THE ROLE OF IMMUNOGLOBULIN ISOTYPES (IgG1-IgG4, IgA AND IgM) IN HIV PREECLAMPTICS ON HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY, IN SOUTH AFRICA.

by

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PREFACE

This study contains original work done by the author, it has not been submitted in any form to any other University.

The research within this dissertation was conducted in the Optics and Imaging Centre, Doris Duke Medical Research Institute, College of Health Science, University of Kwa-Zulu Natal, Durban, South Africa under the supervision of Professor T. Naicker.

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DECLARATION

I, Mikaila Moodley (214558958) declare that:

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

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

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DEDICATION

Hands that held my heart,

food that nurtured my soul,

lessons that moulded my character,

love that is ever flowing reaching through time and space.

That's what a resilient woman has given me.

To my late grandmother-Everything I am today, I owe to you.

Lord for you believeth in me, that I believeth in myself.

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- To my friends and colleagues for making this journey enjoyable and for the ever-willing support.

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Disease Syndrome
BREC	Biomedical Research Ethics Committee
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human Immunodeficiency Virus
Ig	Immunoglobulin
kDa	Kilo Daltons
LMIC	Low-middle income countries
PE	Preeclampsia
MBL	Mannose-binding Lectin
vs	Versus
WHO	World Health Organization

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ABSTRACT

Background: The epicentre of a successful pregnancy lies within the placenta and is nurtured by suppressed immune responses. However, the fragile balance between maternal inflammatory responses and the regulation of maternal immunoglobulins is distorted by HIV and Preeclampsia (PE). Preeclampsia and co-morbid diseases such as HIV infections are major contributors to maternal morbidity and mortality, worldwide. Furthermore, the effects of Highly Active Antiretroviral therapy (HAART) on the reconstitution of immunity in HIV preeclamptics remain obscure. Therefore, the current study aims to investigate the role of immunoglobulins in HIV infected preeclamptics and elucidate the effects of HAART on immunoglobulin levels in HIV infected PE.

Method: Ethical clearance was granted by the Biomedical Research Ethics Committee (BREC)**.** Serum samples of 38 normotensive and 38 preeclamptic pregnancies were collected at a regional hospital and further categorized based on HIV status. The serum samples were then subjected to the analysis of immunoglobulin (IgG1-IgG4, IgA and IgM) concentration (ng/dl). The Bio-Plex immunoassay technique of analysis was used to investigate the concentration of immunoglobulin isotypes in the sample population. Immunoglobulin concentrations were considered significant when $p < 0.05$.

Results: Immunoglobulin concentration was evaluated in pregnancy type irrespective of HIV status. A non-significant down-regulated trend of IgG1, IgG3 and IgG4 was observed, whilst IgG2 and IgM showed a non-significant up-regulation. On the contrary, IgA levels presented a significant increase in preeclamptics irrespective of HIV status. However, in HIV infected pregnancies irrespective of pregnancy type IgG1 presented a non-significant up-regulated trend, whilst IgG3 and IgG4 showed non-significant down-regulatory trends. Nonetheless, IgG2, IgA and IgM demonstrated a significant down-regulation in HIV infected pregnancies, irrespective of pregnancy type. Furthermore, IgG1, IgG3, IgG4 and IgM showed a non-significant difference when analysed according to pregnancy type and HIV status. However, IgG2 and IgA presented a significant up-regulation in HIV negative PE.

Conclusion: This study highlights the importance of the maternal-fetal transfer of immunoglobulins (IgG subclass) in pregnancy. We report a significant up-regulation of IgG2, indicating its role in activating the classical complement pathway thereby, exacerbating the inflammatory response in PE. The up-regulation of IgA is an ingenerate anti-inflammatory response, which inhibits the exacerbated inflammatory cascade *via* classical complement activation in PE. In HIV infection the down-regulation of IgG2, IgA and IgM maybe due to

HAART. In addition, IgA showed an up-regulation in HIV associated PE, suggesting that the reconstitution of HAART is insufficient in neutralizing the exaggerated inflammatory response in PE. To further understand the role of IgG2 and IgA in HIV associated PE, larger studies are warranted.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Maternal Mortality

"Maternal mortality is the death of a woman whilst pregnant or within 42 days of delivery or termination of pregnancy, from any cause related to, or aggravated by pregnancy or its management, but excluding deaths from incidental or accidental causes."

(World Health Organization, 1992)

The health of a woman during pregnancy and the puerperium is essential to the well-being of a society (Moran and Moodley, 2012). However, every day approximately 800 women die from childbirth or pregnancy related complications, worldwide (World Health Organization, 2014). Sub-Saharan Africa and southern Asia account for 83.8% of maternal deaths in the world (Say *et al.*, 2014). According to the World Health Organization (WHO), hypertensive disorders of pregnancy such as preeclampsia (PE) are the most common cause of maternal mortality (2.7%), followed by other complications and diseases (2.5%), hemorrhagic disorders (1.1%) and infectious diseases (0.6%) (Vogel *et al.*, 2014).

In South Africa, the impact of HIV infections and hypertensive disorders such as PE on maternal mortality prevalence rates, remain prominent (Moodley *et al*., 2016; Phoswa *et al*., 2018). Progress to reduce maternal mortality rates depend on competent hospital care, strong health systems and understanding the cause of diseases responsible for maternal deaths (Alkema *et al.*, 2016). Hence, this study intends to provide data on HIV infected women with PE in, KwaZulu-Natal, South Africa.

1.2 Human immunodeficiency virus (HIV)

1.2.1 The epidemiology and etiology

First identified in the 1980's, HIV infection has become a leading global health crisis (Gallo and Montagnier, 2003; Nakagawa *et al.*, 2013). HIV has unique characteristics such as persistent infection, vertical transmission from mother to child, transmission through body secretions, and a variability that allows it to escape immunity and antiretroviral drugs (Maartens *et al.*, 2014).

It has been estimated that 13.1% South Africans are HIV positive, of whom one fifth are women of child bearing age (15-49 years) (Statistics South Africa, 2018). The risk of maternal death for

a HIV infected pregnant woman is approximately 10 times higher than that of a HIV negative pregnant woman (Moran and Moodley, 2012). These statistics incite research on this maternal epidemic in South Africa.

1.2.2 The pathogenesis of HIV

HIV exists as an enveloped glycoprotein complex that targets CD4 (T-helper cell) cell receptors (Lizeng *et al*., 2003). Once inside the target cell, HIV uses the viral enzyme-reverse transcriptase to produce DNA from viral RNA (Warnock *et al.*, 2011). The new viral DNA called a provirus, inserts itself into the target cell's nucleus (Cooper *et al.*, 2013). The provirus then directs the host cell into producing new copies of viral RNA proteins, enabling the multiplication of HIV (Stacey *et al.*, 2009).

Although B cells and T-helper cells initially mount a vigorous response to viral exposure, over time HIV destroys these cells together with other body cells such as macrophages, monocytes, astrocytes and dendritic cells (Liu *et al*., 2004; Maartens *et al*., 2014). Consequently, the resultant profound deficit in B cells and T-helper cells have a domino effect, causing the collapse of an efficient immune system (Moir and Fauci, 2017). This leads to high viral mutation rates and changing resistance to anti-retroviral drugs (Maartens *et al*., 2014).

1.3 Preeclampsia- "The disease of theories"

1.3.1 The epidemiology and etiology

Preeclampsia is an exaggerated inflammatory disease that occurs in almost 7-10% of all pregnancies and is responsible for more than 50 000 deaths /annum worldwide (Ghulmiyyah and Sibai, 2012; Loewendorf *et al*., 2015). A higher prevalence of PE is reported in low-middle income countries (LMIC) as opposed to developed countries, due to the lack of robust prenatal care and health services (World Health Organization, 2005).

Preeclampsia is typically characterized by new onset-hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) and protein excretion ≥ 300 mg in 24 hours, within 20 weeks of gestation (Hutcheon *et al.*, 2011). This pregnancy specific disorder involves multiple organ systems including the kidney, liver, lungs, heart, pulmonary and neurological systems (Young *et al*., 2010; Arulkumaran and Lightstone, 2013). In addition, PE may lead to the

development of eclampsia, fetal growth restriction and low birth weight babies (Ghulmiyyah and Sibai, 2012; Gathiram and Moodley, 2016).

Despite being the primary reason for intensive care unit admittances during the puerperal period, the multifactorial etiology of PE remains unclear (Khaliq *et al*., 2018). However, various risk factors for PE have been reported (Phoswa *et al*., 2018). According to Mol *et al*. (2016) a maternal or paternal family history of PE, maternal age, obesity and multiple pregnancies predispose women to PE. Similarly, women who suffer from pre-existing conditions such as chronic hypertension, autoimmune disease, kidney disease or infertility are at a higher risk of developing PE (Uzan *et al*., 2011). In addition, the primi-paternity hypothesis states that the risk of PE increases in women with limited exposure to their partners sperm (Hutcheon *et al*., 2011).

1.3.2 Placental origins of normotensive pregnancies

During early human pregnancy a depressed maternal immune system causes extra villous cytotrophoblasts to invade the uterus (Naicker *et al.*, 2013). This invasion transforms spiral arteries into large sinusoidal-like vessels of low resistance thus enhancing placental perfusion to meet the utero-placental unit's metabolic demands (Whitley and Cartwright, 2010). The transformation of these vessels are referred to as the physiological re-modelling of spiral arteries (Harris, 2011).

1.3.3 Placental origins and pathogenesis of preeclampsia

The pathogenesis of PE remains surreptitious thus its name "the disease of theories" (Pipikin and Rubin, 1994). However, few would deny that the fundamental cause of PE involves the placenta (Jeffocate, 1966). In PE, endovascular invasion of the uterus is limited to the decidua, (Lyall *et al.*, 2013). Therefore, physiological re-modelling of spiral arteries in the myometrium is ineffective (Cerdeira and Karumanchi, 2012). This results in smaller myometrial arterial diameters, eventuating in trophoblast cell death (Young *et al*., 2010). Consequently, the death of trophoblast cells create a hypoxic environment for the placental and fetal tissue (Huang *et al.*, 2010). These abnormalities allow the interaction between activated immune cells and pro-inflammatory cytokines to persist (Shamshirsaz *et al.*, 2012). This eventuates in placental oxidative stress and unremitting elevated inflammatory responses (Szarka *et al.*, 2010).

