The risk and severity of preeclampsia secondary to iodine deficiency and iodine deficiency-mediated subclinical hypothyroidism: mechanisms and early cardiovascular consequences

Bitamazire Charles Businge

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy (Medicine)

At the

Department of Medicine, Faculty of Helth Sciences, University of Cape Town

February 2022

Supervisor: Professor Andre Pascal Kengne

Abstract

Background

Iodine deficiency affects about 2 billion people globally and is the leading cause of Subclinical (SCH) and overt (OH) hypothyroidism that are some of the risk factors of preeclampsia. Iodine deficiency in pregnancy, which can be corrected by supplementation, has been suggested in the past three decades as a risk factor for preeclampsia. It is uncertain if this is mainly because of elevated thyroid-stimulating hormone (TSH) in women with iodine deficiency complicated by hypothyroidism. Alternatively, it may be because of reduced serum antioxidant capacity and other pathways that are associated with the more prevalent inadequate iodine intake in pregnancy, or both.

Aims

The current thesis had three major objectives: 1) To determine the burden of iodine deficiency in pregnancy in Africa; 2) To find out if there is an association between iodine deficiency in pregnancy, subclinical hypothyroidism, endothelial dysfunction and preeclampsia; 3) To find out possible mechanisms through which iodine deficiency in pregnancy may predispose to preeclampsia, eclampsia, endothelial dysfunction and early cardiovascular pathological changes.

Methods

In the first part of this thesis, two systematic reviews are presented. The first was carried out in order to estimate the burden of iodine deficiency in pregnancy in Africa. This was complemented with a regression analysis using the more readily available school-age children (SAC) median urinary iodine concentration (UIC) and the limited pregnancy median UIC data to estimate the level of iodine nutrition in pregnancy in all the African countries. The second systematic review was carried out to find out the relationship between inadequate iodine intake in pregnancy and preeclampsia.

In the second part of this thesis, we present results of case-control studies in which women with preeclampsia, severe preeclampsia, eclampsia and normotensive pregnant controls were enrolled at Nelson Mandela Academic Hospital (NMAH) and Mthatha Regional Hospital (MRH) in Eastern Cape South Africa. Their iodine nutrition status, thyroid function, endothelial dysfunction and arterial stiffness were compared by assaying the urinary iodine concentration (UIC), thyroglobulin (Tg), thyroid-stimulating hormone (TSH), triiodothyronine

(T3), thyroxine (T4), nitric oxide (NO), oxidised low-density lipoprotein (oxLDL), pulse wave velocity (PWV) and aortic augmentation index (AI).

In the third part of the thesis, two studies aimed at exploring mechanisms through which iodine and other micronutrient deficiencies together with environmental factors predispose to preeclampsia and eclampsia are presented. The first is a study using Factor Analysis to find out how various nutritional and inflammatory markers interact in different pathways to predispose to preeclampsia using data collected at Lomo Medical Centre, Kinshasa Province, Democratic Republic of Congo. The second is a case-control study in which we compared the thyroid function status, urinary iodine and serum potassium of women with eclampsia, severe preeclampsia and normotensive pregnant controls to find out if iodine deficiency, the resultant thyroid dysfunction and serum potassium levels were associated with the risk of eclampsia.

Results

Prevalence of iodine deficiency in pregnancy in Africa: results from systematic reviews and meta-analyses and pregnancy median UIC estimated from SAC median UIC

Pregnancy iodine nutrition status data was available for 23/54 African countries. Between 2005 and 2020 a few African countries had sufficient (pregnancy median urinary iodine concentration [pMUIC] 150 - 250 μg/L), most had mildly inadequate (pMUIC 100 - 149 μg/L), and some moderate-to-severe inadequate iodine nutrition in pregnancy (pMUIC <50 - 99 μg/L). The pooled pregnancy median UIC was 145 μg/L (95% CI 126–172). Using the more available SAC median UIC, pregnancy median UIC was derived for 49/54 African countries, 22 (45%) of these which had insufficient iodine intake during pregnancy (i.e., <150 μg/L).

In the second systematic review and meta-analysis the pooled mean UIC from 3 eligible cross-sectional studies of 210 normotensive pregnant women was significantly higher than that of 254 preeclamptic women. The mean UIC difference was 164.4 µg/L (95% CI 45.1–283.6, $p < 0.01$, $I^2 > 50$). There was a non-significant risk of preeclampsia for women with UIC <150 µg/L in two eligible cohort studies, with a pooled risk ratio of 2.85 (95% CI 0.42–20.05, $p = 0.09$, $I^2 < 25$).

Iodine nutrition status and preeclampsia

Eastern Cape women with severe preeclampsia/eclampsia had significantly lower UIC (a measure of recent iodine intake) and significantly higher serum thyroglobulin (a measure of long-term iodine intake) than both (uncomplicated) preeclampsia and normotensive-pregnant participants. The median values for severe preeclampsia/eclampsia, preeclampsia and normotensive pregnant participants were UIC (µg/L) 98.8, 127.7, and 217.1 $p=0.005$; Thyroglobulin (µg/L) 32.9, 21.4, 19.4 $p=0.001$. Pregnant women with hypertensive disease had less than adequate iodine intake in pregnancy (median UIC ≤ 150 µg/L) and higher than normal serum thyroglobulin (≥ 20 µg/L), the values were worse for women with severe preeclampsia/eclampsia compared to women with uncomplicated preeclampsia.

Measures of endothelial dysfunction

The median Aortic augmentation index was 7.5, 19.0 and 21.0 ($p < 0.001$) and the Pulse Wave Velocity 5.1, 5.7 and 6.3 m/s respectively for Eastern Cape normotensive, preeclampsia and severe preeclampsia women ($p<0.001$ for both). Women with severe preeclampsia had significantly higher median TSH than normotensive and preeclamptic women without severe features (3.0, 2.3 and 2.3 IU/L, respectively). A similar trend was observed for oxidised LDL (1.2, 1.0 and 1.0 IU/L for normotensive, preeclampsia and severe preeclampsia participants, $p < 0.05$). In linear regressions, TSH, age and hypertensive disease were independent predictors of elevated PWV which is associated with endothelial dysfunction and future cardiovascular disease.

Possible mechanisms in the pathophysiology of preeclampsia associated with iodine deficiency, resultant thyroid dysfunction and other nutritional or inflammatory factors

Using data of normotensive and preeclamptic women from Kinshasa Province, Democratic Republic of Congo, we have characterised four main pathophysiological pathways through which low iodine intake in pregnancy may predispose to preeclampsia. These are the interactions between selenium/iodine deficiency and elevated serum TSH, leading to endothelial dysfunction; serum ferritin, gamma-glutamyl transferase (GGT), C-reactive protein (CRP) and low urinary iodine excretion precipitating inflammatory oxidative stress. The others are elevated serum high sense-CRP (hs-CRP) and Rheumatoid factor subclinical inflammation and immune cell activation and high T3/T4 ratio acute TSH stimulation of thyroid with low thyroid iodine stores that may lead to excessive superoxide and hydroxyl production further exacerbating oxidative stress and endothelial dysfunction.

