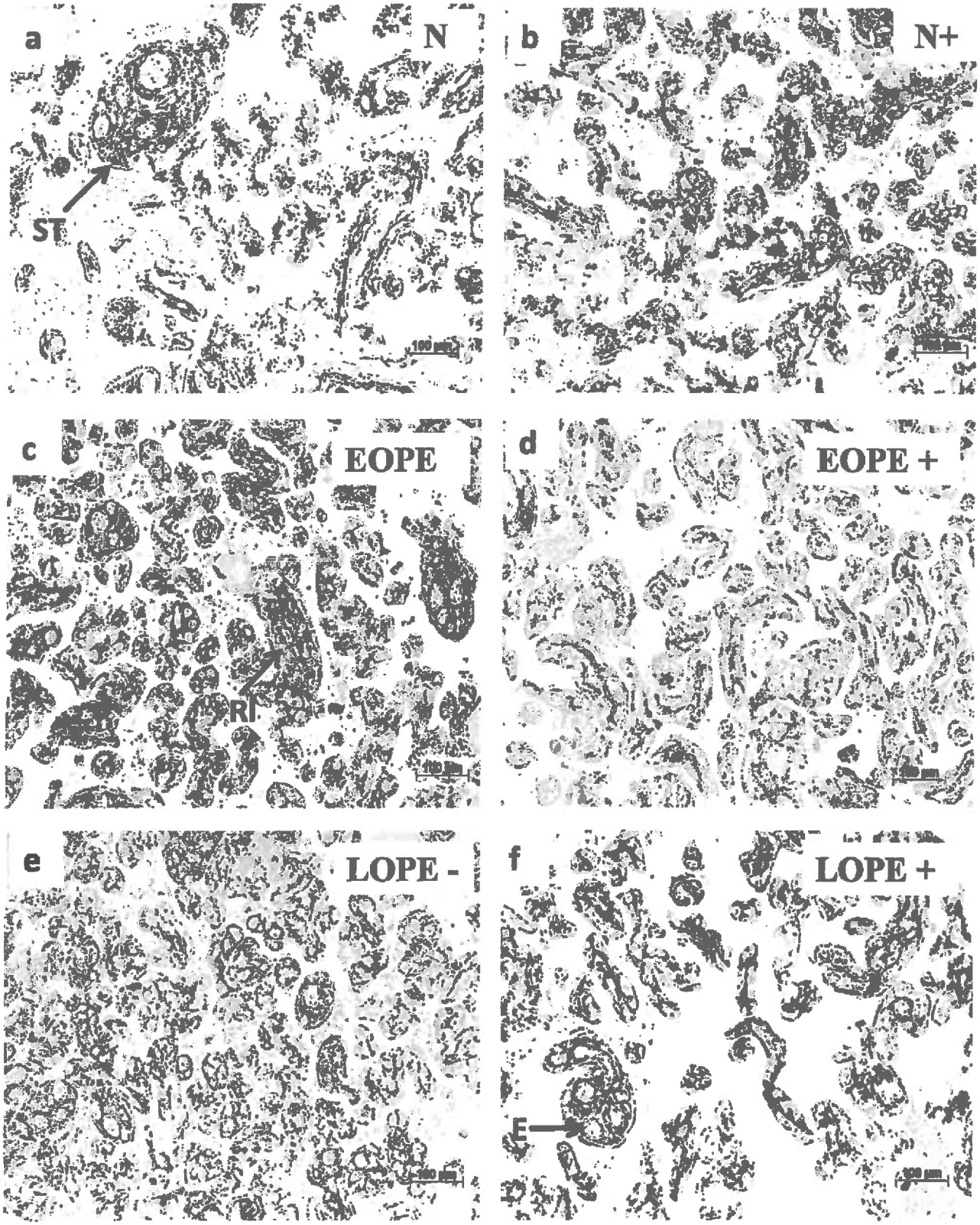
10. Govender, N., Moodley, J., Gathiram, P., Naicker, T., 2014. Soluble fms-like tyrosine kinase-1 in HIV infected pre-eclamptic South African Black women. Placenta 35, 618-624.
11. Govender, N., Naicker, T., Moodley, J., 2015. Endoglin in HIV-associated preeclamptic placentae. Hypertens. Pregnancy 34, 342-354.
12. Graf, R., Schönfelder, G., Mühlberger, M., Gutsmann, M., 1995. The perivascular contractile sheath of human placental stem villi: its isolation and characterization. Placenta 16, 57-66.
13. Gramberg, T., Soilleux, E., Fisch, T., Lalor, P.F., Hofmann, H., Wheeldon, S., Cotterill, A., Wegele, A., Winkler, T., Adams, D.H., 2008. Interactions of L-sectin and DC-SIGN/DC-SIGNR with viral ligands: Differential pH dependence, internalization and virion binding. Virology 373, 189-201.
14. Hultgård-Ekwall, A.-K., Mayerl, C., Rubin, K., Wick, G., Rask-Andersen, H., 2006. An interstitial network of podoplanin-expressing cells in the human endolymphatic duct. J Assoc. Res. Otolaryngol. 7, 38-47.
15. Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T., Ishii, K.J., 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441, 101-105.
16. Kerjaschki, D., Regele, H.M., Moosberger, I., Nagy-Bojarski, K., Watschinger, B., Soleiman, A., Birner, P., Krieger, S., Hovorka, A., Silberhumer, G., 2004. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. J Am Soc Nephrol. 15, 603-612.
17. Kitano, H., Kageyama, S.-I., Hewitt, S.M., Hayashi, R., Doki, Y., Ozaki, Y., Fujino, S., Takikita, M., Kubo, H., Fukuoka, J., 2010. Podoplanin expression in cancerous stroma induces lymphangiogenesis and predicts lymphatic spread and patient survival. Arch. Pathol. Lab. Med. 134, 1520-1527.
18. Laresgoiti-Servitje, E., Gómez-López, N., Olson, D.M., 2010. An immunological insight into the origins of pre-eclampsia. Hum Reprod. Update 16, 510-524.
19. Lee, S.K., Kim, J.Y., Lee, M., Gilman-Sachs, A., Kwak-Kim, J., 2012. Th17 and regulatory T cells in women with recurrent pregnancy loss. Am. J. Reprod. Immunol. 67, 311-318.

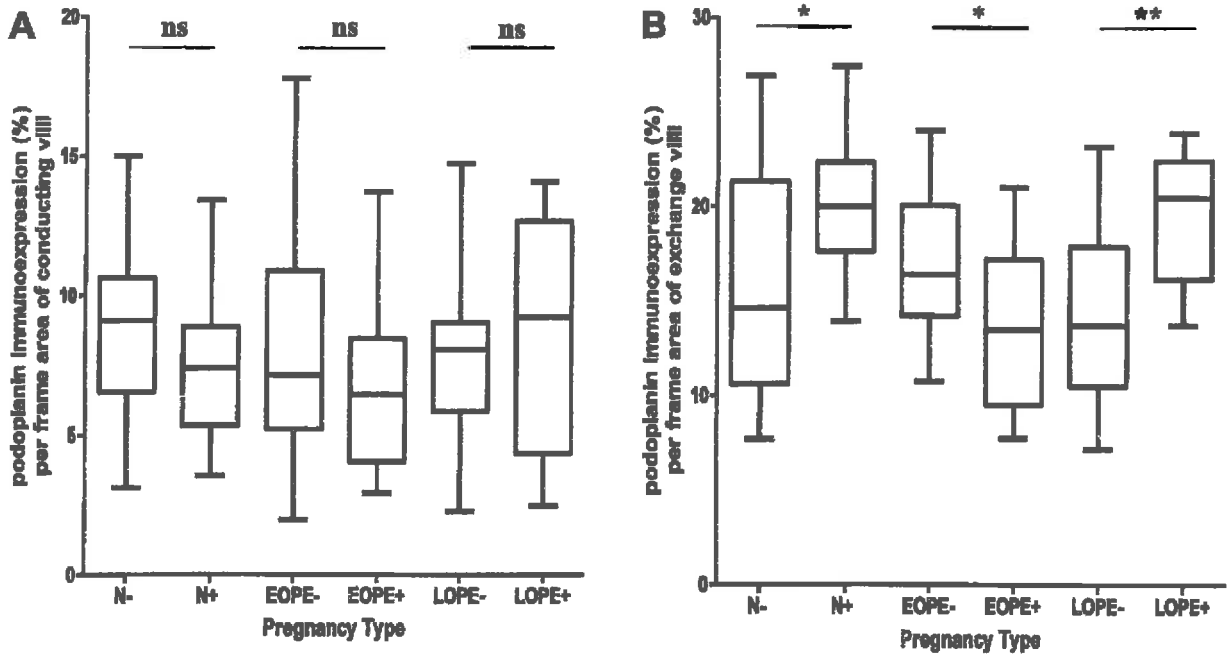
20. Liu, H., Li, Y., Zhang, J., Rao, M., Liang, H., Liu, G., 2015. The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia. Placenta 36, 279-286.
21. Martel, C., Li, W., Fulp, B., Platt, A.M., Gautier, E.L., Westerterp, M., Bittman, R., Tall, A.R., Chen, S.-H., Thomas, M.J., 2013. Lymphatic vasculature mediates macrophage reverse cholesterol transport in mice. J Clin Invest. 123, 1571-1579.
22. Martín-Villar, E., Megías, D., Castel, S., Yurrita, M.M., Vilaró, S., Quintanilla, M., 2006. Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. J Cell Sci. 119, 4541-4553.
23. Moodley, J., Onyangunga, O.A., Maharaj, N.R., 2016. Hypertensive disorders in primigravidas black South African women. A one year descriptive analysis. Hypertens. Pregnancy 8, 1-7.
24. Navarro-Núñez, L., Langan, S.A., Nash, G.B., Watson, S.P., 2013. The physiological and pathophysiological roles of platelet CLEC-2. Thromb. Haemost. 109, 991-998.
25. Normén, C., Tammela, T., Petrova, T.V., Alitalo, K., 2011. Biological basis of therapeutic lymphangiogenesis. Circulation 123, 1335-1351.
26. Onyangunga, O.A., Moodley, J., Merhar, V., Ofusori, D.A., Naicker, T., 2016. Lymphatic vascular endothelial hyaluronan receptor-1 immunoexpression in placenta of HIV infected pre-eclamptic women. J Reprod. Immunol. 117, 81-88.
27. Ordóñez, N.G., 2005. D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. Hum. Pathol. 36, 372-380.
28. Peters, A., Burkett, P.R., Sobel, R.A., Buckley, C.D., Watson, S.P., Bettelli, E., Kuchroo, V.K., 2015. Podoplanin negatively regulates CD4+ effector T cell responses. J Clin. Invest. 125, 129-140.
29. Peters, A., Lee, Y., Kuchroo, V.K., 2011. The many faces of Th17 cells. Curr. Opin. Immunol. 23, 702-706.
30. Pinheiro, M.B., Martins-Filho, O.A., Mota, A.P.L., Alpoim, P.N., Godoi, L.C., Silveira, A.C.O., Teixeira-Carvalho, A., Gomes, K.B., Dusse, L.M., 2013. Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state. Cytokine 62, 165-173.

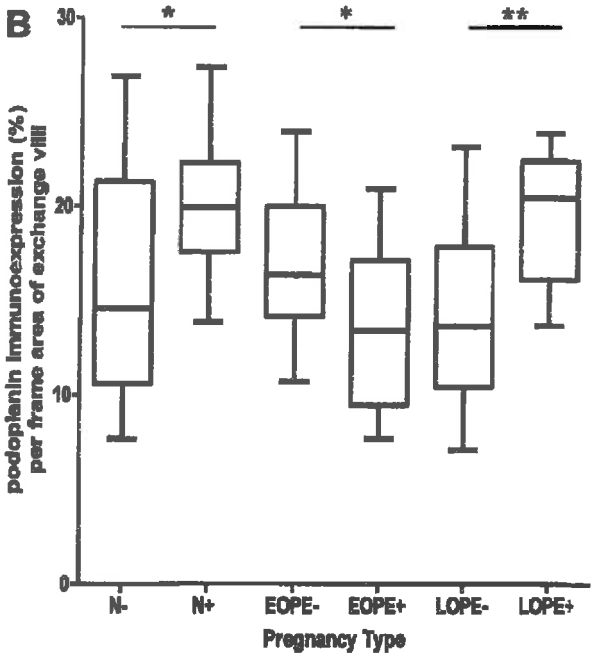
31. Portmann-Lanz, C.B., Schoeberlein, A., Huber, A., Sager, R., Malek, A., Holzgreve, W., Surbek, D.V., 2006. Placental mesenchymal stem cells as potential autologous graft for pre-and perinatal neuroregeneration. Am J Obs. Gynecol. 194, 664-673.
32. Ramirez, M.I., Millien, G., Hinds, A., Cao, Y., Seldin, D.C., Williams, M.C., 2003. T1α, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. Develop. Biol. 256, 62-73.
33. Redman, C., Tannetta, D., Southcombe, J., Dragovic, B., Gardiner, C., Collett, G., Sargent, I., 2012. The dangerous placenta and pre-eclampsia, A Review. Placenta 33, A12-A12.
34. Saito, S., 2010. Th17 cells and regulatory T cells: new light on pathophysiology of preeclampsia. Immunol. Cell Biol. 88, 615.
35. Scaradavou, A., 2002. HIV-related thrombocytopenia. Blood reviews, 16, 73-76.
36. Schmid, K., Birner, P., Gravenhorst, V., End, A., Geleff, S., 2005. Prognostic value of lymphatic and blood vessel invasion in neuroendocrine tumors of the lung. Am. J. Surgic. Pathol. 29, 324-328.
37. Schwartz, D.A., Sungkarat, S., Shaffer, N., Laosakkitiboran, J., Supapol, W., Charoenpanich, P., Chuangsuwanich, T., Mastro, T.D., 2000. Placental abnormalities associated with human immunodeficiency virus type 1 infection and perinatal transmission in Bangkok, Thailand. J. Infect. Dis. 182, 1652-1657.
38. Shen, Y.-M.P., Frenkel, E.P., 2004. Thrombosis and a hypercoagulable state in HIV-infected patients. Clin. Appl. Thromb. Hemost. 10, 277-280.
39. Suzuki-Inoue, K., Kato, Y., Inoue, O., Kaneko, M.K., Mishima, K., Yatomi, Y., Yamazaki, Y., Narimatsu, H., Ozaki, Y., 2007. Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. J. Biol. Chem. 282, 25993-26001.
40. Valensise, H., Vasapollo, B., Gagliardi, G., Novelli, G.P., 2008. Early and late preeclampsia two different maternal hemodynamic states in the latent phase of the disease. Hypertension 52, 873-880.
41. Wang, Y., Sun, J., Gu, Y., Zhao, S., Groome, L.J., Alexander, J.S., 2011. D2-40/podoplanin expression in the human placenta. Placenta 32, 27-32.

