

**TRACE ELEMENT ANALYSIS OF BIOLOGICAL SAMPLES FROM
NORMOTENSIVE AND PRE-ECLAMPTIC SOUTH AFRICAN
PREGNANT WOMEN**

by

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submitted in partial fulfillment of the requirements for the degree of

MASTER OF MEDICAL SCIENCE

in the

Discipline of Optics and Imaging

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PREFACE

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

The research described in this dissertation was carried out in the Optics & Imaging Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor T Naicker, and co-supervision of Dr K Maduray and Professor J Moodley.

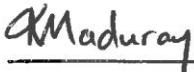


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DECLARATION

I, Cassandra Soobramoney 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.
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DEDICATION

To my late grandma Krishnavalli Naidoo

*The epitome of a beautiful soul
You will live in my heart forever, until we meet again*

PUBLICATIONS

- C Soobramoney, K Maduray, J Moodley, R Moodley and T Naicker (2016). Elemental analysis of nails from normotensive and pre-eclamptic Black South African women: A clinical study. *Journal of Obstetrics and Gynecology*. Submitted-Manuscript ID: 2016-OG-18459
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LIST OF ABBREVIATIONS

As	Arsenic
Ca	Calcium
Mg	Magnesium
Se	Selenium
Pb	Lead
Ni	Nickel
Cu	Copper
Co	Cobalt
Zn	Zinc
Mn	Manganese
Cr	Chromium
Fe	Iron
Cd	Cadmium
PE	Pre-eclampsia
IUGR	Intrauterine growth restriction

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ABSTRACT

Elemental deficiency is a causative factor in a number of pregnancy related conditions such as pre-eclampsia (PE). Its greatest impact is in developing countries, where the dietary intake of essential minerals and vitamins are poor. Therefore, the main aim of this study was to compare the concentrations of thirteen different trace elements in nail samples from pre-eclamptic and normotensive women. Also, this study aimed to compare the nail concentrations of these thirteen different trace elements to hair and serum concentrations in pre-eclamptic and normotensive women. The selected study site was a large regional hospital in Durban, Kwa-Zulu Natal. Nail clippings were sampled from normotensive ($n = 33$) and pre-eclamptic ($n = 33$) pregnant women. Nail (0.02 g), pubic hair (0.01 g) and serum (0.5 mL) samples were processed using the wet acid digestion method. Each of the digested samples were analyzed for Zinc (Zn), Selenium (Se), Lead (Pb), Nickel (Ni), Magnesium (Mg), Manganese (Mn), Iron (Fe), Copper (Cu), Calcium (Ca), Cobalt (Co), Cadmium (Cd), Chromium (Cr) and Arsenic (As) using the inductively coupled plasma-optical emission spectrometry.

There was a significant decrease in the nail concentration of Co ($p = 0.0001$), Mn ($p = 0.022$) and Cd ($p = 0.004$) in the PE group. Also in the pre-eclamptic group, pubic hair samples yielded a significant decrease in Co ($p = 0.02$), Cr ($p = 0.01$) and Zn ($p = 0.05$) while Co ($p = 0.0001$), Mn ($p = 0.03$), Cu ($p = 0.001$), Ca ($p = 0.02$), Mg ($p = 0.0001$), Se ($p = 0.001$) and Zn ($p = 0.01$) was significantly lower as compared to the normotensive group. Significantly decreased levels of Co were observed across all three biological samples whilst decreased levels of Mn were only observed in the serum and nail samples in the pre-eclamptic compared to the normotensive group. In conclusion, these dual studies observed a possible link between pre-eclampsia and macro- as well as micronutrients. Therefore, more extensive research on the role of these elements may aid in reducing the risk of developing PE. Also, it may be used as a potential indicator or biomarker for early diagnosis of pre-eclampsia.

CHAPTER 1

Background and Literature

1.1.Pre-eclampsia

Pre-eclampsia (PE) is a relatively common condition that affects 3-10% of pregnancies worldwide, and it is one of the leading causes of fetal and maternal morbidity and mortality. The occurrence of maternal and fetal morbidity in developed countries is less common due to patient access to better health facilities (Jeyabalan, 2013), and more frequent in middle to low income countries (*i.e.* sub-Saharan Africa and the Indian subcontinent) (Dadelszen *et al.*, 2012). Studies have shown that PE affects various organ systems. Therefore, determining the precise etiology of this condition has been a major challenge and remains unknown (Jeyabalan, 2013).

1.1.1. Clinical Features of Pre-eclampsia

Pre-eclampsia can be classified as either early or late onset. Early onset PE refers to occurrence of the condition before or at 33 weeks of gestation while late onset PE occurs at or after 34 weeks of gestation (Madazli *et al.*, 2014). The occurrence of PE is more common during the second half of a pregnancy, with a new onset of hypertension and proteinuria. The main clinical features of PE are a systolic blood pressure of ≥ 140 mmHg and a diastolic blood pressure of ≥ 90 mmHg as well as the presence of proteinuria (≥ 300 mg) in a 24 hr urine sample obtained from pregnant women (Young *et al.*, 2010). It is often characterized as either mild or severe, depending on the clinic features and complications that may occur. The characteristics of severe PE includes; a blood pressure of ≥ 160 mmHg systolic and/or ≥ 110 mmHg diastolic, renal and hepatic dysfunction as well as placental abruption and fetal growth restrictions (Jeyabalan, 2013).

In pre-eclampsia, the maternal uterine spiral arterioles arteries fail to convert into wide sinusoids. Also, the trophoblastic invasion of the uterine vessel is incomplete which gives rise to restricted blood supply and this causes fetoplacental hypoxia. Subsequently, placental factors are released to compensate for the compromised blood flow. These placental factors include angiogenic agents, cytokines, products of lipid peroxidation, autoantibodies and placental cell debris. One of the contributing factors of endothelial dysfunction is an ischemic placenta which alters the balance between circulating levels of angiogenic and anti-angiogenic growth factors. These growth factors include vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase-1 (sFlt-1). sFlt-1 has been shown to bind to and inhibit the VEGFR activity. Both sFlt-1 and endoglin (also an anti-angiogenic factor), have

been implicated in the pathogenesis of pre-eclampsia indicating elevated levels in the maternal circulation before the onset of the disease. Pre-eclampsia has been suggested to be an inflammatory response to pregnancy. An excess of immune complexes is unable to be cleared by the maternal immune system, hence they are deposited in the various endothelial layers causing pro-inflammatory cytokines and oxidative stress. The maternal oxidative stress in turn stimulates further placental apoptosis and necrosis which leads to varying severities of pre-eclampsia (Sanjay *et al.*, 2014). If the condition is not properly managed, it could lead to the development of eclampsia, which is characterized with severe seizures and the possibility of the individual going into a coma (Young *et al.*, 2010). The sudden development of edema can also be an indicator of PE, however it is known as a non-specific clinical feature (Alghazali *et al.*, 2014).

1.1.2. Risk factors

Medical conditions such as chronic hypertension, diabetes, renal disease and obesity are associated with risks of developing PE and it predisposes both mother and child to cardiovascular disease after the pregnancy (Alghazali *et al.*, 2014). Gestational diabetes has been thought to be linked to PE with the severity of gestational diabetes being influenced by the progressive rate of PE (Shamsi *et al.*, 2013). Furthermore, PE is more common during a woman's first pregnancy however there is an increased risk of the condition in multiparous pregnant women and in women who frequently change partners. Also, mild PE has been noted in pregnancies involving the delivery of a male child and this may be due to the increased levels of testosterone in the body. In addition, studies have shown that a prior abortion confers a weak protective effect against PE while a prior pregnancy confers a strong protective effect (Shamsi *et al.*, 2013).

A family history of the condition increases the risk of developing severe PE (Young *et al.*, 2010). Also, one of the major maternal risk factors includes the age of the mother. Some studies have shown that women above the age of 35 years have an increased risk of developing PE while other studies show that younger women (< 25 years) are more likely to develop this disorder. This makes advanced maternal age as well as young maternal age a risk factor. It has been observed that women above the age of 40 are two times more likely get the disease (Shamsi *et al.*, 2013). Also, researchers have found that the time between pregnancies is a risk factor, with findings showing that women with more than 5 years between pregnancies are at a greater risk of developing PE than those who had less than 2 years between their pregnancies (Shamsi *et al.*, 2013). Additionally, PE can be considered to be a

risk factor for other maternal and fetal complications later on in life. The mother and child have an increased risk of having a stroke or developing hypertension while the mother also has an increased risk of coronary heart disease (English *et al.*, 2015). It has been noted that the timely delivery of the child does not reduce the risk of life altering complications (e.g. stroke, renal failure etc) of PE (Dadelszen *et al.*, 2012).

1.1.3. Prevention and Management

The most common cure for PE is the birth of the baby and delivery of placenta. This indicates that the placenta plays a major role in the pathogenesis of PE (Young *et al.*, 2010). The management of PE focuses mainly on early detection and delivering the child in a timely manner to prevent serious complications and even death (English *et al.*, 2015). There are two factors that should be considered at diagnosis before delivery and these include the severity of the PE and gestational age of the fetus (Uzan *et al.*, 2011). However, there are drugs available to help manage PE, depending on the severity. These include; magnesium sulphate and antihypertensive medication such as aspirin and nifedipine.

Magnesium sulphate is used to treat patients with severe PE and it is a well-established drug to be used in the reduction of complications from eclampsia for both the mother and fetus. However, this treatment must be closely monitored as misuse (*i.e.* overdose, high concentrations) could lead to organ failure (Uzan *et al.*, 2011). Antihypertensive medications such as aspirin and nifedipine have been used to treat patients with PE. Studies have shown that antiplatelet agents such as aspirin has been effective in preventing PE in women who were at moderate to high risk of developing the disease (Bartsch *et al.*, 2016), while nifedipine is used to treat severe hypertension during pregnancy and to prevent delivery preterm (Uzan *et al.*, 2011).

Another method of managing or maybe even preventing PE is the use of vitamin and mineral supplements. Calcium supplementation administered from mid-pregnancy has shown to significantly decrease the risk of developing PE especially in women with low calcium (Ca) dietary intake. However studies have shown that the use of supplements, especially Ca, increases the risk of the myocardial infarctions (Dadelszen *et al.*, 2012). It is currently recommended by WHO that calcium supplementation is taken by women before pregnancy especially in women who are at high risk for PE and other hypertension disorders (Dadelszen *et al.*, 2012). Furthermore, the ingestion of isolated vitamins such as vitamin C and E does not confer a protected effect against PE, as opposed to a diet rich in antioxidants. This means that a nutrient rich, balanced diet is essential for both the mother and the fetus. A healthy diet can

not only reduce the risk of PE but also reduce the severity of this condition as well (Dadelszen *et al.*, 2012).

1.1.4. Vitamins and minerals

Vitamins and minerals play an important role in the development of the growing fetus and the overall health of the mother (E.Hassan *et al.*, 2014). Pre-eclampsia, together with other conditions such as placental abruption, still birth and low birth weight are considered to be linked to or are the result of macro and trace element deficiencies during pregnancy (Alghazali *et al.*, 2014). Minerals and elements (i.e. calcium, zinc, iron etc) are usually provided by dietary intake during pregnancy. Pregnant women from developing countries are likely to have an inadequate dietary intake of these micronutrients due to their poor socio-economic status. Therefore, nutritional deficiencies are common amongst pregnant women in developing countries and this could play a role in the development of PE (Wang *et al.*, 2009).

1.2. The Role of Macro and Trace Elements in Pregnancy and Pre-eclampsia

Macro and trace elements have been shown to be crucial to the development of the fetus and a deficiency of these micronutrients could affect delivery and outcome of a pregnancy as well as overall development in childhood and adulthood (Pathak and Kapil, 2004). Certain trace elements function as catalytic components in various reactions while others play a vital role in the structural function of molecules such as enzymes and hormones (Al-Jameil *et al.*, 2015). Interactions between certain trace elements results in their dependency on one another. For example, Zn absorption is impaired by iron (Fe) deficiency in the intestinal mucosa while copper (Cu) competes with Zn at the intestinal absorption level. The absorption of Cu is reduced by increased Zn dietary intake however increased levels of Cu do not impair Zn absorption. Essential elements required during pregnancy for maintaining the health of the mother and development of the fetus include Ca, Zn, magnesium (Mg), iron (Fe), Cu, selenium (Se), manganese (Mn), chromium (Cr) and cobalt (Co). In contrast, toxic elements such as lead (Pb), cadmium (Cd) and arsenic (As) can negatively impact fetal growth. The deficiency or excess of these elements has been associated with cell damage, fetal growth restriction and maternal complications. Therefore, elements such as Zn, Fe and Ca should be taken daily by pregnant women as it is necessary for proper development and health (Elind, 2016).

1.2.1. Zinc

Zinc plays a major role in the proper development of the brain of the fetus and it also aids the mother during labor (Rasthore *et al.*, 2011). The concentration of serum Zn has been reported to be decreased in PE as compared to normotensive women (Elind, 2016). Zinc is a cofactor of DNA and RNA synthesis as well as various other enzymes. Zinc deficiency in pregnant women can be either mild or severe. Congenital malformation and spontaneous abortions are associated with a severe Zn deficiency while low birth weight, intrauterine growth restriction (IUGR) and preterm delivery are the outcome of milder forms of Zn deficiency (Awadallah *et al.*, 2004).

1.2.2. Iron

Iron deficiency and anemia are recognized globally as the most common nutritional deficiency problem (Awadallah *et al.*, 2004). Iron deficiency can negatively impact the growth of the fetus by causing preterm delivery. It can also affect the early life of the child by altering the brain biochemistry as well as the development of the brain, furthermore it can lead to the development of cardiovascular disease in adulthood (Gambling *et al.*, 2003).

The Fe requirement for an expectant mother increases from 0.8 mg per day in the first trimester to 6-7 mg per day in the second trimester in order to ensure proper development of the fetus and overall health of the mother. Iron has a particularly important role as it is essential for hemoglobin synthesis and the additional production of erythrocytes which are required during pregnancy (Allen, 2000). During pregnancy maternal Fe absorption is increased. The placenta mediates the transfer of Fe from the mother to fetus for all the unborn child's developmental needs. In PE women, serum iron levels are elevated which results in lipid peroxidation and damage to endothelial cells (Elind, 2016). Trials conducted have shown that the birth weight of newborns was higher in the mothers who had taken iron supplements as compared to non-supplemented pregnancies. Pre-term babies are more likely to have developmental problems such as stunted growth and poor Fe stores (Allen, 2000).

1.2.3. Copper

Copper is involved in the function of many cuproenzymes which are necessary for life (Pathak and Kapil, 2004). It is required for the absorption of Fe as it aids in the formation of ceruloplasmin which is a Cu carrying protein that plays a role in Fe metabolism (Ozden *et al.*,

2015). Copper deficiencies in fetuses have been shown to cause structural and biochemical abnormalities (Pathak and Kapil, 2004). Also, studies have shown that the concentration of Cu in pregnant women is twice that of normal adults. This suggests that Cu plays an important role in maintaining a healthy pregnancy as low levels of this element increases the risk of preterm birth and the premature rupture of membranes (Gambling *et al.*, 2003). Additionally, increased levels of Cu are associated with PE as it is an important antioxidant component required to reduce the effects of oxidative stress (Elind, 2016).