Iodine deficiency, thyroid dysfunction in the pathophysiology of eclamptic fits

Eclamptic women in the Eastern Cape (South Africa) had significantly lower urinary iodine concentration (UIC), free triiodothyronine (FT3), median serum potassium (K), but higher serum thyroglobulin (Tg) than women with severe preeclampsia and normotensive pregnant controls. The median values respectively for participants with eclampsia, severe preeclampsia and normotensive controls were UIC 69.5, 95.7, and 169.5 µg/L; FT3 3.8, 4.4 and 4.7 pmol/L; K 3.7, 4.2, 4.3 mmol/L; and Tg 39.0, 22.4, 19.5 µg/L. Low serum T3 and T4 levels coupled with a preferential transfer of T4 across the blood-brain barrier alter the physiological T3/T4 ratio in the CNS attenuating the inhibitory effects of GABA while the excitatory function of glutamate remains intact. Low serum potassium further attenuates GABA_B receptor mediated tonic extra-synaptic inhibition resulting in net motor neurone stimulation and increased predisposition to the involuntary tonic-clonic convulsions observed in eclampsia.

Conclusion

There is still a high prevalence of inadequate iodine intake in pregnancy in Africa estimated to affect 45% of the African nations 25 years after commencement of iodine fortification of salt and other foodstuffs. While the complication of goitre and cretinism may have reduced, pregnant women in Africa may still be prone to high risk of preeclampsia, eclampsia, future cardiovascular disease and various degrees of reduced psychomotor development of the foetus depending on the severity and duration of insufficient iodine intake in pregnancy. Alternative measures need to be considered to mitigate the persistently high prevalence of iodine deficiency in pregnancy in Africa and other areas around the globe despite national salt iodization programs and other iodine fortification efforts that have been implemented for several decades. This will help reduce the risk of preeclampsia, eclampsia and related complications.

Keywords

Africa

C-Reactive Protein

Eclampsia

Endothelial dysfunction

Gamma glutamyl transferase

Inflammation

Insufficient iodine intake

Iodine

Iodine deficiency

Low serum potassium

Low serum triiodothyronine

Meta-analysis

Oxidative stress

Preeclampsia

Pregnancy

Pulse Wave Velocity

Selenium

Systematic Review

Thyroid-stimulating hormone

Urinary iodine concentration

DEDICATION

This thesis is dedicated to all the participants who took part in various studies that form part of this thesis.

DECLARATION

I, BITAMAZIRE CHARLES BUSINGE, declare that "The risk and severity of preeclampsia secondary to Iodine deficiency and iodine deficiency-mediated subclinical hypothyroidism: mechanisms and early cardiovascular consequences" is my own work, and that all the sources I have used or cited have been indicated and acknowledged as complete references. The thesis is submitted to the University of Cape Town in fulfilment of the requirement for the degree of Doctor of Philosophy (PhD) in Medicine, through Faculty of Health Sciences. This work has not been submitted for any other degree in this or another university.

Signed Date:

Declaration on Inclusion of Publications in the PhD Thesis

"I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication (s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication (s)":

Businge, C.B., Madini, N., Longo-Mbenza, B and Kengne, A.P. (2019). Insufficient iodine nutrition status and the risk of pre-eclampsia: a protocol for systematic review and meta-analysis. BMJ Open 9(5). http://dx.doi.org/10.1136/bmjopen-2018-025573.

Businge, C.B., Longo-Mbenza, B and Kengne, A.P. (2019). The prevalence of insufficient iodine intake in pregnancy in Africa: protocol for a systematic review and meta-analysis. *Systematic Reviews*, 8:209. https://doi.org/10.1186/s13643-019-1092-7.

Businge CB, Longo-Mbenza B, Kengne AP. Iodine nutrition status in Africa: potentially high prevalence of iodine deficiency in pregnancy even in countries classified as iodine sufficient. Public Health Nutr. 2020 Aug 3:1-6. doi: 10.1017/S1368980020002384.

Businge CB, Usenbo A, Longo-Mbenza B, Kengne AP. Insufficient iodine nutrition status and the risk of pre-eclampsia: a systemic review and meta-analysis. BMJ Open. 2021 Feb 10;11(2):e043505. doi: 10.1136/bmjopen-2020-043505.

Businge CB, Longo-Mbenza B, Kengne AP. Iodine deficiency in pregnancy along a concentration gradient is associated with increased severity of preeclampsia in rural Eastern Cape, South Africa. BMC Pregnancy Childbirth. 2022;22(1):98. doi: 10.1186/s12884-021-04356-6.

Businge CB, Longo-Mbenza B, Kengne AP. Mildly elevated thyroid-stimulating hormone is associated with endothelial dysfunction and severe preeclampsia among pregnant women with insufficient iodine intake in Eastern Cape province, South Africa. Ann Med. 2021;53(1):1083-1089.

Businge CB, Longo-Mbenza B, Kengne AP. Exploration of the Underlying Nutritional, Inflammatory and Oxidative Stress Pathological Mechanisms in Preeclampsia Using Principal Component Analysis. EJMED. 2021; 3: 19-24.

Businge CB, Longo-Mbenza B, Kengne AP. Low serum triiodothyronine and potassium levels are associated with increased risk of eclampsia among women in the Eastern Cape Province of South Africa. Clin Exp Obstet Gynecol. 2022; 49(2): 032 http://doi.org/10.31083/j.ceog4902032.

Signature: Signed by candidate Date: 8th Februaary 2022

Student Name: Bitamazire Charles Businge **Sudent Number**: BSNBIT001/ 1655198

ACKNOWLEDGEMENTS

I am very pleased to register my gratitude without reservation to Prof Andre Pascal Kengne who not only accepted to commit his time to supervise my PhD studies and thesis, but has without hesitation provided a thorough critique of my work at various stages, advice and mentorship as well as for his patience and encouragement.

My deep gratitude too to Prof Longo Mbenza for helping to acquire data on iodine and other micronutrient nutrition in pregnancy from Kinshasa province, Democratic Republic of Congo and for his encouragement and critical review of the manuscripts.

My profound gratitude to the Discovery Foundation (South Africa) for the Rural Fellowship grant number 038372 that supported the laboratory tests for the various studies in this thesis.

I am grateful to Walter Sisulu University, my employer who provided part of my school dues.

I appreciate the support the encouragement of Prof ML Mdaka the current head and Prof GAB Buga the emeritus head of Obstetrics and Gynaecology at Walter Sisulu University and all my colleagues in the Department and University for their encouragement and support during my period of study.

I would also like to thank the Dr Anthony Usembo and Dr Hannibal T Musarurwa, each for contributing to and co-authoring one the systematic reviews and Ms Namhla Madini for her contribution and co-authoring one systematic review protocol.

My thanks too to Dr Ambrose Namugowa providing the SphygmaCor Machine for obtaining the measures of endothelial dysfunction. My gratitude too to Prof Constance Rusike for conducting the oxidative stress analyses.

With joy I would like to appreciate the unwavering support of my wife Mary Juliet, and the support and example from my children Esther and Gabriel, whose prayers and determination at their own studies were a regular source of motivation for me.

I wish to give glory to God who not only enabled me to study while attending very busy work schedules at my work station in addition to challenges paused by the covid-19 pandemic, but also who helped me find the most appropriate supervisor and all the people and organisations whose support has enabled me to complete my studies. To Him be the glory now and forever more, Amen.