Many studies attribute trophoblast cell death to immunological factors such as a deviant immunologic tolerance to pregnancy, exaggerated inflammatory responses, increased circulating anti-angiogenic factors, the perturbation of the renin-angiotensin axis II and decreased placental nitric oxide production (Venkatesha *et al*., 2006; Matsubara *et al*., 2015). Whist other studies hypothesize that genetic susceptibility to aberrant placentation is the cause of PE. (Thakoordeen *et al*., 2018). Despite the innumerable pathogenic theories, PE remains incurable and the only recognized treatment is delivery of the placenta (Young *et al*., 2010).

1.3.4 The role of inflammation and oxidative stress in preeclampsia

Pregnancy poses a metabolic and immune challenge for the mother who must accommodate a semi-allogenic fetus (Kalagiri *et al.*, 2016). During healthy pregnancy a subdued maternal immune system elicits a placental production of immunomodulatory hormones, anti-inflammatory cytokines and moderated pro-inflammatory cytokines into the maternal blood stream (Cornelius *et al.*, 2018). This in turn suppresses the inflammatory cascade, to avoid rejection of the fetus, whilst simultaneously protecting maternal immunity and apposite placentation (Eiland *et al.*, 2012; Harmon *et al.*, 2016). These immunomodulatory hormones and cytokines also act as mediators of morphologic changes during spiral artery conversion (Ducray *et al*., 2011).

However, since PE is associated with an exaggerated inflammatory cascade, an amplified placental production of pro-inflammatory cytokines transpires (Boij *et al*., 2012). This impairs the regulation of arterial supply systems, cytotrophoblast invasion and endovascular proliferation in the placenta (Harmon *et al.*, 2016). The aberrant production of pro-inflammatory cytokines instigates an oxidative burst from Reactive Oxygen Species (ROS), which exceed their antioxidant capacity (Shamshiraz *et al*., 2012). Thereby, resulting in vascular oxidative stress during PE (Bowen *et al*., 2001). This in turn, enhances hypoxia in fetal and placental tissues (LaMarca *et al.*, 2007). Therefore, it is believed that a disturbance of the maternal immunologic system exacerbates a chronic inflammatory cascade, which may lead to pregnancy loss during PE (Ahmad and Khidir, 2018; Black and Horowitz, 2018).

1.4 Impact of HIV on the incidence of Preeclampsia

One of the pathogenic explanations for PE development, is the aggravation of the maternal immune tolerance to pregnancy (Kalumba *et al.*, 2013). However, HIV infection is associated

with immune suppression (Browne *et al.*, 2015). Therefore, it has been postulated that HIV infection would inhibit immune hyperactivity and thus prevent the development of PE (Frank *et al.*, 2004). However, PE research among HIV-infected women are limited (Powis *et al.*, 2013). There is ongoing debate as to whether HIV-infected women have a lower or higher susceptibility to developing PE, compared to HIV negative women (Landi *et al.*, 2014). Furthermore, the use of Highly Active Anti-Retroviral Therapy (HAART) in HIV infected individuals has been proposed to reestablish excessive immune responses (Landi *et al*, 2014). Therefore, it is has been hypothesized that HAART may predispose HIV infected pregnant women to PE development (Phoswa *et al.*, 2018).

1.5 Immunoglobulins

1.5.1 Mechanism of action in the human immune system

Humans have a sophisticated immune system, which includes several types of immune cells and an array of specialized protein molecules (Holborow and Reeves, 1977). These proteins belong to a specific group of antibodies known as immunoglobulins, that are produced by B cells or plasma cells (Berkowska *et al*., 2011). In 1939, immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were discovered as three patently recognized groups of proteins (Schroeder and Cavacini, 2010). A few decades later, immunoglobulin D (IgD) and immunoglobulin E (IgE) antibody isotypes were discovered (Amarasekera, 2011; Chen and Cerutti, 2011). Immunoglobulins defend the immune system as natural antibodies, through antigen-antibody interactions (Zhou *et al*., 2007). These complexes inactivate antigens *via* their direct neutralization, agglutination, precipitation, lysis, phagocytosis and indirect complement fixation mechanisms (Figure 1.1) (Panda and Ding, 2015).

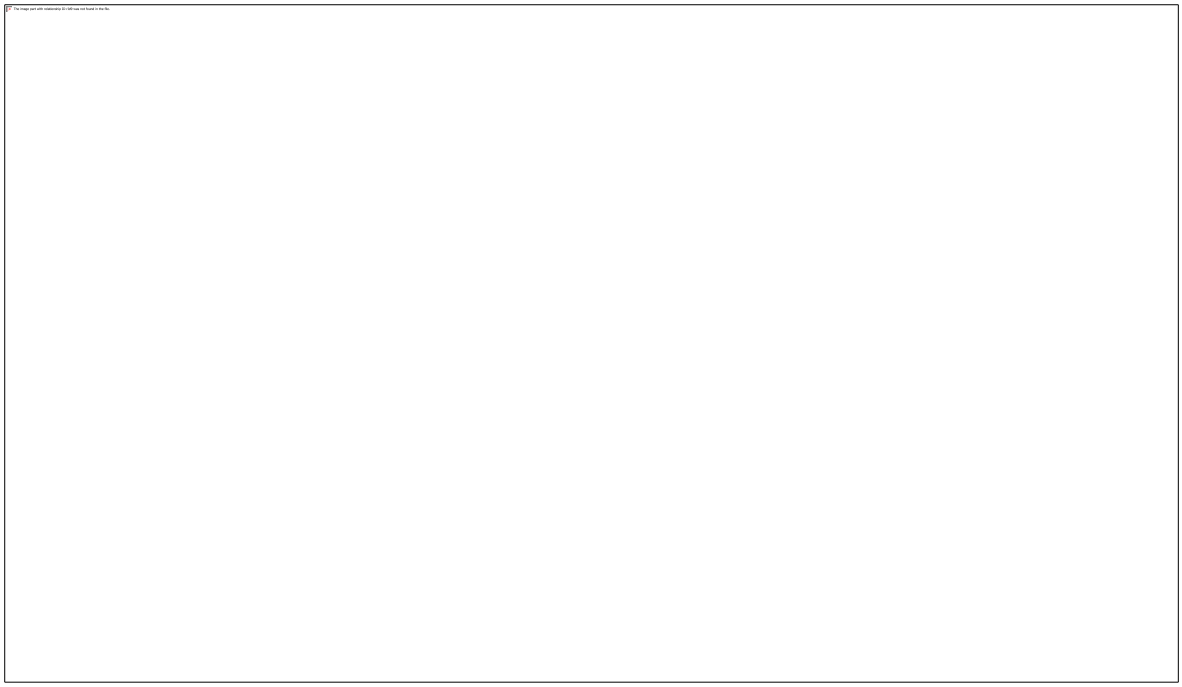

Figure 1.1: Mechanism of antibody action in human immunity-Adapted from Marieb and Hoehn, (2007).

1.5.2 The structure of immunoglobulins

In humans, there are five immunoglobulin classes:

- α – IgA
- δ – IgD
- γ – IgG
- ε – IgE
- μ – IgM

Immunoglobulins are composed of 82-96% proteins and 4-18% carbohydrates (Vidarsson *et al*., 2014). These globulins have molecular weights between 160 000 and 970 000 kDa (Marieb and Hoehn, 2007) . All immunoglobulins have a similar basic structure consisting of four looping peptide chains with disulfide bonds, which form a molecule known as an antibody monomer (Williams and Barclay, 1988; Malek, 2013). These monomers structurally take up either a Y or T shape (Figure 1.2) (Bengten *et al.*, 2000).

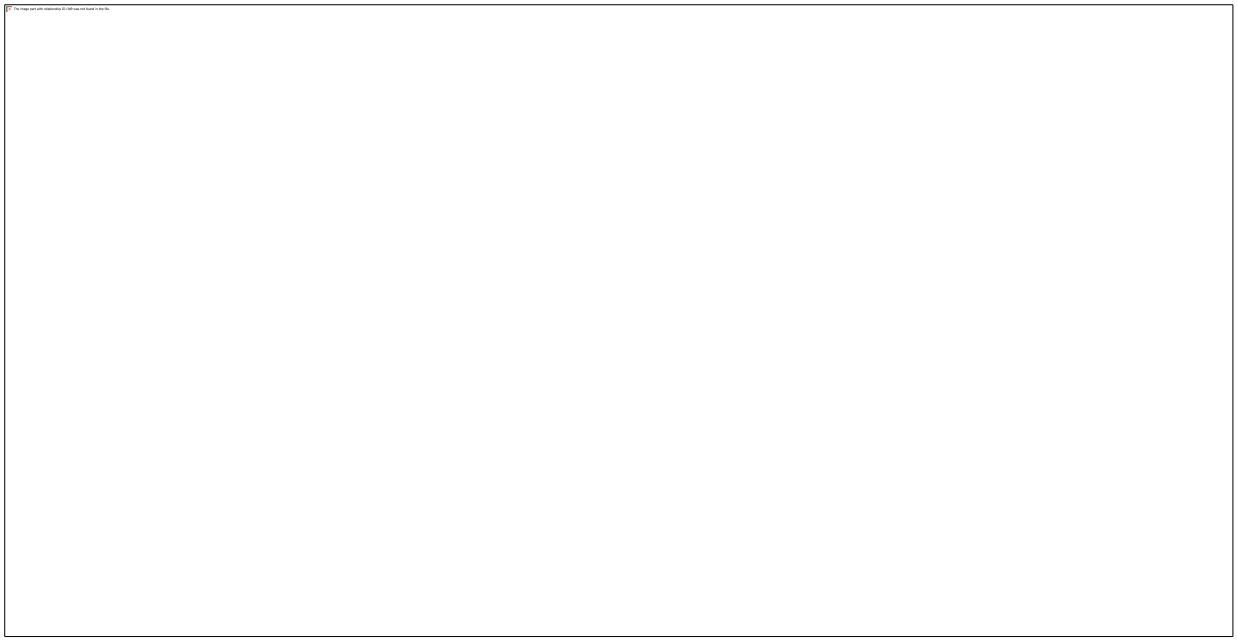

Figure 1.2: Schematic diagram of a typical antibody showing two Ig heavy chains (yellow) linked by disulfide bonds to two Ig light chains (blue)-Adapted from Schroeder and Cavacini, (2010).