1.2.4. Magnesium

Trials conducted have shown that women with PE have reduced levels of Mg and Ca in their bodies. The treatment of choice is usually magnesium supplementation (Ephraim *et al.*, 2014). Magnesium is essential to the structure and function of the human body, making it one of the most abundant minerals in the body. Approximately 50% of the body's total Mg is located in the bones with majority of the remaining Mg being found in tissues, organs and cells. Only a minuscule proportion of Mg is found in the blood stream (Baloch *et al.*, 2012). Additionally, Mg plays a significant role in peripheral vasodilation and neurochemical transmission (Al-Jameil *et al.*, 2015). In the growing fetus Mg is essential for cell multiplication and ensuring a balanced neuro muscular system. This element plays a role in proper fetal development and bone formation also acting as a cofactor for various enzymes (Baloch *et al.*, 2012). A deficiency of Mg results in an increase in the production of reactive oxygen species which is associated with endothelial dysfunction and PE (Elind, 2016).

1.2.5. Calcium

During pregnancy the maternal requirement of Ca is around 300mg per day (Ritchie and King, 2000). It has an important role in water balance of the cells and muscle contraction (Al-Jameil *et al.*, 2015). Notably, Ca deficiencies in pregnant women can lead to the fetus being born prematurely. Thus, calcium supplementation can lead to the prevention of preterm birth and the development of PE. There has been evidence to suggest that an excessive amount of calcium supplementation may have harmful effects on mother and fetus (Dadelszen and Magee, 2014). Calcium supplementation, which does not include dietary Ca, may cause postnatal bone demineralization and it has been associated with an increased risk of myocardial infarction (Dadelszen and Magee, 2014). A study conducted showed that a low dose of Ca supplements decreased the risk of developing PE (Dadelszen and Magee, 2014), while another demonstrated that a high intake of dietary Ca increases the likelihood of proper

bone development of the infant and it can also prevent bone loss to the mother during pregnancy (Hacker *et al.*, 2012).

1.2.6. *Selenium*

Selenium activates anticarcinogenic factors and prevents cardiovascular diseases. It stimulates the immune system to act antagonistically towards heavy metals such as Pb, Cd etc. Selenium also protects cells against genetic damage (Pieczynska and Grajeta, 2015). During pregnancy a Se deficiency can lead to dysfunctions in the nervous system of the fetus as well as neural tube defects especially anencephaly rachischisis (a type of birth defect that causes abnormal formation of the spinal column). Selenium is essential for preventing insulin resistance and promoting proper glucose uptake and the regulation of cellular absorption of glucose (Pieczynska and Grajeta, 2015). Low serum Se levels have been observed in women with PE. A decrease in selenoprotein production (including endogenous antioxidants) have also been reported during PE pregnancies which could lead to an increase in oxidative stress (Elind, 2016).

1.2.7. *Manganese*

Manganese is required for the metabolism of carbohydrates, lipids and proteins as well as for various cellular metabolic mechanisms such as protection against lipid peroxidation, making it an essential trace element. Studies have shown that the Fe stores in the body affect Mn absorption, whereby women with low Fe stores absorb approximately 5% of dietary Mn while women with normal Fe, stores approximately 1% of dietary Mn (Wood, 2009). A dietary deficiency of Mn can lead to bone abnormalities, abnormal metabolism of carbohydrates and lipids as well as congenital ataxia in the newborn child. It may also lead to an individual being susceptible to certain diseases such as osteoporosis and epilepsy. In contrast, excess exposure to Mn can have neurotoxic effects (Takser *et al.*, 2004). Manganese is classified as an important antioxidant component, and a deficiency of this element can result in an increase in oxidative stress which could lead to the development of PE (Ikaraoha *et al.*, 2016).

1.2.8. *Other Beneficial Trace Elements*

Another micronutrient involved in glucose uptake is Cr. Chromium acts as a cofactor for insulin and may be important for glucose homeostasis. It is found in many different foods such as cheese, vegetables, fish etc (Woods *et al.*, 2008). While Co, on the other hand, benefits human health as it is part of vitamin B₁₂, and it has been used to treat anemia as it induces red blood cell production. Decreased levels of Cr and Co may play a role in the

development of PE as these elements cause an increase in oxidative stress, inflammation, endothelial dysfunction and even DNA damage (Elind, 2016). While most trace elements are beneficial and necessary to the health of pregnant women and their developing fetuses, exposure to some trace elements such as As and Cd can have detrimental effects.

1.2.9. *Lead*

Lead is an environmental contaminant which is used in many products, such as paint on a daily basis. It accumulates in soil or dust and can be easily ingested by children leading to elevated levels in the blood. Elevated levels of Pb can lead to a decrease in IQ scores, violent or aggressive behaviors and even anemia, with these effects being irreversible. Furthermore, studies have shown that Pb exposure to pregnant women negatively impacts the developmental of the fetus. It could also lead to the abnormal development of vital organs, low birth weight and have severe neurological effects (Haman *et al.*, 2015). This toxic metal increases oxidative stress thereby increasing the risk of developing PE as this disorder is associated with oxidative stress (Haman *et al.*, 2015).

1.2.10. *Cadmium*

Cadmium exposure occurs when humans come in contact with foods such as seafood, offals, cereals as well as the inhalation of tobacco smoke. Cadmium has a relatively long half-life once it has been absorbed, especially in the kidneys (Kippler *et al.*, 2012). Cadmium has been shown to negatively impact the development of children as it accumulates in the placenta of humans (Kippler *et al.*, 2012). This can lead to reduced size at birth as well as pre-term delivery (Nishijo *et al.*, 2002). It has been reported that Cd may impair transport of Zn to the fetus as the levels of this element has been shown to be elevated in the placenta of PE women when compared to a normotensive population (Kippler *et al.*, 2012).

1.2.11. *Other Harmful Trace Elements*

Arsenic is a common environmental pollutant and is also a known carcinogen and neurotoxic element. It has been reported that high levels of arsenic (> 50 ug/L) found in drinking water can result in low birth weight pregnancies (Tofail *et al.*, 2009) an this element has been shown to be the cause of stillbirths and spontaneous abortion (Ahmad *et al.*, 2001). Oxidative stress has been reported to be an essential component of As toxicity. Additionally, nickel (Ni) has been shown to have various adverse effects on the health of exposed individuals. These

range from skin problems and depression to cancer, heart attacks and even kidney dysfunction (Abdulrahman *et al.*, 2012).

1.3. *Aims and Objectives*

The role of these elements on the development of PE has not been properly established. Therefore, the aim of this study is to analyze the concentration of Zn, Fe, Ca, Cu, Cr, Co, Pb, Se, As, Mg, Mn, Ni and Cd in the biological samples (nail, hair and serum) of normotensive and pre-eclamptic Black South African women.

The specific objectives include quantifying the levels of these elements in the nails (finger and toe nails) of the pregnant women using inductively coupled plasma-optical emission spectrometry. These results will then be compared to the values obtained for the hair and serum samples in order to determine if there are any differences or similarities among the data.

CHAPTER 2

Manuscript 1

***Elemental analysis of nails from normotensive and pre-eclamptic Black South
African women: A clinical study***

Submitted to the Journal of Obstetrics and Gynecology

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Detailed Status Information

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Running Title	Elemental Analysis of Nails in Pre-eclamptic Women
Manuscript Type	Main Research Article
"Theme Issue"	N/A
Clinical Category	General Gynaecology
Corresponding Author	Cassandra Soobramoney (University of Kwa-Zulu Natal)
Contributing Authors	Kaminee Maduray , J Moodley , Roshila Moodley , Thajasvarie Naicker
Abstract	<p>Objective: To compare the concentrations of thirteen different elements in nail samples from pre-eclamptic and normotensive pregnant women. Setting: The study site was a regional hospital in Durban, Kwa-Zulu Natal. Population: Nail samples were collected from normotensive (n=33) and pre-eclamptic (n=33) pregnant women. Method: Approximately 0.02 g of nail samples were digested in 70% nitric acid and analyzed using inductively coupled plasma-optical emission spectrometry. Main Outcome Measures: Analytes of interest were the following essential elements calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), selenium (Se) and Zinc (Zn) as well as toxic elements, arsenic (As), cadmium (Cd) and lead (Pb). Results: The levels of Ca (3467.4 197.42 vs. 2897.0 189.82 g/g) and Mg (736.10 60.98 vs. 694.83 59.36 g/g) were higher in the normotensive compared to the pre-eclamptic group, however it was not significant. In contrast, the concentration of Cd (3.07 0.29 g/g) was significantly lower in the pre-eclamptic compared to the normotensive group (p = 0.004). Also, there was a significant difference between the normotensive and pre-eclamptic group for Co (p = 0.0001) and Mn (p = 0.022). Conclusion: This study demonstrated significantly lower levels of Cd, Co and Mn in pre-eclampsia which justifies the need for further research on these elements towards the effective management or prevention of pre-eclampsia which could ultimately aid in establishing its pathogenesis. Keywords: Pre-eclampsia, essential nutrients, toxic elements, pregnancy, nails</p>
Tweetable abstract	A significant decrease was observed in the levels of Co, Cd and Mn in the pre-eclamptic group of the study
Keywords	pre-eclampsia
Methodology	LABORATORY STUDIES
Disclosure of Interests	No, there are no conflict of interests that I should disclose, having read the above statement.
Section Verification	Yes

**Elemental analysis of nails from normotensive and pre-eclamptic Black South
African women: A clinical study**

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Running title: Elemental Analysis of Nails in Pre-eclamptic Women

Abstract

Objective: To compare the concentrations of thirteen different elements in nail samples from pre-eclamptic and normotensive pregnant women.

Setting: The study site was a regional hospital in Durban, Kwa-Zulu Natal.

Population: Nail samples were collected from normotensive (n=33) and pre-eclamptic (n=33) pregnant women.

Method: Approximately 0.02 g of nail samples were digested in 70% nitric acid and analyzed using inductively coupled plasma-optical emission spectrometry.

Main Outcome Measures: Analytes of interest were the following essential elements calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), selenium (Se) and Zinc (Zn) as well as toxic elements, arsenic (As), cadmium (Cd) and lead (Pb).

Results: The levels of Ca (3467.4 ± 197.42 vs. 2897.0 ± 189.82 $\mu\text{g/g}$) and Mg (736.10 ± 60.98 vs. 694.83 ± 59.36 $\mu\text{g/g}$) were higher in the normotensive compared to the pre-eclamptic group, however it was not significant. In contrast, the concentration of Cd (3.07 ± 0.29 $\mu\text{g/g}$) was significantly lower in the pre-eclamptic compared to the normotensive group ($p = 0.004$). Also, there was a significant difference between the normotensive and pre-eclamptic group for Co ($p = 0.0001$) and Mn ($p = 0.022$).

Conclusion: This study demonstrated significantly lower levels of Cd, Co and Mn in pre-eclampsia which justifies the need for further research on these elements towards the effective management or prevention of pre-eclampsia which could ultimately aid in establishing its pathogenesis.

Keywords: Pre-eclampsia, essential nutrients, toxic elements, pregnancy, nails

Introduction

In pregnancy, macro and trace elements are crucial for various biochemical and metabolic processes, which are involved in the development of a healthy fetus. Interactions between the various elements allow for appropriate intrauterine fetal development with elements, such as Ca, Fe, Zn and Mg being fundamental to fetal growth (Pathak and Kapil, 2004). Certain elements also function as catalytic components in these processes or play a vital role in the structure and function of molecules such as enzymes and hormones (Al-Jameil *et al.*, 2015). Therefore, adopting a balanced diet during pregnancy ensures adequate minerals, macro and trace elements for both the mother and fetus. In low and middle income countries (LMIC) an insufficient intake of essential nutrients can result in various deficiencies and nutrition related conditions in women (Pathak and Kapil, 2004).

Pre-eclampsia, together with other pathological conditions such as placental abruption, stillbirth and low birth weight are associated with a deficiency of elements in the body (E.Hassan *et al.*, 2014). Notably this may augment the development of pre-eclampsia in LMIC such as South Africa. In such populations where Ca intake is low, Ca supplementation as part of the antenatal care is recommended for reducing the risk of developing pre-eclampsia (Dadelszen *et al.*, 2012). However, the ingestion of isolated vitamins such as vitamin C and E supplementation does not confer a protected effect against pre-eclampsia, as opposed to a diet rich in antioxidant components such as Se, Mn and Zn. Thus a nutrient rich dietary intake is not only beneficial for the health of the mother and the fetus but may reduce the risk and the severity of pre-eclampsia (Dadelszen *et al.*, 2012).

Unfortunately, there is a paucity of data on the role of essential elements in the development pre-eclampsia. Therefore, this study evaluates the concentrations of essential elements calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg),

manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn) as well as toxic elements arsenic (As), cadmium (Cd) and lead (Pb) in the nail clippings of Black South African pregnant women with pre-eclampsia and their normotensive controls.

Methods

Study population

This study was carried out at the Optics and Imaging Centre, University of Kwa-Zulu Natal. Following informed consent and institutional ethics approval (BE: 092/16) a total of 66 pregnant women were recruited from the antenatal clinic of a regional hospital in Durban, Kwa-Zulu Natal Province, South Africa. Nail samples were collected from normotensive (n = 33) and pre-eclamptic pregnant women (n = 33). Pre-eclampsia was clinically defined as blood pressure of 140/90 mmHg taken twice at least two hours apart, systolic blood pressure of ≥ 160 mmHg, a rise of 30 mmHg and 15 of mmHg over baseline values and a proteinuria of 1 + (30 – 100 mg/dL) or greater (Amin *et al.*, 2014). Additionally, dietary information of all patients was collected via a questionnaire and expressed as a percentage (%) of women that consumed the different types of food products. Pregnant women infected with HIV or had other medical conditions such as gestational diabetes, epilepsy, chronic asthma, cardiac, thyroid and chronic renal diseases were excluded from the study.

Collection and analysis of samples

Nail samples included both finger and toe-nail clippings, and were obtained using a sterile stainless steel nail clipper. Both the certified reference material and nail clippings (0.02 g) were digested using the wet acid digestion method (Kuboyama *et al.*, 2005). Samples were boiled in 10 mL of nitric acid (HNO₃) on a hot plate. Thereafter, the digests were transferred to 25 mL volumetric flasks and distilled water was added to reach volume capacity. Digested samples were analyzed for As, Ca, Cd, Cr, Co, Cu, Fe, Mg, Mn, Ni, Pb, Se and Zn using an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima™ 5300 DV,

USA) and reported as μg per gram ($\mu\text{g/g}$), dry matter. Working standards were prepared from 1000 ppm elemental standards that were supplied by Sigma Aldrich (St Louis, USA) and were prepared in 70% HNO_3 to match the sample matrix. The three most sensitive analytical wavelengths were initially chosen for analysis and the line with minimal spectral interferences and maximum analytical performance was finally selected.