Abbreviations

ALT	Alanine Transaminase
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AST	Aspartate Transaminase
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CRP	C-Reactive Protein
DBP	Diastolic Blood Pressure
ECSECC	Eastern Cape Socio Economic Consultative Council
FPG	Fasting Plasma Glucose
FT3	Free Serum Triiodothyronine
FT4	Free Serum Thyroxine
GA	Gestational Age
GABA	Gamma Aminobutyric Acid
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IGN	Iodine Global Network
K+	Serum Potassium
LDL	Low Density Lipoprotein
mUIC	Median Urinary Iodine Concentration
NMDA	N-methyl-D-aspartate
OR	Odds Ratio
OxLDL	Oxidised Low-density Lipoprotein
PICO	Population, Intervention/exposure, Comparison and Outcome
pMUIC	Pregnancy Median Urinary Iodine concentration

PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analysis
PRISMA-P	Preferred Reporting Items for Systematic reviews and Meta-Analysis protocols
PROSPERO	International Prospective Register of Systematic Reviews
PWV	Pulse Wave Velocity
RF	Rheumatoid Factor
SAC	School Age Children
SBP	Systolic Blood Pressure
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
T3	Triiodothyronine
T4	Thyroxine
Tg	Thyroglobulin
TSH	Thyroid-Stimulating Hormone
UIC	Urinary Iodine Concentration
UNICEF	United Nations Children’s Education Fund
USI	Universal Salt Iodization
WHO	World Health Organisation
WOA	Weeks of Amenorrhoea

Table of contents

LIST OF TABLES

LIST OF FIGURES

THE CANDIDATE’S CONTRIBUTION

The candidate conceived the research idea, wrote the proposal for the research project, collected biological specimens as well as clinical and demographic data with the help of a research assistant. He then wrote and published protocols for the two systematic reviews with guidance of the supervisor. The candidate then participated in searching and screening articles for inclusion in the systematic reviews, as well as data extraction. He then carried out data analysis for all the manuscripts, wrote the first drafts and revisions and is also lead and corresponding author of all the manuscripts included in the various chapters of this thesis.

Chapter 1

General introduction

1.1 Background

Preeclampsia complicates 5 to 10% of all pregnancies and is a known cause of severe maternal and perinatal morbidity and mortality (1). Subclinical hypothyroidism (SCH), one of the established risk factors of preeclampsia, is a medical condition characterised by serum thyroid-stimulating hormone (TSH) levels higher than the upper limit despite normal thyroxine levels (2). The upper limit of normal serum TSH is 2.5 mIU/L in the first trimester and 3.0 mIU/L in the second and third trimesters (2). Globally, the prevalence of SCH in pregnancy varies between 2.5% and 17% according to geographical location and ethnicity (3-6). Most of the affected women do not present with characteristic symptoms of hypothyroidism. However, SCH in pregnancy is associated with many other adverse maternal and offspring complications apart from preeclampsia, such as abruptio placenta, preterm delivery, neuro-cognitive deficiency and delayed psychomotor maturation (7-10).

Worldwide the most common cause of SCH is iodine deficiency, particularly in the highland areas of central Africa, Asia and South America (11-13). In iodine replete areas, autoimmune thyroiditis, a condition associated with reduced thyroid gland parenchyma, is the most common cause of SCH (11, 12). The World Health Organisation (WHO) estimates that about 2 billion people live in areas with iodine insufficiency (14). Even in first world countries, which are considered as iodine replete, mild-to-moderate iodine deficiency is the most widespread cause of maternal hypothyroxinaemia, a herald of hypothyroidism (15). Hence, iodine deficiency is much more prevalent than SCH. Over the past three decades, a few studies reported an association between iodine deficiency and preeclampsia (16, 17). Some have reported that iodine deficiency reduces serum antioxidant capacity, hence precipitating endothelial dysfunction (17). Potentially, this makes iodine deficiency in pregnancy a more prevalent risk factor for preeclampsia than SCH worth studying.

Iodine deficiency, a major public health problem throughout Africa, is the most common cause of thyroid disorders on the continent (18). Iodine deficiency disorders are prevalent in both mountainous and non-mountainous Africa countries (19). Inadequate supply of iodine from the diet is thought to be the major contributory factor to iodine deficiency related diseases in Africa. However, there is an established association between iodine deficiency and goitrogens, the most significant being poorly detoxified cassava, which is rich in thiocyanate (20).

Before the implementation of universal salt iodization in South Africa in 1995, endemic goitre was common. Hence, the population was predisposed to detrimental effects of iodine deficiency and hypothyroidism in pregnancy (13, 21). A nutrition survey conducted in 1998 revealed adequate iodine nutrition in seven of the nine provinces of South Africa (with median urinary iodine concentration >100μ/L) (22). The other two provinces (Limpopo and Mpumalanga) had more than adequate iodine nutrition. However, national or provincial statistics can mask local and at-risk group-specific health situations (23, 24). For instance, more than 86% of the households in South Africa used iodised salt but only 62.4% used adequately iodized salt with less than 50% of the households in Limpopo, Mpumalanga, and Northwest using adequately iodised salt (22). Between 8% and 37% of households in South Africa at the time used non-iodised salt that was procured from distributors of agricultural salt and small retail shops (25). These were mainly households with poor socioeconomic status. Although South Africa was on the verge of eliminating iodine deficiency by 2007, over 30% of the population still had no access to adequately iodised salt (25).

The increased demand of iodine might offset the corrective effect of iodine supplementation on the effects of endemic iodine deficiency among women in reproductive age and nutrition transition, especially among the increasing population of rural to urban migrants (26). Rural to urban migration, commonly accompanied by adoption of western diets and increased dependence on processed foods and restaurants, is associated with inconsistent utilisation of iodized salt (24, 26). Only 25% of the processed foodstuffs in South Africa used iodized salt according to the results of one study (27). In addition, the marketing of foodstuffs through multinational food stores that penetrate into local communities has the potential for nationwide distribution of foodstuffs with high concentrations of nitrates and perchlorate from abroad or from commercial agricultural farms within South Africa (28).

The prevalence of overt iodine deficiency disorders in South Africa, such as endemic goitre have remarkably decreased (25). However, there is a possible high prevalence of thyroxinaemia and subclinical hypothyroidism from iodine deficiency among population groups in South Africa at risk of inadequate iodine intake such as pregnant women, breastfeeding babies, rural-to-urban migrants, rural inhabitants who cannot access iodized salt.

Although some studies have reported an association between iodine deficiency and hypertensive disease in pregnancy (16, 17), there is a paucity of data on the relationship between iodine deficiency and or subclinical hypothyroidism and hypertensive disease in pregnancy in Africa and around the world. It is not yet clear if iodine deficiency is an independent risk factor of preeclampsia. Neither are the mechanisms through which iodine deficiency may increase the risk of preeclampsia certain.

The increasing prevalence of iodine deficiency in areas originally thought to be iodine sufficient has been attributed to inadequate fortification of foodstuffs with iodine in the background of high levels of perchlorate and thiocyanate in water sources and the diet (29-31). Both perchlorate and thiocyanate significantly diminish the uptake of iodine by the thyroid gland especially in states of increased thyroid stimulation by TSH secondary to iodine deficiency (30, 31).

Iodine is one of the most potent exogenous antioxidants whose deficiency is associated with oxidant imbalance and endothelial dysfunction (32), hence may increase the risk of preeclampsia even without concurrent TSH elevation or subclinical or overt hypothyroidism. Prolonged elevation of serum TSH in severe iodine deficiency, SCH or OH leads to thyroid hyper-stimulation with increased output of reactive oxygen radicals (superoxide and hydrogen peroxide) especially in the presence of selenium and haeme deficiency leading to further oxidant stress (33). Furthermore, elevated serum TSH diminishes the production of nitric oxide (NO) from the endothelium by acting through its extra-thyroidal receptors (34). These processes taken together not only predispose to endothelial dysfunction and hypertension, but might lead to placenta ischemia and atherosis; and could potentially interfere with endometrial and myometrial spiral artery trophoblastic invasion, all of which are thought to herald the onset of preeclampsia (35, 36). This could also account for the early pregnancy complications associated with SCH and OH. The placenta is one organ that physiologically has high iodine storage (37-40). Therefore, iodine deficiency may interfere with normal placental development and function.