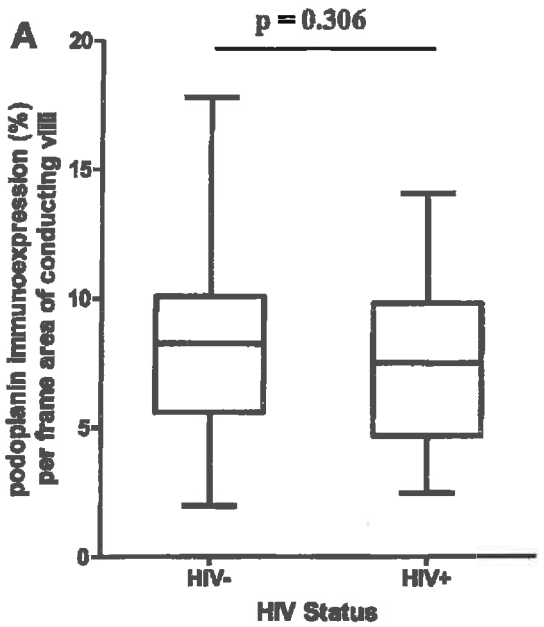
42. Wegmann, T.G., Lin, H., Guilbert, L., Mosmann, T.R., 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a T H 2 phenomenon? Immunol. Today 14, 353-356.
43. Zumsteg, A., Christofori, G., 2012. Myeloid cells and lymphangiogenesis. *Cold Spring Harb. Perspect. Med.* 2, a006494.

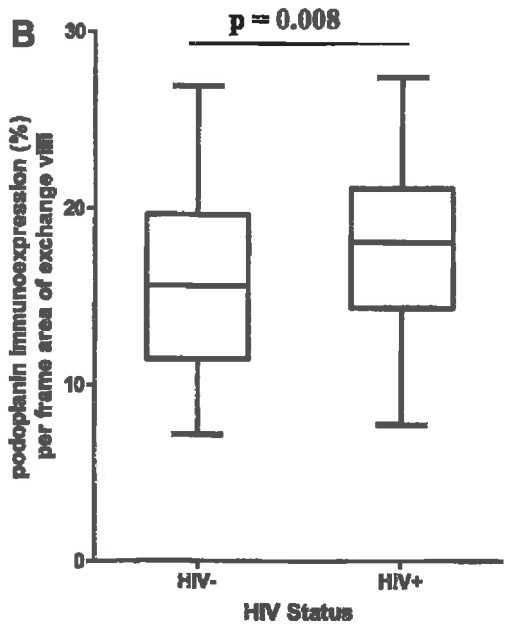

a
N
b
N+
c
EOPE
Rl
100 µm
d
EOPE
E
e
LOPE
f
LOPE
E
100 µm

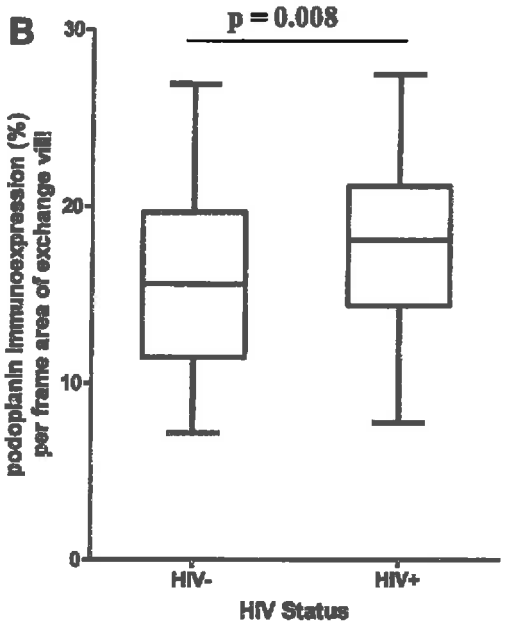

a
N
ST
b
N+
c
EOPE
RI
d
EOPE +
e
LOPE -
f
LOPE +
E

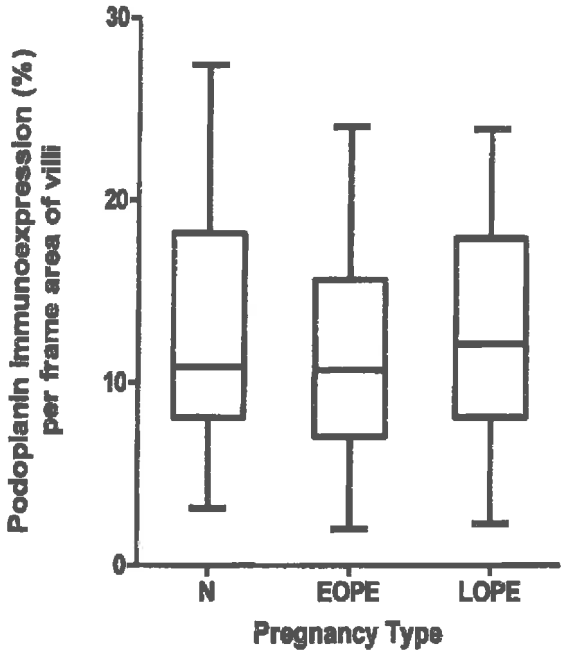

A
ns
ns
ns
podoplanin immunoexpression (%) per frame area of conducting villi
20
15
10
5
0
N-
N+
EOPE-
EOPE+
LOPE-
LOPE+
Pregnancy Type
B
*
*
**
podoplanin immunoexpression (%) per frame area of exchange villi
30
20
10
0
N-
N+
EOPE-
EOPE+
LOPE-
LOPE+
Pregnancy Type

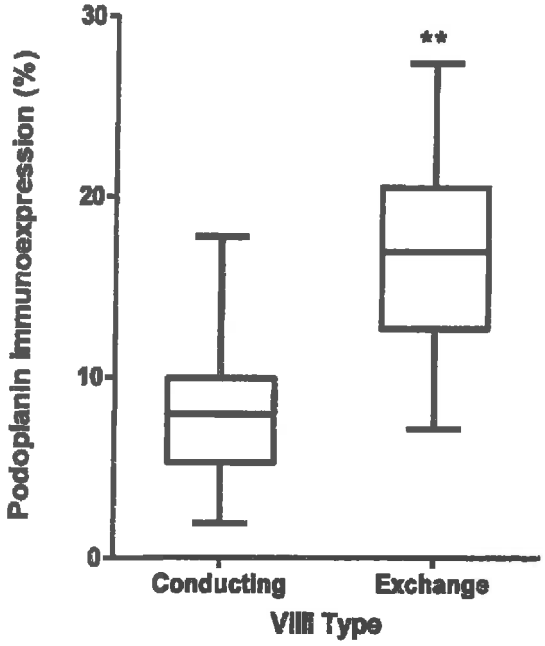

B
30
20
10
0
*
*
**
podoplanin immunoexpression (%)
per frame area of exchange villi
N-
N+
EOPE-
EOPE+
LOPE-
LOPE+
Pregnancy Type

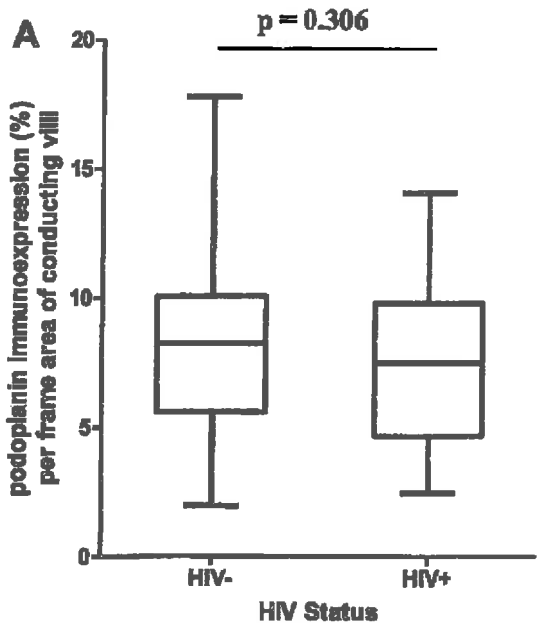

A
p = 0.306
podoplanin immunoexpression (%)
per frame area of conducting villi
20
15
10
5
0
HIV-
HIV+
HIV Status

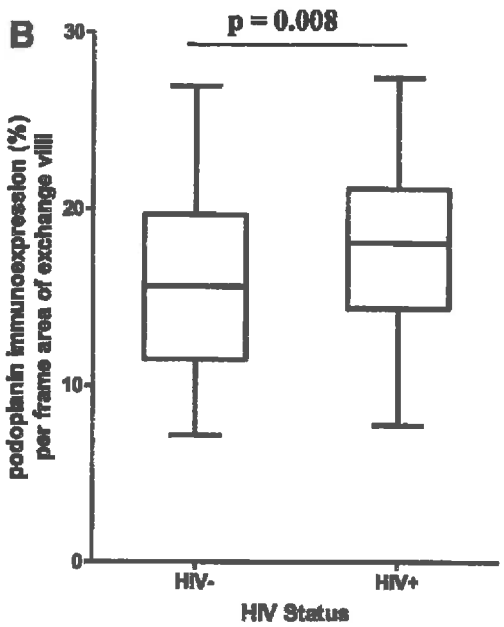

B
p = 0.008
podoplanin immunoexpression (%)
per frame area of exchange villi
30
20
10
0
HIV-
HIV+
HIV Status

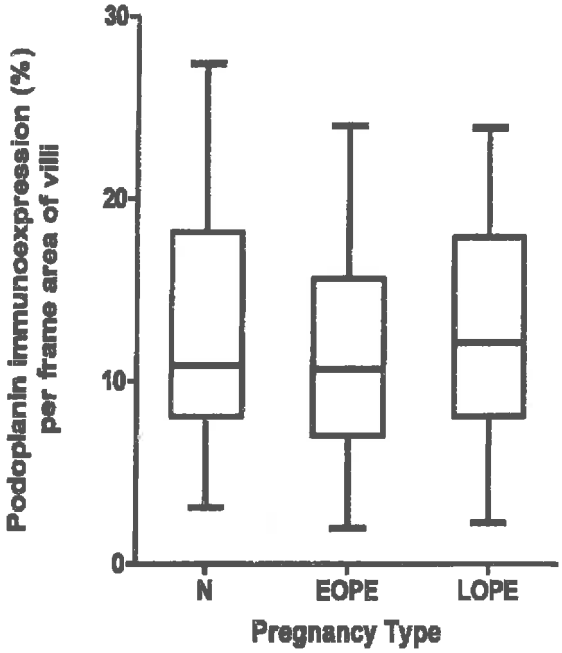

B
p = 0.008
podoplanin immunoexpression (%)
per frame area of exchange villi
30
20
10
0
HIV-
HIV+
HIV Status


Podoplanin immunoexpression (%)
per frame area of villi
30
20
10
0
N
EOPE
LOPE
Pregnancy Type


30
20
10
0
**
Podoplanin Immunoexpression (%)
Conducting
Exchange
VIII Type


A
p = 0.306
podoplanin immunoexpression (%)
per frame area of conducting villi
20
15
10
5
0
HIV-
HIV+
HIV Status


B
p = 0.008
podoplanin immunoexpression (%)
per frame area of exchange villi
30
20
10
0
HIV-
HIV+
HIV Status


Podoplanin immunoexpression (%)
per frame area of villi
30
20
10
0
N
EOPE
LOPE
Pregnancy Type

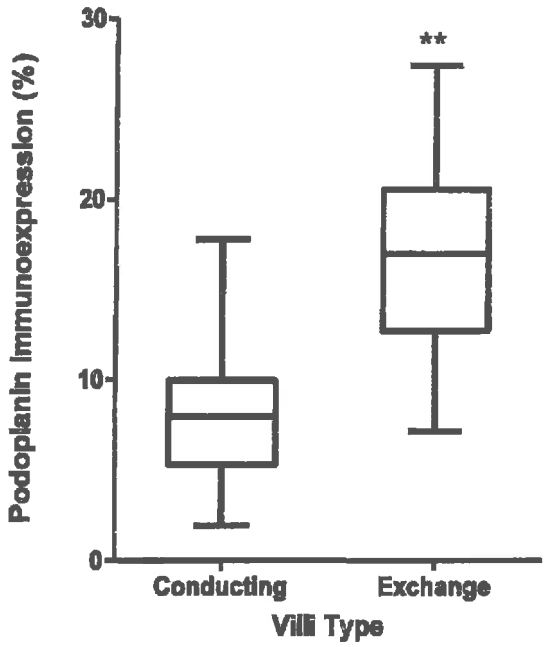

30
20
10
0
**
Podoplanin Immunoexpression (%)
Conducting
Exchange
Villi Type

FIGURE CAPTIONS

Fig. 1. Podolanin immunostained conducting villi across study groups; Rlc- Reticular-like complex; E- Endothelial cells; ST- Syncytrophoboblast.

Fig. 2. Podoplanin immunostained exchange villi across study groups; Rlc- Reticular-like complex; E- Endothelial cells; ST- Syncytrophoboblast.

Fig. 3. Podoplanin immunoexpression in conducting (A) and exchange villi (B) across all study groups (n= 30 per category). *$p<0.05$; ns= non-significant.

Fig.4. Podoplanin immunoexpression according to HIV status (N-, EOPE-, LOPE-; n=90 and N+, EOPE+, LOPE+; n=90) within conducting (A) and exchange (B) villi.

Fig.5. Podoplanin immunoexpression based on the pregnancy type (N, EOPE and LOPE) within conducting and exchange villi.

Fig.6. Podoplanin immunoexpression within conducting (n=180) and exchange (n=180) villi across of study group

Fig. S1. Composite image of Podoplanin immunostained placental villi of normotensive positive stem (SV), intermediate (IV) and terminal (TV) villi, artery (arrow); initial magnification x5.