Statistical analysis

The data obtained was analyzed using IBM Statistical Package for the Social Science (PASW Statistics, Version 23, IBM Corporation, Cornell, New York). The clinical data was expressed as mean \pm standard deviation (SD), the elemental concentrations were expressed as median \pm standard error of mean (S.E.M) and the dietary information was presented as percentages. The independent T-test and Mann-Whitney tests were used to determine the p values of the parametric and non-parametric data. All data with a p value ≤ 0.05 was considered significant. Pearson's correlation was used to identify significant relationships between the data.

Results

Quality Assurance

The experimental results obtained for the certified reference material (White clover – BCR – 402, Institute for Reference Materials and Measurements, European Commission, Joint Research Centre, Belgium) was compared to the certified values from the Certificate of Analysis (Table 1). The investigation showed measured results to be within the acceptable range of that stipulated for the certified reference material at the 95% confidence interval.

Table 1: Experimental values and certified values ($\mu\text{g/g}$, dry, mass, median \pm S.E.M, 95% confidence interval, $n = 4$) for the certified reference material (White clover – BCR 402)

Element	Certified value	Experimental value
Cr	5.19	5.10 ± 0.170
Fe	244	237 ± 18.0
Se	6.70 ± 0.25	6.81 ± 1.40
Zn	25.2	30.3 ± 6.37

Demographic data

The clinical data of the study population is represented as mean \pm SD in Table 2. The systolic ($p = 0.0001$) and diastolic ($p = 0.0001$) blood pressure levels were significantly higher in the pre-eclamptic as compared to normotensive group, with values of 155.88 ± 11.98 mmHg and 99.09 ± 7.27 mmHg, respectively. The maternal age ($p = 0.870$), body mass index ($p = 0.989$), hemoglobin count ($p = 0.983$), white blood cell count ($p = 0.819$), red blood cell count ($p = 0.176$) and platelet count ($p = 0.084$) were similar between the groups. However, baby weight was significantly lower in the pre-eclamptic as compared to the normotensive group (2.81 ± 0.74 versus 3.30 ± 0.71 kg respectively; $p=0.030$). A significant negative correlation was observed for baby weight and diastolic blood pressure ($r = -0.603$; $p = 0.004$) in the pre-eclamptic group. It should be noted that patients received ferrous sulphate (200 mg), folic acid (5 mg) and Ca (500 mg) supplementation during the antenatal period.

Table 2: Clinical data of normotensive (n = 33) and pre-eclamptic (n = 33) women

	Normotensive	Pre-eclampsia	p-value
Systolic BP (mmHg)	113.39 ±14.29	155.88 ±11.98	*0.0001
Diastolic BP (mmHg)	69.06 ±10.12	99.09 ±7.27	*0.0001
Proteinuria (+)	0.00 ±0.00	2.0 ±0.777	*0.0001
BMI (kg/m²)	32.29 ±6.46	32.27 ±7.60	0.989
Maternal Age (yrs)	26.14 ±6.54	26.32 ±6.31	0.870
Baby Weight (kg)	3.30 ±0.71	2.81 ±0.74	*0.030
Gestational Age (wks)	35.61 ±4.89	33.55 ±5.59	0.069
HB (g/dL)	10.15 ±3.47	10.95 ±1.29	0.983
WBC (L)	8.60 ±2.94	8.86 ±3.48	0.819
RBC (L)	3.92 ±0.63	3.64 ±0.43	0.176
PLT (L)	184.01 ±100.01	230.70 ±67.98	0.084

BP: Blood Pressure; BMI: Body Mass Index; HB: Hemoglobin; WBC: White blood cell; RBC: Red blood cells; PLT: Platelet, *Level of significance: ($p \leq 0.05$)

Patient dietary information

The dietary information of the study population is presented in Table 3. The dietary intake of fish ($p = 0.489$) and meat ($p = 0.664$) were similar between the study groups whilst a significant difference in the number of women consuming cheese ($p = 0.004$), green vegetables ($p = 0.001$), fruits ($p = 0.0001$) and cereal ($p = 0.0001$) was observed between the groups.

Table 3: Dietary information of normotensive (n = 33) and pre-eclamptic (n = 33) women

	Normotensive				Pre-eclampsia				
	Daily	>once a	<once a	Never	Daily	>once a	<once a	Never	<i>P</i> value
	(%)	week	week	(%)	(%)	week	week	(%)	(%)
Coffee	45.45	3.03	24.24	27.27	54.54			45.45	*0.011
Rooibos	54.54	3.03	15.15	27.27	84.85			15.15	0.173
Tea									
Milk	69.70	3.03	21.21	6.06	87.88			12.12	*0.003
Cheese	69.70	3.03	21.21	6.06	90.91			9.09	*0.004
Yoghurt	69.70	3.03	24.24	3.03	93.94			6.06	*0.001
Greens	66.67	3.03	27.27	3.03	87.88			12.21	*0.001
Fruits	69.70	3.03	27.27		96.97			3.03	*0.0001
Cereal	72.73	3.03	21.21	3.03	87.88			12.21	*0.0001
Fish	3.03	36.36	48.48	12.13	6.06	36.36	54.55	3.03	0.489
Meat	6.06	63.64	18.18	3.03	6.06	78.79	15.15		0.664

*Level of significance ($p \leq 0.05$)*Elemental concentrations*

Elemental concentrations are represented as median \pm S.E.M in Table 4. If present, concentrations of As were found to be below the instrument detection limit in both study groups. Except for Cr, Fe and Zn, median concentrations of all elements studied were found to be higher in the normotensive compared to the pre-eclamptic group. The concentrations of Cd ($p = 0.004$), Co ($p = 0.0001$) and Mn ($p = 0.022$) were found to be significantly higher in the normotensive compared to the pre-eclamptic group. In both the normotensive and pre-eclamptic group, concentrations of essential elements were found to be in decreasing order of Ca > Fe > Mg > Zn > Cu > Ni > Se > Mn/Cr > Co and concentrations of toxic elements were

in decreasing order of Pb > Cd > As. The normotensive and pre-eclamptic groups showed significant positive correlations between Ca and Mg ($r = 0.745$, $p = 0.0001$ and $r = 0.932$; $p = 0.0001$, respectively) and Zn and Fe ($r = 0.746$, $p = 0.0001$ and $r = 0.386$, $p = 0.026$, respectively).

Table 4: Elemental concentrations (median \pm S.E.M) in nail samples of normotensive (n = 33) and pre-eclamptic (n = 33) patients

	Normotensive	Pre-eclampsia	<i>P</i> value
Ca	3467.4 \pm 197.42	2897.0 \pm 189.82	0.133
Cd	3.07 \pm 0.29	1.56 \pm 0.40	*0.004
Cr	5.21 \pm 1.57	5.24 \pm 12.42	0.331
Co	2.75 \pm 0.32	ND	*0.0001
Mg	736.10 \pm 60.98	694.83 \pm 59.36	0.523
Mn	6.96 \pm 1.28	3.70 \pm 0.76	*0.022
Fe	284.01 \pm 165.36	292.82 \pm 51.52	0.517
Cu	54.18 \pm 278.72	43.60 \pm 23.57	0.125
Pb	51.70 \pm 25.16	36.75 \pm 23.15	0.352
Se	10.11 \pm 1.51	9.81 \pm 1.47	0.831
Ni	13.62 \pm 3.43	11.83 \pm 2.44	0.172
Zn	205.98 \pm 63.88	296.79 \pm 34.19	0.394

As: Arsenic, Ca: Calcium, Cd: Cadmium, Cr: Chromium, Co: Cobalt, Mg: Magnesium, Mn: Manganese, Fe: Iron, Cu:

Copper, Pb: Lead, Se: Selenium, Ni: Nickel, Zn: Zinc. *Level of significance ($p \leq 0.05$), ND – not detected.

Discussion

Main Findings

This study demonstrates elevated levels of Ca and Mg in normotensive compared to pre-eclamptic group with significant positive correlations between Ca and Mg across both groups. The levels of Se and Mn were lower in the pre-eclamptic group, with only a significant decrease observed in Mn ($p = 0.022$) levels compared to the normotensive group. A significant decrease in both Co ($p = 0.000$) and Cd ($p = 0.004$) concentrations was observed in the pre-eclamptic group. Furthermore, a positive correlation between these two trace elements ($r = 0.846$, $p = 0.000$) was also noted in the pre-eclamptic group. Findings from the dietary information revealed that the intake of cheese ($p = 0.004$), green vegetables ($p = 0.001$), fruits ($p = 0.0001$) and yoghurt ($p = 0.001$) were less common in the normotensive group compared to the pre-eclamptic. The clinical data showed strong evidence of lower baby weight in the pre-eclamptic pregnancies.

Strengths and Limitations

A major strength of this study is that elemental analysis of nails from pre-eclamptic women is novel. This study also analyzes thirteen different elements, including toxic element levels and recorded dietary intake data in a South African population. We however, did not investigate the adherence to ferrous sulphate, Ca and folic acid supplementation; neither did we take into consideration the population exposure to different toxic elements in the environment. In addition, though we found that fetal weights in the pre-eclamptic group were lower than that in the normotensive group, we had no information as to whether these babies had intrauterine growth restriction (IUGR).

Interpretation

During gestation the excess or deficiency of trace elements may contribute to endothelial dysfunction resulting in complications such as pre-eclampsia. The decreased level of Ca is known to lead to the constriction of smooth muscles in the blood vessels, which leads to increased vascular resistance and consequential blood pressure elevation. Also, low Ca levels may increase blood pressure by stimulating the release of parathyroid hormone and renin which then increases intracellular Ca in smooth muscles, resulting in vasoconstriction (Ephraim *et al.*, 2014, Elind, 2016). It is mandatory practice for patients to receive Ca supplementation including ferrous sulphate and folic acid supplementation; however Mg either as a tablet or in a multivitamin preparation was not prescribed as standard care in our study. Our study found Co levels were reduced in the pre-eclamptic compared to the normotensive group. Cobalt benefits human health as it is a component of vitamin B₁₂ and low levels of B₁₂ can cause complications such as anemia, infections, inflammation and pre-eclampsia (Huwait *et al.*, 2015).

The mechanism by which pre-eclampsia develops is linked to an increase in oxidative stress. Oxidative stress is defined as an imbalance between excess cellular generation of reactive oxygen species (ROS) and antioxidants (Ikaraoha *et al.*, 2016, Elind, 2016). Studies have recorded a decrease in the production of antioxidants in pre-eclamptic women (Ikaraoha *et al.*, 2016, Elind, 2016). Our study found decreased levels of Cu and increased levels of Zn in the pre-eclamptic compared to normotensive group. Notably, Zn protects cells from free radical injury and is a fundamental component of the antioxidant enzyme, superoxide dismutase. Zinc supplementation has been shown to increase plasma antioxidant power, decrease plasma inflammatory cytokines and oxidative stress. Zinc is involved in the defense against oxidative stress and is an essential cofactor for thymulin which modulates cytokine release and induces proliferation (Maggini *et al.*, 2007). Copper and Zn are the essential components of the antioxidant enzyme, copper-zinc superoxide dismutase which is responsible for the removal of free radicals (Al-Jameil *et al.*, 2015). In our study, Mn levels were reduced in the pre-eclamptic group; this element is an important cofactor in a wide range of antioxidant enzymes. This finding further implicates a possible decrease in the antioxidant levels and increase in oxidative stress. Recent studies have also associated low maternal blood Mn concentration with poor birth outcomes such as increased risk of IUGR and low birth weight (Wood, 2009) whereas, low Se status was associated with a greater risk of the development of pre-eclampsia (Rayman *et al.*, 2014). Our results also show reduced Se levels in the pre-eclamptic group. A finding similar to that of Rayman *et al* (2003) who found significantly

lower ($p = 0.001$) median toenail Se concentrations in a pre-eclamptic group (0.56 mg/kg) compared to matched controls (0.62 mg/kg) (Rayman *et al.*, 2014). This study also observed that lower Se levels was significantly related to the severity of pre-eclampsia ($p = 0.029$) adjudged by delivery before 32 weeks (Rayman *et al.*, 2014). Selenium is required for optimum immune response and influences the innate and acquired immune response systems. It plays a key role in the removal of excess damaging free radicals which are produced during oxidative stress (Maggini., 2007). However, not all free radicals are harmful, as nitric oxide (NO) is a potent vasodilator that causes relaxation of the smooth muscles. It mediates endothelial function by regulating vascular tone and smooth muscle cell development. In placenta, endothelial nitric oxide synthase (eNOS) is associated with the differentiation of cytotrophoblast to syncytiotrophoblast. Additionally, eNOS uncoupling has been observed to be a source of superoxide formation and is related to reduce NO production. Various inflammation modulators such as TNF- α is increased in plasma and placenta of pre-eclamptic women. TNF- α downregulates eNOS which leads to elevated levels of ROS and an increase in oxidative stress (Aranguren et al., 2014). Using a rat model, Wang *et al* (2014), found that prenatal exposure to low levels of Cd induces the phenotypical characteristics of pre-eclampsia such as hypertension, proteinuria and IUGR. This study also showed evidence that Cd exposure during pregnancy causes the synthesis of placental glucocorticoid (Wang *et al.*, 2014a).

Conclusion

This study demonstrates a significant decrease in the levels Cd, Co and Mn in pre-eclampsia. Therefore, increasing the dietary intake of essential nutrients may reduce the risk of pre-eclampsia development and ensure the delivery of a healthy baby. More extensive studies on these elements may confirm the exact role of nutrition in the pathogenesis of pre-eclampsia.

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Disclosure

The authors of this study declare that there are no conflicts of interest present.

Authors' contribution

Professor T Naicker and Professor J Moodley contributed to the concept and design of the study. C Soobramoney, Dr K Maduray, Dr R Moodley in the analysis of the trace elements and statistical analysis. All of the authors contributed to compiling and editing the manuscript.

Ethics

Ethical approval for this study was obtained from the University of Kwa-Zulu Natal Biomedical Research Ethics Committee on 20/04/16. The BREC reference number allocated to this study is BE092/16.

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References

1. Pathak P, Kapil U. Role of Trace Elements Zinc, Copper and Magnesium During Pregnancy and its Outcome. *Ind J Pediat.* 2004;71(11):1003-5.
2. Al-Jameil N, Tabassum H, Ali MN, Qadeer MA, Khan FA, Al-Rashed M. Correlation between serum trace elements and risk of preeclampsia: A case controlled study in Riyadh, Saudi Arabia. *Saudi J Biol Sci.* 2015:1-7.
3. E.Hassan E, Elhhatim WS, M.Bakhit S, Shrif NEMA, A.Huneif M. Assessment of trace elements in Sudanese preeclamotic pregnant women. *European Journal of Biomedical and Pharmaceutical Sciences.* 2014;1(2):13-21.