One half of the four chains are heavy chains, whilst the other half are light chains (Williams and Barclay, 1988). Immunoglobulin isotypes have different heavy chain regions and effector functions (Leder, 1982; Tonegawa, 1983). Both heavy and light chain regions are divided based on the variability in their amino acid sequence (Schroeder *et al.*, 2008). These are the:

- **Light Chain** – Variable region (110 amino acids) and Constant region (110 amino acids).
- **Heavy Chain** – Variable region (110 amino acids) and Constant region (330-440 amino acids).

Each chain that forms an antibody has a variable (V) region at one terminal, and a constant (C) region at the opposite terminal (Casali and Schettino, 1996). These variable regions form the antigen-antibody binding sites (Schroeder and Cavacini, 2010). The expression of a specific isotype determines the function of an antibody, *via* the specific binding to Fc receptor molecules on different isotype cells (Smith *et al*., 2004).

1.5.3 The role of complement fixation in inflammation

Complement fixation is the chief antibody defense mechanism employed against foreign cell agents in the body (Nayak *et al*., 2010). Complement activation occurs *via* three pathways each

activated by specific antibody or antigen complexes *viz*: classical, lectin and alternative pathways (Regal *et al.*, 2015). The systemic action of the classical pathway is activated through the binding of immunoglobulins (IgG subclasses and IgM) to antigen cells, and then to C1 complement molecules (Nayak *et al*., 2010). However IgG2 and IgG4 activate classical complement less efficiently and only under certain conditions (Vidarsson *et al.*, 2014). The lectin pathway is activated by antibody binding to mannose-binding lectin (MBL) molecules, whereas, the alternative pathway does not require an exogenic trigger, and is activated at low levels (Collard *et al*., 2000; Carroll, 2004).

Essentially, all three pathways lead to the deposition of C3b to further opsonize the target and form membrane attack complexes -C5-C9 (Figure 1.3) (Regal *et al*., 2015). Many of the proteins released from the complement pathway are enzyme precursors (Nayak *et al.*, 2010). The release of these proteins amplify the inflammatory response, and promotes phagocytosis, cell lysis and anomalous placental development, during pregnancy (Shamshirsaz *et al.*, 2012).

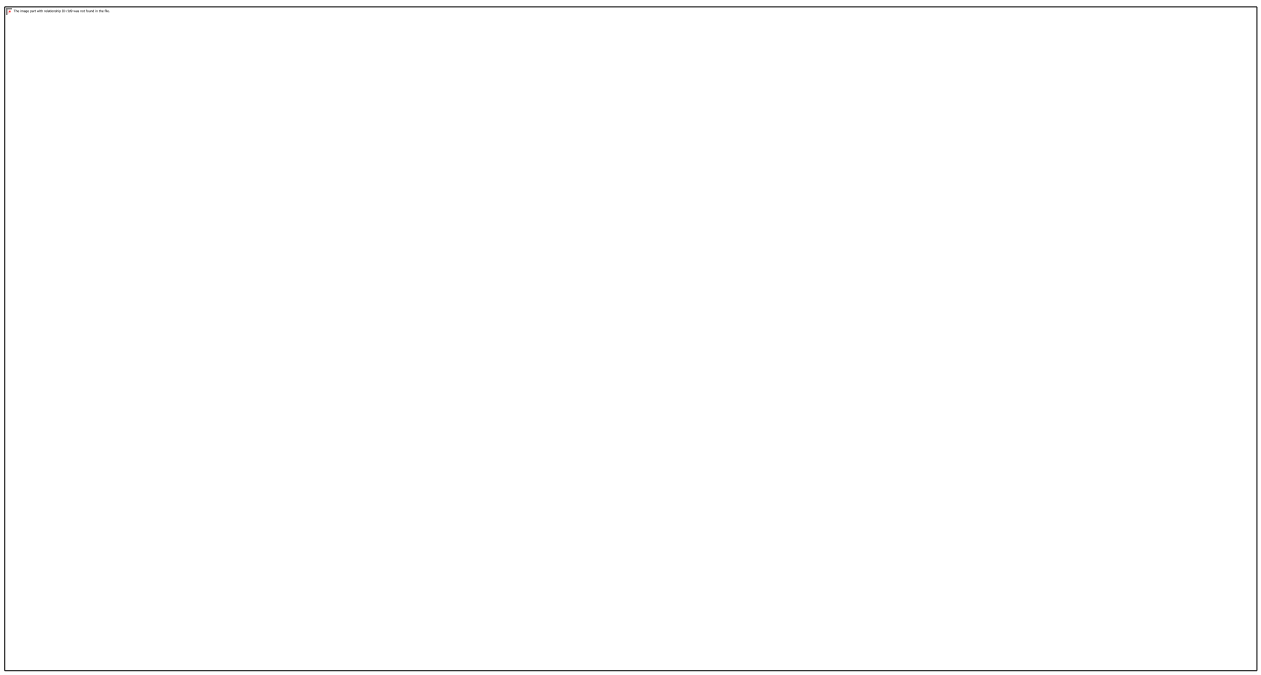

Figure 1.3: The Antigen-Antibody complex during the activation of the classic pathway of complement- Adapted from Arthur *et al*. (2000).

1.5.4 Immunoglobulin isotypes and their biological roles in humans

IgM: Immunoglobulin M is the first immunoglobulin to be released by plasma cells as a primary response to pathogen invasion and is indicative of a current infection (Gronwall *et al*., 2012). IgM is a good agglutinating agent and is produced in considerable serum quantities in humans (400-2300 µg/ml) (Wang *et al*., 2016). If prolonged high levels of this immunoglobulin exist, then a prolonged exposure to parasites may be assumed (Raccine and Winslow, 2009).

IgG: Immunoglobulin G is the secondary protective action that occurs against a foreign antigen, and it is the most abundant serum protein in humans (Vidarsson *et al*., 2014). This specific immunoglobulin isotype contains four subclasses such as IgG1, IgG2, IgG3 and IgG4, that are named according to their decreasing levels within human serum (Einarsdottir *et al.*, 2014). Although the IgG subclasses have identical amino acid sequences, each subclass consists of different constant regions, that are involved in binding to IgGFc receptors (FcγR) and C1 molecules of classical complement (Vidarrsson *et al.*, 2014). This results in different effector functions amongst the subclasses, regarding activation of the complement pathway, phagocytosis or antibody-dependent cell-mediated cytotoxicity (Kapur *et al*., 2014). The activation of a specific subclass is sometimes dependent on the specificity of the antigen present (Vlug *et al*., 1994; Pan and Hammarstrom, 2000). The chemical composition of the antigen may also cause preferential subclass switching within the IgG group (Pone *et al*., 2010; Pone *et al*., 2012). In addition, selective subclass deficiencies are not detrimental, but they may lead to an enhanced susceptibility towards specific pathogenic classes (Jefferis and Kumararatne, 1990).

IgA: Immunoglobulin A is abundant in human body secretions such as saliva, sweat, intestinal juice and milk (Lopez *et al*., 2018). However, serum IgA exists in limited amounts in plasma, and accounts for 20% of all serum immunoglobulins (Pabst, 2012). Therefore, secretory IgA and serum IgA are molecules with different biochemical and immunochemical properties (Kerr, 1990). IgA functions to thwart pathogenic attachment to epithelial cell surfaces (including neuron membranes and the epidermis), it is also believed that IgA may possess anti-inflammatory effects (Woof and Russell, 2011).

IgD: Immunoglobulin D represents 0.25% of the total immunoglobulins in human serum (Rogentine *et al*., 1966). Despite its discovery five decades ago, the specific function of IgD remains enigmatic (Chen and Cerutti, 2011).

IgE: The immunoglobulin E isotype protects the host from parasitic infections that induce allergies (Amarasekera., 2011; Xiong *et al*., 2012). In human blood IgE exists in trivial quantities, unless stimulated by allergic antigens (Unal *et al*., 2017). During allergic reactions IgE binds to the surface membrane of blood basophils and tissue mast cells (Fish *et al*., 2005). It is believed that IgE evolved to protect the host from helminth infections (Snow *et al*., 1996).

1.5.5 Placental transfer of maternal immunoglobulins to the fetus

An undeveloped fetal immune system requires immune protection from the maternal-to-fetal transfer of antibodies across the placenta (Simister, 2003). During pregnancy, the predominant antibody in the maternal-fetal transport process is IgG and its subclasses (Kane and Acquah, 2009). The concentration of IgG and its subclass in a maternal-to-fetal direction is regarded as: IgG1> IgG4> IgG3> IgG2 (Malek, 1996). The placental transfer of IgG and its subclasses depend on maternal levels (Palmeira *et al.*, 2011). Nonetheless, existing IgM in the fetus is of fetal origin, whilst no transfer activity of IgA in the maternal-to-fetal placental transfer mechanism has been reported to-date (Malek, 2013). Relatedly, IgD and IgE isotypes have not been reported in this transfer process (Holt and Jones, 2000). Therefore, the current study involves the detection of antibodies, specifically IgG1, IgG2, IgG3, IgG4, IgA and IgM in maternal serum.

1.6 The role of immunoglobulins in normotensive pregnancies

During healthy human pregnancy, complement activation produces a judicious inflammatory response that succors the continuation of pregnancy (Salmon and Girardi, 2008). However, the role of immunoglobulins in activating a suppressed complement cascade has yet to be fully elucidated (Nayak, *et al*., 2010). Khirwadkar and Kher (1991) studied IgG, IgA and IgM levels in healthy pregnant women compared to non-pregnant women. Healthy pregnant women reported a decrease in IgG and IgA, throughout pregnancy although, IgM appeared to increase in the third trimester (Khirwadkar and Kher, 1991).

1.7 The role of immunoglobulins in preeclampsia

Preeclampsia involves the maladaptation of the maternal immune system, which results in a hyperbolic complement activation and systemic inflammatory response (Derzy *et al*., 2010). This is sequentially associated with placental insufficiency, maternal endothelial dysfunction and fetal growth restriction that is characteristic of PE (Lynch and Salmon., 2010).

A few studies have investigated the role of antibodies in the heightened inflammatory response of PE (Arinola *et al.*, 2006; [b]Amah-Tariah *et al.*, 2016). A significantly lower IgG level in preeclamptic pregnancies have been reported (Fialova *et al.*, 2004; [b]Amah-Tariah *et al.*, 2016). In contrast, Biró *et al.* (2007) reported no significant differences in IgG levels during PE. In addition, no significant difference in IgM concentrations have been reported between normotensive and preeclamptic pregnancies (Fialova *et al.*, 2004; Arinola *et al.*, 2006). However, data on IgM levels in PE are variable, as [b]Amah-Tariah *et al.* (2016) reported significantly lower levels in preeclamptic, diabetic Nigerian women with significantly higher IgA levels in preeclamptic pregnancies (Arinola *et al.*, 2006).