Parameters	Normotensive		EOPE		LOPE	
	HIV- (n=30)	HIV+ (n=30)	HIV- (n=30)	HIV+ (n=30)	HIV- (n=30)	HIV+ (n=30)
Maternal Age (years)	26.5 ± 6.4	27.7 ± 5.5	25.2 ± 6.5**	31.8 ± 5.9**	24.2 ± 6.4	26.7 ± 4.6
Gestational Age (wks)	38.5 ±1.1	38.7 ± 0.7	32.9 ± 2.6*	33 ± 3.60*	37.1 ± 6.1	37.4 ±1.2
Baby Weight (g)	3346 ± 321	3255 ± 396	2187 ± 799*	2083 ± 852*	3078 ± 457*	2962 ± 277*
Baby Sex (M:F)	14:16	14:16	14:16	12:18	15:15	20:10

*$p < 0.05$; **$p=0.001$; wks- weeks

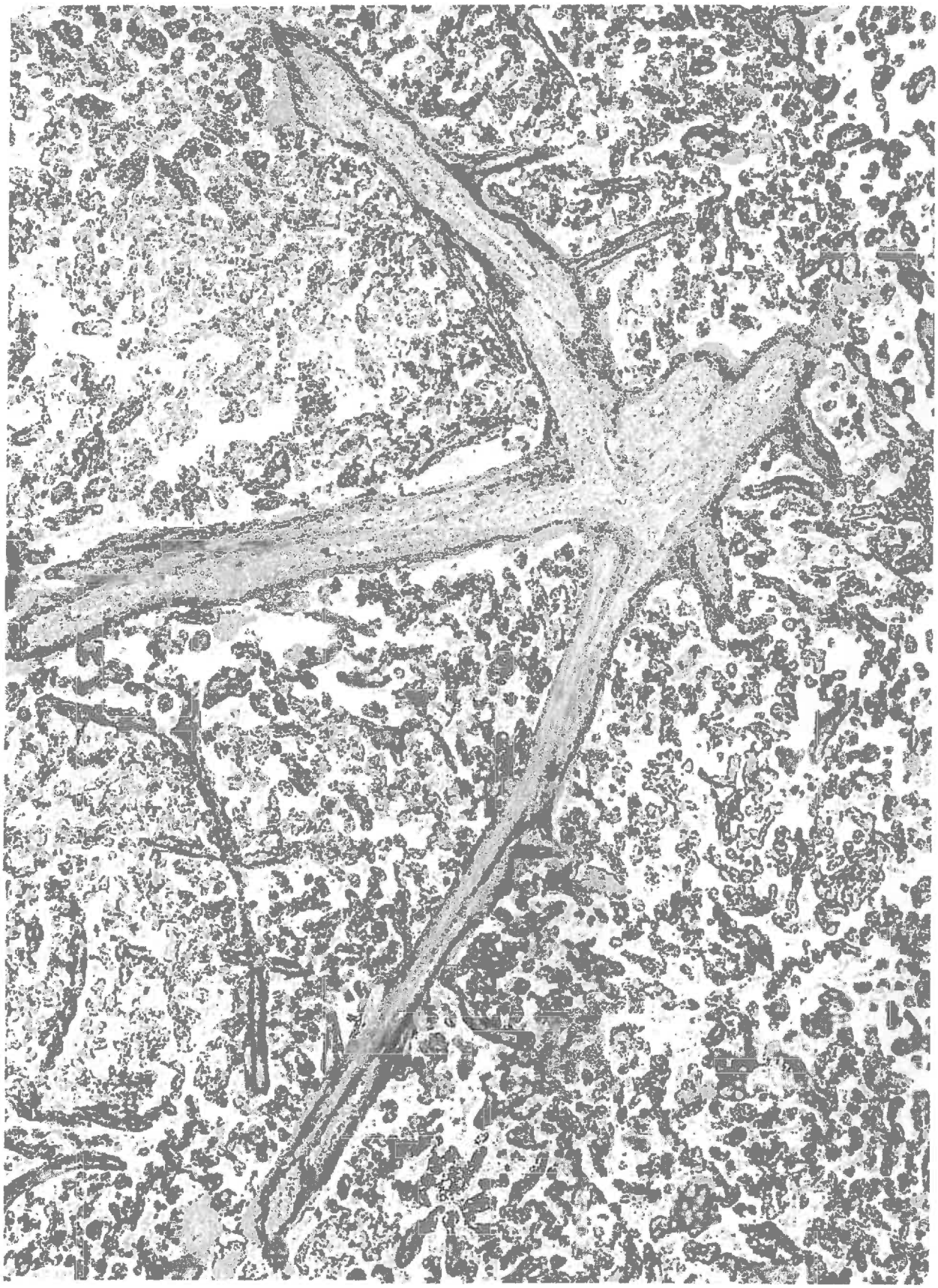
Villi Type	Normotensive		EOPE		LOPE	
	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-
Conducting	7.55 ± 2.59	8.92 ± 3.18*	6.77 ± 2.78	7.92 ± 4.00	8.69 ± 3.97	7.74 ± 2.84
Exchange	20.02 ± 4.02*	15.69 ± 6.19	13.47 ± 4.10	17.11 ± 3.66**	19.64 ± 3.2**	14.23 ± 4.50

Significance: *$p < 0.05$; **$p \leq 0.001$; $n = 180$; parametric data expressed as mean ± SD.

LIST OF TABLES

Table 1. Patient demographics across study groups

Table 2. Immunoreactivity of podoplanin in conducting and exchange villi across the groups



HIGHLIGHTS

- Absence of lymphatic vessels within the placenta.
- Podoplanin expression is up-regulated in HIV positive pregnancies.
- Podoplanin was immunolocalized in a reticular-like stroma complex within the placental villi and maintained fluid homeostasis.
- Podoplanin immunoexpression was down-regulated in the exchange villi of early onset pre-eclampsia.

Its immunoexpression was significantly up-regulated in the exchange versus conducting villi (p = 0.0001) irrespective of the pregnancy type and HIV status. Podoplanin was down-regulated in the EOPE group compared to LOPE in the exchange villi (p = 0.05). PDPN was up-regulated in HIV+ *vs* HIV- groups regardless of pregnancy and villi type. Based on the HIV status, PDPN immunoexpression within conducting villi was higher in the HIV- (8.19 ± 3.37%) versus HIV+ (7.67 ± 3.23%) group albeit non-significantly (p = 0.306). Conversely regardless of pregnancy type, PDPN immunoexpression within exchange villi was different (p = 0.008) between the HIV+ and HIV- groups.

Conclusion: However,

CHAPTER 6

ABSTRACTS IN DOHET APPROVED INTERNATIONAL JOURNALS

1. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley, and Thajasvarie Naicker (2015). Analysis of Lymphatic vessel endothelial hyaluronan receptor-1 in the placental bed of HIV associated pre-eclampsia. Placenta 36(9), A56. Impact factor 2.71.

2. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley, and Thajasvarie Naicker (2015). Vascular endothelial growth factor receptor-3 in placenta of HIV associated normotensive and pre-eclamptic pregnancies. Placenta 36(9), A57. Impact factor 2.71.

3. **Onankoy Atshakala Onyangunga**, Jagidesa Moodley, and Thajasvarie Naicker (2015). Placental lymphatic vascular endothelial hyaluronan receptor-1 in HIV associated pre-eclampsia. Placenta 36(9), A57. Impact factor 2.71.

Volume 36, Issue 9, September 2015 ISSN 0143-4004

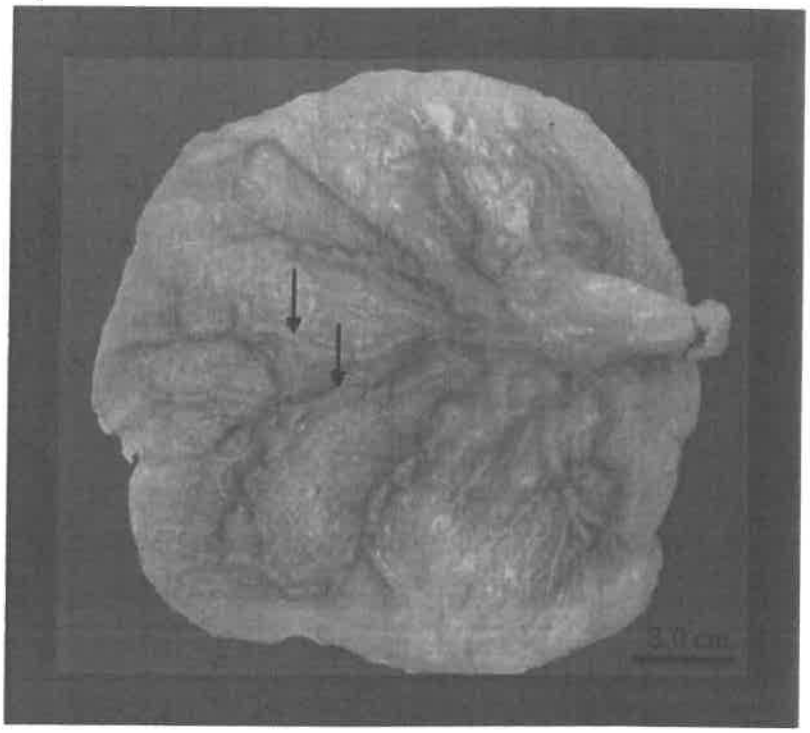
ELSEVIER

PLACENTA

THE FRONTIER BETWEEN MOTHER AND FETUS

IN THIS ISSUE:

Creation of plastinated placentas as a novel teaching resource for medical education in obstetrics and gynaecology



Karalyn E. McRae, Gregory A.L. Davies, Ronald A. Easteal, Graeme N. Smith

Available online at
ScienceDirect
www.sciencedirect.com

The Official Journal of the
International Federation of Placenta Associations
IFPA
International Federation of Placenta Associations

IFPA
INTERNATIONAL FEDERATION OF PLACENTA ASSOCIATIONS

PLACENTA

The Official Journal of the International Federation of Placenta Associations (incorporating the Australia and New Zealand Placenta Research Association, the European Placenta Group, the Japanese Placenta Association, the Placenta Association of the Americas

Editors

Graham J. Burton
Director
Centre for Trophoblast Research
Physiological Laboratory
Downing Street
Cambridge, CB2 3EG
UK

Vicki L. Clifton
NHMRC Senior research Fellow
and Associate Professor
Robinson Institute
The University of Adelaide
Lyell McEwin Hospital
Haydown Road
Elizabeth Vale
Adelaide
South Australia
5122 Australia

Yoel Sadovsky
Director
Magee-Womens Research Institute
Professor of OBGYN, Microbiology and Molecular Genetics
University of Pittsburgh
204 Craft Avenue
Pittsburgh, PA 15213
USA

Editors Emeritus

Gernot Desoye, *Austria*

D. Michael Nelson, *USA*

Trophoblast Research Editor

Gendie E. Lash
Reproductive and Vascular Biology Group
Institute of Cellular Medicine
Newcastle University
UK

Editorial Board

Vikki M. Abrahams, *USA*
Christian Albrecht, *Switzerland*
Julie Baker, *USA*
Anthony M. Carter, *Denmark*
D. Stephen Charnock-Jones, *UK*
Brian Cox, *Canada*
Gernot Desoye, *Austria*
Gordon Douglas, *USA*
Caroline Dunk, *Canada*
Jan Jaap H.M. Erwich, *The Netherlands*
Thierry Fournier, *France*
Penny Gowland, *UK*
Stefan Hansson, *Sweden*
Alicia Jawerbaum, *Argentina*
Yee Khong, *Australia*
Terence Lao, *Hong Kong*
Rohan Lewis, *UK*
Ofer Mandelboim, *Israel*
Udo Markert, *Germany*
Padma Murthi, *Australia*
Nihar Nayak, *USA*
Michal Neeman, *Israel*
Augustine Rajakumar, *USA*
John Robinson, *USA*
Henning Schneider, *Switzerland*
Neil Sebire, *UK*
Mike Soares, *USA*
Michael Stark, *Australia*
Masaomi Takayama, *Japan*
Tullia Todros, *Italy*
Yan-Ling Wang, *China*
Melissa Westwood, *UK*
Derek Wildman, *USA*
Aureo Yamada, *Brazil*

alone (n = 28), FGR alone (n = 20) or both PE/FGR combined (n = 34) compared to gestational age-matched controls (n = 23).
Results: Flt-1, VEGF, and TGF-β were mainly located on trophoblast cells, while IDO was located on endothelial cells in villous stroma. Flt-1 and TGF-β were strongly expressed in PE alone, FGR alone, and PE/FGR combined groups compared to control group. Furthermore, PE/FGR combined group showed higher Flt-1 expression compared to FGR alone group. PE alone group showed significantly lower IDO expression compared to FGR alone group. Among patients with PE, high Flt-1 expression was concerned with the existence of maternal nephropathy, but not with hypertention. There was no significant difference in VEGF expression among the groups.
Conclusion: Higher Flt-1/lower IDO expression seems to be related to PE, and these findings suggest that both angiogenic and immunosuppressive factors may play a role in pathophysiology of PE complicatedly.

P2.60.
SERUM HTRA1 IS DIFFERENTIALLY REGULATED BETWEEN EARLY-ONSET AND LATE-ONSET PREECLAMPSIA

Sonia Soo Yee Teoh[1,2], Min Zhao[3], Yao Wang[1,2], Qi Chen[3,4], Guiying Nie[1,2]. [1] *Implantation and Placental Development Laboratory, Centre for Reproductive Health, MIMR-PHI Institute of Medical Research, Clayton, Victoria, Australia;* [2] *Monash University, Clayton, Victoria, Australia;* [3] *Wuxi Maternity and Children's Health Hospital, Nanjing Medical University, Jiangsu, China;* [4] *Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand*

The High temperature requirement A (HtrA) proteases are a family of serine proteases conserved from bacteria to mammals, and are known to have important functions in protecting cells from stress conditions. There are four mammalian HtrA paralogs (HtrA1–4). HtrA1 is involved in apoptosis and anoikis, and is proposed to function as a tumour suppressor. HtrA1 has also been implicated in the development of other diseases such as arthritis, Alzheimer's disease, age-related macular degeneration, and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy.
Objectives: We have previously shown that the placenta expresses the highest level of HtrA1 compared to other tissues. HtrA1 has recently been reported to be up-regulated in preeclampsia (PE), a pregnancy-specific systemic disorder primarily involving hypertension and proteinuria, typically occurring after 20 weeks of gestation in previously normotensive women. However, it is unclear whether placental HtrA1 is secreted into the maternal circulation and whether serum HtrA1 is altered in preeclamptic pregnancies.
Methods: In this study, we developed an enzyme linked immunosorbent assay (ELISA) that is highly specific to HtrA1 and suitable for detecting serum HtrA1.
Results: Using this assay, we discovered that serum HtrA1 levels increased progressively with increasing gestation in normal pregnancies. However, this trend was perturbed in women with PE, and the perturbation was highly distinct between early-onset (occurring <34 weeks) and late-onset (>34 weeks) PE. Compared to gestation-age matched normal pregnancies, serum HtrA1 was significantly increased in early-onset PE, but significantly reduced in late-onset PE.
Conclusion: This is the first report to show a clear increase of HtrA1 in the maternal circulation during normal pregnancy, consistent with HtrA1 being highly expressed in the placenta. This study also highlights the complex regulation of HtrA1 in different subsets of PE.

P2.61.
THE ROLE OF PERIPHERAL NATURAL KILLER CELLS IN HIV ASSOCIATED PRE-ECLAMPSIA IN SOUTH AFRICA

Anushka Ajith, Jagidesa Moodley, Thajasvarie Naicker. *University of KwaZulu-Natal, Durban, South Africa*

HIV and pre-eclampsia are the leading causes of maternal morbidity and mortality in South Africa. The effect of HIV on NK cells and peripheral NK cells in pre-eclampsia is not well documented.
Objective: This study attempts to elucidate the role of peripheral NK cells in HIV associated pre-eclampsia.
Methods: Blood samples were obtained from 106 pre-eclamptic (47 HIV infected and 59 HIV uninfected) and 60 normotensive pregnant (30 HIV infected and 30 HIV uninfected) Black South Africans (> 18 years) at Prince Mshiyeni Memorial Hospital, KwaZulu-Natal. Whole blood was lysed and stained with CD3 BD Horizon V500, CD56 Pe-Cy7 and CD16 FITC. Samples were analysed on the BDLSRFortessa™ flow cytometer and the data analysed using FlojoV10. Lymphocytes were identified using FSC/SSC and the $CD3^-$ population was identified. Peripheral NK cells were further classified as $CD56^{dim}$ and $CD16^+$.
Results: Significant differences were observed for systolic and diastolic pressure, dipstick, gestational age at birth and baby weight across all cohorts, irrespective of HIV status. Differences were also noted between NK cells and peripheral NK cells across all cohorts. There was a significant increase in NK cells in the HIV positive group compared to the HIV negative group in both the normotensive and pre-eclamptic groups. However, there was a decrease in peripheral NK cells in HIV positive compared to the negative group in both the normotensive and pre-eclamptic groups.
Conclusions: NK cells are the first line of defense against pathogens. This study confirms their role as an increased accumulation of NK cells were observed in the HIV positive compared to the HIV negative group. However, this study demonstrated a significant decrease in peripheral NK cells in the HIV positive group compared to the negative group. HIV may have a mechanism preventing the cytotoxic properties of peripheral NK cells.