4. Dadelszen Pv, Firoz T, Donnay F, Gordon R, Hofmeyr GJ, Lalani S, *et al.* Pre-eclampsia in Low and Middle Income Countries-Health Services Lessons Learned From the PRE-EMPT(PRE-Eclampsia-Eclampsia Monitoring, Prevention & Treatment) Project. *J Obstet Gynaecol.* 2012;34(10):917-26.
5. Amin SV, Illipilla S, Hebbar S, Rai L, Kumar P, Pai MV. Quantifying Proteinuria in Hypertensive Disorders of Pregnancy. *Int J Hypertens.* 2014;8:1-11.
6. Kuboyama K, Sasaki N, Nakagome Y, Kataoka M. Wet digestion. *Analytical Chemistry.* 2005;360:184-91.
7. Ephraim RKD, Osakunor DNM, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy-induced hypertension: a case-control study in the Cape Coast metropolis, Ghana. *BMC Pregnancy and Childbirth.* 2014;14(390):1-8.
8. Elind A-HO. Trace Elements as Potential Biomarkers of Pre-eclampsia. *Ann Res Rev Biol.* 2016;9(1):1-10.
9. Huwait EA, Kumosani TA, Moselhy SS, Mosaoa RM, S.Yaghmoor S. Relationship between soil cobalt and vitamin B12 levels in the liver of livestock in Saudi Arabia: role of competing elements in soils. *Afr Health Sci.* 2015;15(3).
10. Ikaraoha IC, Mbadiwe NC, Anetor JI, Ojareva IA. Serum trace elements in Pre-eclamptic Nigerians. *As J Med Sci.* 2016;7(3):78-83.
11. Wood RJ. Manganese and birth outcome. *Nutri Rev.* 2009;67(7):416-20.
12. Rayman MP, Searle E, Kelly L, Johnsen S, Bodman-Smith K, Bath SC, *et al.* Effect of selenium on markers of risk of pre-eclampsia in UK pregnant women: a randomised, controlled pilot trial. *Br J Nutr.* 2014;112(1):99-111.
13. Wang F, Zhang Q, Zhang X, Luo S, Ye D, Guo Y, *et al.* Preeclampsia induced by cadmium in rats is related to abnormal local glucocorticoid synthesis in placenta. *Reprod Biol Endocrinol.* 2014;12(77):1-9.

CHAPTER 3

Manuscript 2

Elemental analysis of serum and hair from pre-eclamptic South African women

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Elemental Analysis of Serum and Hair from Pre-eclamptic South African Women

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Abbreviations: Arsenic (As); Calcium (Ca); Cadmium (Cd); Chromium (Cr); Cobalt (Co); Magnesium (Mg); Manganese (Mn); Iron (Fe); Copper (Cu); Lead (Pb); Selenium (Se); Nickel (Ni); Zinc (Zn).

Abstract

Pre-eclampsia is a hypertensive disorder that is associated with adverse maternal and perinatal outcomes. It has been proposed that specific trace and macro elements associated with antioxidant activities may also play a contributory role in aetiology of pre-eclampsia. The aim of this study was to measure the concentrations of thirteen different elements in hair and serum samples from women with a diagnosis of pre-eclampsia. Venous blood and pubic hair samples were collected from forty-three pre-eclamptic and twenty-three normotensive pregnant women. In each sample, the concentration of arsenic (As); calcium (Ca); cadmium (Cd); chromium (Cr); cobalt (Co); magnesium (Mg); manganese (Mn); iron (Fe); copper (Cu); lead (Pb); selenium (Se); nickel (Ni); zinc (Zn) were measured using inductively coupled plasma-optical emission spectrometry. Cobalt concentration in hair was significantly lower in the pre-eclampsia group ($1.56 \pm 0.74 \mu\text{g/g}$) compared to the normotensive group ($2.89 \pm 4.99 \mu\text{g/g}$) ($p = 0.02$). The concentrations of Zn and Cr were significantly higher in hair samples from the pre-eclamptic group, compared to the normotensive control (Zn, 395.99 ± 48.60 vs $330.88 \pm 29.70 \mu\text{g/g}$; Cr, 13.31 ± 2.67 vs $11.05 \pm 7.62 \mu\text{g/g}$; $p \leq 0.05$). There were no significant differences in the hair levels of other elements between groups. Serum Zn was significantly higher in the pre-eclamptic group ($0.16 - 253.4 \text{ mg/L}$) compared to the normotensive group ($0.2 - 48.4 \text{ mg/L}$) ($p = 0.01$). Serum Ca, Co, Cu, Mg, Mn and Se levels were found to be significantly lower in the pre-eclamptic group compared to the normotensive group ($p < 0.05$). This study confirms the association between pre-eclampsia and maternal trace as well as macro element levels.

Keywords: Pre-eclampsia, trace elements, macronutrients, pregnancy, hypertension, blood pressure

Introduction

The nutritional status of women of reproductive age and during pregnancy is important for their own health and healthy birth outcomes [1-2]. It is well documented that pregnancy is the time during which increased nutrients are most needed by the mother to support fetal growth and development while maintaining maternal homeostasis and preparing the mother for lactation [3]. It is also a period of small, continuous gestation-related physiologic changes that affect the metabolism of all nutrients. For instance, within several weeks of conception an endocrine organ (placenta) is formed which secretes hormones that impacts the metabolism of all nutrients [3]. Thereafter, the fetus is dependent on the mother to deliver its nutrients via the placenta. Since the placenta carries oxygenated, nutrient-rich blood to the fetus, it is considered to be rich in micro-nutrient-requiring antioxidant enzymes. These include glutathione peroxidases (selenium) and superoxide dismutases (copper, zinc and manganese), which are vital for protecting the embryo and placenta from oxidative stress. Inadequate antioxidant activity has been postulated to be related to reduction in placental vascularization and blood supply to the fetus. This may potentially result in hypoxia and ischaemia, and is likely to contribute to pre-eclampsia and poor fetal growth [4]. Therefore, deficiencies of macronutrients or micronutrients can increase a women's risk for pregnancy complications such as pre-eclampsia [5].

Pre-eclampsia is a multi-systemic condition occurring after 20 weeks of gestation. It is clinically characterised by new onset hypertension (systolic blood pressure ≥ 140 mmHg; diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 300 mg/24 h) [6]. Globally, pre-eclampsia is one of the major causes of maternal and neonatal morbidity and mortality. In South Africa, pre-eclampsia is also the commonest direct cause of maternal deaths [7]. Despite continuing research, the pathogenesis of this disorder is still unclear and delivery of the placenta remains the only cure [6]. Presently, a large number of other studies suggest that nutritional factors may also play a major role in blood pressure regulation, cardiovascular disease, hypertension and pre-eclampsia development [8-10].

Magnesium (Mg) and calcium (Ca) deficiency has been implicated as a possible cause of pre-eclampsia [11] since its supplementation during pregnancy may reduce the incidence of high blood pressure, pre-eclampsia low birth weight and pre-term birth [12]. In addition, iron (Fe) supplements and increased Fe stores in the third trimester are associated with increased oxidative stress and the risk of pre-eclampsia development [13]. Other trace element deficiencies such as zinc (Zn), copper (Cu) and selenium (Se) have been related to various reproductive problems (e.g. infertility, placental abruption, premature rupture of membranes,

stillbirths and low birth weight) including pre-eclampsia [14]. A number of studies have reported on the implication of toxic metals such as lead Pb and cadmium (Cd) in pre-eclampsia. Human exposure to excess toxic metals in the environment and a deficiency of bio-elements essential for antioxidant defense mechanisms causes oxidative stress, which leads to pre-eclampsia [15-18]. However, further epidemiological and clinical studies utilizing diverse biological samples in defined populations may help understand the role of various essential and toxic metals in pre-eclampsia. Therefore, the aim of this study was to evaluate the concentrations of thirteen different elements in hair and serum samples from women with a diagnosis of pre-eclampsia.

Material and Methods

Study design

Institutional ethics approval (BE092/16) was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal and informed consent was obtained from all women. This study was conducted in a large urban regional hospital. The study population consisted of normotensive (n = 23) and pre-eclamptic (n = 63) women. Clinical and demographic data were recorded by a research nurse.

Hair and serum sample collection

Hair and serum samples were collected from the same women for each study group. Hair samples were collected from the pubic area by using a sterile shaving razor and stored in sterile plastic bags at room temperature. Blood was drawn from the ante-cubital vein and centrifuged for 10 min at 3000 rpm to extract serum.

Digestion of hair and serum samples

To each sample (0.01 g of hair or 0.5 mL of serum), 10 mL of 70% nitric acid was added and heated until complete digestion occurred. Thereafter, digests were diluted aqueously in a 25 mL volumetric flask. For method validation, the certified reference material (CRM), lyophilised serum control for trace elements (Ref No: 8880; ClinChek®, Munich, Germany), was used and was reconstituted as per instruction manual and digested under the same conditions as the samples. All samples were transferred into polytetrafluoroethylene (PTFE) bottles and stored in the refrigerator until elemental analysis.

Elemental Analysis of samples

Elemental analysis of digested hair and serum samples were conducted by inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima™ 5300 DV) at different wavelengths for each of the analytes. Wavelength selection was based on minimum spectral interferences and the wavelengths selected were: As (197 nm), Ca (393 nm), Cd (227 nm), Co (231 nm), Cr (268 nm), Cu (325 nm), Fe (260 nm), Mg (280nm), Mn (261 nm), Ni (232 nm), Pb (220 nm), Se (196 nm) and Zn (214 nm). The instrument detection limits for each analyte was As (4.29 ppb), Cd (0.607 ppb), Co (0.346 ppb), Cr (0.744 ppb), Cu (1.41 ppb), Mn (0.095 nm), Ni (0.839 ppb), Pb (2.21 ppb) and Se (2.54 ppb).

Statistical Analysis

The Statistical package for the Social Sciences (PASW Statistics 23, IBM Corporation, Cornell, New York) was used for statistical analyses. Parametric data was expressed as mean \pm standard deviation and non-parametric data expressed as median \pm SEM. Statistical significance of the data was determined using Independent T-test and Mann-Whitney. Pearson correlation coefficient was used to find the relationship among various study parameters. A p-value ≤ 0.05 was considered statistically significant.

Results

Quality assurance

The experimental values obtained for the CRM ($n = 6$, $p = 0.05$) were (in $\mu\text{g/L}$) 4.57, 7.37, 3.85, 10.42, 61.97, 796.8, 1334, 12382, 1345 for Cd, Cr, Mn, Ni, Se, Cu, Fe, Mg and Zn; compared to certified value ranges (in $\mu\text{g/L}$) (3.63-5.45), (5.99-8.99), (3.81-5.71), (7.08-10.6), (52.9-79.3), (681-921), (1330-1790), (11200-13600) and (1120-1520). Experimental values compared well with certified values thereby validating the method.

Clinical and demographic data

The clinical and socio-demographic parameters of the study population are summarized in Table 1. The mean maternal age, weight and body mass index (BMI) of the pre-eclamptic group were not significantly different from those of the normotensive group ($p > 0.05$). There were significant differences between the groups for mean diastolic blood pressure ($p = 0.0001$), systolic blood pressure ($p = 0.0001$), proteinuria ($p = 0.0001$) and gestational age ($p = 0.004$). There was a statistically significant lower rate of delivery by elective cesarean delivery in the normotensive (22%) group compared to the pre-eclamptic (47%) group ($p = 0.05$), with normal vaginal delivery being the predominant mode of delivery in both groups.

There were no stillbirths or maternal deaths after delivery in both groups. The pre-eclamptic (2.40 \pm 0.94 kg) group had significantly lower mean birth weight compared to the normotensive (3.19 \pm 0.39 kg) group ($p = 0.004$). A strong positive Pearson correlation coefficient was observed between BMI and maternal weight ($r = 0.96$, $p = 0.0001$); BMI and diastolic pressure ($r = 0.5$, $p = 0.007$) as well gestational age at delivery and baby weight ($r = 0.7$, $p = 0.0001$) in the pre-eclamptic group.

Table 1 Demographic and clinical data of normotensive control and pre-eclamptic groups (n = 66).

Parameters	Normotensive Group (n = 23)	Pre-eclamptic Group (n = 43)	Significance (P-value)
Unemployment Rate (%)	100	81	0.04*
Maternal Age (years)	24 \pm 5	25 \pm 5	0.36
Maternal Weight (kg)	71 \pm 16	74 \pm 20	0.65
BMI (kg/m ²)	43 \pm 7	31 \pm 8	0.69
Systolic Blood Pressure (mmHg)	107.87 \pm 15.59	158.05 \pm 14.16	0.0001*
Diastolic Blood Pressure (mmHg)	67.91 \pm 11.67	103.14 \pm 12.23	0.0001*
[#] Proteinuria (+)	0 \pm 0	1 \pm 0	0.0001*
Gestational Age at Delivery (weeks)	37 \pm 5	33 \pm 5	0.004*
Caesarean Delivery (%)	22	47	0.05*
Baby Weight (kg)	3.19 \pm 0.39	2.40 \pm 0.94	0.004*

[#]Quantification of proteinuria using the urinary dipstick testing is as follows: 0 = 0 mg/dL; 1 = 30 to 100 mg/dL. Abbreviations: n, number of patients; BMI, body mass index. Data are mean \pm standard deviation. *Compared with normotensive control group, $p \leq 0.05$.

Elemental concentrations in hair

The concentrations of 13 different elements in hair samples from both the pre-eclamptic and normotensive groups are shown in Table 2. Although median concentrations in hair for As, Ca, Cd, Fe, Mg, Pb and Se were higher in the pre-eclamptic group compared to the normotensive group, this was not significantly different ($p > 0.05$). Median Cu, Mn and Ni concentrations in the hair were lower in the pre-eclamptic group compared to the normotensive group ($p > 0.05$). The median concentration of Co was significantly lower ($p = 0.02$) while the median concentrations of Zn and Cr were significantly higher ($p \leq 0.05$) in hair samples of pre-eclamptic women relative to the normotensive control.

Table 2 The levels of different elements in hair samples from the pre-eclampsia and control groups (n = 66).