1.8 The role of immunoglobulins in HIV infected pregnancies

The consequence of HIV infection on the human immune system, is progressive immunodeficiency (Huber *et al*., 2011). This deficiency leaves the body vulnerable to pathogens and opportunistic malignancies (Josh *et al*., 2011). Over-time HIV destroys the antibody immune response, through the depletion of CD4 T-cell count and the impairment of B cell function (Prendergast *et al*., 2010; Cooper *et al*., 2013). Subsequently, HIV infection during pregnancy may result in aberrant immunoglobulin levels, dysregulated compliment activation and cell-mediated immunity, which disturbs the precarious immune state in pregnancies (Khan *et al*., 2016).

However, a Nigerian study has reported that HIV viral load and serum immunoglobulin concentrations have no statistically significant correlations, irrespective of pregnancy (Akinpelu *et al*., 2012). Conversely, HIV infection is known to impair B lymphocyte activity with a resultant increase in overall immunoglobulin levels (Aucouturier *et al.*, 1986). In a recent study, [a]Amah-Tariah *et al.* (2016) assessed immunoglobulin levels in HIV positive pregnant women with malaria and reported an overall increase in IgG, IgM and IgA levels over the three trimesters of pregnancy. The increase in immunoglobulin levels may lead to adverse pregnancy outcomes ([a]Amah-Tariah *et al.*, 2016).

1.9 The role of immunoglobulins in HIV infected preeclampsia

Preeclampsia is a gestational precondition that involves the hyperactivity of the immune system and inflammatory mechanisms, whilst HIV eventuates in a chronic inhibition of the immune system (Akinpelu *et al.*, 2012; Harmon *et al.*, 2016).

The significant decrease of IgG levels during PE could be explained by the placental transfer of immunoglobulins, or proteinuria loss of IgG subclasses ([a]Amah-Tariah *et al.*, 2016). However, elevated IgG levels in HIV infected pregnancies may be the result of increased B cell activation to control the high viral load (Akinpelu *et al.*, 2012). Furthermore, the maternal decrease of IgA and IgM may be attributed to significant proteinuria loss during PE (Arinola *et al*., 2006). Nevertheless, damage to the vascular walls in PE, initiates intense narrowing of blood vessels, resulting in placental ischemia (Eiland *et al.*, 2012). This leads to an increased production of white blood cells and IgM antibodies, which mount a rapid stimulation of inflammatory responses, possibly validating the conflicting IgM increase in PE ([a]Amah-Tariah *et al.*, 2016).

In addition, increased IgA levels during pregnancy may be result of a fostered neutralization activity against antigens during PE or HIV infection ([a]Amah-Tariah *et al*., 2016). Similarly, increased IgM levels may signify existing pathogenic activity, and HIV disease progression ([a]Amah-Tariah *et al.*, 2016).

Despite the few studies on immunological parameters in South Africa, there is a paucity of data on the effects of immunoglobulins in HIV infected preeclamptics. Therefore, this study aims to investigate immunoglobulin isotypes in HIV associated preeclamptics receiving HAART in a South African setting.

1.10 Aims and objectives

The exploration of serum markers in HIV infected pregnant women on HAART and PE, steer the direction of research on preventative strategies. The fundamental objective behind such studies is the global need to improve overall antenatal and perinatal care. Therefore, this study targeted the response of immunoglobulins in immune activation, which prompt the release of pro-inflammatory cytokines, disrupting placentation during PE.

Aim

To investigate the role of immunoglobulins (IgG subclasses, IgA and IgM) in HIV associated PE on HAART.

Objectives

- To analyse the concentration of IgG subclasses, IgA and IgM, and examine their role in inflammatory responses based on pregnancy type (Normotensive pregnant *vs* PE) and HIV status (HIV negative *vs* HIV positive).

- To analyse the concentration of IgG subclasses, IgA and IgM, and evaluate their role in immune responses during HIV associated PE.

- To examine the effect of HAART on the concentration of IgG subclasses, IgA and IgM in HIV associated PE.

CHAPTER 2

PROOF OF SUBMISSION

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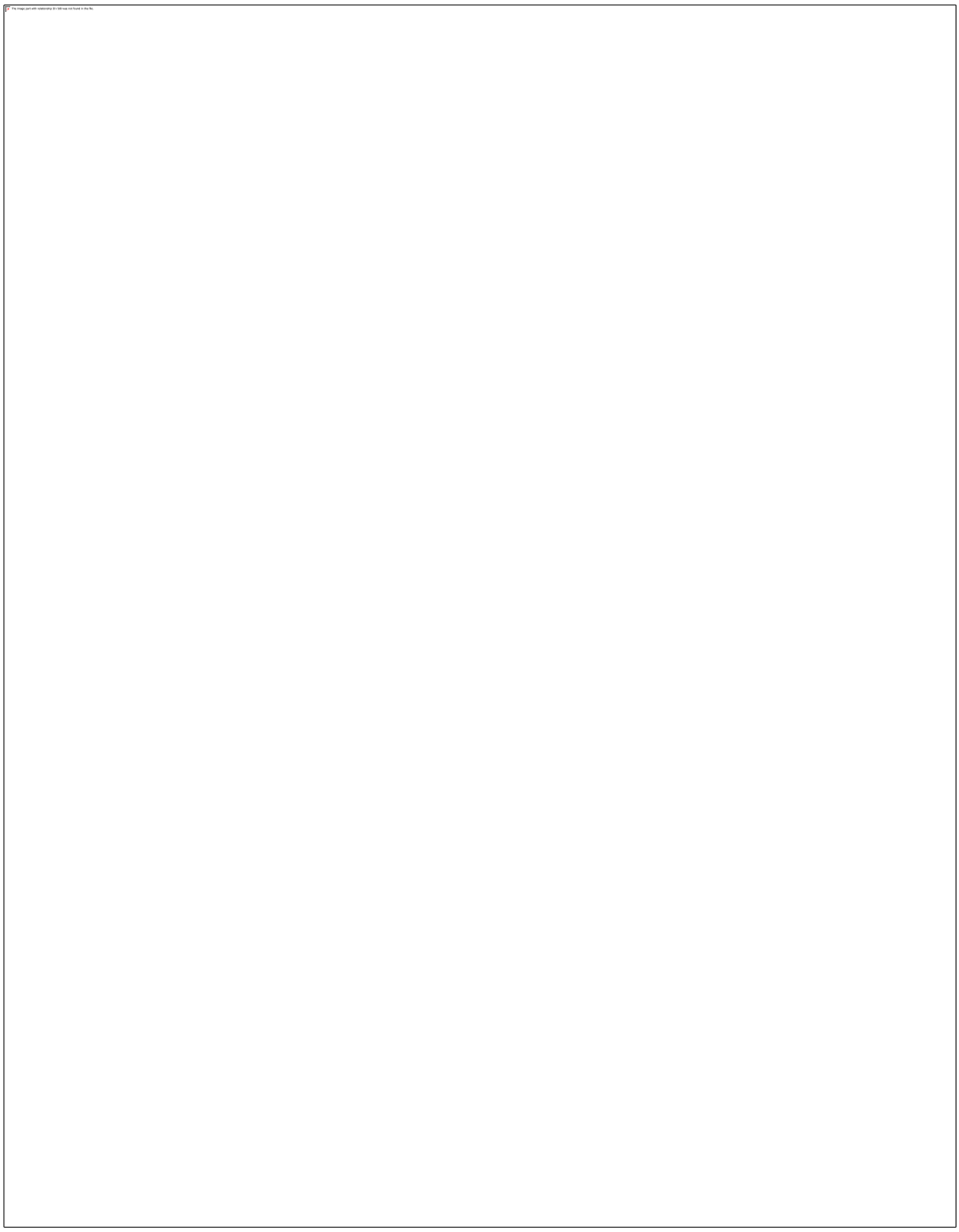

THE ROLE OF IMMUNOGLOBULIN ISOTYPES (IgG1-IgG4, IgA AND IgM) IN HIV ASSOCIATED PREECLAMPSIA IN SOUTH AFRICAN WOMEN ON HAART.

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ABSTRACT

The fragile balance of immunity that exists in pregnancy is altered during pregnancy complications, such as preeclampsia and HIV infection. Immune responses are coordinated by the regulation of immunoglobulins, which is dysregulated during pregnancy. Preeclampsia involves the exacerbation of an immune response. However, HIV infection suppresses the immune system. This study aims to elucidate the role of immunoglobulins in HIV associated normotensive versus preeclamptic pregnancies, receiving HAART. Post informed consent, 38 normotensive and 38 preeclamptic women were sub-categorised by HIV status. Maternal serum was analysed using the Bio-Plex immunoassay technique. IgA was significantly up-regulated in PE compared to normotensive pregnancies. Additionally, IgG2, IgA and IgM were significantly down-regulated in HIV positive pregnancies. IgG2 and IgA expressed significant up-regulations in HIV negative PE. The significant difference of IgG2 and IgA suggest an association between the hyper-inflammatory milieu of PE and HIV infection in neutralising immunity, irrespective of HAART.

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INTRODUCTION

The epidemiology of the Human immunodeficiency virus (HIV) infection and hypertensive disorders such as preeclampsia (PE), implicate both disorders as major contributors to maternal mortality in South Africa (Maharaj *et al.*, 2017). However, global prevalence data on PE among HIV infected pregnant women remain discrepant (Machado *et al.*, 2014).

Preeclampsia is defined as a multi-organ hypertensive disorder of pregnancy, that is associated with a generalized systemic inflammatory response (Wu *et al.*, 2017). The etiology of PE is unclear therefore, treatment remains empiric (Khaliq *et al*., 2018). The modern understanding of the pathogenesis of PE involves the exploration of possible alterations in immunological processes (Maharaj *et al.*, 2017).