P2.62.
ANALYSIS OF LYMPHATIC VESSEL ENDOTHELIAL HYALURON RECEPTOR-1 IN THE PLACENTAL BED OF HIV ASSOCIATED PRE-ECLAMPSIA

Onankoy Atshakala Onyangunga, Jagidesa Moodley, Thajasvarie Naicker. *University of KwaZulu-Natal, Durban, South Africa*

The lymphatic vascular system plays a crucial role to maintain the homeostasis of tissue fluid drainage and immune-assistance in both normal and pathological conditions[1]. In pregnancy, the blood circulatory system needs the lymphatic system to collect and transfer macromolecules, lymphocytes, and protein-rich fluid back to the blood circulation. A paucity of data of lymphatic vessels in HIV associated pre-eclampsia exists.
Objective: The objective of this study was to examine the expression of lymphatic vessel endothelial hyaluron receptor-1 (LYVE-1) in the placental bed of HIV associated normotensive and pre-eclamptic pregnancies.
Methods: Placental bed biopsies were obtained from 180 women undergoing caesarean section at Prince Mshiyeni Memorial Regional Hospital. The pre-eclamptic participants were stratified according to early-onset and late-onset presentation. Pre-eclamptic and normotensive were further sub-divided according to their HIV status. H&E staining confirmed the presence of a true placental bed. Immunohistochemical staining was performed. The anti-human mouse LYVE-1 antibody (1:160) was 4–5 fold amplified using a linker and visualised with the diaminobenzidine chromogen. All samples were viewed on a Zeiss Axioscope microscope interfaced with the Axiovision image analysis software.
Results: LYVE-1 was present in all endothelial cells lining lymphatic vessels across all groups. LYVE-1 expression predominated within the decidua but was also observed within the myometrium. Endometrial glands within decidua and myometrium were not stained. Endothelial cells lining myometrial converted and unconverted spiral arteries were positive. Extravillous cytotrophoblasts were weakly stained.
Conclusion: LYVE-1 is a reliable marker for lymphatic vessels in the placental bed. Lymphatic vessels serve as important regulators of fluid homeostasis in pregnancy. The qualitative decline in expression of LYVE-1 observed in the pre-eclamptic group of our study supports the idea that LYVE-1 may play a role in the regulation of hyaluron metabolism in trophoblasts and the possibility of lymphatic-lineage specific molecules in the placental bed vasculature.

P2.63.

VASCULAR ENDOTHELIAL GROWTH FACTOR-RECEPTOR-3 IN PLACENTA OF HIV ASSOCIATED NORMOTENSIVE AND PRE-ECLAMPTIC PREGNANCIES

Onankoy Atshakala Onyangunga, Jagidesa Moodley, Thajasvarie Naicker. *University of KwaZulu-Natal, Durban, South Africa*

The last decade has seen important progress in understanding the pathogenesis of pre-eclampsia. Indeed pre-eclampsia, a human pregnancy specific condition is a result of an imbalance between angiogenic and anti-angiogenic factors[1]. Anti-angiogenic factors are highly elevated in pre-eclampsia. Pre-eclampsia is characterised by a circulating pro-lymphangiogenic state as evident by a decrease in soluble VEGF-R3, slightly decreased soluble VEGF-R2, increased VEGF-C and dramatically lower ratio of soluble VEGF-R2+R3/VEGF-C. HIV infection is a systemic infection affecting all tissues including lymphatic endothelial cells. Current knowledge of the effects of HIV associated hyper permeability is limited to disruption of the integrity of vascular structures and/or enhancement of inflammatory reactions.The co-morbidity of HIV infection and pre-eclampsia on this lymphangiogenic disparity is poorly understood.

Objective: In the present study, we investigated the expression of the cell membrane receptor tyrosine kinase VEGF-R3 in HIV associated normotensive and pre-eclamptic placenta.

Methods: We analysed VEGF-R3 in term placenta in normotensive and pre-eclamptic women from Prince Mshiyeni Memorial hospital, KwaZulu-Natal. Groups were further stratified by CD4 count and early or late presentation of elevated blood pressure. Immunocytochemistry was performed using mouse anti-human antibody (dilution1:10). Sections were viewed on the Zeiss Axioscope A1 microscope.

Results: VEGFR-3 was expressed within the invasive extravillous cell population and VEGFR-2 to the syncytiotrophoblast and cytotrophoblast cell populations in all villi types across all study groups. The endothelium lining all capillaries and large placental blood vessels were stained. The placenta was used as a positive control. The negative control showed no staining.

Conclusion: This study demonstrates a qualitative lower expression of the receptor protein levels in pre-eclampsia compared with levels in the normotensive group. However morphometric image analysis is underway to elucidate differences between groups. These findings demonstrate a dysregulation of placental expression of VEGFR-3, a novel finding in the pathological maternal co-morbidity of HIV and pre-eclampsia.

P2.64.

PLACENTAL LYMPHATIC VASCULAR ENDOTHELIAL HYALURONIC RECEPTOR-1 IN HIV ASSOCIATED PRE-ECLAMPSIA

Onankoy Atshakala Onyangunga, Jagidesa Moodley, Thajasvarie Naicker. *University of KwaZulu-Natal, Durban, South Africa*

The lymphatic vascular system serves as a conduit for interstitial fluid extravasated from blood vessels. It plays an important role in diseases resulting from over-reactive (pre-eclampsia) and under-reactive (HIV) immune responses. Lymphatic vascular endothelial hyaluronan receptor-1 (LYVE-1) is a selective marker for lymphatic endothelium and a homolog of CD44, the hyaluronan receptor.

Objective: The study aims to examine lymphangiogenesis in immunostained placenta with LYVE-1 in HIV associated normotensive versus pre-eclamptic pregnancies.

Methods: Post institutional ethics approval, term placental tissue was obtained at Prince Mshiyeni Memorial district hospital in KwaZulu-Natal. Samples were divided into normotensive (HIV+ve and HIV-ve) and pre-eclamptic (HIV+ve and HIV-ve) cohorts and further stratified according to CD4 count ≤350 or ≥350 as well as early (<34 weeks gestation) or late presentation (>34weeks gestation). Pre-eclampsia was defined as new onset hypertension (>140/90mmHg) with proteinuria. Immuno-histochemistry was performed using the Envision kit (DAKO) with the anti-human mouse LYVE-1 (1:160; R&D Systems) antibody. Slides were viewed on the Zeiss Axioscope A1 microscope interfaced with the Axiovision software for image analysis.

Results: Positive staining of LYVE-1 was present in the exchange (terminal, intermediate) and the conducting (stem) villous fetal endothelium. LYVE-1 staining was predominantly absent in the syncytiotrophoblast layer of the placenta, however, focal areas were occasionally positive. Morphometric analysis of the intensity of LYVE-1 expression is currently being performed. LYVE-1 was not found within the villous mesenchymal core. Positive controls of lymph nodes displayed staining whilst substitution of the primary antibody with a non-immune serum of the same IgG class produced no staining.

Conclusion: This study demonstrates a decline in LYVE-1 staining in the HIV+ pre-eclamptic group compared to the other groups. The presence of LYVE-1 in the trophoblasts and villous fetal endothelial cells indicate that LYVE-1 may perform special functions in the placenta beyond those described in lymphatic endothelial cells.

P2.65.

CHARACTERIZING THE HEMATOPOIETIC STEM/PROGENITOR CELLS IN THE PLACENTA AND UMBILICAL CORD BLOOD FROM NORMAL AND PREECLAMPTIC SUBJECTS

Zahra Masoumi[1], Mary Familari[2], Mattias Magnusson[3], Eva Mezey[4], Stefan Hansson[1]. [1]*Division of Obstetrics and Gynecology, Department of Clinical Sciences, Lund University Hospital, Lund University, Lund, Sweden;* [2]*Department of Zoology, University of Melbourne, Melbourne, Australia;* [3]*Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Lund, Sweden;* [4]*Adult Stem Cell Section, CSDB, NIDCR, National Institutes of Health, Bethesda, MD, USA*

Objective: Preeclampsia (PE) is a pregnancy-related syndrome affecting about 3–8% of pregnancies, causing high maternal and fetal mortality. Several groups have reported a significant increase in free fetal hemoglobin (HbF) in the placenta and maternal blood in PE. The placenta is a hematopoietic organ during development from where at a certain embryonic stage the hematopoietic stem/progenitor cells (HSPCs) migrate to the fetal liver and bone marrow. Looking for the source of increased HbF levels in PE, we studied the HSPCs in the placenta and umbilical cord blood (UCB).

Methods: HSPCs ($CD34^+$ $CD45^{low}$) were isolated from the placenta and UCB from both normal and PE pregnancies. The surface expressions of specific adhesion molecules (AMs) associated with cell adhesion and differentiation were evaluated by flow cytometry.

Two-tailed Student's T-test was used and p values ≤0.05 were considered significant.

Results: We detected significantly ($p \leq 0.05$) higher expression of CD11a (LFA-1), CD49d (VLA-4) and CD44 on HSPCs from normal UCB compared to placenta. These markers are important in cell commitment and myeloid and/or erythroid differentiation. Interestingly, levels of these AMs were not significantly different among HSPCs from placenta vs. UCB in PE. However, comparing the UCB and placenta HSPCs in both PE and normal patients indicated a significantly ($p \leq 0.001$) higher expression of CD62L (L-selectin) among the UCB HSPCs. Moreover, comparing placenta HSPCs from PE and normal patients demonstrated a trend ($p \leq 0.05$) toward higher expression of CD11a, CD44 and CD49e in PE HSPCs.

Conclusion: HSPCs from normal UCB seem to be more committed towards myeloid and/or erythroid lineages when compared to normal placenta HSPCs. Higher CD62L on both PE and normal UCB HSPCs suggest their higher engraftment capacities compared to placenta HSPCs. Comparing normal and PE placenta HSPCs demonstrates a commitment towards myeloid and/or erythroid lineages in PE.

P2.66.

EGF DECREASES SFLT1 SECRETION AND ENDOTHELIAL DYSFUNCTION: A POTENTIAL THERAPEUTIC FOR PREECLAMPSIA

Roxanne Hastie[1,2], Stephen Tong[1,2], Ping Cannon[1,2], Fiona Brownfoot[1,2], Natalie Hannan[1,2], Tu'uhevaha Kaitu'u-Lino[1,2]. [1]*University*

CHAPTER 7

MICROSCOPY SOCIETY OF SOUTHERN AFRICA

PROCEEDINGS

VOLUME 44 2014

PROTEA HOTEL, STELLENBOSCH CONFERENCE

LYMPHATIC VESSEL ENDOTHELIAL HYALURON RECEPTOR-1 IN THE PLACENTAL BED OF NORMOTENSIVE AND PRE-ECLAMPTIC PREGNANCIES

O.A. Onyangunga[1], T. Naicker[1], D.A. Ofusori[1], and J. Moodley[2]

[1]Optics & Imaging Centre, [2]Woman's Health & HIV Research Centre, University of KwaZulu-Natal, Durban

The human pregnancy is characterised by a specific remodelling of the spiral artery during implantation of the trophoblast. The lymphatic vascular system plays a crucial role to maintain the homeostasis of tissue fluid drainage and immune-assistance in both normal and pathological conditions[1]. In pregnancy, the blood circulatory system needs the lymphatic system to collect and transfer macromolecules, lymphocytes, and protein-rich fluid back to the blood circulation. New lymphatic vessels have been recently identified in the placental bed of normal and pre-eclamptic pregnancy[2-3]. A paucity of data of lymphatic vessels in HIV associated pre-eclampsia exists. Since lymphatic vessel endothelial hyaluron receptor-1 (LYVE-1) is a specific marker for lymphatic endothelial cells, a study of LYVE-1 provides new insights on the lymphatic vascular character of the placental bed that is important for pregnancy and fetal development. The objective of this novel study was to examine the expression of LYVE-1 in the placental bed of HIV associated normotensive and pre-eclamptic pregnancies.

Placental bed biopsies were obtained from 180 women undergoing caesarean section at Prince Mshiyeni Memorial Regional Hospital. The pre-eclamptic participants were stratified according to early-onset and late-onset presentation. Pre-eclamptic and normotensive were further sub-divided according to their HIV status. H&E staining confirmed the presence of a true placental bed.

Immunohistochemical staining was performed. Endogenous peroxidase was blocked and the antigen was retrieved using a retrieval kit (high pH, DAKO, Denmark). The anti-human mouse LYVE-1 antibody (1:160) was 4-5 fold amplified using a linker and visualised with the di-aminobenzidine chromogen. All samples were viewed on a Zeiss Axioscope microscope interfaced with the Axiovision image analysis (Zeiss) software.

LYVE-1 was present in all endothelial cells lining lymphatic vessels across all groups. LYVE-1 expression predominated within the decidua (Fig. 1) but was also observed within the myometrium. Endometrial glands within decidua and myometrium were negative for LYVE-1 expression. Endothelial cells lining myometrial converted and unconverted (Fig. 2) spiral arteries were positive for LYVE-1. Extravillous cytotrophoblasts were weakly stained. Morphometric analysis of LYVE-1 expression within spiral arteries is in process.

LYVE-1 is a reliable marker for lymphatic vessels in the placental bed. Lymphatic vessels serve as important regulators of fluid homeostasis in pregnancy. The qualitative decline in expression of LYVE-1 observed in the pre-eclamptic group of our study supports the idea that LYVE-1 may play a role in the regulation of hyaluron metabolism in trophoblasts and the possibility of lymphatic-lineage specific molecules in the placental bed vasculature.

References

1. Cirpan, T., *et al.* (2007) Clin Exp Obst Gynaecol 34(4), 228.
2. Volchek M., *et al.* (2010) Hum Reprod 00(0), 1.
3. Red-Horse, K., (2008) Placenta 22, s55-s59.

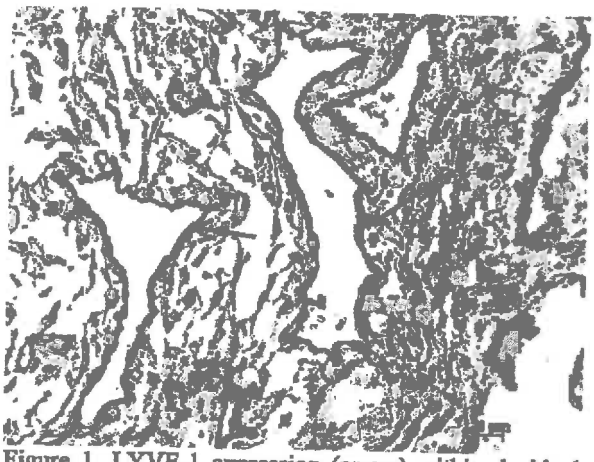


Figure 1. LYVE-1 expression (arrow) within decidual lymphatic vessels in decidua from HIV associated early onset pre-eclampsia.

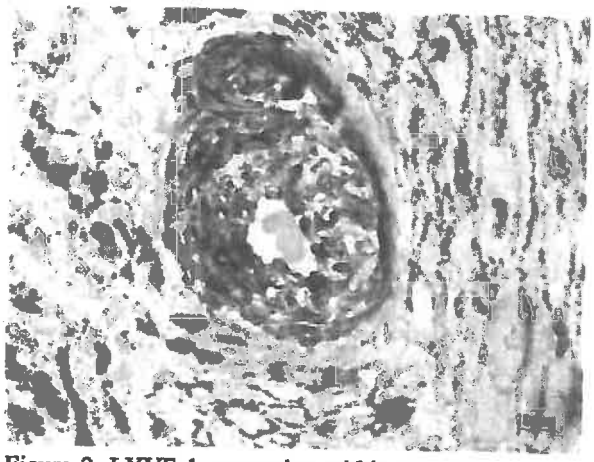


Figure 2. LYVE-1 expression within myometrial spiral arteries in HIV associated early onset pre-eclampsia.