Iodine also has been shown to alleviate cytotoxic effects associated with Helicobacter pylori infection, which is a known risk factor of endothelial dysfunction and increased carotid media-intima thickness, cardiovascular disease and preeclampsia among women in reproductive age group (41-44).

The thyroid responds to iodine deficiency by preferential production of T3 instead of T4 (45). The brain derives most of its T3 by deiodination of T4 that is transported to the brain by transthyretin (15, 45). Hence, serum T4 deficiency reduces the availability of T3 to the brain, which can result in various clinical and subclinical manifestations in both pregnant women and their progeny. T4 not only regulates metabolism in the brain by acting on its nuclear receptors, but is also a required substrate of deiodinase type 2 for the production of cytosolic T3, which modulates synaptic neurotransmission (15, 45). Therefore, SCH secondary to iodine deficiency may not only increase the risk of preeclampsia but also that of eclampsia.

Pregnancy is a state of increased iodine demand due to the physiological increase in thyroid hormone output. This is because of the oestrogen mediated increase in thyroid binding globulin that progressively diminishes the free T4 in the serum; transfer of iodine to the foetus; and increased renal iodine clearance (46). Therefore, women with mild-to-moderate iodine deficiency may develop severe iodine deficiency in pregnancy with resultant SCH, overt hypothyroidism (OH) or isolated T4 deficiency with resultant maternal and offspring short term and long-term complications (46-48).

Only about 30% of SCH patients are symptomatic and given the non-specific nature of the symptoms, most patients with SCH are never diagnosed (49). Diagnosis and management of SCH is potentially of value in the preconception period or early in the first trimester when probable intervention would be of benefit (2, 50, 51).

Although several observational studies have reported that SCH is associated with adverse maternal and foetal outcomes (11), universal screening and treatment of SCH pregnancy with thyroxine has not yet been adopted due to lack of enough evidence from randomised control trials. Vissenberg et al. (52) in a meta-analysis found no enough evidence for the benefit of L-T4 supplementation in pregnancy for treatment of women with SCH. Iodine deficiency was not investigated as the probable cause of SCH, hence supplementation with iodine in addition to L-T4 was not considered.

1.2 Statement of the problem

Preeclampsia is one of the leading causes of maternal and perinatal morbidity and mortality globally as well as in South Africa (53, 54). SCH hypothyroidism in pregnancy, one of risk factors of preeclampsia is likely prevalent in geographical settings with endemic iodine deficiency and among population groups with low dietary iodine intake (48). The physiological changes of pregnancy exacerbate the degree of iodine deficiency in pregnancy (46). This might increase the risk of preeclampsia through defective trophoblastic spiral artery invasion, placenta ischaemia, oxidative imbalance and endothelial dysfunction due to elevated TSH, iodine deficiency and increased release of vaso-active factors from the ischaemic placenta (32). Since iodine deficiency is more prevalent than subclinical or overt hypothyroidism and predisposes to serum antioxidant deficit and endothelial dysfunction, it may be a more common independent risk factor for preeclampsia than overt or SCH. There is paucity of data on the relationship between iodine deficiency, SCH and preeclampsia in South Africa, a country formerly with endemic goitre and potential high prevalence of iodine deficiency in pregnancy.

1.3 Aims

The current thesis had three principal aims: 1) To determine the burden of iodine deficiency in pregnancy in Africa; 2) To find out if there is an association between iodine deficiency in pregnancy, subclinical hypothyroidism, endothelial dysfunction and preeclampsia; 3) To investigate the possible mechanisms through which iodine deficiency in pregnancy may predispose to preeclampsia, eclampsia, endothelial dysfunction and early cardiovascular pathological changes.

1.3.1 Specific objectives

Part 1: Assessment of the trends in prevalence of iodine deficiency in pregnancy in Africa since implementing national iodine fortification programs in 1995

- To assess the trend and current state of iodine deficiency in pregnancy in Africa since 1995 through a systematic literature review and meta-analysis.
- To determine the level of insufficient iodine intake in pregnancy in all African countries using pregnancy median urinary iodine concentration (pMUIC) estimated from the more available school-age children (SAC) mUIC.

Part 2: Assessment of the relationship between iodine nutrition state in pregnancy, thyroid dysfunction and preeclampsia

- To carry out a systematic review and meta-analysis of observational studies on the association between iodine nutrition status in pregnancy and preeclampsia.
- To ascertain the relationship between iodine nutrition status in pregnancy and preeclampsia among women receiving care at the maternity units of Nelson Mandela Academic Hospital (NMAH) and Mthatha Regional Hospital (MRH) in Eastern Cape South Africa.
- To correlate the degree of iodine deficiency and SCH with the severity of endothelial dysfunction and preeclampsia among women receiving care at the maternity units of NMAH and MRH.

Part 3: Investigating possible mechanisms through which iodine deficiency predisposes to various degrees of preeclampsia and eclampsia

- To investigate the mechanisms through which iodine deficiency with other nutritional and environmental factors may increase the risk of preeclampsia using previously collected data from Democratic Republic of Congo (DRC) and newly collected data from Eastern Cape, South Africa.

1.4. Justification

Before 1995, iodine deficiency was endemic throughout Africa and as the commonest cause of thyroid disorders on the continent, necessitated iodine fortification at national level (13, 18, 55). The main underlying factors for iodine deficiency in Africa, iodine deficient soils and staple diets rich in goitrogens, have not changed and may still predispose large populations to iodine deficiency disorders especially in settings and populations groups with limited access to iodine fortified foodstuffs (20, 56).

South Africa is one of the sub-Saharan countries with iodine deficient soils (25). Although salt iodization has been in practice since 1995, about 30% of the population are not yet adequately reached (25). In addition, rural communities and subpopulations that depend on processed foods such as urban migrants are likely to be exposed to diets deficient in iodized salt (27, 28). Environmental factors such as high levels of nitrates in the soil or perchlorate in water sources and petroleum pollutants and thiocyanate in cigarette smoke may further reduce the bioavailability of iodine from the diet (30, 31). Hence, there is likely a significant proportion of expectant mothers with iodine deficiency and SCH secondary to iodine deficiency. SCH hypothyroidism is asymptomatic in about 70% of cases and when symptoms are present, they mimic pregnancy symptoms (49). Hence SCH not only goes unrecognised in the general population but is likely to remain unrecognised and untreated, yet it is associated with short term and long-term complications to mothers and their progeny, especially in populations at risk of iodine deficiency and exposure to inhibitors of iodide uptake by the thyroid gland.

My preliminary research from the Democratic Republic of Congo (DRC), a country with soils deficient in iodine and selenium, showed that iodine deficiency was not only associated with preeclampsia but also with the severity of preeclampsia (57). The data also showed that urinary iodine excretion and serum TSH were associated with the incidence and severity of preeclampsia. If these findings can be corroborated in the South African study population, screening all pregnant women for iodine deficiency using urine dipstick followed by iodine supplementation may be one of the cost-effective measures to reduce the incidence, maternal and foetal mortality and morbidity, and risk of future cardiovascular disease and metabolic syndrome associated with preeclampsia.