	Normotensive Control Group (n = 23)	Pre-eclamptic Group (n = 43)	P-value
	(µg/g)	(µg/g)	
As	5.47 ±2.79 (0.06, 49.23)	7.63 ±1.32 (0.44, 19.59)	0.50
Ca	2184.91 ±160.69 (1213.65, 4674.34)	2394.75 ±124.77 (1550.53, 4925.18)	0.12
Cd	3.75 ±0.64 (2.78, 17.50)	3.96 ±0.87 (2.03, 34.60)	0.12
Co	2.89 ±4.99 (0.14, 36.51)	1.56 ±0.74 (0.14, 11.32)	0.02*
Cr	11.05 ±7.62 (3.25, 158.96)	13.31 ±2.67 (5.81, 88.08)	0.01*
Cu	78.78 ±28.21 (1.08, 668.97)	58.87 ±17.32 (4.84, 590.98)	0.53
Fe	449.39 ±78.36 (191, 1453.89)	613.54 ±107.29 (232.69, 4433.83)	0.20
Mg	451.71 ±36.79 (278.02, 983.81)	549.56 ±29.34 (345.18, 1264.08)	0.08
Mn	13.57 ±1.13 (8.20, 29.05)	13.07 ±0.95 (8.19, 32.55)	0.86
Ni	8.40 ±1.31 (3.81, 35.64)	6.86 ±0.81 (0.79, 23.38)	0.85
Pb	58.77 ±37.04 (33.04, 891.94)	72.27 ±19.82 (23.94, 773.97)	0.15
Se	23.93 ±2.62 (0.30, 41.66)	24.42 ±1.78 (4.30, 45.48)	0.66
Zn	330.88 ±29.70 (121.79, 668.70)	395.99 ±48.60 (138.85, 1718.49)	0.05*

Abbreviations: n, number of patients; As, Arsenic; Ca, Calcium; Cd, Cadmium; Cr, Chromium; Co, Cobalt; Mg, Magnesium; Mn, Manganese; Fe, Iron; Cu, Copper; Pb, Lead; Se, Selenium; Ni, Nickel; Zn, Zinc. Values are median ± SEM (minimum, maximum). * Compared with normotensive control group, $p \leq 0.05$.

Elemental concentrations in serum

The concentrations of 13 different elements in serum samples from the pre-eclamptic and normotensive groups are shown in Table 3. Although serum Zn levels was significantly higher in the pre-eclamptic group (0.16 – 253.4 mg/L) compared to the normotensive group (0.2 – 48.4 mg/L) ($p = 0.01$), note that Zn was below the instrument detection limit for some measurements in each of the study groups. Serum Ca, Co, Cu, Mg, Mn and Se was significantly lower in the pre-eclamptic group relative to the normotensive control group ($p < 0.05$). Statistically, there were no significant differences in serum As, Cd, Cr, Fe, Ni and Pb levels between the two study groups (Table 3).

Table 3 Serum levels of different elements across study groups (n = 66).

	Normotensive Control Group (n = 23)	Pre-eclamptic Group (n = 43)	P-value	Lab Ref Values	Reference
	(mg/L)	(mg/L)		(mg/L)	
As	0.49 ±0.0 (0.01, 0.13)	0.06 ±0.0 (0.06, 0.06)	0.81	0.002-0.023	[19]
Ca	107.61 ±6.30 (85.47, 222.34)	75.44 ±2.06 (56.58, 102.81)	0.02*	82-97 [#]	[20]
Cd	0.10 ±0.3 (0.01, 0.34)	0.05 ±0.04 (0.01, 0.96)	0.14	0.001-0.005	[19]
Co	0.02 ±0.0 (0.0, 0.03)	ND	0.0001*	<0.001	[21]
Cr	0.05 ±0.03 (0.0, 0.24)	0.24 ±0.01 (0.0, 0.37)	0.26	< 0.0-0.0021	[19]
Cu	5.19 ±3.09 (0.91, 58.59)	2.27 ±0.25 (0.42, 8.21)	0.001*	1.3-2.4 [#]	[20]
Fe	179.94 ±47.31 (25.31, 829.83)	142.64 ±129.00 (0.99, 4891.3)	0.32	0.3-1.93 [#]	[20]
Mg	38.73 ±1.44 (31.09, 61.80)	29.93 ±0.41 (24.27, 36.50)	0.0001*	11-22 [#]	[20]
Mn	0.03 ±0.0 (0.0, 0.16)	0.02 ±0.0 (0.01, 0.03)	0.03*	0.004-0.012	[19]
Ni	0.14 ±0.0 (0.0, 0.09)	0.02 ±0.0 (0.0, 0.06)	0.16	0.001-0.005	[19]
Pb	0.16 ±0.21 (0.0, 3.0)	0.20 ±0.17 (0.04, 5.49)	0.22	<0.25	[19]
Se	0.14 ±0.01 (0.04, 0.21)	0.06 ±0.01 (0.0, 0.16)	0.001*	0.71-1.33 [#]	[20]
Zn	2.13 ±3.01 (0.20, 48.36)	18.03 ± 30.28 (0.16, 253.40)	0.01*	0.5-0.77 [#]	[20]

Abbreviations: n, number of patients; ND, not detected (measurements were below the instrument detection limit); As, Arsenic; Ca, Calcium; Cd, Cadmium; Cr, Chromium; Co, Cobalt; Mg, Magnesium; Mn, Manganese; Fe, Iron; Cu, Copper; Pb, Lead; Se, Selenium; Ni, Nickel; Zn, Zinc. Values are median ± SEM (minimum, maximum). * Compared with the normotensive control group, $p \leq 0.05$. [#]Laboratory (Lab) reference (Ref) values for pregnant women.

Correlation analysis

Table 4 shows the correlation between selected clinical parameters (maternal and fetal) and hair elemental concentrations in the pre-eclampsia. A significant positive correlation between hair Se concentrations and gestational age at delivery ($r = 0.31$, $p = 0.05$) was found (Table 4). Table 5 shows strong significant positive correlations between maternal age and serum Ni concentrations ($r = 0.45$, $p = 0.05$); serum Ni concentrations and diastolic blood pressure ($r = 0.3$, $p = 0.05$); maternal weight and serum Zn concentrations ($r = 0.88$, $p = 0.05$); BMI and serum Zn concentrations ($r = 0.80$, $p = 0.01$); as well as baby weight and serum Zn concentrations ($r = 0.6$, $p = 0.6$) in the pre-eclamptic group. Also, significant positive correlations between maternal weight and serum Cr concentrations ($r = 0.33$, $p = 0.05$); BMI and serum Cr concentrations ($r = 0.38$, $p = 0.05$); diastolic blood pressure and serum Cr concentrations ($r = 0.34$, $p = 0.05$) were observed in the pre-eclamptic group. Moreover, there were significant negative correlations between serum Ni concentrations with gestational age

($r = -0.5$, $p = 0.01$) and baby weight ($r = -0.35$, $p = 0.05$) were noticed in the pre-eclamptic group.

Table 4 Correlations between hair elemental levels ($\mu\text{g/g}$), maternal and fetal parameters in the pre-eclamptic group ($n = 43$).

Parameters	As	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Se	Zn
Mat Age	-0.23	0.29	-0.07	0.46	-0.16	0.20	-0.20	0.31	-0.03	0.07	-0.08	0.03	0.08
Mat weight	-0.06	-0.23	-0.12	0.33	-0.21	0.00	-0.16	0.06	-0.21	-0.17	-0.17	0.26	-0.26
BMI	0.11	-0.26	-0.14	0.41	-0.11	-0.07	-0.24	0.06	-0.31	-0.06	-0.18	0.09	-0.22
Gest Age	0.26	0.14	0.14	0.18	0.18	0.11	-0.20	0.13	0.1	-0.03	0.14	0.31*	-0.01
DBP	0.11	0.00	0.07	-0.20	-0.16	-0.12	0.07	0.10	-0.18	-0.08	0.11	-0.15	-0.19
SBP	0.11	0.09	-0.03	0.17	-0.08	-0.02	0.25	0.01	0.04	0.04	0.01	0.0	-0.0
Baby Weight	-0.12	0.02	0.12	0.49	0.14	-0.10	0.13	-0.7	0.21	-0.25	0.12	0.30	-0.051

Pearson correlation (r); * Correlation is significant at the 0.05 level (2-tailed).

Table 5 Correlations between serum elemental levels (mg/l), maternal and fetal parameters in the pre-eclamptic group ($n = 43$).

Parameters	As	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Se	Zn
Mat Age	0.0	-0.08	-0.16	-0.03	0.01	0.04	0.08	0.02	-0.02	0.45**	0.23	-0.13	0.48
Mat weight	0.0	0.19	-0.11	0.27	0.33*	-0.08	0.17	0.12	-0.1	-0.04	-0.10	-0.09	0.88**
BMI	0.0	0.14	-0.07	0.30	0.38*	-0.15	0.16	0.09	-0.2	-0.07	-0.11	-0.16	0.80*
Gest Age	0.0	-0.08	-0.16	-0.03	0.01	0.04	0.08	0.02	-0.02	-0.5**	0.23	-0.13	0.48
DBP	0.0	0.30	0.18	-0.06	0.34*	-0.11	-0.05	0.20	0.04	0.3*	-0.18	-0.25	0.06
SBP	0.0	0.17	0.25	-0.1	-0.24	-0.07	0.16	0.13	0.00	-0.01	-0.06	-0.04	-0.1
Baby Weight	0.0	-0.08	-0.34	0.0*	0.05	0.03	0.19	0.05	0.11	-0.35*	0.21	0.03	0.6

Pearson correlation (r); *Correlation is significant at the 0.05. ** Correlation is significant at the 0.01 level (2-tailed).

Discussion

In this study, the median value of Zn in the hair samples was significantly higher in the pre-eclamptic group ($395.99 \mu\text{g/g}$) compared to the normotensive control group ($330.88 \mu\text{g/g}$) ($p = 0.05$). Previous studies have reported either no statistically significant change, statistically significant higher or lower serum Zn levels in pre-eclamptic compared to normotensive pregnant women [22-27]. Elevated serum Zn levels may play an etiological role in women with primary hypertension. In this case, it is possible that excess serum Zn levels may cause an increase in intracellular Zn, which results in an increase of free Ca^{2+} levels in the smooth

muscular layer of the blood vessels with consequent elevated blood pressure [28]. In addition, high serum Zn levels with decreased serum Cu levels may also lead to elevation in systemic blood pressure in healthy individuals, because of the Zn-Cu antagonistic interactions that exist in the body. Therefore, this increased serum Zn and decreased serum Cu level reduces the activity of superoxide dismutase (SOD), which creates oxidative stress [28]. Interestingly, placental oxidative stress and maternal vascular dysfunction is a well-known phenomenon in pre-eclampsia [28]. In our study, the levels of Cu were also lower and the levels of Zn were higher in both hair and serum from the pre-eclamptic group compared to the normotensive control group. We also found a negative correlation serum Zn and serum Cu concentrations ($r = -0.37$, $p = 0.37$) were noted. Previous studies have also found significantly lower levels of Cu in serum [29-32] and hair [9, 30, 33] samples from the pre-eclamptic group compared to the control group. Similarly, hair and serum levels of Mn were also found to be significantly ($p \leq 0.05$) lower in the pre-eclamptic group ($13.07 \pm 0.95 \mu\text{g/g}$ and $0.02 \pm 0.0 \text{ mg/L}$ respectively) in comparison to the control group ($13.57 \pm 1.13 \mu\text{g/g}$ and $0.03 \pm 0.0 \text{ mg/L}$ respectively). A similar study by Al-Jameil *et al.*, (2014) reported on low serum Mn level in pre-eclampsia (0.072 mg/L) [29]. The current literature states that low serum Mn levels have a defined role in the impairment of endothelial function during pre-eclampsia [29]. Since, Mn is an active component of arginine, which is one of the most versatile amino acids that serve as a precursor for the synthesis of nitric oxide, and nitric acid is regarded as the key determinant of endothelial function [34-35]. It seems that the altered balance of nitric acid and reactive oxygen species (ROS) play a critical role in the pathogenesis of pre-eclampsia by causing endothelial dysfunction. Furthermore, it is known that nitric acid and ROS accumulates in placental tissue and pre-eclampsia is known to be cured by the removal of the placenta [34-35]. Also, Mn is required for the proper functioning of enzymes like SOD. Hence decreased levels of Mn may affect the antioxidant potential of cells by the resultant decreasing SOD activity as well as increased lipid peroxidation, causing an increase in blood pressure [29].

Calcium and its relationship to pre-eclampsia have been widely explored [36]. During pregnancy, the Ca requirements are between 600 to 1300 mg/L per day and a deficiency of Ca increases the risk of pre-eclampsia development [9]. In our study, significantly low serum Ca levels in the pre-eclamptic group ($75.44 \pm 2.06 \text{ mg/L}$) group compared to control group ($107.61 \pm 6.30 \text{ mg/L}$) ($p = 0.02$) was noted. These findings confirm the findings of several other studies [9, 37-39]. It seems that serum levels of Ca play a role in the development of pre-eclampsia, as it has been observed that reduced Ca levels in the serum causes an increase in the amount of parathyroid hormone and renin release, which causes an increase in intracellular Ca in vascular smooth muscle. These events are responsible for increased

vascular resistance and vasoconstriction, which lead to a rise in blood pressure [9]. Magnesium also plays a vital role in blood pressure regulation. Whilst Ca is needed for blood vessel contraction, Mg is needed for muscle relaxation and opening. Therefore, it is not surprising that reduced levels of Mg levels in the blood are associated with pre-eclampsia. During pregnancy, the Mg requirement is 400 mg/L per day. Lower levels of Mg in the serum of the pre-eclamptic group (29.93 ± 0.41 mg/L) compared to the normotensive group (38.73 ± 1.44 mg/L) were also noted in our study [9, 34]. These findings are in agreement with several studies which report that serum Mg levels are lower in pre-eclamptic pregnant women [9, 28, 36].

Selenium is another element found to be deficient in pregnant women with pre-eclampsia. It is a vital component of different seleno-enzymes, which are needed for defense against oxidative stress or ROS that cause damage to the endothelial cells. During gestation an increase of Se by 10 µg per day is required in order to maintain adequate levels (100 µg/L per day) of Se to support the increased Se requirements of the fetus, newborn and to prevent pre-eclampsia [9]. Other elements that have been associated with pre-eclampsia are Co and Cr. Kolusari *et al.*, (2008) reported that Co serum levels were lower in pre-eclamptic (1.27 µg/L) compared to normotensive (2.23 µg/L) women [40]. In contrast, another study reported slight increase in Co levels in hair from pre-eclamptic women (0.033 µg/g) compared to normotensive women (0.017 µg/g) and suggested that hair may be an active route of Co excretion [33]. Whereas, this study shows a decrease in Co and an increase in Cr levels in hair and serum samples from pre-eclamptic women.

Noteworthy, in this study the hair levels of Zn were significantly higher in the pre-eclamptic group compared to the normotensive group, and that Ca and Cu were significantly lower. Concentrations of all elements studied were higher in hair samples than serum samples. This variability may be attributed to the factor that blood elemental analysis measures the components that are absorbed and temporarily circulating in the body before excretion and/or during storage. Whereas, hair elemental analysis reflects the elements stored in the body, body's nutritional imbalances and presence of toxic metals in the body [41]. This study shows concentrations of elements in serum to be in decreasing order of $\text{Co} > \text{Cd} > \text{Ni} \sim \text{Mn} > \text{As} \sim \text{Se} > \text{Pb} > \text{Cr} > \text{Cu} > \text{Zn} > \text{Mg} > \text{Ca} > \text{Fe}$ and concentrations of elements in hair to be in decreasing order of $\text{Co} > \text{Cd} > \text{Ni} > \text{As} > \text{Mn} > \text{Cr} > \text{Se} > \text{Cu} > \text{Pb} > \text{Zn} > \text{Mg} > \text{Fe} > \text{Ca}$.