Corresponding author: *felyonya@yahoo.com*

IMMUNOLOCALISATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-RECEPTOR-3 IN PLACENTA OF NORMOTENSIVE AND PRE-ECLAMPTIC PREGNANCIES

O.A. Onyangunga[1], J. Moodley[2], D.A. Ofusori[1], D.A. Margolis[1] and T. Naicker[1]

[1]Optics and Imaging Centre, [2]Woman's Health & HIV Research Centre, University of KwaZulu-Natal, Durban

The last decade has seen important progress in understanding the pathogenesis of pre-eclampsia. Indeed pre-eclampsia, a human pregnancy specific condition is a result of an imbalance between angiogenic and anti-angiogenic factors[1]. Anti-angiogenic factors are highly elevated in pre-eclampsia. Pre-eclampsia is characterised by a circulating pro-lymphangiogenic state as evident by a decrease in soluble VEGF-R3, slightly decreased soluble VEGF-R2, increased VEGF-C and dramatically lower ratio of soluble VEGF-R2+R3/VEGF-C[2-3].

HIV infection is a systemic infection affecting all tissues including lymphatic endothelial cells. Current knowledge of the effects of HIV associated hyper permeability is limited to disruption of the integrity of vascular structures and/or enhancement of inflammatory reactions. Therefore the co-morbidity of HIV infection and pre-eclampsia on this lymphangiogenic disparity is poorly understood. In the present study, we investigated the expression of the cell membrane receptor tyrosine kinase VEGF-R3 (also designated FLT-4) in HIV associated normotensive and pre-eclamptic placenta.

We analysed VEGF-R3 in term placenta in normotensive and pre-eclamptic women from Prince Mshiyeni Memorial hospital in KwaZulu-Natal. Groups were further stratified by CD4 count and early or late presentation of elevated blood pressure. Immunocytochemistry was performed using mouse anti-human antibody (clone #54703; dilution1:10; DAKO, Denmark). A two-step technique (Envision, DAKO, Glostrup) was used for visualisation with diaminobenzidine as the chromogen. Sections were counterstained with Mayer's haematoxylin and viewed on the Zeiss Axioscope A1 microscope.

VEGFR-3 was expressed within the invasive extravillous cell population (Fig. 1) and VEGFR-2 to the syncytiotrophoblast (Fig. 2) and cytotrophoblast (Fig. 2) cell populations in all villi types across all study groups. The endothelium lining all capillaries and large placental blood vessels were stained (Fig. 3). The placenta was used as a positive control. The negative control showed no staining.

This study demonstrates a qualitative lower expression of the receptor protein levels in pre-eclampsia compared with levels in the normotensive group. However morphometric image analysis is underway to elucidate differences between groups. These findings demonstrate a dysregulation of placental expression of VEGFR-3, a novel finding in the pathological maternal co-morbidity of HIV and pre-eclampsia.

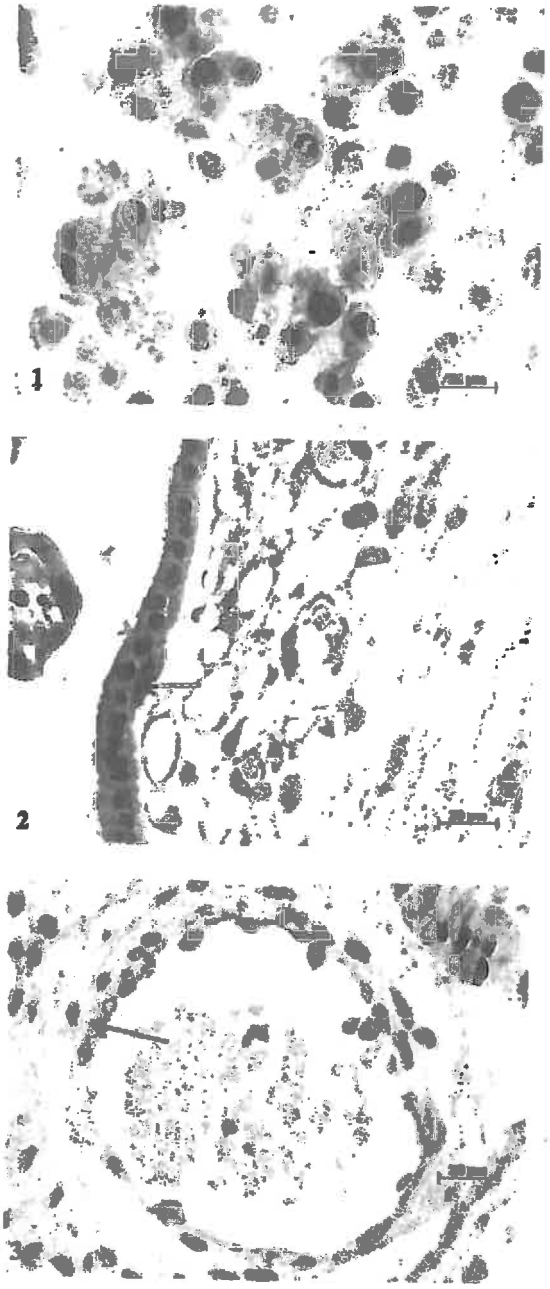


Figure 1-3: Extravillous trophoblast (1), syncytiotrophoblast (2) and cytotrohoblast (2; arrow) cell populations. Also note positive staining of endothelial cells (3; arrow).

References

1. Govender, N., Moodley, J. and Naicker, T. (2013) Cardiovas J Afr 24(5), 1.
2. Lely, A.T., *et al.*, (2013) Hypertens Pregn 32(1), 42.
3. Tamela, T. and Alitalo, K. (2010) Cell 140, 460.

Corresponding author: *felyonya@yahoo.com*

CHAPTER 8

SYNTHESIS

HIV/AIDS contributes to 40.5% of maternal deaths in South Africa (Saving Mothers, 2012). The province of KwaZulu-Natal (23.1%) is the epicenter of the HIV global pandemic. It is a serious obstetric dilemma that young women of reproductive age are at risk of HIV infection (Muula, 2008). Pre-eclampsia is predominantly a condition associated with primigravidae with a incidence of 12% within KwaZulu-Natal (Panday et al., 2004). The infection of HIV superimposed upon induced hypertensive pregnancies occur frequently in our province hence presents a unique paradigm to study these two co-morbidities.

MANUSCRIPT 1: Hypertensive Disorders in Primigravid Black South African women: A one year descriptive analysis (published).

To describe the incidence and obstetric and perinatal outcomes in Black South Africans with hypertension disorders of pregnancy, we examined 5860 primigravidae deliveries. Of these, 12.5% had hypertensive disorders of pregnancies. A diagnosis of gestational hypertension occurred in 6.7% of primigravidae and was the commonest type of hypertensive disorders in pregnancy. Mild to moderate PE or non-severe PE, severe PE and eclampsia occurred in 5.75% of the study population. Severe PE occurred in 1.43% of the primigravidae. The rate of caesarian deliveries in woman with PE was 50%. No perinatal death in the gestational hypertension group, but the overall perinatal mortality in PE group was 5.9%. Our findings are in keeping with the previous study of the overall incidence of hypertension in regional hospitals located in South Africa (Panday et al., 2004). Therefore, we can reliably state that the frequency of hypertensive disorders of pregnancy in the Durban area is approximately 12%.

Primiparity has been reported as a high risk factor for pre-eclampsia development (Dekker and Sibai, 2001; Duckitt and Harrington, 2005; Noor et al., 2008). The high incidence of pre-eclampsia-eclampsia syndrome in primigravidae in our environment is of obvious concern. Our data indicates that the primigravidae age of less than 24 years is associated with increased risk of complication.

MANUSCRIPT 2: Maternal and perinatal outcomes after caesarean delivery in HIV infected early and late onset pre-eclampsia in South African population (submitted)

In light of the high HIV infection in our province, we further analyzed the maternal and perinatal outcomes of HIV negative and positive pregnant women who had scheduled caesarean deliveries for early and late onset pre-eclampsia at a regional hospital in Durban, South Africa. The study looked at maternal and fetal outcomes in the two clinical entities. Women with early onset pre-eclampsia were older compared to those with late onset pre-eclampsia (p=0.0001). Also the HIV positive women were older compared to the HIV negative in both early- and late-onset pre-eclampsia (p=0.03). These results are similar to Lisonkwa and Joseph (2013). However, multiparity and primiparity were more representative in early and late onset pre-eclampsia respectively. The poor fetal outcome in EOPE is probably the result of severe hypoxia in the placenta microenvironment (Andraweera et al, 2014; Mitsui et al., 2015). The severity of hypertension and the HIV status did not differentiate the 2 groups. Overall, maternal complications (eclampsia, persistent postpartum hypertension, HELLP syndrome, maternal death) and poor fetal outcomes occurred predominately in early onset pre-eclampsia.

MANUSCRIPT 3: Lymphatic vascular endothelial hyaluronan receptor-1 immunoexpression in placenta of HIV infected pre-eclamptic women (published).

A functioning lymphatic system is necessary for maternal-fetal immunity to help fight infections, maternal tolerance and maintain tissue fluid homeostasis (Lamont et al., 2003). It is well known that human non-pregnant endometrium lacks lymphatic vessels (Naz et al., 2004). Moreover, to-date there are conflicting reports of the presence or absence of a lymphatic drainage within the placenta (Gu et al., 2006; Volchek et al., 2010; Castro et al., 2011; Bellini et al., 2012; Lee et al., 2013; Liu et al., 2015). This novel study was the first to outline the immunolocalisation of lymphatic vascular endothelial hyaluronan receptor-1, a selective marker for lymphatic endothelium and a homolog of CD44, hyaluronan receptor in the placenta of HIV associated normotensive and pre-eclamptic pregnancies.

We report an absence of lymphatic vessels within the fetal placenta. LYVE-1 immunostaining was localised to endothelial cells, fibroblast-like cells and medial cells lining fetal vessels of both

occurs in the EOPE group (Valensise et al, 2008). Also, hypoxia and inflammation are known to contribute to induction of lymphangiogenesis (Van der Auwera et al., 2004; Baluk et al., 2005; Morfoisse et al., 2015).

In acute inflammation, PDPN has an inhibitory function on T cell response where it controls Th17 response by reducing sensitivity to IL-7 survival signals (Peters et al., 2015). It is plausible that PDPN serves to shut-off Th17 response thereby limiting the pathogenicity of these cells. This action may be mediated via the interaction of PDPN with its 3 ligands viz., CLEC-2, galectin-8 and the chemokine CCL21 (Suzuki et al., 2007; Cueni and Detmar, 2009; Kerjaschki et al., 2004). During the acute inflammatory state such as PE, PDPN probably binds to either CLEC-2 or galectin-8 thereby reducing CD4 effector T cells. In the chronic state of HIV infection, PDPN interacts with CCL21, thus may promote chronic inflammation (Peters et al., 2015). The dual paradigm of the effect of PDPN on T cell response in HIV infection and pregnancy pathologies differ hence the functional role of PDPN *via* its ligand interaction requires further investigation.

CONCLUSION

We report a 12.5% incidence of primigravidae amongst hypertensive disorders in pregnancy in Black South African women. Moreover, we confirm the heterogeneity of pre-eclampsia and show that the timing of onset of this pregnancy disorder is important to disease severity. Furthermore HIV status seems to influence maternal and neonatal outcomes. We also demonstrate a decrease in lymphatic vascular endothelial hyaluronan receptor-1 staining in HIV positive pre-eclamptic group compared to HIV negative and normotensive groups. The presence of lymphatic vascular endothelial hyaluronan receptor-1 staining in the trophoblast and villous fetal endothelial cells indicate that lymphatic vascular endothelial hyaluronan receptor-1 may perform special functions in the placenta beyond those performed by lymphatic endothelial cells. Using PDPN, the presence of a reticular-lymphatic like conductive network within the placenta stroma is involved in fluid homeostasis. This network was up-regulated in HIV infection. The dual paradigm of the effect of PDPN on T cell response in HIV infection and pregnancy pathologies differ hence, the functional role of PDPN via its ligand interaction requires further investigation.

CHAPTER 9

REFERENCES

1. Abramson, D., 2012. *Blood vessels and lymphatics in organ systems*. Academic Press.
2. Adams, R.H. and Alitalo, K., 2007. Molecular regulation of angiogenesis and lymphangiogenesis. *Nature reviews Molecular cell biology*, *8*(6), pp.464-478.
3. Akolekar, R., Syngelaki, A., Sarquis, R., Zvanca, M. and Nicolaides, K.H., 2011. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11–13 weeks. *Prenatal diagnosis*, *31*(1), pp.66-74.
4. Alitalo, K., Tammela, T. and Petrova, T.V., 2005. Lymphangiogenesis in development and human disease. *Nature*, *438*(7070), pp.946-953.
5. Andraweera, P.H., Dekker, G.A. and Roberts, C.T., 2012. The vascular endothelial growth factor family in adverse pregnancy outcomes. *Human reproduction update*, *18*(4), pp.436-457.
6. Andraweera, P.H., Dekker, G.A., Thompson, S.D., Dissanayake, V.H.W., Jayasekara, R.W. and Roberts, C.T., 2014. Hypoxia-inducible factor-1α gene polymorphisms in early and late onset preeclampsia in Sinhalese women. *Placenta*, *35*(7), pp.491-495.
7. Appay, V. and Sauce, D., 2008. Immune activation and inflammation in HIV-1 infection: causes and consequences. *The Journal of pathology*, *214*(2), pp.231-241.
8. Astarita, J.L., Acton, S.E. and Turley, S.J., 2012. Podoplanin: emerging functions in development, the immune system, and cancer. *Frontiers in immunology*, *3*, p.283.
9. Baluk, P., Tammela, T., Ator, E., Lyubynska, N., Achen, M.G., Hicklin, D.J., Jeltsch, M., Petrova, T.V., Pytowski, B., Stacker, S.A. and Ylä-Herttuala, S., 2005. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. *The Journal of clinical investigation*, *115*(2), pp.247-257.
10. Banerji, S., Hide, B.R., James, J.R., Noble, M.E. and Jackson, D.G., 2010. Distinctive properties of the hyaluronan-binding domain in the lymphatic endothelial receptor Lyve-1 and their implications for receptor function. *Journal of Biological Chemistry*, *285*(14), pp.10724-10735.
11. Banerji, S., Ni, J., Wang, S.X., Clasper, S., Su, J., Tammi, R., Jones, M. and Jackson, D.G., 1999. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *The Journal of cell biology*, *144*(4), pp.789-801.

12. Basta, D., Latinovic, O., Lafferty, M.K., Sun, L., Bryant, J., Lu, W., Caccuri, F., Caruso, A., Gallo, R. and Garzino-Demo, A., 2015. Angiogenic, lymphangiogenic and adipogenic effects of HIV-1 matrix protein p17. *Pathogens and disease*, *73*(8): ftv062.

13. Bell, M.J., 2010. A historical overview of preeclampsia-eclampsia. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*, *39*(5), pp.510-518.