1.5 Hypotheses

- Urinary iodine excretion (UIC) will vary inversely while serum thyroglobulin and TSH will vary directly with the severity of preeclampsia
- The degree of endothelial dysfunction will be higher and serum nitric oxide lower among women with preeclampsia

1.6 Conceptual framework for the thesis

The conceptual framework for this research project (Figure1.1) was synthesized from literature on the impact of iodine deficiency on pregnancy. Iodine deficiency predisposes to defective trophoblast proliferation and migration which are some mechanisms proposed in the aetiology of placental type-preeclampsia (32, 58). The placenta is a high-metabolic organ with potential for production of reactive oxygen species. It is also one of the organs with high physiological concentrations of iodine whose antioxidant effect reduces lipid peroxide formation and may ensure normal placentation and function (39, 40, 59).

Placental and foetal hypoxia following defective placentation in preeclampsia results in increased production of reactive oxygen species (60-62). This predisposes in the short term to intrauterine growth restriction (IUGR) and acute cardiovascular, renal and CNS injuries.

In addition, it predisposes the developing foetus to cardiac, vascular and metabolic illnesses in adult life (62-64). Excess ROS lead to oxidation and disruption of the normal structure of phospholipids, proteins and DNA (65).

Chronic iodine deficiency results in low thyroid iodine stores and diminished synthesis of thyroid hormones, reduced T3/T4 negative feedback on the pituitary and hypothalamus a situation that persistently activates TSH secretion from the pituitary gland (48).

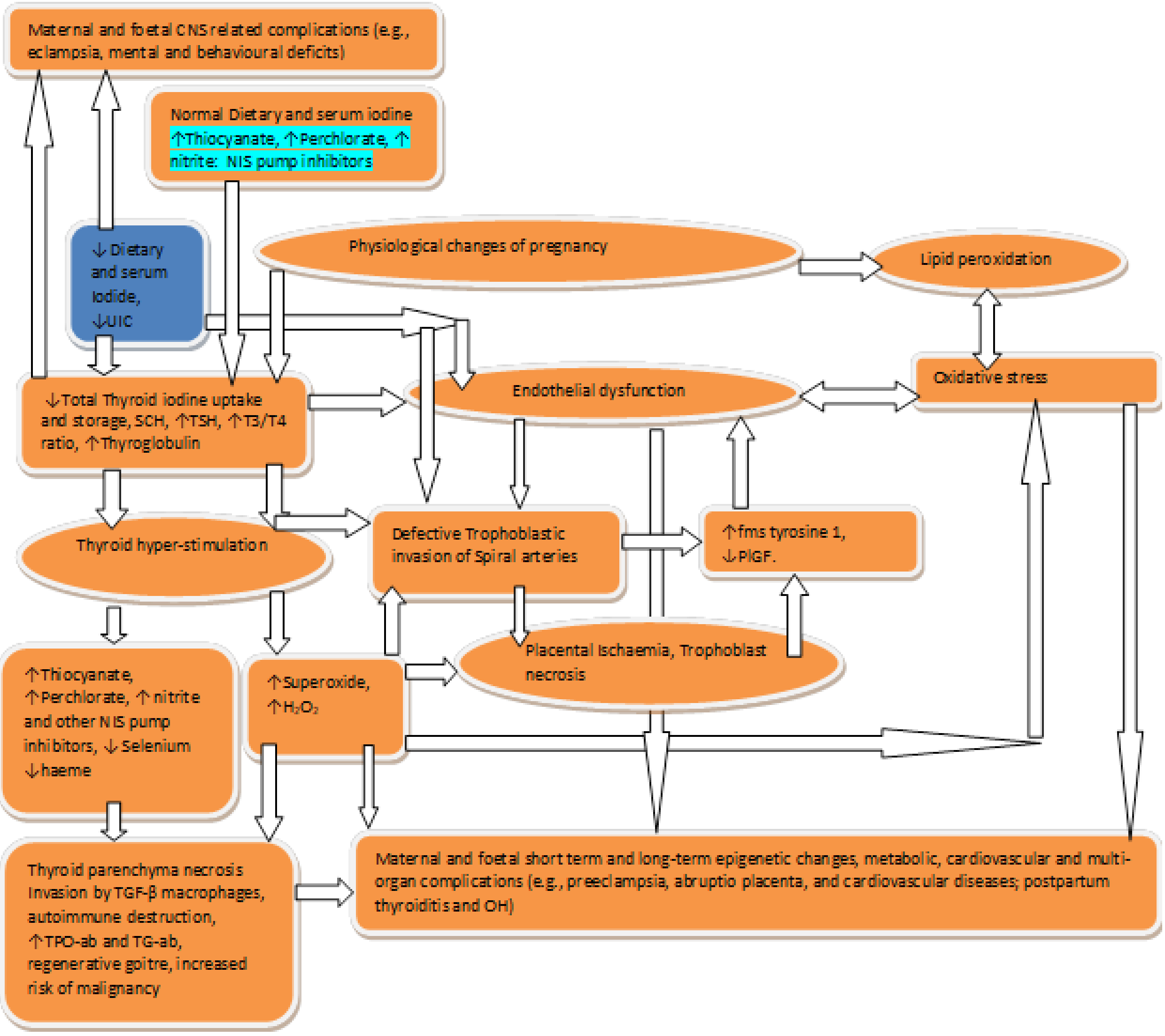


Figure 1. 1: Conceptual framework

UIC: urinary iodine concentration; TGF- β: transforming growth factor; TPO-ab: thyroid peroxidase antibody; TG-ab: Thyroglobulin antibody; NIS: Sodium iodide symporter; H_2O_2: Hydrogen peroxide; OH: hydroxyl radical; PlGF: Placenta growth factor.

This results in hyper-stimulation of the thyroid gland, increased thyroglobulin synthesis and iodine clearance by up to 60% and increased thyroid volume and goiter in states (48). Secondly, excessive TSH stimulation will lead to increased hydrogen peroxide (H_2O_2) production despite insufficient iodide uptake by the thyroid. If selenium is also insufficient, the superoxide radical (O_2^{-}), a more potent reactive oxygen species than H_2O_2, accumulates because the insufficient amounts of the selenium dependent superoxide dismutase (SOD) cannot convert all the O_2^{-} to H_2O_2 (66). If iron is also deficient, the haeme-dependent thyroid peroxidase (TPO) will not be available in adequate levels to catalyze iodide and reduce H_2O_2. Therefore, H_2O_2 will also accumulate, leading to (oxidative) damage to the thyroid follicular cells, necrosis and inflammation that could trigger autoimmune thyroid disease.