The strength of the current study was that it used a homogenous population of Black South African women, to prevent the confounding effects of ethnicity. Also, elemental analysis was performed on pubic hair in this study alleviating cosmetic factors of hair straightening and

bleaching as well as environmental factors that interfere with the endogenous content in hair. A limitation of this study was that parathyroid hormone levels were not measured as elevated serum parathyroid hormone levels could be a marker for low Ca levels. Low Ca levels have previously been associated with increased risk of developing pre-eclampsia.

Conclusion

The present study reveals that maternal serum levels of Ca, Co, Cu, Mg, Mn and Se in pre-eclamptic women are significantly lower than normotensive women. Similarly, significantly lower levels of Co were also observed in hair from pre-eclamptic women. Thus, the evidence in this present study corroborated an association between pre-eclampsia and essential elements (both macro and micro). Even though, there are a number of other studies demonstrating a significant association between pre-eclampsia and trace elements, further studies are required to determine the levels of these elements in all three trimesters of pre-eclamptic pregnancies. Monitoring trace and macro elements may assist in understanding their role in the pathogenesis of pre-eclampsia, using them as biomarkers or prophylaxis interventions during pregnancy.

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Declaration of Interest

The authors report no conflicts of interest.

References

1. J.E. Chavarro, J.W. Rich-Edwards, B.A. Rosner, W.C. Willett, Iron intake and risk of ovulatory infertility, *Obstet Gynecol.* 108 (5) (2006) 1145-1152.
2. I.M. Ebisch, C.M. Thomas, W.H. Peters, D.D. Braat, R.P. Steegers-Theunissen, The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility, *Hum Reprod Update.* 13 (2) (2007) 163-174.
3. J.C. King, Physiology of pregnancy and nutrient metabolism, *Am J Clin Nutr.* 71 (2000) 1218S-25S.
4. H.D. Mistry, P.J. Williams, The Importance of Antioxidant Micronutrients in Pregnancy, *Oxidative Med and Cell Longevity* 2011 (2011) 1-12.
5. J.M. Roberts, J.L. Balk, L.M. Bodnar, J.M. Belizán, E. Bergel, A. Martinez, Nutrient involvement in preeclampsia, *J Nutr.* 133 (2) (2003) 1684S-1692S.
6. G. Lambert, J.F. Brichant, G. Hartstein, V. Bonhomme, P.Y. Dewandre, Preeclampsia: an update, *Acta Anaesthesiol Belg.* 65 (4) (2014) 137-149.
7. K. Daniels, S. Lewin, Translating research into maternal health care policy: a qualitative case study of the use of evidence in policies for the treatment of eclampsia and pre-eclampsia in South Africa, *Hlth Res Pol Syst.* 6 (2008) 1-13.
8. J. Uzan, M. Carbonnel, O. Piconne, R. Asmar, J. Ayoubi, Pre-eclampsia: pathophysiology, diagnosis, and management, *Vasc Hlth Risk Manag.* 7 (2011) 467-474.
9. O.A. Elind, Trace Elements as Potential Biomarkers of Preeclampsia, *Ann Res & Rev Biol* 9 (1) (2016) 1-10.
10. C.M. Champagne, Magnesium in hypertension, cardiovascular disease, metabolic syndrome, and other conditions: a review, *Nutr Clin Pract.* 23 (2) (2008) 142-51.
11. S. Sandip, K. Asha, G. Paulin, S. Hiren, S. Gagandeep, V.A. Amit, Comparative Study of Serum Uric Acid, Calcium and Magnesium in Preeclampsia and Normal Pregnancy, *J Adv Res Biol in Sci* 5 (1) (2013) 55-58.
12. G.J. Hofmeyr, A. Roodt, A.N. Atallah, L. Duley, Calcium supplementation to prevent pre-eclampsia - a systematic review, *S Afr Med J.* 93 (3) (2003) 224-8.
13. T.O. Scholl, Iron status during pregnancy: setting the stage for mother and infant, *Am J Clin Nutr.* 81 (5) (2005) 1218S-1222S.
14. R.E. Black, Micronutrients in pregnancy, *Br J Nutr.* 85(2) (2001) S193-S197.
15. K. Ebrahim, A. Ashtarinezhad, The Association of Amniotic Fluid Cadmium Levels with the Risk of Preeclampsia, Prematurity and Low Birth Weight, *Iran Neonato* 6 (2) (2015) 1-6.

16. D.A. Kennedy, C. Woodland, G. Koren, Lead exposure, gestational hypertension and pre-eclampsia: a systematic review of cause and effect, *J Obstet Gynaecol.* 32 (6) (2012) 512-517.
17. A.M. Ismail, A.M. Abbas, S.M. George, A.H. Mohamed, Environmental exposure to lead and cadmium in patients with preeclampsia; A case-control study, *Asian J Pharmacol and Toxicol* 3 (12) (2015) 27-33.
18. S.M. Motawei, S.M. Attalla, H.E. Gouda, M.A. El-Harouny, A.M. El-Mansoury, Lead level in pregnant women suffering from pre-eclampsia in Dakahlia, Egypt, *J Occup Environ Med.* 4 (1) (2013) 36-44.
19. J.T. Queenan, C.Y. Spong, C.J. Lockwood, *Protocols for high-risk pregnancies: An evidence-based approach*, sixth ed., Wiley Blackwell, USA, 2015.
20. S. Duh, J.D. Cook, Laboratory reference range values. <http://www.stedmansonline.com/webFiles/Dict-Stedmans28/APP17.pdf>, 2005.
21. M. Laposata, *Laboratory Medicine: The diagnosis of disease in the clinical laboratory*. second ed, Graw-Hill Global Education Holdings, New York, 2010.
22. M.S. Sarwar, S. Ahmed, M.S. Ullah, H. Kabir, G.K. Rahman, A. Hasnat, M.S. Islam, Comparative study of serum zinc, copper, manganese, and iron in preeclamptic pregnant women, *Biol. Trace Elem Res.* 154 (2013) 14–20.
23. N. Ilhan, M. Simsek, The changes of trace elements, malondialdehyde levels and superoxide dismutase activities in pregnancy with or without preeclampsia, *Clin. Biochem.* 35 (2002) 393–397.
24. L. Farzin, F. Sajadi, Comparison of serum trace element levels in patients with or without pre-eclampsia, *J. Res. Med. Sci.* 17 (2012) 938–941.
25. M. Harma, A. Kocyigit, Correlation between maternal plasma homocysteine and zinc levels in preeclamptic women, *Biol. Trace Elem. Res.* 104 (2005) 97–105.
26. O. Katz, O. Paz-Tal, T. Lazer, B. Aricha-Tamir, M. Mazor, A. Wiznitzer, E. Sheiner, Severe pre-eclampsia is associated with abnormal trace elements concentrations in maternal and fetal blood, *J. Matern. Fetal Neonat Med.* 25 (2012) 1127–1130.
27. K. Mahomed K, M.A. Williams, G.B. Woelk, S. Mudzamiri, S. Madzime, I.B. King, D.D. Bankson, Leukocyte selenium, zinc and copper concentration in preeclamptic and normotensive pregnant women, *Biol. Trace Elem. Res.* 75 (2000) 107–118.
28. K.B. Singh, Molecular Basis of Hypertension: A Systematic Review on the Role of Metal Ions for Increase Prevalence of Hypertension in India, *J Biosci Med.* 4 (2016) 12-22.
29. N. Al-Jameil, H. Tabassum, H. Al-Mayouf, N.D. Alenzi, S.M. Hijazy, F.A. Khan, Analysis of serum trace elements-copper, manganese and zinc in preeclamptic

- pregnant women by inductively coupled plasma optical emission spectrometry: a prospective case controlled study in Riyadh, Saudi Arabia, *Int J Clin Exp Pathol.* 7 (5) (2014) 1900-1910.
30. H.M. Huang, P.L. Leung, D.Z. Sun, M.G. Zhu, Hair and serum calcium, iron, copper, and zinc levels during normal pregnancy at three trimesters, *Biol Trace Elem Res.* 69 (2) (1999) 111-1120.
 31. H.H. Al-Shalah, N. Mudher Al-Hilli, M.A. Hasan, The Association of Serum Iron, Zinc, and Copper Levels with Preeclampsia, *Med J Babylon* 12 (2016) 1027-1036.
 32. D.V. Kanagal, A. Rajesh, K. Rao, H. Shetty, P.K. Shetty, H. Ullal, Zinc and copper levels in preeclampsia: a study from coastal South India, *Int J Reprod Contracept Obstet Gynecol.* 3 (2) (2014) 370-373.
 33. M.J. González-Muñoz, F.J. Sánchez-Muniz, S. Ródenas, M.I. Sevillano, M.L. MaríaTeresa, S. Bastida, Differences in metal and metalloid content in the hair of normo- and hypertensive postmenopausal women, *Hyperten Res.* 33 (2010) 219–224.
 34. W.U. Guoyao, M. Sidney, J.R. Morris, Arginine metabolism: nitric oxide and beyond. *Biochem. J.* 336 (1998) 1–17.
 35. K. Matsubara, T. Higaki, Y. Matsubara, A. Nawa, Nitric Oxide and Reactive Oxygen Species in the Pathogenesis of Preeclampsia, *Int. J. Mol. Sci.* 16 (2015) 4600-4614.
 36. B.H. Spencer, J.J. Vanderlelie, A.V. Perkins, Essentiality of Trace Element Micro nutrition in Human Pregnancy: A Systematic Review, *J Preg Child Hlth.* 2 (3) (2015) 1-7.
 37. S. Akhtar, S. Begum, S. Ferdousi, Calcium and Zinc Deficiency in Preeclamptic Women, *J Bangladesh Soc Physiol.* 6 (2) (2011) 94-99.
 38. S. Kumru, S. Aydin, M. Simsek, K. Sahin, M. Yaman, G. Ay, Comparison of serum copper, zinc, calcium, and magnesium levels in preeclamptic and healthy pregnant women, *Biol Trace Elem Res.* 94 (2) (2003) 105-112.
 39. M. Kosch, M. Hausberg, F. Louwen, M. Barenbrock, K.H. Rahm, K. Kisters, Alterations of plasma calcium and intracellular and membrane calcium in erythrocytes of patients with pre-eclampsia, *J Hum Hypertens.* 14 (5) (2000) 333-336.
 40. A. Kulusari, M. Kurdoglu, R. Yildizhan, E. Adali, T. Edirne, A. Cebi, H. Demir, I.H. Yoruk, Catalase activity, serum trace element and heavy metal concentrations, and vitamin A, D and E levels in pre-eclampsia, *J Int Med Res.* 36 (6) (2008) 1335-1341.
 41. G. Ahmad, H.D. Kuhi, A. Mohit, A review Hair tissue analysis: An analytical method for determining essential elements, toxic elements, hormones and drug use and abuse, *Intl. Res. J. Appl. Basic. Sci.* 4 (11) (2013) 3675-3688.

CHAPTER 4

Synthesis and Conclusion

Pre-eclampsia (PE) is a condition that can affect the mother and the fetus. In developing countries, PE accounts for 15-20% of maternal deaths and complicates 2-8% of all pregnancies (Shamsi *et al.*, 2013). Whereas, reports from developed countries propose that 15% of preterm births together with perinatal mortality is 5 times more likely to occur in PE pregnancies. Despite major medical advances, the only known treatment intervention for PE is the delivery of the baby and placenta (English *et al.*, 2015).

Numerous studies on PE have suggested that this disease occurs in two stages (Hladunewich *et al.*, 2007). The first stage is described by reduced perfusion linked to abnormal placentation with impaired trophoblast invasion and insufficient remodeling of the uterine spiral arteries. The second stage is the maternal systemic manifestations with combined inflammatory, metabolic, and thrombotic responses converging to change vascular function which can result in multi-organ damage (Hladunewich *et al.*, 2007). This results in ROS and placenta oxidative stress, during pregnancy, delivery and the period following birth (Jain, 1986). The defective trophoblast invasion associated with pre-eclampsia may be a result of immune intolerance. In pre-eclampsia, failure of trophoblast invasion results in reduced uterine perfusion pressure and placental ischemia. This may induce the release pro-inflammatory cytokine with may contribute to mediate the endothelial damage (Maggini *et al.*, 2007). Also, trace element deficiencies are common amongst pregnant women with recent findings now supporting the possibility that deficiency of some trace elements may make women susceptible to the development of PE (Roberts *et al.*, 2003). Some of these trace elements, known as essential elements (i.e. Cu and Zn), can regulate the balance between free radicals and antioxidants (Roberts *et al.*, 2003).

Copper is an important enzymatic antioxidant system in the body as it catalyzes the formation of ROS. The levels of Cu as well as Zn are reduced in PE patients (Akinloye *et al.*, 2010, Kumru *et al.*, 2003) while another study found that only Cu was statistically different as compared to the normotensive patients (Ugwuja *et al.*, 2010). Also, studies have shown increased serum Zn concentration in PE patients compared to healthy pregnant women (Diaz *et al.*, 2002, Harma *et al.*, 2005). In our study, decreased levels of Cu as well as other important antioxidant components such as Se and Mn were found in the PE group. This may decrease antioxidant enzyme activity thereby increasing oxidative stress. Nutrients have the ability to increase or decrease the presence of free radicals or antioxidants thereby having a direct affect on oxidative stress. Therefore a diet

rich in fat could also be a contributing factor to the development of PE as lipids are comprehensively involved in the generation of free radicals (Roberts *et al.*, 2003). Recent studies have also associated low maternal blood Mn concentration with poor birth outcomes such as increased risk of IUGR and low birth weight (Wood, 2009). A study done by Rayman *et al* (2003) found significantly lower ($p = 0.001$) median toenail Se concentrations in a PE group (0.56 mg/kg) compared to matched controls (0.62 mg/kg). Our study also observed that lower Se levels were significantly related to the severity of PE ($p = 0.029$) and early delivery before 32 weeks (Rayman *et al.*, 2014). In another study carried by Ikaraoha *et al* (2016) a significantly lower serum level of Se ($p = 0.0001$), Zn ($p = 0.001$), Cu ($p = 0.001$), Co ($p = 0.0001$) and Mn ($p = 0.0001$) were reported (Ikaraoha *et al.*, 2016). Also, Al-Jameil *et al* (2015) found significantly lower levels of Zn, Mg and Ca ($p < 0.001$) in serum samples of PE when compared to normotensive women (Al-Jameil *et al.*, 2015). These results corroborate with the significantly decreased serum levels observed in our study. Calcium is regarded as the best studied element in PE its supplementation is vital to maintaining normal Ca levels during pregnancy (Roberts *et al.*, 2003). Low Ca intakes can lead to the development of hypertension by stimulating the release of parathyroid hormone and rennin. This in turn leads to an increase in intracellular Ca which results in vasoconstriction and vascular resistance (Spencer *et al.*, 2015). Calcium together with Mg plays a crucial role in blood pressure regulation. Magnesium produces arterial relaxation and lowers arterial blood pressure during pregnancy. The onset of PE and progression to labour may be initiated by Mg. A proposed marker for women who are at a high risk for preterm delivery is low serum Mg levels, with Mg supplementation used as a preventative measure against preterm delivery (Spencer *et al.*, 2015). Magnesium is also administered to manage and reduce the risk of PE during pregnancy (Roberts *et al.*, 2003).