During healthy pregnancy, a suppressed immune response evades maternal rejection of the fetal allograft thereby, ensuring pregnancy success (Kalagiri *et al.*, 2016). However, in PE a decreased cytotrophoblast invasion, is followed by a resultant non-physiological transformation of myometrial spiral arteries (Thakoordeen *et al*., 2018). This leads to the development of small-bore high resistance capacitance vessels with reduced blood flow, creating a hypoxic microenvironment that contributes to maternal morbidity and mortality (Redman and Sargent, 2009; Costantine and Cleary, 2013). Moreover, an activation of pro-inflammatory cytokines, exacerbates the inflammatory cascade in PE (Cornelius *et al*., 2018).

In addition, HIV infection involves cell invasion by the virus, to produce viral RNA (Maartens *et al.*, 2014). This enables the multiplication of HIV and eventually results in the destruction of T-helper and B cells (Stacey *et al.*, 2009; Cooper *et al*., 2013). The efficient production of antibodies is thus distressed causing a suppression of the immune system and exposing cells to opportunistic viral infections (Huber *et al*., 2011).

The role of the immune system is fundamental to both PE development and HIV infection (Girardi *et al*., 2006; Landi *et al.*, 2014). Therefore, knowledge on antibody activation of the immune inflammatory response is pertinent, when attempting to understand the pathophysiology and treatment of both PE and HIV infection.

Immunoglobulins are a group of antibody cells produced by B cells, that activate the complement pathway (Ziegler *et al*., 2018). Immunoglobulin activation of the classical complement pathway initiates cell lysis, phagocytosis of foreign antigen cells and inflammatory responses (Shamshirsaz *et al.*, 2012). During healthy pregnancy, the maternal-fetal placental transfer of immunoglobulins

simultaneously contributes to the suppression of the immune system and protection of the fetus (Fouda *et al.*, 2018). Immunoglobulin G (IgG) and its subclasses are the predominant immunoglobulin in the maternal transmission of antibodies (Holt and Jones, 2000). Despite the role of IgM and IgA in the complement pathway, these immunoglobulins are omitted from the maternal-fetal transfer of immunity (Malek, 2013).

In PE, a significant decrease in the concentration of IgG with a concomitant increase in IgA and IgM have been reported (Arinola *et al.*, 2006). In contrast, Biró *et al.* (2007) observed no significant difference in IgG and IgM levels between preeclamptic compared to normotensive healthy pregnancies. Therefore, data on immunoglobulin concentrations in PE are conflicting (Table 1). Nonetheless, a significant increase in IgG, IgA and IgM was observed in HIV positive pregnancies ([a]Amah-Tariah *et al.*, 2016).

To our knowledge, there is no available data on immunoglobulin isotype levels in HIV associated normotensive and preeclamptic pregnancies. Moreover, there is a paucity of data on immunoglobulin levels in HIV infected pregnancies receiving HAART. Therefore, the aim of this study is to investigate the concentration of immunoglobulin isotypes (IgG1-IgG4, IgA and IgM) in HIV infected women with PE, receiving HAART.

Table 1: Immunoglobulin concentrations in normotensive, preeclamptic and HIV infected pregnancies, extracted from previous studies.

Author (year)	Population & Sample	HIV status	Gestational age	IgG	IgA	IgM
Khirwadkar and Kher., (1991)	India Serum 100	−	Trimester 1 Trimester 2 Trimester 3	1817.64 mg/dl 1687.93 mg/dl 1623.33 mg/dl	197.14 mg/dl 205.64 mg/dl 213.70 mg/dl	188.70 mg/dl 207.98 mg/dl 251.46 mg/dl
				(p < 0.001)	***(p < 0.05)***	***(p < 0.001)***
Fialova *et al.*, (2004)	Czech Republic Serum 21	−	Trimester 3	3.3±4.20 mpl 2.70±3.40 mpl*		1.1±1.7 mpl 0.50±1.60 mpl*
				(p < 0.05)		***(p > 0.05)***
Arinola *et al.*, (2006)	Nigeria Serum 63	−	Trimester 3	1311.80±97.00 mg/dl 519.20±29.90 mg/dl*	50.80±43.90 mg/dl 48.80±36.80 mg/dl*	72.80±43.00 mg/dl 69.00±48.70 mg/dl*
				(p < 0.05)	***(p < 0.05)***	***(p > 0.05)***
Biró *et al*., (2008)	Netherlands Serum microparticles 30	–	Trimester 2	6.6±10.1 g//l 4.5±7.4 g/l*		0.6±1.7 g/l 0.5±1.8 g/l*
				(p > 0.05)		***(p > 0.05)***
[b]Amah-Tariah *et al.*, (2016)	Nigeria Serum 100	−	Trimester 3	1529.00±88.11mg/dl 1366.67±67.26mg/dl*	130.24±14.00mg/dl 132.08±15.99mg/dl*	136.20±23.89mg/dl 172.67±29.19 mg/dl*
				(p < 0.05)	***(p > 0.05)***	***(p < 0.05)***
[a]Amah-Tariah *et al.*, (2016)	Nigeria Serum 100	− +	Trimester 1	1529.00±88.11mg/dl 1694.00±170.79mg/dl[#]	130.24±14.00mg/dl 141.10±18.98mg/dl[#]	136.20±23.89mg/dl 162.40±34.41mg/dl[#]
				(p < 0.05)	***(p < 0.05)***	***(p < 0.05)***

Key: Preeclampsia,* HIV positive,[#] g/l -grams per litre, mg/dl- milligrams per decilitre, mpl-microparticles per litre, *P<0.05* = significant.

METHODS AND MATERIAL

Ethics approval and study population

This prospective study was conducted at the University of KwaZulu-Natal. Institutional ethics clearance was obtained from the Biomedical Research Ethics Committee (BREC-BE 338/17). Participants were recruited in the antenatal period (trimesters 2 and 3) from a large regional hospital in Durban, South Africa. The study population (n=76) was determined in consultation with a biostatistician using the Fischer's test, and consisted of a normotensive group (n=38) and a PE group (n=38). Each group was further categorized into HIV positive (n=19) and HIV negative (n=19) women. Following written informed consent, venous blood samples were collected in K3-EDTA tubes, serum was then extracted and stored in cryovials at -80°C, until immunoassay. PE was defined as a systolic pressure (mmHg) of ≥140 and a diastolic pressure ≥90 (mmHg), whilst proteinuria measured ≥2 on a urine dipstick after 20 weeks of pregnancy (Cnossen *et al.*, 2006). All diagnosed HIV positive women were initiated on anti-retroviral therapy (HAART). Healthy normotensive and HIV negative pregnant women served as control groups. The exclusion criteria were based on pregnant women with: chorioamnionitis, eclampsia, polycystic ovarian syndrome, abruptio-placentae, intrauterine death, sickle cell disease, chronic renal disease, cardiac disease, unknown HIV status, pre-existing seizure disorders and asthma.

Bio-plex immunoassay

Differences in serum immunoglobulin isotypes were assessed using the Bio-Plex® Pro™ Human isotyping Panel 6-Multiplex assay (catalogue #171A3100M; Bio-Rad laboratories Inc., South Africa) according to the manufacturer's instructions. Serum IgG1-IgG4, IgA and IgM (1:40 000 dilution) were distinguished by capture antibody-coupled magnetic beads. This immunoassay was similar to the sandwich ELISA technique. Fluorescent SA-PE was used to bind and capture antibody-coupled magnetic beads. After washing, detected differences in fluorescent intensity and concentration of captured coupled magnetic beads of the serum isotypes were carried out, using Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad laboratories Inc, USA).

The Bio-Plex Manager™ software version 4.1. was used to obtain the observed concentration of serum immunoglobulin isotypes based on pregnancy type (normotensive *vs* PE) and HIV status (HIV negative *vs* HIV positive).

Statistical Analysis

Data was analyzed using GraphPad Prism 5.00 for windows (GraphPad Software, San Diego, California USA). Statistical analysis across all groups, HIV status and pregnancy type were performed using the One-way ANOVA (Bonferroni *post hoc* and *Kruskal Wallis)* multiple comparison tests. For individual analysis, a student's T-test was performed. The level of significance was considered as a probability level of $p < 0.05$.

RESULTS

Patient demographics (maternal age, gestational age, systolic pressure, diastolic pressure and weight) are outlined in Table 2.

Table 2: Patient demographics across study population**.**

	HIV negative Normotensive	**HIV positive Normotensive**	**HIV negative Preeclampsia**	**HIV positive Preeclampsia**	***P*-value**
Maternal age (years)	25.32±5.76	28.00±6.37	28.53±6.66	29.95±6.23	0.1517
Gestational age (weeks)	38.42±1.87	39.44±1.82	28.11±2.26	28.21±2.26	0.0001
Systolic BP (mmHg)	113.26±12.09	120.70±10.59	159.42±12.35	155.84±8.90	0.0001
Diastolic BP (mmHg)	70.89±8.87	72.65±9.90	101.32±8.90	105.05±11.96	0.0001
Maternal weight (kg)	78.02±15.38	77.36±12.95	72.52±16.68	75.66±18.10	0.5593

Results are represented as mean ± standard deviation. Key: mmHg-millimeter mercury, Kg-kilogram, BP-blood pressure.

Table 3: Concentration of immunoglobulins between preeclamptic and normotensive pregnancies.

Pregnancy type	**IgG1**	**IgG2**	**IgG3**	**IgG4**	**IgA**	**IgM**
Pre-eclampsia	323.90±163.70	187.20±129.60	34.39±18.92	11.34±10.95	131.20±121.60	80.11±66.07
Normotensive	377.50±178.70	163.10±127.00	40.92±31.91	19.21±21.55	65.10±33.63	66.11±32.34
P-value	0.2965	0.3062	0.7278	0.2531	0.0245	0.6969

Results are represented as mean ± standard deviation (ng/dl)

Table 4: Concentration of immunoglobulins between HIV positive and HIV negative pregnancies.

HIV status	**IgG1**	**IgG2**	**IgG3**	**IgG4**	**IgA**	**IgM**
HIV positive	366.90±191.50	128.30±81.87	36.42±30.25	12.65±14.88	74.21±68.62	61.05±41.53
HIV negative	334.60±151.70	222.00±148.40	38.89±21.90	17.90±19.51	122.10±110.80	85.18±59.06
P-value	0.4175	0.0011	0.1819	0.1487	0.0038	0.0176

Results are represented as mean ± standard deviation (ng/dl)

Table 5: Concentration of immunoglobulins between four subject groups inclusive of HIV status and pregnancy type.