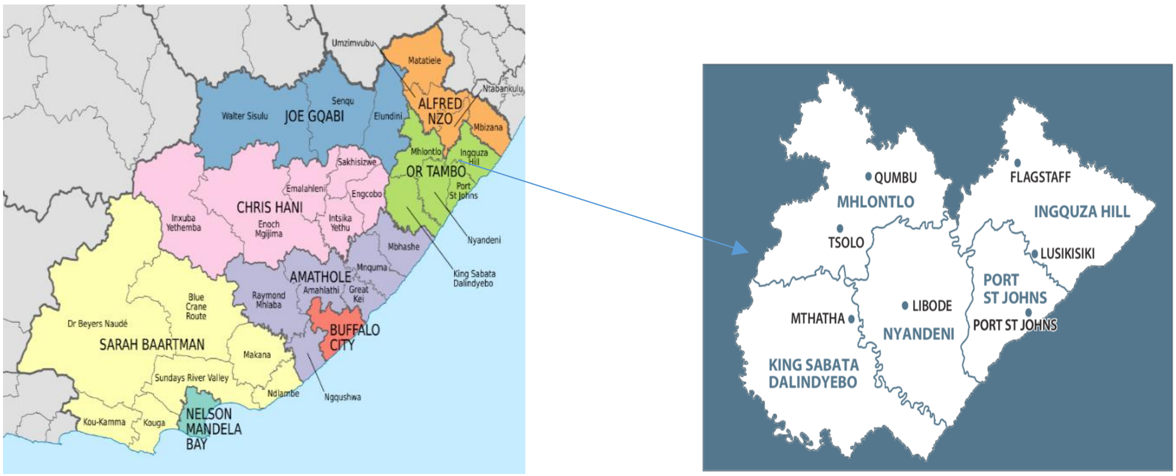
The increased circulation of superoxide and hydrogen peroxide radicals react with and rapidly diminish the levels of Nitric Oxide (NO) forming peroxynitrite (ONOO-) a more potent oxidant and inducer of inflammation and endothelial dysfunction (66). This further accentuates oxidative stress and endothelial dysfunction, thus increasing the risk of preeclampsia (66-68). Elevated serum TSH is also a known stimulant of endothelial dysfunction by inhibition of endothelial NO synthase (69). This further reduces serum NO and accentuates the reduction in flow mediated dilatation and endothelial activation, which are well-known features of preeclampsia (70). Endothelial dysfunction has also been found to promote atherosclerosis and to increase the risk of cardiovascular disease (71-73).

Dietary perchlorate, nitrate and thiocyanate have structural similarity to halides and competitively inhibit the trapping and oxidation of iodide ions by the sodium/iodide symporter and thyroid peroxidase respectively (30, 74, 75). This phenomenon is enhanced in states of increased thyroid stimulation/high serum TSH secondary to underlying iodine deficiency or secondary to physiological changes of pregnancy. Subsequently there is depletion of thyroid iodine stores and increased renal loss of the un-trapped iodide, further aggravating the iodine deficiency and predisposing to oxidant imbalance, endothelial dysfunction, goitre and other adverse outcomes of iodine deficiency (30, 33, 48).

1.7 Setting for the case-control studies

This part of the research project was conducted at Nelson Mandela Academic and Mthatha Regional Hospitals located at Mthatha Town in King Sabata Dalindyebo District. The catchment area for the two hospitals comprises mainly of O.R. Tambo District Municipality (ORTDM) as well as the bordering local districts of Chris Hani, Joe Gqabi and Alfred Nzo municipalities (Figure 2). ORTDM occupies the eastern coastal portion of the Eastern Cape Province, South Africa (76). It is bordered by KwaZulu-Natal province to the north, and by other Eastern Cape district municipalities of Amatole, Chris Hani, Joe Gqabi and Alfred Nzo. ORTDM extends over 15,946.84 Km^2 and incorporates five local municipalities (King Sabata Dalidyebo, Mhlontlo, Nyandeni, Ngquza Hill and Port St Johns. In ORTDM women make up

53.3% while 59.2% of the population are under 25 years of age. Over 74% of the population live below the poverty line (76).



Source: en.wikipedia.org and researchgate.net

Figure 1. 2 : Map of the Eastern Cape Province, South Africa, showing OR Tambo and other district municipalities. Inset is a map showing the various local districts that comprise OR Tambo district municipality.

References

1. Vest AR, Cho LS. Hypertension in pregnancy. Curr Atheroscler Rep. 2014;16(3):395.
2. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid. 2011;21(10):1081-125.
3. Casey BM, Dashe JS, Wells CE, McIntire DD, Byrd W, Leveno KJ, et al. Subclinical hypothyroidism and pregnancy outcomes. Obstet Gynecol. 2005;105(2):239-45.
4. Mosso L, Martínez A, Rojas MP, Margozzini P, Solari S, Lyng T, et al. [Frequency of subclinical thyroid problems among women during the first trimester of pregnancy]. Rev Med Chil. 2012;140(11):1401-8.
5. Dhanwal D, Bajaj S, Rajput R, Subramaniam KAV, Chowdhury S, Bhandari R, et al. Prevalence of hypothyroidism in pregnancy: An epidemiological study from 11 cities in 9 states of India. 2016;20(3):387-90.
6. Ezzeddine D, Ezzeddine D, Hamadi C, Abbas HA, Nassar A, Abiad M, et al. Prevalence and Correlation of Hypothyroidism With Pregnancy Outcomes Among Lebanese Women. Journal of the Endocrine Society. 2017;1(5):415-22.
7. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999;341(8):549-55.
8. Klein RZ, Sargent JD, Larsen PR, Waisbren SE, Haddow JE, Mitchell ML. Relation of severity of maternal hypothyroidism to cognitive development of offspring. J Med Screen. 2001;8(1):18-20.
9. Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, et al. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. Clin Endocrinol (Oxf). 2010;72(6):825-9.
10. van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JA, Goddijn M, et al. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update. 2011;17(5):605-19.
11. Lazarus JH. Screening for thyroid dysfunction in pregnancy: is it worthwhile? J Thyroid Res. 2011;2011:397012.
12. Jameson JL, Mandel SJ, Weetman AP. Disorders of the Thyroid Gland. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson JL, Loscalzo J, eds. Harrison's Principles of Internal Medicine, 19e. New York, NY: McGraw-Hill Education; 2014.
13. WHO. Assessment of iodine deficiency disorders and monitoring their elimination : a guide for programme managers. 3rd ed. Geneva: World Health Organization; 2007.
14. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744-50.
15. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. Eur J Endocrinol. 2004;151 Suppl 3:U25-37.
16. Gulaboglu M, Borekci B, Delibas I. Urine iodine levels in preeclamptic and normal pregnant women. Biol Trace Elem Res. 2010;136(3):249-57.

17. Cuellar-Rufino S, Navarro-Meza M, Garcia-Solis P, Xochihua-Rosas I, Arroyo O. Iodine levels are associated with oxidative stress and antioxidant status in pregnant women with hypertensive disease. Nutrición Hospitalaria. 2017;34:661.

18. Tsegaye B, Ergete W. Histopathologic pattern of thyroid disease. East Afr Med J. 2003;80(10):525-8.

19. Kishosha PA, Galukande M, Gakwaya AM. Selenium deficiency a factor in endemic goiter persistence in sub-Saharan Africa. World J Surg. 2011;35(7):1540-5.

20. Taga I, Oumbe VA, Johns R, Zaidi MA, Yonkeu NJ, Altosaar I. Youth of West Cameroon are at high risk of developing IDD due to low dietary iodine and high dietary thiocyanate. Afr Health Sci. 2008;8(4):227-33.

21. Kalk WJ, Paiker J, van Arb MG, Pick W. Dietary iodine deficiency in South Africa. Surveys before the introduction of universal salt iodisation. S Afr Med J. 1998;88(3 Endocrinology):357-8.

22. Jooste PL, Weight MJ, Lombard CJ. Iodine concentration in household salt in South Africa. Bull World Health Organ. 2001;79(6):534-40.

23. Lazarus JH. Iodine status in europe in 2014. Eur Thyroid J. 2014;3(1):3-6.

24. Pizzorno L. Iodine: the Next Vitamin D? Part I. Longevity Medicine Review 2010.

25. Jooste P, Zimmermann M. Progress towards eliminating iodine deficiency in South Africa. South African Journal of Clinical Nutrition. 2008;21(1):8-14.