14. Bellini, C., Rutigliani, M., Boccardo, F., Campisi, C., Bellini, T., Bonioli, E. and Fulcheri, E., 2012. Are there lymphatic vessels in the placenta? *Lymphology*, *45*(1), pp.34-36.

15. Böckle, B.C., Sölder, E., Kind, S., Romani, N. and Sepp, N.T., 2008. DC-sign+ CD163+ macrophages expressing hyaluronan receptor LYVE-1 are located within chorion villi of the placenta. *Placenta*, *29*(2), pp.187-192.

16. Breiteneder-Geleff, S., Matsui, K., Soleiman, A., Meraner, P., Poczewski, H., Kalt, R., Schaffner, G., and Kerjaschki, D (1997). Podoplanin, novel 43-kdmembraneprotein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. *Am. J. Pathol.* 151, 1141–1152.

17. Cerdeira, A.S. and Karumanchi, S.A., 2012. Angiogenic factors in preeclampsia and related disorders. *Cold Spring Harbor perspectives in medicine*, *2*(11), p.006585.

18. Cerrato, E., Calcagno, A., D'Ascenzo, F., Biondi-Zoccai, G., Mancone, M., Marra, W.G., Demarie, D., Omedè, P., Abbate, A., Bonora, S. and DiNicolantonio, J.J., 2015. Cardiovascular disease in HIV patients: from bench to bedside and backwards. *Open heart*, *2*(1), pe000174.

19. Chaipan, C., Steffen, I., Tsegaye, T.S., Bertram, S., Glowacka, I., Kato, Y., Schmökel, J., Münch, J., Simmons, G., Gerardy-Schahn, R. and Pöhlmann, S., 2010. Incorporation of podoplanin into HIV released from HEK-293T cells, but not PBMC, is required for efficient binding to the attachment factor CLEC-2. *Retrovirology*, *7*(1), p.1.

20. Chen, Y. and Zhang, Z., 2012. Placenta related pathogenic factors for preeclampsia.

21. Choi, I., Lee, S. and Hong, Y.K., 2012. The new era of the lymphatic system: no longer secondary to the blood vascular system. *Cold Spring Harbor perspectives in medicine*, *2*(4), pa006445.

22. Conde-Agudelo, A., Villar, J. and Lindheimer, M., 2008. Maternal infection and risk of preeclampsia: systematic review and metaanalysis. *American journal of obstetrics and gynecology*, *198*(1), pp.7-22.

23. Cueni, L.N. and Detmar, M., 2009. Galectin-8 interacts with podoplanin and modulates lymphatic endothelial cell functions. *Experimental cell research*, *315*(10), pp.1715-1723.

24. Dekker, G. and Sibai, B., 2001. Primary, secondary, and tertiary prevention of pre-eclampsia. *The Lancet*, *357*(9251), pp.209-215.

25. Dekker, G., 2002. The partners' role in the etiology of preeclampsia. J. Reprod. Immunol., 57: 203-215

26. Derrien, M., Faye, A., Dolcini, G., Chaouat, G., Barré-Sinoussi, F. and Menu, E., 2005. Impact of the placental cytokine-chemokine balance on regulation of cell-cell contact-induced human immunodeficiency virus type 1 translocation across a trophoblastic barrier in vitro. *Journal of virology*, *79*(19), pp.12304-12310.

27. Duckitt, K. and Harrington, D., 2005. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *Bmj*, *330*(7491), p.565.

28. Dvorak, H.F., 2003. How tumors make bad blood vessels and stroma. *The American journal of pathology*, *162*(6), pp.1747-1757.

29. Edwards, D.R.V., Romero, R., Kusanovic, J.P., Hassan, S.S., Mazaki-Tovi, S., Vaisbuch, E., Kim, C.J., Erez, O., Chaiworapongsa, T., Pearce, B.D. and Bartlett, J., 2011. Polymorphisms in maternal and fetal genes encoding for proteins involved in extracellular matrix metabolism alter the risk for small-for-gestational-age. *The Journal of Maternal-Fetal & Neonatal Medicine*, *24*(2), pp.362-380.

30. El-Dayem, T.M.A., Elagwany, A.S., Khamis, M.Y., Kamha, E.S. and Hassan, A.M.M., 2016. Correlation of serum soluble endoglin to the severity of pre-eclampsia and its effect on the pregnancy outcome. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *5*(5), pp.1593-1600.

31. Elias, A., Nelson, B., Oputiri, D. and Geoffrey, O.B.P., 2013. Antiretroviral toxicity and oxidative stress. *American Journal of Pharmacology and Toxicology*, *8*(4), p.187.

32. English, F.A., Kenny, L.C. and McCarthy, F.P., 2015. Risk factors and effective management of preeclampsia. *Integrated blood pressure control*, *8*, p.7.

33. Evanko, S.P., Tammi, M.I., Tammi, R.H., Wight, T.N., 2007. Hyaluronan-dependent pericellular matrix. Adv. Drug Deliv. Rev. 59, 1351–1365.

34. Fang, R., Dawson, A., Lohsoonthorn, V. and Williams, M.A., 2009. Risk factors of early and late onset preeclampsia among Thai women. *Asian biomedicine: research, reviews and news*, *3*(5), p.477.

35. Farr, A., Nelson, A. and Hosier, S. (1992). Characterization of an antigenic determinant preferentially expressed by type I epithelial cells in the murine thymus. *J. Histochem. Cytochem.* 40, 651–664.

36. Ferrara, N., Gerber, H.P. and LeCouter, J., 2003. The biology of VEGF and its receptors. *Nature medicine*, *9*(6), pp.669-676.

37. Freitag, N., Tirado-González, I., Barrientos, G., Herse, F., Thijssen, V.L., Weedon-Fekjær, S.M., Schulz, H., Waliukat, G., Klapp, B.F., Nevers, T. and Sharma, S., 2013. Interfering with Gal-1–mediated angiogenesis contributes to the pathogenesis of preeclampsia. *Proceedings of the National Academy of Sciences*, *110*(28), pp.11451-11456.

38. Fu, J., Gerhardt, H., McDaniel, J.M., Xia, B., Liu, X., Ivanciu, L., Ny, A., Hermans, K., Silasi-Mansat, R., Mcgee, S., Nye, E., Ju, T., Ramirez, M. I., Carmeliet, P., Cummings, R. D., Lupu, F. and Xia, L. (2008). Endothelial cell O-glycan deficiency causes blood/lymphatic misconnections and consequent fatty liver disease in mice. *J. Clin. Invest.* 118, 3725–3737.

39. Gebhardt, G.S., Fawcus, S., Moodley, J. and Farina, Z., 2015. Maternal death and caesarean section in South Africa: results from the 2011-2013 saving mothers report of the national committee for confidential enquiries into maternal deaths. *SAMJ: South African Medical Journal*, *105*(4), pp.287-291.

40. Ghulmiyyah, L. and Sibai, B., 2012, February. Maternal mortality from preeclampsia/eclampsia. In *Seminars in perinatology* (Vol. 36, No. 1, pp. 56-59). WB Saunders.

41. Gibellini, L., De Biasi, S., Pinti, M., Nasi, M., Riccio, M., Carnevale, G., Cavallini, G.M., de Oyanguren, F.J.S., O'Connor, J.E., Mussini, C. and De Pol, A., 2012. The protease inhibitor atazanavir triggers autophagy and mitophagy in human preadipocytes. *Aids*, *26*(16), pp.2017-2026.

42. Gordon, E.J., Gale, N.W. and Harvey, N.L., 2008. Expression of the hyaluronan receptor LYVE-1 is not restricted to the lymphatic vasculature; LYVE-1 is also expressed on embryonic blood vessels. *Developmental Dynamics*, *237*(7), pp.1901-1909.

43. Govender, N., Naicker, T. and Moodley, J., 2015. Endoglin in HIV-associated preeclamptic placentae. *Hypertension in pregnancy*, *34*(3), pp.342-354.

44. Gu, B., Alexander, J.S., Gu, Y., Zhang, Y., Lewis, D.F. and Wang, Y., 2006. Expression of lymphatic vascular endothelial hyaluronan receptor-1 (LYVE-1) in the human placenta. *Lymphatic research and biology*, *4*(1), pp.11-17.

45. Gupta, S.K., Shen, C., Moe, S.M., Kamendulis, L.M., Goldman, M. and Dubé, M.P., 2012. Worsening endothelial function with efavirenz compared to protease inhibitors: a 12-month prospective study. *PloS one*, *7*(9), pe45716

46. Hall, D., Gebhardt, S., Theron, G. and Grové, D., 2014. Pre-eclampsia and gestational hypertension are less common in HIV infected women. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *4*(1), pp.91-96.

47. Haram, K., Mortensen, J.H. and Nagy, B., 2014. Genetic aspects of preeclampsia and the HELLP syndrome. *Journal of pregnancy, 2014.*

48. Hess, P.R., Rawnsley, D.R., Jakus, Z., Yang, Y., Sweet, D.T., Fu, J., Herzog, B., Lu, M., Nieswandt, B., Oliver, G. and Makinen, T., 2014. Platelets mediate lymphovenous hemostasis to maintain blood-lymphatic separation throughout life. *The Journal of clinical investigation*, *124*(1), pp.273-284.

49. Hofmeyr, G.J., Lawrie, T.A., Atallah, Á.N., Duley, L. and Torloni, M.R., 2014. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev*, *6*(6).

50. Jao, J. and Abrams, E.J., 2014. Metabolic complications of in utero maternal HIV and antiretroviral exposure in HIV-exposed infants. *The Pediatric infectious disease journal*, *33*(7), p.734.

51. Jeyabalan, A., 2013. Epidemiology of preeclampsia: impact of obesity. *Nutrition reviews*, *71*(suppl 1), pp. S18-S25.

52. Ji, R.-C. (2006). Lymphatic endothelial cells, tumor lymphangiogenesis and metastasis: new insights into intratumoral and peritumoral lymphatics. *Cancer Metastasis Rev.* 25, 677–694.

53. Jiang, D., Liang, J. and Noble, P.W., 2007. Hyaluronan in tissue injury and repair. *Annu. Rev. Cell Dev. Biol.*, *23*, pp.435-461.

54. Junus, K., Centlow, M., Wikström, A.K., Larsson, I., Hansson, S.R. and Olovsson, M., 2012. Gene expression profiling of placentae from women with early-and late-onset pre-eclampsia: down-regulation of the angiogenesis-related genes ACVRL1 and EGFL7 in early-onset disease. *Molecular human reproduction*, *18*(3), pp.146-155.

55. Jussila, L. and Alitalo, K., 2002. Vascular growth factors and lymphangiogenesis. *Physiological Reviews*, *82*(3), pp.673-700.

56. Kalkunte, S.S., Neubeck, S., Norris, W.E., Cheng, S.B., Kostadinov, S., Hoang, D.V., Ahmed, A., von Eggeling, F., Shaikh, Z., Padbury, J. and Berg, G., 2013. Transthyretin is dysregulated in preeclampsia, and its native form prevents the onset of disease in a preclinical mouse model. *The American journal of pathology*, *183*(5), pp.1425-1436.

57. Kalumba, V.M.S., Moodley, J. and Naidoo, T.D., 2013. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. *Cardiovasc J Afr*, *24*(24), p.7.

58. Kane, S. C., Costa, F.D.S. and Brennecke, S.P., 2013. New directions in the prediction of pre-eclampsia. *Australian and New Zealand Journal of Obstetrics and Gynaecology, 54(2),* pp.101-107.

59. Karumanchi, S.A., 2012. Angiogenesis and preeclampsia. *Journal of Reproductive Immunology*, *94*(1), pp.29-30.

60. Karumanchi, S.A., 2016. Angiogenic Factors in Preeclampsia. *Hypertension*, *67*(6), pp.1072-1079.

61. Kato, A., Katada, S. and Touhara, K., 2008. Amino acids involved in conformational dynamics and G protein coupling of an odorant receptor: targeting gain-of-function mutation. *Journal of neurochemistry*, *107*(5), pp.1261-1270.

62. Kerjaschki, D., Regele, H.M., Moosberger, I., Nagy-Bojarski, K., Watschinger, B., Soleiman, A., Birner, P., Krieger, S., Hovorka, A., Silberhumer, G. and Laakkonen, P., 2004. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *Journal of the American Society of Nephrology*, *15*(3), pp.603-612.

63. Khan, K.S., Wojdyla, D., Say, L., Gülmezoglu, A.M. and Van Look, P.F., 2006. WHO analysis of causes of maternal death: a systematic review. *The lancet*, *367*(9516), pp.1066-1074.

64. Lamont, R.F., 2003. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. *BJOG: An International Journal of Obstetrics & Gynaecology*, *110*(s20), pp.71-75.

65. Laresgoiti-Servitje, E., 2013. A leading role for the immune system in the pathophysiology of preeclampsia. Journal of leukocyte biology, 94(2), pp.247-257.

66. Lee, W.Y., Wang, C.J., Lin, T.Y., Hsiao, C.L. and Luo, C.W., 2013. CXCL17, an orphan chemokine, acts as a novel angiogenic and anti-inflammatory factor. *American Journal of Physiology-Endocrinology and Metabolism*, *304*(1), pp.E32-E40.

67. Lely, A.T., Salahuddin, S., Holwerda, K.M., Karumanchi, S.A. and Rana, S., 2013. Circulating lymphangiogenic factors in preeclampsia. *Hypertension in pregnancy*, *32*(1), pp.42-49.

68. Leung, C., Saaid, R., Pedersen, L., Park, F., Poon, L. and Hyett, J., 2015. Demographic factors that can be used to predict early-onset pre-eclampsia. *The Journal of Maternal-Fetal & Neonatal Medicine*, *28*(5), pp.535-539.

69. Liang, X., Yang, D., Hu, J., Hao, X., Gao, J. and MAO, Z., 2008. Hypoxia inducible factor-1alpha expression correlates with vascular endothelial growth factor-C expression and lymphangiogenesis/angiogenesis in oral squamous cell carcinoma. *Anticancer research*, *28*(3A), pp.1659-1666.

70. Lisonkova, S. and Joseph, K.S., 2013. Incidence of preeclampsia: risk factors and outcomes associated with early-versus late-onset disease. *American journal of obstetrics and gynecology*, *209*(6), pp.544-e1.

71. Liu, H., Li, Y., Zhang, J., Rao, M., Liang, H. and Liu, G., 2015. The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia. *Placenta*, *36*(3), pp.279-286.

72. Lowe, K.L., Navarro-Nunez, L. and Watson, S.P., 2012. Platelet CLEC-2 and podoplanin in cancer metastasis. *Thrombosis research*, *129*, pp.S30-S37.

73. Machado, E.S., Krauss, M.R., Megazzini, K., Coutinho, C.M., Kreitchmann, R., Melo, V.H., Pilotto, J.H., Ceriotto, M., Hofer, C.B., Siberry, G.K. and Watts, D.H., 2014. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. *Journal of Infection*, *68*(6), pp.572-580.

74. Madazli, R., Yuksel, M.A., Imamoglu, M., Tuten, A., Oncul, M., Aydin, B. and Demirayak, G., 2014. Comparison of clinical and perinatal outcomes in early-and late-onset preeclampsia. *Archives of gynecology and obstetrics*, *290*(1), pp.53-57.

75. Matejevic, D., Neudeck, H., Graf, R., Müller, T. and Dietl, J., 2001. Localization of hyaluronan with a hyaluronan-specific hyaluronic acid binding protein in the placenta in pre-eclampsia. *Gynecologic and obstetric investigation*, *52*(4), pp.257-259.