HIV status & pregnancy type	**IgG1**	**IgG2**	**IgG3**	**IgG4**	**IgA**	**IgM**
HIV positive Preeclampsia	315.60±174.60	131.60±94.97	29.03±17.97	10.36±10.54	94.80±87.82	61.10±50.58
HIV negative Preeclampsia	332.30±156.40	242.80±137.80	39.74±18.77	12.31±11.55	167.80±141.02	99.13±75.17
HIV positive Normotensive	418.20±198.30	125.00±68.81	43.80±37.99	14.94±18.25	53.61±32.75	61.00±31.42
HIV negative Normotensive	336.80±151.10	201.30±159.30	38.03±25.14	23.49±24.15	76.60±31.20	71.22±33.28
P-value	0.6533	0.0084	0.3653	0.2770	0.0008	0.0758

Results are represented as mean ± standard deviation (ng/dl)

Immunoglobulins in pregnancy type*:*

Table 3 outlines the mean ±standard deviation (ng/dl) of immunoglobulins based on pregnancy type *i.e.* pre-eclamptic *vs* normotensive pregnancies. A significant up-regulation of IgA was observed in PE (131.20±121.60 ng/dl) compared to normotensive (65.10±33.63 ng/dl) ($p < 0.05$) pregnancies, regardless of HIV status. However, no significant difference was noted for IgG1-IgG4 and IgM ($p > 0.05$) based on pregnancy type.

Immunoglobulins in HIV status specific pregnancy:

The immunoglobulin expression between HIV positive *vs* HIV negative pregnancies, irrespective of pregnancy type is shown in Table 4. The expression of IgG2 (128.30±81.87 *vs* 222.00±148.40 ng/dl), IgA (74.21±68.62 *vs* 122.10±110.80 ng/dl) and IgM (61.05±41.53 *vs* 85.18±59.06 ng/dl) was significantly downregulated in HIV positive compared to HIV negative pregnancies ($p < 0.05$). However, IgG1, IgG3 and IgG4 expressions were similar between HIV positive *vs* HIV negative women ($p > 0.05$).

Immunoglobulins across all subject groups inclusive of HIV status and pregnancy type*:*

Table 5 shows the mean ± standard deviation (ng/dl) of immunoglobulin (IgG1-IgG4, IgA and IgM) concentrations across all subject groups. The elevated trends of IgG1 and IgG3 (418.20±198.30 and 43.80±37.99 ng/dl) was noted in HIV positive normotensive pregnancies respectively, albeit non-significantly ($p > 0.05$). IgG2 and IgG4 levels showed highest in HIV negative PE and normotensive pregnancies (242.80±137.80 and 23.49±24.15 ng/dl) respectively,

compared to HIV positive normotensive and preeclamptic pregnancies. IgG2 showed significance across all groups ($p < 0.05$), whilst no significance in IgG4 levels existed across the groups ($p > 0.05$). IgA and IgM were higher in HIV negative preeclamptic pregnancies (167.80±141.02 ng/dl and 99.13±75.17 ng/dl) than in HIV negative normotensive, and HIV positive normotensive and preeclamptic pregnancies. However, IgM showed no significant difference amongst the groups ($p > 0.05$) whilst, IgA showed a significant difference across all subject groups ($p < 0.05$).

DISCUSSION

Pregnancy is a complex journey with intricate transitional developments in the immune system of the mother, ensuring a successful pregnancy (Regal *et al.*, 2015). Immunoglobulins play an important role in maintaining the balance of the immune system between mother and child (Malek *et al.*, 1994). During pregnancy, IgG and IgM activate the classical complement pathway, initiating a depressed placental production of pro-inflammatory cells and cytokines (Panda and Ding, 2015; Khan *et al.*, 2016). This induces a suppressed inflammatory response to assure a successful pregnancy (Cornelius *et al*., 2018). However, the role of IgA in complement activation is controversial (Wolf *et al.*, 1994). Some studies observed no effect on the complement pathway, whilst others highlight that IgA is active only in the alternative and lectin complement pathways (Mestecky and McGhee, 1987; Russell *et al.*, 1989). In a study in India, healthy pregnant women expressed a significant decrease in IgG and IgA, with an up-regulation of IgM when compared to healthy non-pregnant women (Khirwadkar and Kher, 1991) (Table 1).

Despite literature validating the disparity in immune levels amongst healthy pregnant and non-pregnant women, the immune system is further modified in severely complicated pregnancies (Kalagiri *et al.*, 2016). Our study report IgG subclasses, IgA and IgM levels in HIV seronegative normotensive pregnancies, and compares them to HIV positive normotensive pregnancies and HIV positive and negative PE. Preeclampsia is associated with inflammatory processes (Fialova *et al.,* 2004). Classical complement activation during PE suggests a proliferated production of pro-inflammatory cytokines that induce chemotaxis, leukocyte and neutrophil activation thus intensifying the inflammatory cascade (LaMarca *et al*., 2007; Khan *et al.*, 2016). Fialova *et al.* (2004), Arinola *et al.* (2006) and later, [b]Amah-Tariah *et al.* (2016) reported significantly low total IgG levels in PE, compared to normotensive pregnancies (Table 1). Nevertheless, in our study IgG1, IgG3 and IgG4 were non-significantly down-regulated in PE compared to normotensive pregnancies, irrespective of HIV status (Table 3). These results corroborate the total IgG level observed by Biró *et al.* (2007). This diminution may be attributed to the role of IgG subclasses in the transfer of passive immunity from mother to child (Kane and Acquah, 2009). The concentration of IgG subclasses in both mother and child exists as: IgG1> IgG4> IgG3> IgG2 (Firan *et al*., 2001). Therefore, IgG1, IgG3 and IgG4 conveyed a down-regulation in PE.

In our study, IgG2 was up-regulated in PE compared to normotensive pregnancies irrespective of HIV status, albeit non-significantly (Table 3). The increase of this specific subclass may be due to IgG2 being the least favored IgG subclass transported across the placenta (Malek *et al.*, 1996). Increased IgG2 levels in PE

could also be a result of subclass switching, that depends on the chemical composition and route taken by an antigen on entering the body (Berkowska *et al.*, 2011). Polysaccharide antigens trigger subclass switching to IgG2, activating classical complement and inducing the inflammatory cascade, when T-cell help is unavailable (Pone *et al.*, 2010). The IgG2 subclass also responds to bacterial antigens thus excessive levels of this subclass may be indicative of bacterial infections (Kuijpers *et al.*, 1992). Pregnant women also have a greater susceptibility to infections, due to a marginally suppressed immunity (Arinola *et al.*, 2006). Therefore, immunizations against communicable bacteria may increase IgG2 levels in pregnant women.

In the present study, IgA was significantly increased in PE compared to normotensive pregnancies, irrespective of HIV status (Table 3). Nonetheless, [b]Amah-Tariah *et al.* (2016) noted a non-significant up-regulated trend of IgA levels in preeclamptic and diabetic pregnancies, compared to normotensive pregnancies (Table 1). Notably, an up-regulated IgA expression could be a result of an underlying pathogenic condition, as IgA acts to neutralize mucosal pathogens (Kilian and Russell, 2015). Furthermore, IgA may act to prevent exaggerated inflammatory responses *via* the lectin and alternative pathways (Wolf *et al.*, 1994; Pasquier *et al.*, 2005). IgA acts to compete with IgG and IgM antibodies for the same antigen, thus preventing IgG and IgM from binding to the C1 complex and inhibiting the classical complement activation (Wilton, 1978). Moreover, serum IgA appears to initiate a down-regulation of pro-inflammatory cytokines thus suppressing the inflammatory cascade (Wolf *et al*., 1994). This specific effector function of IgA might justify the high serum levels shown in hyper-inflammatory PE. Contrastingly, Arinola *et al.* (2006) reported significantly low IgA levels in PE when compared to normotensive pregnancies (Table 1). The down-regulation of IgA may be explained by significant proteinuria loss that occurs during PE, as a result of increased glomerular filtration or reduced reabsorption (Murakami *et al.*, 2000).

In the current study, IgM levels were up-regulated (albeit non-significantly) in PE compared to normotensive pregnancies, irrespective of HIV status (Table 3). Consistently, previous studies also report a non-significant up-regulation in IgM levels in PE (Fialova *et al.*, 2004; Arinola *et al.*, 2006; Biró *et al.*, 2007). Nonetheless, [b]Amah-Tariah *et al.* (2016) observed a significant up-regulation of IgM in PE versus normotensive pregnancies (Table 1). IgM antibodies are the first line of defense in pathogenic invasion hence, underlying infections such as the HIV infection may explain the observed upregulation (Wang *et al.*, 2016). IgM is also a principal activator of the C1 complex in classical complement and may be increased during PE (Ziegler *et al*., 2018). Moreover, IgA and IgM are omitted from the maternal-fetal immune transfer hence, the observed up-regulation of these immunoglobulins (Malek, 2013; Rakoff-Nahoum, 2016).

HIV infected pregnant women undergo severely complicated pregnancies, as the immune system is directly affected and modified by the virus itself (Huber *et al.*, 2011). Initially direct activation of classical complement by HIV remains complex however, the immune system induces neutralization mechanisms to reduce viral infectivity (Scherl *et al.*, 2006). In line with the latter, an up-regulated trend of IgG1 was noted in HIV infection, albeit non-significantly (Table 4). Nevertheless, a significant increase of total IgG in HIV infected pregnant women has been reported by [a]Amah-Tariah *et al.* 2016 (Table 1). IgG1 is the most abundant IgG subclass in serum, and it appears during viral infections to initiate inflammatory responses (Einarsdottir *et al.*, 2014; Kapur *et al*., 2014). Additionally, when specific protein antigens induce B cell activation in the presence of T-helper cells subclass switching may favor IgG1, to activate the classical complement pathway (Pone *et al.*, 2010). Thus up-regulated IgG1 levels in our study may be conceivable.