26. Stern R, Puoane T, Tsolekile L. An exploration into the determinants of noncommunicable diseases among rural-to-urban migrants in periurban South Africa. Prev Chronic Dis. 2010;7(6):A131.

27. Harris MJ, Jooste PL, Charlton KE. The use of iodised salt in the manufacturing of processed foods in South Africa: bread and bread premixes, margarine, and flavourants of salty snacks. Int J Food Sci Nutr. 2003;54(1):13-9.

28. Friel S, Chopra M, Satcher D. Unequal weight: equity oriented policy responses to the global obesity epidemic. Bmj. 2007;335(7632):1241-3.

29. Zimmermann MB. Iodine deficiency in industrialized countries. Clin Endocrinol (Oxf). 2011;75(3):287-8.

30. De Groef B, Decallonne BR, Van der Geyten S, Darras VM, Bouillon R. Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. Eur J Endocrinol. 2006;155(1):17-25.

31. Steinmaus C, Miller MD, Howd R. Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001-2002 national health and nutrition examination survey. Environ Health Perspect. 2007;115(9):1333-8.

32. Vidal ZEO, Rufino SC, Tlaxcalteco EH, Trejo CH, Campos RM, Meza MN, et al. Oxidative Stress Increased in Pregnant Women with Iodine Deficiency. Biological Trace Element Research. 2014;157(3):211-7.

33. La Vignera S, Condorelli R, Vicari E, Calogero AE. Endothelial dysfunction and subclinical hypothyroidism: a brief review. J Endocrinol Invest. 2012;35(1):96-103.

34. Lu M, Yang CB, Gao L, Zhao JJ. Mechanism of subclinical hypothyroidism accelerating endothelial dysfunction (Review). Exp Ther Med. 2015;9(1):3-10.

35. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308(5728):1592-4.

36. Redman CW, Sargent IL**.** Immunology of pre-eclampsia. Am J Reprod Immunol. 2010;63(6):534-43.

37. Patrick L**.** Iodine: deficiency and therapeutic considerations. Altern Med Rev. 2008;13(2):116-27.

38. Burns R, Azizi F, Hedayati M, Mirmiran P, O'Herlihy C, Smyth PPA**.** Is placental iodine content related to dietary iodine intake? Clinical endocrinology. 2011;75(2):261-4.

39. Miller D**.** Extrathyroidal Benefits of Iodine. Journal of American Physicians and Surgeons. 2006;11.

40. Aceves C, Anguiano B, Delgado G**.** The extrathyronine actions of iodine as antioxidant, apoptotic, and differentiation factor in various tissues. Thyroid. 2013;23(8):938-46.

41. Ma F, Zhao W, Kudo M, Aoki K, Misumi J**.** Inhibition of vacuolation toxin activity of Helicobacter pylori by iodine, nitrite and potentiation by sodium chloride, sterigmatocystin and fluoride. Toxicol In Vitro. 2002;16(5):531-7.

42. Karbasi-Afshar R, Khedmat H, Izadi M**.** Helicobacter pylori Infection and atherosclerosis: a systematic review. Acta Med Iran. 2015;53(2):78-88.

43. Tersigni C, Franceschi F, Todros T, Cardaropoli S, Scambia G, Di Simone N**.** Insights into the Role of Helicobacter pylori Infection in Preeclampsia: From the Bench to the Bedside. Frontiers in Immunology. 2014;5:484.

44. Glinoer D**.** The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev. 1997;18(3):404-33.

45. Kundu S RK**.** Thyroid Hormone Homeostasis in Adult Mammalian Brain: A Novel Mechanism for Functional Preservation of Cerebral T3 Content During Initial Peripheral Hypothyroidism. Al Ameen Journal of Medical Sciences. 2010;3(1):5 - 20.

46. Zantour B AW, Marmouch H, Chebbi W Hypothyroidism in Pregnancy. In: E P, ed. Current Topics in Hypothyroidism with Focus on Development. Rijeka, Croatia: In Tech 2013: 29-62.

47. Glinoer D**.** The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. Best Pract Res Clin Endocrinol Metab. 2004;18(2):133-52.

48. Lazarus J**.** Thyroid Regulation and Dysfunction in the Pregnant Patient. In: Feingold KR AB, Boyce A, et al, ed. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2000; 2016.

49. Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, et al. Impaired endothelium-dependent vasodilatation in subclinical hypothyroidism: beneficial effect of levothyroxine therapy. J Clin Endocrinol Metab. 2003;88(8):3731-7.

50. Lopez M**.** Hypothyroxinemia. In: JM N, ed. Pregnancy is Related with Attention Deficit Hyperactivity Disorder, Contemporary Trends in ADHD Research. Rijeka, Croatia: In Tech; 2012:57 - 69.

51. De Groot L, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(8):2543-65.

52. Vissenberg R, van den Boogaard E, van Wely M, van der Post JA, Fliers E, Bisschop PH, et al. Treatment of thyroid disorders before conception and in early pregnancy: a systematic review. Hum Reprod Update. 2012;18(4):360-73.

53. Burton GJ, Redman CW, Roberts JM, Moffett A**.** Pre-eclampsia: pathophysiology and clinical implications. Bmj. 2019;366:l2381.

54. DOH**.** Saving Mothers 2014-2016: Seventh triennial report on confidential. Pretoria: Department of Health; 2018.

55. Andersson M, Takkouche B, Egli I, Allen HE, de Benoist B**.** Current global iodine status and progress over the last decade towards the elimination of iodine deficiency. Bull World Health Organ. 2005;83(7):518-25.

56. Zimmermann MB**.** Iodine deficiency. Endocr Rev. 2009;30(4):376-408.

57. Businge C, Longo-Mbenza B, Adeniyi V, Babeki V, Kaboka A, Muaka M, et al. Iodine Deficiency in Pregnancy as a Predictor of Sub-clinical Hypothyroidism, Preeclampsia and Future Cardiovascular Disease. Asian Journal of Clinical Nutrition. 2017;9:118-23.

58. Redman C, Sargent I**.** Preeclampsia and the systemic inflammatory response. Seminars in nephrology. 2004;24:565-70.

59. Chen C-Y, Chen C-P, Lin K-H**.** Biological Functions of Thyroid Hormone in Placenta. International Journal of Molecular Sciences. 2015;16:4161-79.

60. Bayhan G, Kocyigit Y, Atamer A, Atamer Y, Akkus Z**.** Potential atherogenic roles of lipids, lipoprotein (a) and lipid peroxidation in preeclampsia. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2005;21:1-6.

61. Atamer Y, Kocyigit Y, Yokus B, Atamer A, Erden A**.** Lipid peroxidation, antioxidant defense, status of trace metals and leptin levels in preeclampsia. European journal of obstetrics, gynecology, and reproductive biology. 2005;119:60-6.

62. Chiavaroli V, Giannini C, D'Adamo E, Giorgis T, Chiarelli F, Angelika M**.** Insulin Resistance and Oxidative Stress in Children Born Small and Large for Gestational Age. Pediatrics. 2009;124:695-702.

63. Franco MCP, Kawamoto EM, Gorjão R, Rastelli VMF, Curi R, Scavone C, et al. Biomarkers of oxidative stress and antioxidant status in children born small for gestational age: evidence of lipid peroxidation. Pediatric research. 2007;62(2):204-8.