76. Mattar, R., Amed, A.M., Lindsey, P.C., Sass, N. and Daher, S., 2004. Preeclampsia and HIV infection. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *117*(2), pp.240-241.

77. Mitsui, T., Masuyama, H., Eto, E., Nobumoto, E., Hayata, K. and Hiramatsu, Y., 2015. Different Fetal and Neonatal Growth between Early-and Late-Onset Preeclampsia. *Open Journal of Obstetrics and Gynecology*, *5*(09), p.516.

78. Moodley, J., 2007. Maternal deaths due to hypertensive disorders in pregnancy: Saving Mothers report 2002-2004. *Cardiovascular journal of Africa*, *18*(6), pp.358-361.

79. Morfoisse, F., Tatin, F., Renaud, E., Hantelys, F., Pujol, F., Adoue, A., Prats, A.C. and Garmy-Susini, B., 2015. 0170: VEGF-D translational regulations promotes tumor lymphatic vessels plasticity. *Archives of Cardiovascular Diseases Supplements*, *7*(2), p.146.

80. Munaut, C., Lorquet, S., Pequeux, C., Blacher, S., Berndt, S., Frankenne, F. and Foidart, J.M., 2008. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. Human reproduction, 23(6), pp.1407-1415.

81. Munaut, C., Lorquet, S., Pequeux, C., Coulon, C., Le Goarant, J., Chantraine, F., Noël, A., Goffin, F., Tsatsaris, V., Subtil, D. and Foidart, J.M., 2012. Differential expression of Vegfr-2 and its soluble form in preeclampsia. *PloS one*, *7*(3), p.e33475.

82. Naljayan, M.V. and Karumanchi, S.A., 2013. New developments in the pathogenesis of preeclampsia. *Advances in chronic kidney disease*, *20*(3), pp.265-270.

83. Naz, R.K., 2004. Modalities for treatment of antisperm antibody mediated infertility: novel perspectives. *American Journal of Reproductive Immunology*, *51*(5), pp.390-397.

84. Necas, J., Bartosikova, L., Brauner, P. and Kolar, J., 2008. Hyaluronic acid (hyaluronan): a review. *Veterinarni medicina*, *53*(8), pp.397-411.

85. Olsson, A.K., Dimberg, A., Kreuger, J. and Claesson-Welsh, L., 2006. VEGF receptor signalling? In control of vascular function. *Nature reviews Molecular cell biology*, *7*(5), pp.359-371.

86. Pan, Y. and Xia, L., 2015. Emerging roles of podoplanin in vascular development and homeostasis. *Frontiers of medicine*, *9*(4), pp.421-430.

87. Panday, M., Mantel, G.D. and Moodley, J., 2004. Audit of severe acute morbidity in hypertensive pregnancies in a developing country. *Journal of Obstetrics and Gynaecology*, *24*(4), pp.387-391.

88. Peters, E., Geraci, S., Heemskerk, S., Wilmer, M.J., Bilos, A., Kraenzlin, B., Gretz, N., Pickkers, P. and Masereeuw, R., 2015. Alkaline phosphatase protects against renal inflammation through dephosphorylation of lipopolysaccharide and adenosine triphosphate. *British journal of pharmacology*, *172*(20), pp.4932-4945.

89. Petla, L.T., Chikkala, R., Ratnakar, K.S., Kodati, V. and Sritharan, V., 2013. Biomarkers for the management of pre-eclampsia in pregnant women. *Indian Journal of Medical Research*, *138*(1), p.60.

90. Ponting, J.M. and Kumar, S., 1995. Localisation and cellular origin of hyaluronectin. *Journal of anatomy*, *187*(Pt 2), p.331.

91. Poon, L.C. and Nicolaides, K.H., 2014. First-trimester maternal factors and biomarker screening for preeclampsia. *Prenatal diagnosis*, *34*(7), pp.618-627.

92. Powe, C.E., Levine, R.J. and Karumanchi, S.A., 2011. Preeclampsia, a disease of the maternal endothelium the role of antiangiogenic factors and implications for later cardiovascular disease. *Circulation*, *123*(24), pp.2856-2869.

93. Raymond, D. and Peterson, E., 2011. A critical review of early-onset and late-onset preeclampsia. *Obstetrical & gynecological survey*, *66*(8), pp.497-506.

94. Red-Horse, K., 2008. Lymphatic vessel dynamics in the uterine wall. Placenta, 29, pp.55-59.

95. Red-Horse, K., Rivera, J., Schanz, A., Zhou, Y., Winn, V., Kapidzic, M., Maltepe, E., Okazaki, K., Kochman, R., Vo, K.C. and Giudice, L., 2006. Cytotrophoblast induction of arterial apoptosis and lymphangiogenesis in an in vivo model of human placentation. *The Journal of clinical investigation*, *116*(10), pp.2643-2652.

96. Redman, C. 2014a. Pre-eclampsia: a complex and variable disease. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *4*(3), pp.241-242.

97. Redman, C.W. and Sargent, I.L., 2010. Review Article: Immunology of Pre-Eclampsia. *American Journal of Reproductive Immunology*, *63*(6), pp.534-543.

98. Redman, C.W.G., 2014b. The six stages of preeclampsia. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *4*(3), pp.241-247.

99. Redman, C.W.G., Tannetta, D.S., Dragovic, R.A., Gardiner, C., Southcombe, J.H., Collett, G.P. and Sargent, I.L., 2012. Review: does size matter? Placental debris and the pathophysiology of pre-eclampsia. *Placenta*, *33*, pp. S48-S54.

100. Roberts, J.M. and Escudero, C., 2012. The placenta in preeclampsia. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *2*(2), pp.72-83.

101. Roberts, J.M. and Gammill, H.S., 2005. Preeclampsia recent insights. Hypertension, 46(6), pp.1243-1249.

102. Robillard, P.Y., Dekker, G., Chaouat, G., Hulsey, T.C. and Saftlas, A., 2011. Epidemiological studies on primipaternity and immunology in preeclampsia–a statement after twelve years of workshops. *Journal of reproductive immunology*, *89*(2), pp.104-117.

103. Rodgers, P.A.W., Donoghue, J.F. and Girling, J.E., 2008. Endometrial lymphangiogensis. *Placenta*, *29*, pp.48-54.

104. Rolfo, A., Many, A., Racano, A., Tal, R., Tagliaferro, A., Ietta, F., Wang, J., Post, M. and Caniggia, I., 2010. Abnormalities in oxygen sensing define early and late onset preeclampsia as distinct pathologies. PloS one, 5(10), p.e13288.

105. Rueda-Clausen, C.F., Campbell, J. and Baker, P.N., 2011. Current challenges in pregnancy-related mortality. *Obstetrics, Gynaecology & Reproductive Medicine*, *21*(8), pp.229-235.

106. Sargent, I.L., Borzychowski, A.M. and Redman, C.W., 2006. Immunoregulation in normal pregnancy and pre-eclampsia: an overview. Reproductive biomedicine online, 13(5), pp.680-686.

107. Sato, W., Kosugi, T., Zhang, L., Roncal, C.A., Heinig, M., Campbell-Thompson, M., Yuzawa, Y., Atkinson, M.A., Grant, M.B., Croker, B.P. and Nakagawa, T., 2008. The pivotal role of VEGF on glomerular macrophage infiltration in advanced diabetic nephropathy. *Laboratory Investigation*, *88*(9), pp.949-961.

108. Saving Mothers 2011-2013: 2015. Sixth Report on the Confidential Enquiries into Maternal Deaths in South Africa. 1-78. http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf

109. Seely, E.W. and Solomon, C.G., 2016. Improving the prediction of preeclampsia. *New England Journal of Medicine*, *374*(1), pp.83-84.

110. Shibuya, M., 2011. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis a crucial target for anti-and pro-angiogenic therapies. *Genes & cancer*, *2*(12), pp.1097-1105.

111. Shibuya, M., 2014. Invited Review: VEGF-VEGFR Signals in Health and Disease. *Biomolecules Therapeutics*, *22*(1), pp.1-9.

112. Sibai, B.M., Publications Committee and Society for Maternal-Fetal Medicine, 2011. Evaluation and management of severe preeclampsia before 34 weeks' gestation. *American journal of obstetrics and gynecology*, *205*(3), pp.191-198.

113. Solis, L.M., Behrens, C., Dong, W., Suraokar, M., Ozburn, N.C., Moran, C.A., Corvalan, A.H., Biswal, S., Swisher, S.G., Bekele, B.N. and Minna, J.D., 2010. Nrf2 and Keap1 abnormalities in non–small cell lung carcinoma and association with clinicopathologic features. *Clinical Cancer Research*, *16*(14), pp.3743-3753.

114. Soto, E., Romero, R., Kusanovic, J.P., Ogge, G., Hussein, Y., Yeo, L., Hassan, S.S., Kim, C.J. and Chaiworapongsa, T., 2012. Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without

placental lesions consistent with maternal underperfusion. *The Journal of Maternal-Fetal & Neonatal Medicine.*

115. Stacker, S.A., Williams, S.P., Karnezis, T., Shayan, R., Fox, S.B. and Achen, M.G., 2014. Lymphangiogenesis and lymphatic vessel remodelling in cancer. *Nature Reviews Cancer*, *14*(3), pp.159-172.

116. Staff, A.C., Benton, S.J., von Dadelszen, P., Roberts, J.M., Taylor, R.N., Powers, R.W., Charnock-Jones, D.S. and Redman, C.W., 2013. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension*, *61*(5), pp.932-942.

117. Steegers, E.A., von Dadelszen, P., Duvekot, J.J. and Pijnenborg, R., 2010. Pre-eclampsia. *The Lancet*, *376*(9741), pp.631-644.

118. Stern R, Asari AA, Sugahara KN (2006) Hyaluronan fragments: an information rich system. Eur J Cell Biol 85 (8): 699–715.

119. Suy, A., Martínez, E., Coll, O., Lonca, M., Palacio, M., de Lazzari, E., Larrousse, M., Milinkovic, A., Hernández, S., Blanco, J.L. and Mallolas, J., 2006. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. *Aids*, *20*(1), pp.59-66.

120. Suzuki, K., Fontaine, A., Hori, Y. and Kobayashi, T., 2007. Versatile Suzuki-Miyaura coupling reaction using diphenylvinylphosphine ligands. *Synlett*, *2007*(20), pp.3206-3208.

121. Suzuki-Inoue, K.,Inoue, O., and Ozaki, Y (2011). Novel platelet activation receptor CLEC-2: from discovery to prospects. *J. Thromb. Haemost.* 9 (Suppl.1), 44–55.

122. Swartz, M.A. and Lund, A.W (2012). Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat. Rev. Cancer* 12, 210–219.

123. Tammi, M.I., Day, A.J. and Turley, E.A., 2002. Hyaluronan and homeostasis: a balancing act. *Journal of Biological Chemistry*, *277*(7), pp.4581-4584.

124. Tenner-Rácz, K., Rácz, P., Schmidt, H., Dietrich, M., Kern, P., Louie, A., Gartner, S. and Popovic, M., 1988. Immunohistochemical, electron microscopic and in situ hybridization evidence for the involvement of lymphatics in the spread of HIV-1. *Aids*, *2*(4), pp.299-311.

125. Torriani, F.J., Rodriguez-Torres, M., Rockstroh, J.K., Lissen, E., Gonzalez-García, J., Lazzarin, A., Carosi, G., Sasadeusz, J., Katlama, C., Montaner, J. and Sette Jr, H., 2004. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *New England Journal of Medicine*, *351*(5), pp.438-450.

126. Torriani, Martin, Bijoy J. Thomas, Robert B. Barlow, Jamie Librizzi, Sara Dolan, and Steven Grinspoon. "Increased intramyocellular lipid accumulation in HIV-infected women with fat redistribution." *Journal of applied physiology*100, no. 2 (2006): 609-614.

127. Tranquilli, A.L., Brown, M.A., Zeeman, G.G., Dekker, G. and Sibai, B.M., 2013. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *3*(1), pp.44-47.

128. Tranquilli, A.L., Dekker, G., Magee, L., Roberts, J., Sibai, B.M., Steyn, W., Zeeman, G.G. and Brown, M.A., 2014. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health, 4(2), pp.97-104.

129. Turley, E.A., Noble, P.W. and Bourguignon, L.Y., 2002. Signaling properties of hyaluronan receptors. *Journal of Biological Chemistry*, *277*(7), pp.4589-4592.

130. Valensise, H., Novelli, G.P. And Vasapollo, B., 2013. Pre-eclampsia: one name, two conditions–the case for early and late disease being different. *Fetal And Maternal Medicine Review*, *24*(01), Pp.32-37.

131. Valensise, H., Vasapollo, B., Gagliardi, G. and Novelli, G.P., 2008. Early and late preeclampsia two different maternal hemodynamic states in the latent phase of the disease. *Hypertension*, *52*(5), pp.873-880.

132. Valenzuela, F.J., Pérez-Sepúlveda, A., Torres, M.J., Correa, P., Repetto, G.M. and Illanes, S.E., 2011. Pathogenesis of preeclampsia: the genetic component. *Journal of pregnancy*, pp.1-8. doi:10.1155/2012/632732.

133. Vallet, J.L, Miles, J.R, Freking, B.A., 2010. Effect of fetal size on fetal placental hyaluronan and hyaluronoglucosaminidases throughout gestation in the pig. *Animal Reproduction Science, 118*, pp.297–309.

134. Van der Auwera, I., Van Laere, S.J., Van den Eynden, G.G., Benoy, I., van Dam, P., Colpaert, C.G., Fox, S.B., Turley, H., Harris, A.L., Van Marck, E.A. and Vermeulen, P.B., 2004. Increased angiogenesis and lymphangiogenesis in inflammatory versus noninflammatory breast cancer by real-time reverse transcriptase-PCR gene expression quantification. *Clinical Cancer Research*, *10*(23), pp.7965-7971.

135. Van der Merwe, J.L., Hall, D.R., Wright, C., Schubert, P. and Grove, D., 2010. Are early and late preeclampsia distinct subclasses of the disease—what does the placenta reveal?. *Hypertension in pregnancy*, *29*(4), pp.457-467.

136. Volchek, M., Girling, J.E., Lash, G.E., Cann, L., Kumar, B., Robson, S.C., Bulmer, J.N. and Rogers, P.A., 2010. Lymphatics in the human endometrium disappear during decidualization. *Human reproduction*, *25*(10), pp.2455-2464.

137. Wang, A., Rana, S. and Karumanchi, S.A., 2009. Preeclampsia: the role of angiogenic factors in its pathogenesis. Physiology, 24(3), pp.147-158.Parrish, M.R., Murphy, S.R., Rutland, S., Wallace, K., Wenzel, K., Wallukat, G., Keiser, S., Ray, L.F., Dechend, R.,

138. Wang, Y. and Oliver, G., 2010. Current views on the function of the lymphatic vasculature in health and disease. *Genes & development*, *24*(19), pp.2115-2126.

139. Wang, Y., Sun, J., Gu, Y., Zhao, S., Groome, L.J. and Alexander, J.S., 2011. D2-40/podoplanin expression in the human placenta. *Placenta*, *32*(1), pp.27-32.

140. Warrington, J.P., George, E.M., Palei, A.C., Spradley, F.T. and Granger, J.P., 2013. Recent advances in the understanding of the pathophysiology of preeclampsia. *Hypertension*, *62*(4), pp.666-673.