Nonetheless, HIV eventually cultivates a resistance to the complement pathway, by acquiring proteins derived from human cells (Huber *et al.*, 2011). Despite the abundance of IgG in HIV infected pregnancies, the opsonized retrovirus ultimately infects macrophages and dendritic cells (Liu *et al*., 2004; Maartens *et al*., 2014). This eventuates in a surge of viral proteins and directs disease progression to AIDS development, because of the failure to control viral activity (Moir and Fauci, 2017). In our study, IgG2 was significantly down-regulated in HIV positive versus HIV negative pregnancies, irrespective of pregnancy type (Table 4). Notably, IgG3 and IgG4 levels were non-significantly down-regulated in HIV positive compared to HIV negative pregnancies, regardless of pregnancy type (Table 4). The decreased IgG2, IgG3 and IgG4 levels may be attributed to a combination of unspecific polyclonal B cell activation and impaired T-helper cell activity, or selective subclass switching (Vidarsson *et al.*, 2014). Additionally, HAART (the standard care for HIV infection in South Africa) is known to reconstitute immunity thus explaining the moderated IgG2, IgG3 and IgG4 expression in our results (Iordache *et al.*, 2017).

In the current study, IgA levels were significantly down-regulated in HIV positive compared to HIV negative pregnancies (Table 4). In contrast, [a]Amah-Tariah *et al.* (2016) reported a significant increase in IgA levels in HIV positive compared to HIV negative pregnancies (Table 1). The neutralization mechanism of IgA induces the elimination of pathogens through phagocytosis, the release of cytokines and activated oxygen species, and antibody dependent cell-mediated cytotoxicity (Woof and Russell, 2011). This neutralizing capacity of IgA may account for high serum levels in HIV positive individuals. Alternatively, the significant down-regulation of IgA in our study may be ascribed to the mechanism of HAART in restoring immune levels.

In our study, IgM significantly declined in HIV infected pregnancies compared to HIV negative pregnancies (Table 4). The decrease in IgM may implicate failure to control viral activity and disease progression to AIDS, following the impairment of non-specific B cell activation. In dissimilarity, [a]Amah-Tariah *et al.* (2016) reported a significant increase of IgM in HIV positive pregnancies. However, previous studies have indicated that varying immunoglobulin levels could be an outcome of genetic and environmental variance in B cell response to HIV infection (Payne *et al.*, 2007; Akinpelu *et al.*, 2012). Furthermore, HAART endeavors to increase CD4+ cell counts, opposing disease progression and reconstituting immunity hence, validating the plausibility of our findings (Fiore *et al.*, 2006; Maharaj *et al.*, 2017).

There is a paucity of information on the role of immunoglobulins in HIV associated PE, receiving HAART. It has been hypothesized, that HIV could counteract the exacerbated immune system response caused by PE, restoring immunoglobulin levels to that of normotensive HIV negative pregnancies (Mattar *et al.*, 2004). This study demonstrates immunoglobulin concentrations across HIV positive, and HIV negative normotensive and preeclamptic pregnancies. We show that IgG1, IgG3, IgG4 and IgM express down-regulatory trends in HIV positive PE, albeit non-significantly (Table 5). It is plausible that regardless of the role of IgG subclasses in maternal-fetal passive immunity, the administration of HAART counteracts uncontrolled B cell activation and high immunoglobulin levels induced by HIV infection. However, IgG2 articulated an overall significant up-regulation in HIV negative PE when compared to HIV negative normotensive, and HIV positive normotensive and preeclamptic pregnancies (Table 5). The distinctive response of this specific IgG subclass may be a result of specific antigen subclass switching, which promotes activation of classical complement by IgG2.

Alternatively, we report significantly higher levels of IgA in HIV negative PE, compared to all other study groups (Table 5). In healthy pregnancies devoid of viral infection, a suppressed maternal immune level is required for the continuation of pregnancy (Khirwadkar and Kher, 1991). During PE, IgA may act explicitly to inhibit the hyper-inflammatory response emanating from classical complement activation. This may be a result of IgA activating the lectin-mannose binding and alternative pathway, attempting to block the activation of the classical complement (Roos *et al.*, 2006). The specific "blocking" effect implicates that IgA may cause a down-regulation of other immunoglobulins during PE, as both IgG and IgM are responsible for activating the classical complement pathway. In addition, IgA also functions to neutralize pathogens like HIV through various elimination techniques (Kerr, 1990). Therefore, a significant up-regulation trend in serum IgA levels is associated with HIV infection (Mazzoli *et al.*, 1999). However, the administration of HAART to HIV infected pregnant women, may

act to reduce elevated IgA levels stimulated by HIV infection. Therefore, IgA was significantly lower in HIV infected PE compared to HIV negative PE (Table 5).

In conclusion we demonstrate the enhancement of IgG2 and IgA in the duality of PE and HIV infection. Our study predicts that whilst IgG2 is the least preferred IgG subclass in the maternal-fetal transfer of passive immunity, it is the preferred subclass to activate complement-mediated inflammatory responses in PE. In addition, it is plausible that IgA acts as an anti-inflammatory marker in the hyper-inflammatory microenvironment of PE. In HIV infection, the down-regulation of IgG2, IgA and IgM may be attributed to HAART. The early detection of up-regulated IgG2 and IgA in pregnancy may serve as a predictive test for PE development. However, further research on the natural anti-inflammatory response of IgA in PE may provide potential protection against the effects of PE. Moreover, the mechanism of IgG2 and IgA in HIV associated PE requires further exploration.

Recommendations and Limitations

These findings highlight the need for further investigation on the role of IgG subclasses and IgA inflammatory responses, in HIV infected preeclamptics on HAART. Further research on the anti-inflammatory effector functions of IgA in PE and HIV infected pregnancies are warranted. Limitations of our study include a small sample size. A larger sample size would enhance the understanding of the role of immunoglobulins in HIV associated PE.

Declaration of interest

There are no conflicts of interest.

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CHAPTER 3

3.1 Synthesis

In South Africa, maternal mortality is a serious public health concern. More than one third (34.7%) of maternal deaths in South Africa emanate from HIV infection in pregnancy (Saving mothers 2011–2013, 2014). In addition, PE is a major contributor of maternal mortality, accounting for 12% of all maternal deaths in South Africa (Saving mothers 2014–2016, 2017). Whilst some epidemiological findings associate PE with genetic and immunological factors, the etiology and pathogenesis of this hypertensive disorder remains a contentious dilemma (Khaliq *et al.*, 2018). Furthermore, literature on the role of HAART in HIV associated PE are few. There is deliberation as to whether HIV infected women on HAART have a lower pre-disposition to obstetric complications, such as PE (Browne *et al*., 2015; Phoswa *et al.*, 2018). The present study aimed to investigate the delicate immune balance that exists between HIV infected preeclamptics, on HAART.

Healthy pregnancy is characterized by suppression of the classical complement pathway, to deter immune responses against the fetus (Ahmad and Khidir, 2018). IgG and IgM bind to C1 complexes of classical complement, prompting the activation of potent anaphylatoxins (C3a and C5a), suppressed pro-inflammatory cytokine production and elevated anti-inflammatory cytokine production (Nayak *et al*., 2010; Vidarrsson *et al*., 2014). The activation of complement proteins persuades cell lysis through the infiltration of a moderated inflammatory cascade (Sarma and Ward, 2011). Corroborating this phenomenon, healthy pregnant women showed a significant down-regulation in IgG and IgA over three trimesters, whilst IgM decreased in the first and second trimesters when compared to non-pregnant women (Khirwadkar and Kher, 1991). However, during PE the immune system is further augmented, as elevated concentrations of complement proteins exist maternally (Derzy *et al*., 2013). The aberrant activation of complement proteins in PE is indicative of a hypertensive inflammatory cascade, which encourages an increased production of pro-inflammatory cytokines and neutrophils (Cornelius *et al.*, 2018).

Although, theoretically an increased IgG level would confirm the amplified inflammatory process that persists during PE, the precedence of fetal needs govern the maternal immune system through transplacental immunity (Fouda *et al*., 2018). The maternal-fetal transfer of immunoglobulins traverses the placenta and favors the IgG4, IgG3 and IgG1 subclasses, providing passive immunity in cumulative levels respectively (Schneider and Miller, 2009). The current study demonstrates a comparison of immunoglobulin levels between PE and normotensive pregnancies, irrespective of HIV status. IgG subclasses (IgG1, IgG3, and IgG4) presented a non-significant down-regulatory trend in PE. Nonetheless, Fialova *et al.* (2004), Arinola *et al.* (2006) and [b]Amah-

Tariah *et al.* (2016) reported a significant down-regulation in overall IgG levels in PE. In addition, we noted a non-significant up-regulated trend of IgG2 in preeclamptic pregnancies compared to normotensive pregnancies, irrespective of HIV status. Although the mechanism of maternal-fetal passive immunity favors IgG subclasses notably, the IgG2 subclass is the least favored form of immune transfer (Palmeira *et al.*, 2011). Furthermore, IgG2 exists in trivial quantities in fetal sera thus explicating the current findings (Malek, 2013). Moreover, our findings allude to IgG2 being the preferred subclass in the activation of classical complement during PE. Alternatively, underlying bacterial infections with a specificity for polysaccharide antigens may distinctively prefer IgG2 antibodies, resulting in an increased B cell production of this subclass (Valenzuela and Schaub, 2018).

Additionally, our study exhibits a non-significant up-regulatory trend in IgM levels, between preeclamptic versus normotensive pregnancies, regardless of HIV status. This observation is supported by Arinola *et al.* (2006) and Biró *et al.* (2007). Nevertheless, [b]Amah-Tariah *et al.* (2016) noted a significantly increased IgM level in PE. The IgM isotype is released as the first line of defense against pathogenic invasion in the human body (Boyden, 1966). In response to extraneous antigens, IgM stimulates the classical complement activation, to direct an inflammatory response against a plethora of pathogens (Baumgarth *et al.*, 2005; Grönwall *et al.*, 2012). The up-regulated trend in IgM levels is anticipated, as PE is an exacerbated inflammatory response and IgM activates classical complement.

Moreover, our study reported significantly higher levels of IgA in preeclamptic compared to normotensive pregnancies, irrespective of HIV status. Nonetheless, [b]Amah-Tariah *et al.* (2016) reported an up-regulation of IgA in preeclamptic diabetic pregnancies, compared to normotensive pregnancies, albeit non-significantly. However, whilst IgG subclasses and IgM activate the classical complement pathway, the role of IgA in complement remains abstruse. Some studies reported no participation of IgA in the complement pathway (Woof and Russell, 2011). However, a few studies report that IgA may inhibit activation of classical complement *via* the lectin and alternative pathways (Wolf *et al*., 1994). The latter is mediated through the binding of carbohydrate recognition receptors on mannose-binding lectin complexes (Matsuda, 1998; Roos *et al.*, 2006). The activation of mannose-binding lectin to IgA is calcium dependent and activates C4 and C3 complement complexes (Dahl *et al.*, 2000). In contrast, the alternative pathway is activated when IgA directly induces C3 activation, in a calcium- independent environment (Hiemstra *et al.*, 1988; Holers, 2008). Activation of the lectin and alternative pathway alludes to IgA as a prohibitor of the hyperbolic inflammatory response by the immune system (Wilton, 1978;

Roos *et al*., 2006). The impediment of classical complement is an attempt by IgA and the immune system to equipoise systemic hypertensive inflammatory responses, as seen in PE. Despite the hyper-inflammatory microenvironment related to PE, substantial proteinuria loss during PE may result in a significant reduction of IgA, as observed by Arinola *et al.* (2006).