We also reported reduced Co levels in the PE compared to the normotensive group in all three biological samples, while Cd levels were slightly elevated in the hair and reduced in the nail and serum samples. Cobalt benefits human health as it is a component of vitamin B₁₂. Low levels of B₁₂ can cause complications such as anemia, infections, inflammation and PE. A study conducted by Wang *et al* (2014), found that prenatal exposure to low levels of Cd induces the phenotypical characteristics of PE such as hypertension, proteinuria and IUGR. Cadmium exposure during pregnancy causes the synthesis of placental glucocorticoid (Wang *et al.*, 2014b).

In our study, the concentration of Fe was elevated in the hair and nails of PE women, while it was decreased in the serum samples. Iron is involved in the regulation of cytokine production and

mechanism of action, and in the activation of protein kinase C, which is essential for phosphorylation of factors regulating cell proliferation. In PE, vasospasms may occur resulting in the destruction of the red blood cells which leads to anemia and elevated serum iron levels. This initiates lipid peroxidation as excess iron react with free radicals of lipoproteins and the cell membrane. Also, increased vascular resistance, hepatic dysfunction and endothelial dysfunction may occur as a result of the changes in the serum activity of ferritin and transferrin induced by elevated Fe levels (Kandi *et al.*, 2014).

Lead levels in our study were increased in hair and serum but decreased in nail samples of the PE group. Lead increases oxidative stress by increasing ROS which results in hypertension. An increase in ROS inactivates nitric oxide (NO) which is a powerful vasodilator. Lead can negatively impact the sodium (Na) gradient pump thus affecting the activity of the Na/Ca exchanger leading to increased intracellular Ca and an increase in smooth muscle contractility. These findings strongly suggest that blood Pb concentrations could be a contributing factor to PE development (Kennedy *et al.*, 2012). Arsenic is also, associated with a wide range of chronic illnesses (*i.e.* diabetes, vascular diseases) including hypertension disorders. In our study, the concentration of As was elevated, decreased and below the detection limit in the hair, serum and nails samples, respectively. Oxidative stress is recognized as a vital mechanism of As toxicity and lipid peroxidation. Furthermore, the presence of As may be the link between increase in oxidative stress and development of PE. In contrast, a novel study carried out by Sandoval-Carrillo *et al* (2016) demonstrated no association between As exposure and PE (Sandoval-Carrillo *et al.*, 2016). Other elements that are reported to be related to PE are; Cr, phosphorus (P), chlorine (Cl) and fluoride (F). These elements cross can cross the placental membrane and are also associated with perinatal risks such as premature rupture of membranes, prematurity, low birth weight and fetal death (Elind, 2016).

In the nails study, a significant decrease of Co ($p = 0.0001$), Mn ($p = 0.022$) and Cd ($p = 0.004$) was recorded in the PE as compared to the normotensive group. In the hair and serum study, the pubic hair samples yielded a significant decrease in Co ($p = 0.02$), Cr ($p = 0.01$) and Zn ($p = 0.05$) while Co ($p = 0.0001$), Mn ($p = 0.03$), Cu ($p = 0.001$), Ca ($p = 0.02$), Mg ($p = 0.0001$), Se ($p = 0.001$) and Zn ($p = 0.01$) was significantly lower in the PE as compared to the normotensive group. Significantly decreased levels of Co were observed across all three biological samples whilst decreased levels of Mn were only observed in the serum and nail clippings samples of PE women. These differences may be attributed to the variation in the biological

properties/characteristics of the different samples, *i.e.* nails, hair and serum. The rate of nail growth is an indicator of the health status of an individual. The shape, discoloration and thickness of nails (both fingers and toe nails) can indicate signs of certain diseases. The elements present in nails reflect the dietary intake of an individual and provide an insight into the exposure period to elements. Therefore, nails carry different nutritional information compared to blood, as nails are ejected from the body once formed. These nail samples can be easily collected as it is non-invasive and conveniently stored at room temperature. Nails are able to integrate trace elements according to dietary intake and environmental exposure over a period of time, making it a good indicator for nutrient related diseases and conditions (He, 2011).

Nails and hair can also be analyzed to determine the concentration and exposure of heavy metals in different environments (Abdulrahman *et al.*, 2012). The use of hair for trace element analysis allows for accuracy and repeatability while it also provides a long term exposure status (Abdulrahman *et al.*, 2012). It does not deteriorate easily and the concentration of trace elements in this type of biological sample is at least 10 times greater than in blood and urine (Krajewski *et al.*, 2009). Hair sampled from the scalp is exposed to various substances such as shampoos and hair dyes. This can affect the elemental concentration found in the hair (Bass *et al.*, 2001), therefore pubic hair was chosen to be used in our study. Conversely, the use of serum is an invasive method and may also be prone to contamination. This type of samples need to be stored correctly and analysis may be time consuming (Hambidge, 2003).

In summary, the present study demonstrates that maternal serum levels of Ca, Co, Cu, Mg, Mn and Se in pre-eclampsia were significantly lower than normotensive women. Similarly, significantly lower levels of Co were observed in hair from pre-eclamptic women. A significant decrease in the levels Cd, Co and Mn in nails of PE women was observed. These results show an imbalance between macro and trace elements in PE. Therefore, more extensive studies on these elements may confirm the exact role of nutrition in the pathogenesis of PE and may be used as potential biomarkers for the detection PE.

CHAPTER 5

References

- Abdulrahman, F. I., Akan, J. C., Chellube, Z. M. & M.Waziri 2012. Levels Of Heavy Metals In Human Hair And Nail Samples From Maiduguri Metropolis, Borno State, Nigeria. *World Environ*, 2, 81-89.
- Ahmad, S. A., Sayed, M. H. S. U., Barua, S., Khan, M. H., Faruquee, M. H., Jalil, A., Hadi, S. A. & Talukder, H. K. 2001. Arsenic In Drinking Water And Pregnancy Outcomes. *Environ Hlth Persp*, 109, 629-631.
- Akinloye, O., Oyewalei, O. J. & Oguntibeju, O. O. 2010. Evaluation Of Trace Elements In Pregnant Women With Preeclampsia.
- Al-Jameil, N., Tabassum, H., Ali, M. N., Qadeer, M. A., Khan, F. A. & Al-Rashed, M. 2015. Correlation Between Serum Trace Elements And Risk Of Preeclampsia: A Case Controlled Study In Riyadh, Saudi Arabia. *Saudi J Biol Sci*, 1-7.
- Alghazali, B. S., Sadiq, A. M. & Hasan, N. N. 2014. The Role Of Trace Elements (Zinc, Copper, Magnesium And Calcium) In Pregnant Women With Preeclampsia In Third Trimester And Fetal Cord Blood After Delivery. *Muthanna Medical Journal*, 1, 12-18.
- Allen, L. H. 2000. Anemia And Iron Deficiency: Effects On Pregnancy Outcome. *Ameri J Clini Nutri*, 71, 1280s-1284s.
- Amin, S. V., Illipilla, S., Hebbar, S., Rai, L., Kumar, P. & Pai, M. V. 2014. Quantifying Proteinuria In Hypertensive Disorders Of Pregnancy. *Int J Hypertens*, 8, 1-11.
- Awadallah, S., Abu-Elteen, K., Elkarmi, A., Quaraein, S., Salem, M. & Mubarak, M. 2004. Maternal And Cord Blood Serum Levels Of Zinc, Copper And Iron In Healthy Pregnant Jordanian Women. *Trace Elements Experi Med*, 17, 1-8.

Baloch, G. H., Shaikh, K., Jaffery, M. H., Abbas, T., Das, C. M., Devrajami, B. R., Shah, S. Z. A. & Fazlani, K. 2012. Serum Magnesium Level During Pregnancy. *World App Sci J* 17, 1005-1008.

Bartsch, E., Medcalf, K. E., Park, A. L. & Ray, J. G. 2016. Clinical Risk Factors For Pre-Eclampsia Determined In Early Pregnancy: Systemic Review And Meta-Analysis Of Large Cohort Studies. *Bmj*, 353, 1-10.

Bass, D. A., Hickok, D., Quig, D. & Urek, K. 2001. Trace Element Analysis In Hair: Factors Determining Accuracy, Precision, And Reliability. *Altern Med Rev* 6, 472-81.

Dadelszen, P. V., Firoz, T., Donnay, F., Gordon, R., Hofmeyr, G. J., Lalani, S., Payne, B. A., Roberts, J. M., Teela, K. C., Vidler, M., Sawchuck, D. & Magee, L. A. 2012. Pre-Eclampsia In Low And Middle Income Countries-Health Services Lessons Learned From The Pre-Empt(Pre-Eclampsia-Eclampsia Monitoring, Prevention & Treatment) Project. *J Obstet Gynaecol*, 34, 917-926.

Dadelszen, P. V. & Magee, L. A. 2014. Pre-Eclampsia: An Update. *Curr Hypertens Rep*, 16, 1-14.

Diaz, E., Halhali, A., Luna, C., Diaz, L., Avila, E. & Larrea, F. 2002. Newborn Birth Weight Correlates With Placental Zinc, Umbilical Insulin-Like Growth Factor , And Leptin Levels In Pre-Eclampsia. *Arch Med Res*, 33, 40-47.

E.Hassan, E., Elhhatim, W. S., M.Bakhit, S., Shrif, N. E. M. A. & A.Huneif, M. 2014. Assessment Of Trace Elements In Sudanese Preeclamptic Pregnant Women. *European Journal Of Biomedical And Pharmaceutical Sciences*, 1, 13-21.

Elind, A.-H. O. 2016. Trace Elements As Potential Biomarkers Of Pre-Eclampsia. *Ann Res Rev Biol*, 9, 1-10.

English, F. A., Kenny, L. C. & McCarthy, F. P. 2015. Risk Factors And Effective Management Of Preeclampsia. *Integr Blood Press Control*, 8, 7-12.

Ephraim, R. K. D., Osakunor, D. N. M., Denkyira, S. W., Eshun, H., Amoah, S. & Anto, E. O. 2014. Serum Calcium And Magnesium Levels In Women Presenting With Pre-Eclampsia And Pregnancy-Induced Hypertension: A Case-Control Study In The Cape Coast Metropolis, Ghana. *Bmc Pregnancy And Childbirth*, 14, 1-8.

Gambling, L., Danzeisen, R., Fosset, C., Andersen, H. S., Dunford, S., S.Srai, S. K. & Mcardle, H. J. 2003. Iron And Copper Interactions In Development And The Effect On Pregnancy Outcome. *J Nutr*, 133, 1554s-6s.

Hacker, A. N., Fung, E. B. & King, J. C. 2012. Role Of Calcium During Pregnancy: Maternal And Fetal Needs. *Nutr Rev*, 70, 397-409.

Haman, T., Mathee, A. & Swart, A. 2015. Low Levels Of Awareness Of Lead Hazards Among Pregnant Women In A High Risk-Johannesburg Neighbourhood. *Int J Environ Res Public Health*, 12, 15022-12027.

Hambidge, M. 2003. Biomarkers Of Trace Mineral Intake And Status. *J Nutr*, 133, 948s-955s.

Harma, M., Harma, M. & Kocyigit 2005. A Correlation Between Maternal Plasma Homocysteine And Zinc Levels In Pre-Eclamptic Women. *Biol Trace Elem Res*, 104, 97-106.

He, K. 2011. Trace Elements In Nails As Biomarkers In Clinical Research. *Eur J Clin Invest*, 41, 98-102.

Hladunewich, M., Karumanchi, S. A. & Lafayette, R. 2007. Pathophysiology Of The Clinical Manifestations Of Pre-Eclampsia. *Clin J Am Soc Nephrol*, 2, 543-549.

Huwait, E. A., Kumosani, T. A., Moselhy, S. S., Mosaoa, R. M. & S.Yaghmoor, S. 2015. Relationship Between Soil Cobalt And Vitamin B12 Levels In The Liver Of Livestock In Saudi Arabia: Role Of Competing Elements In Soils. *Afr Health Sci*, 15.

- Ikaraoha, I. C., Mbadiwe, N. C., Anetor, J. I. & Ojareva, I. A. 2016. Serum Trace Elements In Pre-Eclamptic Nigerians. *As J Med Sci*, 7, 78-83.
- Jain, S. 1986. Membrane Lipid Peroxidation In Erythrocytes Of The Newborn. *Clin Chem Acta*, 16, 301e6.
- Jeyabalan, A. 2013. Epidemiology Of Pre-Eclampsia: Impact Of Obesity *Nutr Rev*, 71, 1-14.
- Kandi, S., T, S., Ramadevi, C., Venugopal, B., Rajkumar, R., Rafi, M. & Ramana, K. V. 2014. Pre Eclampsia And Iron Status: A Review. *American Journal Of Medical And Biological Research*, 2, 121-123.
- Kennedy, D. A., Woodland, C. & Koren, G. 2012. Lead Exposure, Gestational Hypertension And Pre-Eclampsia: A Systematic Review Of Cause And Effect. *J Obstet Gynaecol*, 32, 512-517.
- Kippler, M., Tofail, F., Gardner, R., Rahman, A., Hamadani, J. D., Bottai, M. & Vahter, M. 2012. Maternal Cadmium Exposure During Pregnancy And Size At Birth: A Prospective Cohort Study. *Environ Hlth Persp*, 120, 284-289.
- Krajewski, P., Chudzik, A., Pokrzywnicka, M., Kalinka, J. & Kwiatkowska, M. 2009. Macro-, Micro- And Trace Elements Concentrations In Mother's And Newborn's Hair And Its Impact On Pregnancy Outcome: A Review. *Archives Of Perinatal Medicine*, 15, 67-71.
- Kuboyama, K., Sasaki, N., Nakagome, Y. & Kataoka, M. 2005. Wet Digestion. *Analytical Chemistry*, 360, 184-191.
- Kumru, S., Aydin, S., Simsek, M., Sahin, K. & Yaman, M. 2003. Comparison Of Serum Copper, Zinc, Calcium And Magnesium Levels In Pre-Eclapmtic And Healthy Pregnant Women. *Biol Trace Elem Res*, 94, 105-112.