141. Wetterwald, A., Hoffstetter, W., Cec-chini, M.G., Lanske, B., Wagner, C., Fleisch, H. and Atkinson, M (1996). Characterization and cloning of the E11 antigen, a marker expressed by rat osteoblasts and osteocytes. *Bone* 18, 125–132.

142. WHO Guidelines Approved by the Guidelines Review Committee, 2011. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia. *Geneva: World Health Organization*, pp.1-4.

143. WHO, 2011. Maternal mortality. http://www.who.int/mediacentre/factsheets/fs348/en/.

144. Williams, P.J. and Pipkin, F.B., 2011. The genetics of pre-eclampsia and other hypertensive disorders of pregnancy. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *25*(4), pp.405-417.

145. Wimalasundera, R.C., Larbalestier, N., Smith, J.H., De Ruiter, A., Thom, S.M., Hughes, A.D., Poulter, N., Regan, L. and Taylor, G.P., 2002. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *The Lancet*, *360*(9340), pp.1152-1154.

146. Wrobbel, K.A., 2005. The university-level academic success of missionary kids educated in second-language host country national schools. *Unpublished dissertation, University of Minnesota, Minneapolis, Minnesota.*

147. Wu, H., Shi, H., Wang, Y., Jia, X., Tang, C., Zhang, J. and Yang, S., 2014. Hyaluronic acid conjugated graphene oxide for targeted drug delivery. *Carbon*, *69*, pp.379-389.

148. Wu, M., Du, Y., Liu, Y., He, Y., Yang, C., Wang, W. and Gao, F., 2014. Low molecular weight hyaluronan induces lymphangiogenesis through LYVE-1-mediated signaling pathways. PloS one, 9(3), p.e92857.

149. Young, B.C., Levine, R.J. and Karumanchi, S.A., 2010. Pathogenesis of preeclampsia. *Annual Review of Pathological Mechanical Disease*, *5*, pp.173-192.

150. Zhu, L., Lv, R., Kong, L., Cheng, H., Lan, F. and Li, X., 2015. Genome-wide mapping of 5mC and 5hmC identified differentially modified genomic regions in late-onset severe preeclampsia: a pilot study. *PloS one*, *10*(7), p.0134119.

CHAPTER 10

APPENDICES

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

10 August 2012

Dr OA Onyangunga
Dept of Obs & Gynae
Prince Mshiyeni Memorial Hospital
Private Bag X7
Mobeni
4060

Dear Dr Onyangunga

PROTOCOL: The role of lymphangiogenesis in the placenta and placental bed of HIV associated pre-eclampsia. REF: BE040/12

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 17 February 2012.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 06 June 2012 to queries raised on 23 May 2012 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 10 August 2012. **Please forward BREC a copy of the Export Permit and MTA Agreement if samples are being exported.**

This approval is valid for one year from **10 August 2012**. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

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

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC

Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

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

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on 11 September2012.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

INCWADI YOLWAZI

Isihloko Socwaningo:
The role of lymphangiogenesis in the placental bed of HIV associated pre-eclampsia

Sawubona, nginguDkt. Onyangunga (noma nginguSista.....osebenza noDkt. Onyangunga).

Usuvele wazisiwe ukuthi unomfutho wegazi ophezulu njengoba ukhulelwe. Ngenza (senza) ucwaningo lokuthola imbangela yomfutho wegazi ophezulu kubantu abakhulelwe. Akekho kulo mhlaba oseke wathola isisusa salokhu. Ngakho-ke ukwelashwa okutholayo okokwehlisa umfutho wegazi kanye nokuthi ubelethe ingane yakho uma sekufanele. Ukuthola isisusa somfutho wegazi ophezulu ngeke kukusize kulokhu kukhulelwa kwakho kepha kuyosiza abanye kanye nawe uma usukhulelwe esikhathini esizayo.

Ngifisa ukubamba iqhaza kulolu cwaningo. Ukubamba iqhaza kulolu cwaningo kubandakanya ukuthi sithathe ithishu encane esibelethweni lapho kukhona umzanyana emva kokubeletha. Isiqeshana sethishu noma isicubu (esilingana nephilisi elincane le aspirin) sithathwa emva kokubeletha futhi ngeke silimaze ingane kanye nawe kanye nokukhulelwa kwangesikhathi esizayo. Lezi zicubu zike zathathwa phambilini futhi abukho ubungozi osebuke babikwa obudalwa ukuthathwa kwazo.

Sicela uqaphele ukuthi lolu cwaningo lwenzelwa olunye futhi ucwaningo oluyokwenziwa lweziqu zobudokotela. Ucwaningo luyingxenye yezifundo zami, imiphumela iyoshicilelwa kepha awekho amagama ayosetshenziswa. Imiphumela yalolu cwaningo ngeke isize ukukhulelwa kwakho kepha iyokusiza ekukhulelweni okulandelayo uma ubuye uba nenkinga efanayo.

Amasampula egazi elithathwa emthanjeni alingana nezipunu ezimbili (10mls) ayothathwa ngesikhathi esifanayo uma kuthathwa igazi ngokujwayelekile. Lokhu kungebe mnandi kepha ngeke kukulimaze wena noma ingane. Uzwa ubuhlungu endaweni okukhishwa kuyo igazi kuphela (emthanjeni). Amasampula egazi esiwathathayo ayothunyelwa phesheya ukuze kulinganiswe izimbangela. Alukho ucwaningo lofuzo oluyokwenziwa futhi onke amasampula ayofakwa amakhodi, ngeke abe namagama abantu. Ukuhlolwa okwenziwe kuyoba okwalolu cwaningo kuphela. Lonke igazi eliyosala liyochithwa. Siyothatha ucezwana lomzanyana ukuze sicubungule imbangela. Emva kokuhlola lokho okuyobe kuthathiwe kuyobe sekubuyiswa kulethwe eSikhungweni se Optics & Imaging saseNyuvesi yaKwaZulu-Natali ukuze kwenziwe olunye ucwaningo isikhathi esiyiminyaka eyishumi. Lolu cwaningo alubandakanyi okuthinta ufuzo (isayensi nokuphilayo) kepha ucwaningo lwangesikhathi esizayo lungahle lulubandakanye. Amasampula ngeke aziwe ukuthi awobani.

Ucwaningo ngeke luhlomulise abantu besifazane ekukhulelweni kwabo kwamanje kepha bangasizakala ekukhulelweni okuzayo kanye nabanye abantu besifazane abakhulelwe uma imiphumela ikhomba ushintsho ekulawulweni komfutho ophezulu kakhulu wegazi kumuntu okhulelwe.

Kumele uthathe isikhathi sakho ucabanga ukuthi ungazibandakanya yini nalolu cwaningo. Khumbula ukuthi ukubamba kwakho iqhaza kulolu cwaningo kungukuzikhethela wena. Uma wenqaba ukubamba iqhaza kulolu cwaningo ngeke uphathwe ngendlela eyehlukile kwezinye iziguli.

Ukungadaluiwa:Kuyokwenziwa yonke imizamo ukugcina imininingwane yombambiqhaza iyimfihlo. Ukungadaluiwa okuphelele ngeke kuqinisekiswe. Imininingwane yombambiqhaza iyokwaziswa uma idingwa umthetho.
Izinhlangano ezingahlola noma zikopishe amarekhodi ocwaningo lwethu ukuqinisekisa ikhwalithi kanye nokucubungula ulwazi zibandakanya amaqembu anjenge Research Ethics Committee, Data Safety Monitoring Committee kanye ne Medicines Control Council (uma kudingekile). Qaphela ukuthi lolu cwaningo lwenzelwa inhloso yocwaningo kanye nokuthi ngithole ezinye iziqu eziphezulu (okungukuthi luzokwandisa ulwazi lwami bese lungiholela ekutholeni ezinye iziqu). Imiphumela yocwaningo iyoshicilelwa, kepha-ke awekho amagama ayosetshenziswa kulolu cwaningo noma ushicilelo.

Imininingwane yabacwaningi uma kudingeka olunye ulwazi /ukubika izithiyo ocwaningweni.

1. Dkt. OA Onyangunga, Department of Obstetrics and Gynaecology, PMMH (031-9078473/ 031-9078111)

2. Slz. J Moodley, Womens Health, DDMRI, NRMSM. (031-2604241)

3. Dkt. NR Maharaj, Department of Obstetrics and Gynaecology, PMMH (031-9078318)

4. Slz. JM Foidart, Obstetrics and Gynaecology, Hospital of la Citradelle and LBTD. (Belgium)

5. Dkt. C Munaut, Laboratory of Tumor Biology and Development (LBTD) (+3243662453)

6. Slz. T Naicker, Optics & Imaging Centre, DDMRI, NRMSM.

Imininingwane ka BREC Administrator noma uSihlalo - ukubika izikhalo/izinkinga:
Biomedical Research Ethics, Research Office, UKZN, Private Bag X54001, Durban 4000
Ucingo: +27 (0) 31 260 4769 / 260 1074
Ifeksi: +27 (0) 31 260 2384
Administrator: Ms P Ngwenya i-email: ngwenyap@ukzn.ac.za
USihlalo: i-email: Prof D R Wassenaar c/o ngwenyap@ukzn.ac.za

INCWADI YEMVUME

Imvume Yokubamba Iqhaza Ocwaningweni

Sawubona:

Ulwazi ngomcwaningi (nikeza) kanye nocwaningo oluhleliwe (nikeza).

Uceiwe ukuba ubambe iqhaza ocwanigweni (chaza).

Wazisiwe ngocwaningo waziswa uDkt. O. A. Onyangunga.

Uma kufanele: Wazisiwe ngesinxephezelo esikhona noma ukwelashwa uma kuba khona ukulimala okuqondene nokuzokwenziwa kulolu cwaningo;

Ungaxhumana no Dkt. O. A. Onyangunga kule nombolo ethi 0720207541 nganoma yisiphi isikhathi uma unemibuzo ngocwaningo noma ulimala ngenxa yocwaningo.

Ungathinta iHhovisi le Biomedical Research Ethics kule nombolo ethi 031-260 4769 noma ethi 260 1074 uma unemibuzo ngamalungelo akho njengombambiqhaza ocwaningweni.

Uzikhethele wena ukubamba iqhaza kulolu cwaningo, ngeke uhlawuliswe noma ulahlekelwe imihlomulo uma unqaba ukubamba iqhaza kulolu cwaningo noma ukhetha ukulushiya nganoma yisiphi isikhathi.

Uma uvuma ukubamba iqhaza uyonikezwa ikhophi esayiniwe yale ncwadi kanye nepheshana lolwazi lombambiqhaza okuyisifingqo esibhaliwe socwaningo.

Ucwaningo, kubandakanya ulwazi olungenhla ngichazelwe lona ngomlomo. Ngiyakuqonda ukubandakanyeka kwami kulolu cwaningo ukuthi kusho ukuthini futhi ngiyazikhethela ukubamba iqhaza kulolu cwaningo. Nginikeziwe ithuba lokubuza noma yimiphi imibuzo engingaba nayo mayelana nokubamba iqhaza kulolu cwaningo.

Isiginesha yoMbambiqhaza	**Usuku**
Isiginesha kaFakazi (Uma kufanele)	**Usuku**
Isiginesha yoMhumushi (Uma kufanele)	**Usuku**

INFORMATION DOCUMENT

Study title:
The role of lymphangiogenesis in the placental bed of HIV associated pre-eclampsia

Good morning I am Dr Onyangunga (or I am sister..... working with Dr Onyangunga).

You have already been informed that you have high blood pressure in pregnancy. I am doing (we are doing) a study to find out what causes high blood pressure in pregnancy. No one in the world has found a cause as yet. Therefore your treatment is usually to give you medicine to lower your blood pressure and to deliver your baby once it is mature. Establishing the cause of high blood pressure, will not help you in this pregnancy but may help others and you in a future pregnancy.

I would like you to participate in this study. Your participation in the study involves us taking a small piece of tissue from the area of the womb in which the placenta (after birth) is situated. The piece of tissue or biopsy (the size of a junior aspirin tablet) is taken afterbirth and will not affect your baby and will not affect you, your baby and future pregnancies. Such biopsies have been done previously and have not been known to cause harm.

Please note that this research is also being done for further studies, called a PhD. The research forms a part of my study and the results will be published but no names will be used. The study results will not help your pregnancy but may be of value for you in future pregnancies if you develop the same condition.

Venous blood samples amounting to 2 tablespoons (10mls) of blood will be obtained at the same time as routine bloods are taken. This may cause a slight discomfit but not cause you nor your baby any harm. You only feel some discomfort from the site at which we take blood sample (vein site). The blood samples we take will be sent overseas for estimation of the factors. No genes study will be done and all samples will be "coded" and not have any names. The tests done will only be for this study research. All remaining blood will be destroyed. We will take as well a piece of placenta for analysis of factors. Following analysis the specimens will be returned and stored at the Optics & Imaging Centre at the University of KwaZulu-Natal for future research for a period of 10 years. The present research doesn't include genetics (science and variation in living organisms) but future research might. Samples will be anonymised.

The study will not benefit women in the current pregnancy but maybe of value in future pregnancies and to other pregnant females if the results indicate change in management of preeclampsia.

You should take your time and decide whether you would like to participate in this study. Remember your participation is on your own free will (voluntary). If you decline to take part you will not be treated differently from other patients.

Confidentiality: Every effort will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law.
Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee, Data Safety Monitoring Committee and the Medicines Control Council (where appropriate). Note that this study is being done for both research purposes and for my higher degree (i.e. the study will improve my knowledge and lead to me getting another qualification). Results of the study will be published, however no names identities will be used in this research or publication.

Contact details of researcher/s - for further information / reporting of study related adverse events.

1. Dr OA Onyangunga, Department of Obstetrics and Gynaecology, PMMH (031-9078473/ 031-9078111)

2. Prof J Moodley, Womens Health, DDMRI, NRMSM. (031-2604241)

3. Dr NR Maharaj, Department of Obstetrics and Gynaecology, PMMH (031-9078318)

4. Prof JM Foidart, Obstetrics and Gynaecology, Hospital of la Citradelle and LBTD. (Belgium)

5. Dr C Munaut, Laboratory of Tumor Biology and Development (LBTD) (+3243662453)

6. Prof T Naicker, Optics & Imaging Centre, DDMRI, NRMSM.

Contact details of BREC Administrator or Chair - for reporting of complaints/ problems:
Biomedical Research Ethics, Research Office, UKZN, Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 4769 / 260 1074
Fax: +27 (0) 31 260 2384
Administrator: Ms P Ngwenya Email: ngwenyap@ukzn.ac.za
Chair: Email: Prof D R Wassenaar c/o ngwenyap@ukzn.ac.za

INFORMED CONSENT DOCUMENT

Consent to Participate in Research

Greeting:

Information about the researcher (provide) and the planned study (provide).

You have been asked to participate in a research study (describe).

You have been informed about the study by Dr O. A. Onyangunga.

Where applicable: You have been informed about any available compensation or medical treatment if injury occurs as a result of study-related procedures;

You may contact Dr O. A. Onyangunga at 0720207541 any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Biomedical Research Ethics Office on 031-260 4769 or 260 1074 if you have questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to stop at any time.

If you agree to participate, you will be given a signed copy of this document and the participant information sheet which is a written summary of the research.

The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate. I have been given an opportunity to ask any questions that I might have about participation in the study.

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**
(Where applicable)

_____ _____

Signature of Translator
(Where applicable

Date