Furthermore, the role of immunoglobulins during HIV infected pregnancies on HAART, is perplexing. HIV infection causes a defect in T-helper cells and polyclonal B cell activation, ensuing a large IgG antigen pool (Huber *et al.*, 2011). This may rationalize the upward trend in IgG1 levels noted in our study in HIV positive compared to HIV negative pregnancies, regardless of pregnancy type. However, [a]Amah-Tariah *et al.* (2016) consequently observed a significant increase in overall IgG levels during HIV infected pregnancies, alluding to HIV disease progression to AIDS.

Our study observed a significant down-regulation in IgG2 levels during HIV positive compared to HIV negative pregnancies, regardless of pregnancy type. Comparably, IgG3 and IgG4 communicated non-significant down-regulatory trends in HIV positive pregnancies. During HIV infection, activation of the complement system occurs, owing to the presence of HIV antigens (Huber *et al.*, 2011). This systemic action is due to the virus directly activating the binding of viral envelope proteins to C1 complexes, in the absence of immunoglobulin-antigen complexes (Ebenbichler *et al*., 1991; Spear *et al.*, 1991). Whilst virus infected cells cause complement activation, HIV has the unique ability as a retrovirus to use the complement system to prevent complement-mediated cell lysis of virions (Cooper *et al*., 2013; Moir and Fauci, 2017). This in turn provokes disease progression to AIDS, through increased dissemination and infectivity of the immunodeficiency virus (Huber *et al*., 2011; Maartens *et al*., 2014). The moderated levels of immunoglobulins in our study may concur with rapid disease progression to AIDS whereas, specific subclass switching favoring IgG1 may also be a causative factor to the overall reduction of immunoglobulins. Moreover, in South Africa, all HIV infected women receive anti-retroviral immune therapy (HAART), a standard care practice to reconstitute immunity (Iordache *et al.*, 2017; Phoswa *et al.*, 2018). Notably, HAART may act to restrain non-specific B cell activation and uncontrolled immunoglobulin production in HIV infections, validating the down-regulatory trends observed (Khan *et al.*, 2016).

In the current study, significantly down-regulated IgA and IgM levels in HIV positive pregnancies were noted when compared to HIV negative pregnancies. In variation, [a]Amah-Tariah *et al.* (2016) reported a significant increase in IgA and IgM levels during HIV infected pregnancies, compared

to HIV negative pregnancies. Up-regulated IgA levels in HIV infected individuals may be a result of specific neutralizing action against pathogens (Singh *et al.*, 2014). Interactions between IgA and FcàRI present on neutrophils, eosinophils, monocytes, dendritic cells and some macrophages induce a neutralizing effect against HIV progression to AIDS (Lopez *et al.*, 2018). Moreover, communication between IgA, the lectin and alternative complement pathways towards reducing increased inflammatory responses during acute HIV infection, may justify observations by [a]Amah-Tariah *et al.* (2016). In addition, the up-regulated IgM levels noted by [a]Amah-Tariah *et al.* (2016) suggest an increased inflammatory reaction to acute HIV infection. However, in our study HIV positive pregnancies receiving HAART, may have influenced the current down-regulation trends in IgA and IgM.

To further understand the role of immunoglobulins in HIV infected PE, our study analyzed isotype levels in HIV positive PE, HIV negative PE, HIV positive and negative normotensive pregnancies. We observed non-significant down-regulated IgG1, IgG3, IgG4 and IgM levels in HIV positive PE when compared to HIV positive normotensive, HIV negative normotensive and preeclamptic pregnancies. Despite immune processes that act to reject the developing fetus during pregnancy complications, down-regulatory trends in specific IgG subclasses (IgG1, IgG3 and IgG4) reiterate the pivotal role of the maternal-fetal immune transfer in PE (Palmeira *et al*., 2011). Conversely, the noted trend in IgG1, IgG3, IgG4 and IgM may be the outcome of HAART in HIV infected preeclamptic pregnancies.

On the contrary, our study demonstrated the significant up-regulation of IgG2 in HIV negative PE, when compared to HIV negative normotensive, HIV positive normotensive and preeclamptic pregnancies. These findings are plausible due to specific antigen subclass switching to IgG2 (Valenzuela and Schaub, 2018). In turn, subclass switching may act to promote IgG2 as an activator of the classical complement pathway during pregnancy. Furthermore, we noted a significant up-regulation of IgA in HIV positive PE, when compared to HIV positive normotensive, HIV positive and negative preeclamptic pregnancies. Whilst IgA has unique neutralizing abilities against pathogens, patients receiving HAART may endure the restraint of HIV, as HAART acts to counteract and restore immunity. Moreover, the inimitable anti-inflammatory ability of IgA may act to possibly inhibit classical complement activation (Wolf *et al.*, 1994). The transpired exaggerated inflammatory response from classical complement, may account for the up-regulated IgA levels in our study.

3.2 Conclusion

Our study displays the reconstitution of IgG2, IgA and IgM in HIV positive pregnant women on HAART. Furthermore, we demonstrate the imperative role of IgG1, IgG3 and IgG4, whilst highlighting the function of IgG2 in the maternal-fetal transfer of immunoglobulins. Our results report an up-regulation of IgG2 in PE, suggesting that this specific subclass activates the classical complement pathway thus exaggerating the inflammatory cascade in PE. In our study, the up-regulation of IgA indicates an anti-inflammatory response to the pathogenesis of PE. Moreover, the up-regulation of IgA in HIV positive PE implicates that immune reestablishment due to HAART may be deficient in stabilizing the exaggerated inflammatory response in PE. However, further research on a larger study population is required to understand the role of IgG2 as a pro-inflammatory molecule and disclose the effects of IgA as an anti-inflammatory marker in PE.

CHAPTER 4

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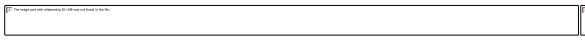
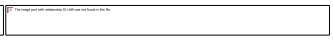
APPENDIX

BREC APPROVAL

RESULTS

Standard curve showing the concentration (ng/dl) of fluorescent intensity (Fl). BioPlex Manager™ software version 4.1.





Regression Type: Logistic - 5PL

Std. Curve: FI = 9.61305 + (20880.5 - 9.61305) / ((1 + (Conc / 206.409)^-1.13181))^0.889675

FitProb. = 0.2254, ResVar. = 1.4522

Regression Type: Logistic - 5PL

Std. Curve: FI = 3.49476 + (7522.71 - 3.49476) / ((1 + (Conc / 1247.14)^-2.2374))^0.486898

FitProb. = 0.6582, ResVar. = 0.1958

Regression Type: Logistic - 5PL

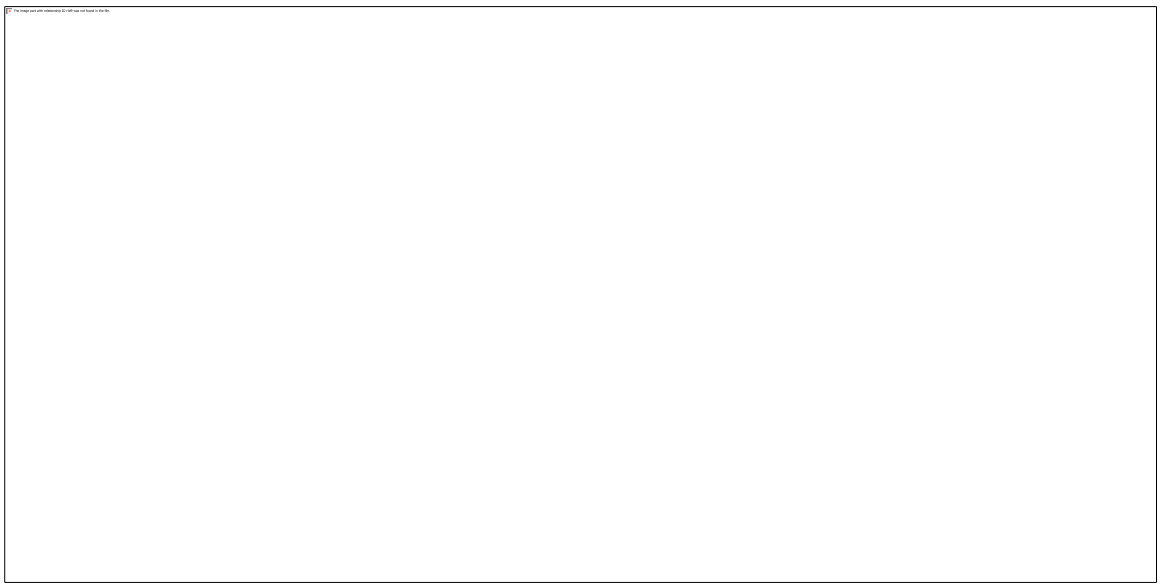
Std. Curve: FI = -6.99762 + (25342.8 + 6.99762) / ((1 + (Conc / 77.4793)^-1.2075))^0.701453

FitProb. = 0.4612, ResVar. = 0.7738

Regression Type: Logistic - 5PL

Std. Curve: FI = 5.57927 + (22502.5 - 5.57927) / ((1 + (Conc / 24.3765)^-1.10428))^0.879445

FitProb. = 0.3027, ResVar. = 1.2142



Regression Type: Logistic - 5PL

Std. Curve: FI = 15.5966 + (20615.6 - 15.5966) / ((1 + (Conc / 12.603)^-1.07737))^1.46774

FitProb. = 0.8560, ResVar. = 0.0329

Regression Type: Logistic - 5PL

Std. Curve: FI = 4.93688 + (19978.2 - 4.93688) / ((1 + (Conc / 906.629)^-1.34072))^0.731065

FitProb. = 0.1374, ResVar. = 1.8400

THE END