- Madazli, R., Yuksel, M. A., Imamoglu, M., Tuten, A., Oncul, M., Aydin, B. & Demirayak, G. 2014. Comparison Of Clinical And Perinatal Outcomes In Early- And Late-Onset Preeclampsia. *Arch Gynecol Obstet*, 290, 53-57.
- Maggini S, Wintergerst ES, Beveridge S & Hornig DH. 2007. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *Br J Nutr*, 98, S29-S35.
- Nishijo, M., Nakagawa, H., Honda, R., Tanebe, K., Saito, S., Teranishi, H., Tawara, K. & Wong, O. 2002. Effects Of Maternal Exposure To Cadmium On Pregnancy Outcome And Breast Milk. *Occup Environ Med*, 59, 394-397.
- Ozden, T. A., Gokcay, G., Cantez, M. S., Durmaz, O., Issever, H., Omer, B. & Saner, G. 2015. Copper, Zinc And Iron Levels In Infants And Their Mothers During The First Year Of Life: A Prospective Study. *Bmc Pediatr*, 15, 1-11.
- Pathak, P. & Kapil, U. 2004. Role Of Trace Elements Zinc, Copper And Magnesium During Pregnancy And Its Outcome. *Ind J Pediatr*, 71, 1003-1005.
- Pieczynska, J. & Grajeta, H. 2015. The Role Of Selenium In Human Conception And Pregnancy. *J Trace Elem Med Biol*, 29, 31-38.
- Rasthore, S., Gupta, A., Batra, H. S. & Rathore, R. 2011. Comparative Study Of Trace Elements And Serum Ceruloplasmin Level In Normal And Preeclamptic Pregnancies With Their Cord Blood. *Biomedical Research*, 22, 209-212.
- Rayman, M. P., Searle, E., Kelly, L., Johnsen, S., Bodman-Smith, K., Bath, S. C., Mao, J. & Redman, C. W. G. 2014. Effect Of Selenium On Markers Of Risk Of Pre-Eclampsia In Uk Pregnant Women: A Randomised, Controlled Pilot Trial. *Br J Nutr*, 112, 99-111.
- Ritchie, L. D. & King, J. C. 2000. Dietary Calcium And Pregnancy-Induced Hypertension: Is There A Relation? *Am J Clin Nutr*, 71, 1371s-4s.
- Roberts, J. M., Balk, J. L., Bodnar, L. M., N, J. M. B., Bergel, E. & Martinez, A. 2003. Nutrient Involvement In Preeclampsia. *J Nutr*, 133, 1684s-1692s.

Sanchez-Aranguren LC, Prada CE, Riano-Medina CE & Lopez M. 2014. Endothelial dysfunction and and pre-eclampsia: role of oxidative stress. *Front Physio*, 5, 1-11.

Sandoval-Carrillo, A., Méndez-Hernández, E. M., Antuna-Salcido, E. I., Salas-Pacheco, S. M., Vázquez-Alaniz, F., Téllez-Valencia, A., Aguilar-Durán, M., Barraza-Salas, M., Castellanos-Juárez, F. X., La Llave-León, O. & Salas-Pacheco, J. M. 2016. Arsenic Exposure And Risk Of Preeclampsia In A Mexican Mestizo Population. *Bmc Pregnancy And Childbirth*, 16, 153.

Sanjay G & Girija W. 2014. Pre-eclampsia-Eclampsia. *J Obstet Gynaecol Ind*, 64(1), 4-13

Shamsi, U., Saleem, S. & Nishter, N. 2013. Epidemiology And Risk Factors Of Pre-Eclampsia; An Overview Of Observational Studies. *Al Ameen J Med Sci*, 6, 292-300.

Spencer, B. H., Vanderlelie, J. J. & Perkins, A. V. 2015. Essentiality Of Trace Element Micronutrients In Human Pregnancy : A Systematic Review. *J Preg Child Health*, 2, 1-7.

Takser, L., Lafond, J., Bouchard, M., St-Amour, G. & Mergler, D. 2004. Manganese Levels During Pregnancy And At Birth: Relation To Environmental Factors And Smoking In A Southwest Quebec Population. *Environ Res*, 95, 119-125.

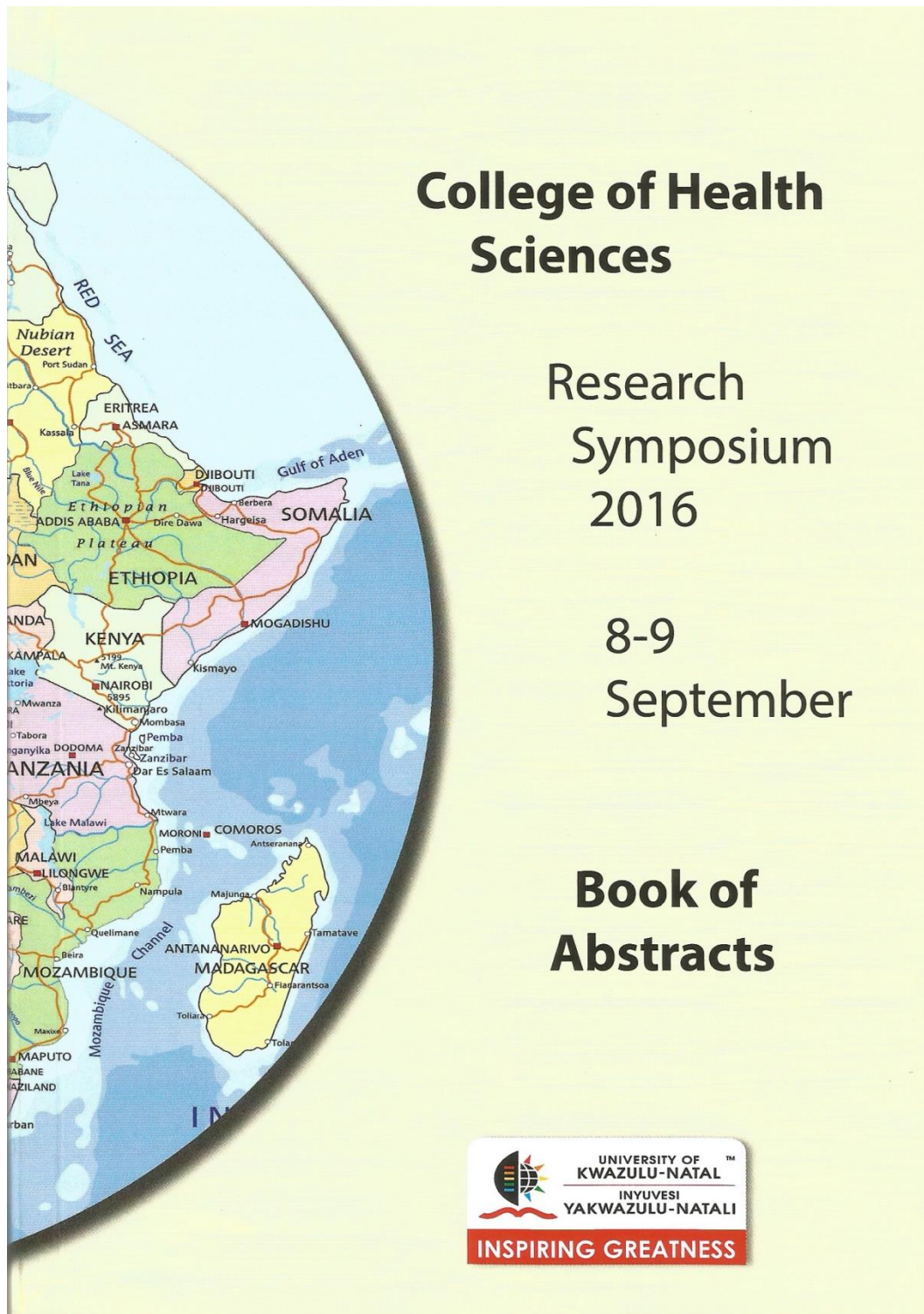
Tofail, F., Vahter, M., Hamadani, J. D., Nermell, B., Huda, S. N., Yunus, M., Rahman, M. & Grantham-Mcgregor, S. M. 2009. Effect Of Arsenic Exposure During Pregnancy On Infant Development At 7 Months In Rural Matlab, Bangladesh. *Environ Hlth Persp*, 117, 288-293.

Ugwuja, E. I., Ejikeme, B. N., Ugwu, N. C., Obeka, N. C., Akubugwo, E. I. & Obidoa, O. 2010. Comparison Of Plasma Copper, Iron And Zinc Levels In Hypertensive And Non-Hypertensive Pregnant Women In Abakaliki, South Eastern Nigeria. *Pak J Nutr*, 9, 1136-1140.

Uzan, J., Carbonnel, M., Piconne, O., Asmar, R. & Ayoubi, J.-M. 2011. Pre-Eclampsia: Pathophysiology, Diagnosis, And Management. *Vasc Hlth Risk Man*, 7, 467-474.

- Wang, A., Rana, S. & Karumanchi, S. A. 2009. Preeclampsia: The Role Of Angiogenic Factors In Its Pathogenesis. *Physiol*, 24, 147-153.
- Wang, F., Zhang, Q., Zhang, X., Luo, S., Ye, D., Guo, Y., Chen, S. & Huang, Y. 2014a. Preeclampsia Induced By Cadmium In Rats Is Related To Abnormal Local Glucocorticoid Synthesis In Placenta. *Reprod Biol Endocrinol*, 12, 1-9.
- Wang, F., Zhang, Q., Zhang, X., Luo, S., Ye, D., Guo, Y., Chen, S. & Huang, Y. 2014b. Preeclampsia Induced By Cadmium In Rats Is Related To Abnormal Local Glucocorticoid Synthesis In Placenta. *Reproductive Biology And Endocrinology*, 12, 1-9.
- Wood, R. J. 2009. Manganese And Birth Outcome. *Nutri Rev*, 67, 416-420.
- Woods, S. E., Ghodsi, V., Engel, A., Miller, J. & James, S. 2008. Serum Chromium And Gestational Diabetes. *Jabfm*, 21, 153-157.
- Young, B. C., Levine, R. S. & Karumanchi, S. A. 2010. Pathogenesis Of Preeclampsia. *Ann Rev Pathol*, 5, 173-192.

APPENDIX



8h00-8h30	: Registration
8h30-10h00	: SESSION 1
8h30-9h00	: Welcome and Introduction of Sponsors Professor Moses Chimbari
9h00-10h00	: KEY-NOTE ADDRESS
Aids	'90-90-90- Learnings From The 21st International Conference' Professor Salim Abdool Karim
10h00-10h30	: TEA
10h30-12h30	: SESSION 2
12h30-13h30	: LUNCH
13h30-15h30	: SESSION 3

FRIDAY, 9TH SEPTEMBER

8h00-10h00	: SESSION 4
10h00-10h30	: TEA
10h30-12h30	: SESSION 5
12h30-13h30	: LUNCH
13h30-15h30	: SESSION 6
13h30- 14h00	: Large Grants recipients
14h00-15h00	: Fractional Professors presentation: Professor Per Arvidsson, Professor Vivienne Russell, Professor Bob Hickner, Professor Hans Peter-Lipp and Professor Taka Mduluza.
15h00-15h30	: PRIZE GIVING and LUCKY DRAWS
15h30	: Vote of Thanks and Closure

SESSION TWO

TRACK ONE : K1

10h30	: Ajonijebu Duyilemi Chris	17
10h45	: Ochieng Oluoch Alfred	92
11h00	: Wellman Amanda	130
11h15	: Madsen Steiner Andre	61
11h30	: Madsen Steiner Andre	74
11h45	: Ngonyoka Anibariki	86
12h00	: Kalicharan Arishka	54
12h15	: Bagwandeem Chauntelle	23

TRACK TWO : K2

10h30	: Roelofse Bianca	103
10h45	: De Gama Zola Brenda	32
11h00	: Soobramoney Cassandra	114
11h15	: Chandrasekaran, B	25
11h30	: Alphonsus Christella Sinthuja	18
11h45	: Amoako Daniel Gyamfi	20
12h00	: Muema Daniel Muli	71
12h15	: Skinner David Lee	111

TRACK THREE: SUSSER AND STEIN

10h30	: Abdulsalam, Y.	16
10h35	: Madsen Steiner Andre	62
10h40	: Bagwandeem Chauntelle	22
10h45	: Nizami Bilal	87
10h50	: Omolo Calvin Andeve	93
10h55	: Naidoo Dhaneshree Bestinee	85
11h00	: Ojwach Doty Brenda Achieng	98
11h05	: Gbalegba N'Guessan Guy Constant	37
11h10	: Hampannavar Girish Appasaheb	46
11h15	: Mavondo Greanious Alfred	67
11h20	: Harerimana Alexis	44
11h25	: January James	50
11h30	: Govender Katya	40
11h35	: Swe-Swe Hans Khine	117
11h40	: Coutts Kim	29
11h45	: Koffi Amoin Jeanne d'Arc	58

COMPARISON OF ELEMENTAL ANALYSIS OF NAILS FROM NORMOTENSIVE AND PRE-ECLAMPTIC BLACK SOUTH AFRICAN WOMEN

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**Optics and Imaging Centre, School of Laboratory Medicine and Medical Science,
College of Health Sciences, University of Kwa-Zulu Natal*

Women Health and HIV Research Unit

*^School of Chemistry and Physics, College of Agriculture, Engineering and Science,
University of Kwa-Zulu Natal*

Introduction

Trace element deficiency is a causative factor in a number of pregnancy related conditions such as pre-eclampsia. Its greatest impact is in developing countries, where the dietary intake of essential minerals and vitamins are low. However, the effect of these trace elements on the development of pre-eclampsia has not been properly established

Aim

The aim of this study was to compare the concentrations of thirteen different trace elements in nail samples from pre-eclamptic and normotensive women

Method

Nail samples were collected from normotensive (n=30) and pre-eclamptic (n=30) pregnant women. Nail samples were weighed and processed using wet acid digestion method. Samples were analyzed for Zinc (Zn), Selenium (Se), Lead (Pb), Nickel(Ni), Magnesium (Mg), Manganese (Mn), Iron (Fe), Copper (Cu), Calcium (Ca), Cobalt (Co), Cadmium (Cd), Chromium (Cr) and Arsenic (As) using the inductively coupled plasma-atomic emission spectrometry

Results

The concentrations of Ca and Mg in the normotensive group were the highest whilst Co and Cd were the lowest. In the pre-eclamptic group, the concentration of Ca and Mg were the highest and As and Co was the lowest. Overall, there was a significant difference between the normotensive and pre-eclamptic group for Cd ($p=0.004$), Co ($p=0.000$) and Mn ($p=0.022$), where by these elements were down-regulated in pre-eclamptic patients

Conclusion

There was a significant decrease in the concentration of Co, Cd and Mn in pre-eclamptic women. This may implicate that an adequate intake of micronutrients is vital for proper development of the fetus and overall health of the mother.

Addendum 2: Biomedical Research Ethics Committee Approval



TM

20 April 2016

Ms C Soobramoney
Discipline of Optics and Imaging
School of Laboratory Science and Medicine Science

Dear Ms Soobramoney

Protocol: Trace element analyses of biological samples from normotensive and pre-eclamptic pregnant women

Degree: MMedSc

BREC reference number: BE092/16

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 29 February 2016.

The study was provisionally approved pending appropriate responses to queries raised. Your response dated 11 April 2016 to queries raised on 09 March 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval**.

This approval is valid for one year from **20 April 2016**. 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 (2015), 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

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 meeting taking place on **10 May 2016**.

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

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor:
Postgrad: dudhraiho@ukzn.ac.za

Biomedical Research Ethics Committee

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