64. Mohn A, Chiavaroli V, Cerruto M, Blasetti A, Giannini C, Bucciarelli T, et al. Increased Oxidative Stress in Prepubertal Children Born Small for Gestational Age. The Journal of Clinical Endocrinology & Metabolism. 2007;92(4):1372-8.

65. Ziech D, Franco R, Pappa A, Panayiotidis M**.** Reactive Oxygen Species (ROS)—Induced genetic and epigenetic alterations in human carcinogenesis. Mutation research. 2011;711:167-73.

66. Smyth P**.** Role of iodine in antioxidant defence in thyroid and breast disease. BioFactors (Oxford, England). 2003;19:121-30.

67. Johansen J, Harris A, Rychly D, Ergul A**.** Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical pratice. Cardiovascular diabetology. 2005;4:5.

68. Lioudaki E, Mavroeidi NG, Mikhailidis DP, Ganotakis ES**.** Subclinical hypothyroidism and vascular risk: an update. Hormones (Athens). 2013;12(4):495-506.

69. Tian L, Zhang L, Liu J, Guo T, Gao C, Ni J**.** Effects of TSH on the function of human umbilical vein endothelial cells. Journal of molecular endocrinology. 2014;52.

70. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant Human Thyrotropin Reduces Endothelium-Dependent Vasodilation in Patients Monitored for Differentiated Thyroid Carcinoma. The Journal of clinical endocrinology and metabolism. 2006;91:4175-8.

71. Suwaidi J, Hamasaki S, Higano S, Nishimura R, Holmes D, Lerman A**.** Long-Term Follow-Up of Patients With Mild Coronary Artery Disease and Endothelial Dysfunction. Circulation. 2000;101:948-54.

72. Schächinger V, Britten M, Zeiher A**.** Prognostic Impact of Coronary Vasodilator Dysfunction on Adverse Long-Term Outcome of Coronary Heart Disease. Circulation. 2000;101:1899-906.

73. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T**.** Endothelial Dysfunction, Oxidative Stress, and Risk of Cardiovascular Events in Patients With Coronary Artery Disease. Circulation. 2001;104:2673-8.

74. Sanchez CA, Crump KS, Krieger RI, Khandaker NR, Gibbs JP**.** Perchlorate and nitrate in leafy vegetables of North America. Environ Sci Technol. 2005;39(24):9391-7.

75. Contempré B, Escobar G, Denef J-f, Dumont J, Many M-C**.** Thiocyanate Induces Cell Necrosis and Fibrosis in Selenium- and Iodine-Deficient Rat Thyroids: A Potential Experimental Model for Myxedematous Endemic Cretinism in Central Africa. Endocrinology. 2004;145:994-1002.

76. ECSECC**.** O.R. Tambo District Municipality Socio-Economic Review and Outlook 2017. Vincent: Eastern Cape Socio Economic Consultative Council; 2017.

Part 1: Assessment of the trends in prevalence of iodine deficiency in pregnancy in Africa since implementing national iodine fortification programs in 1995

This part comprises the following two chapters:

Chapter 2: The prevalence of insufficient iodine intake in pregnancy in Africa: a systematic review and meta-analysis

Chapter 3: Iodine nutrition status in Africa: potentially high prevalence of iodine deficiency in pregnancy even in countries classified as iodine sufficient

CHAPTER 2

The prevalence of insufficient iodine intake in pregnancy in Africa: a systematic review and meta-analysis

Charles Bitamazire Businge, Hannibal Tafadzwa Musarurwa, Benjamin Longo-Mbenza, Andre Pascal Kengne

(The manuscript is currently under peer review)

The protocol for this systematic review was published in the Systematic Reviews journal:

Businge CB, Longo-Mbenza B, Kengne AP. The prevalence of insufficient iodine intake in pregnancy in Africa: protocol for a systematic review and meta-analysis. Systematic Reviews. 2019;8(1):209.

It is included at the end of this thesis as Appendix IVa.

Abstract

Background: Fortification of foodstuffs with iodine, mainly through iodization of salt, which began in several African countries after 1995 is the primary method for mitigating iodine deficiency in Africa. We assessed the degree of iodine nutrition in pregnancy across Africa before and after implementing national iodine fortification programs (CRD42018099434).

Methods: We searched Electronic databases and grey literature for baseline data before implementation of population-based iodine supplementation and for follow-up data up to September 2020. R-metamedian and metamean packages were used to pool country-specific median UIC estimates and derived mean UIC from studies with similar features.

Results: Of 54 African countries, 23 had data on iodine nutrition in pregnancy mostly from subnational samples. Data before 1995 showed that severe iodine deficiency was prevalent in pregnancy with a pooled pregnancy median UIC of 28.6 µg/L (95% CI 7.6–49.5). By 2005, five studies revealed a trend towards improvement in iodine nutrition state in pregnancy with a pooled pregnancy median UIC of 174.1 µg/L (95% CI 90.4—257.7). Between 2005 and 2020 increased numbers of national and subnational studies revealed that few African countries had sufficient, while most had mildly inadequate, and some severely inadequate iodine nutrition in pregnancy. The pooled pregnancy median UIC was 145 µg/L (95% CI 126–172).

Conclusion: Improvement in iodine nutrition status in pregnancy following the introduction of fortification of foodstuffs with iodine in Africa is sub-optimal, exposing a large proportion of pregnant women to the risk of iodine deficiency and associated disorders.

keywords

Iodine, insufficiency, pregnancy, Africa

2.1 Background

Iodine deficiency has a spectrum of consequences that not only affect pregnancy outcomes but also subsequent childhood and maternal health (1-3). Foetal and maternal complications include spontaneous miscarriages, growth restriction, still birth, maternal postpartum thyroiditis and in cases of severe or persistent iodine deficiency, subclinical and overt hypothyroidism, stunted growth, altered serum lipids, mental and motor deficits that can affect both mother and child (1-3). The risk of these complications is higher in settings with endemic iodine deficiency like most countries in Africa in the early 1990s before the initiation of iodine fortification. The degree of iodine deficiency deteriorates in pregnancy due to physiological increase in renal iodine filtration and subsequent loss in urine (4, 5).

Before programs encouraging the fortification of salt and other foodstuffs in Africa, it was estimated that only 10% of the population on the African continent had adequate iodine nutrition (6-8). This was attributed to low soil iodine content and high thiocyanate levels, one of the major goitrogens on the continent (9). By 2019, surveys using national or subnational samples of SAC yielded median UIC consistent with adequate iodine intake in most African countries (10). Because of variation in dietary habits and iodine metabolism of school-age children and pregnant women, the school-age children median UIC does not accurately predict the degree of iodine nutrition among pregnant women from the same setting (11, 12). Of the eleven African countries with data on iodine nutrition in pregnancy by 2017, five had insufficient, four adequate and two more than adequate iodine intake in pregnancy (13).

A daily iodine intake of at least 200 µg, up from the recommended 100-150 µg in non-pregnant women, is necessary to cater for the physiological requirements of pregnancy and compensate for the elevated renal losses (14). In pregnancy a median UIC of < 150 µg/l reflects insufficient intake while UIC of 150-249 µg/l adequate, 250-499 µg/l more than adequate and UIC>500 µg/l reflecting excessive iodine intake (15). It is uncertain if the iodine fortification efforts have had a significant and sustainable impact on the iodine nutrition status in pregnancy in Africa (16).

2.2 Rationale

We conducted this systematic review and meta-analysis to ascertain the trend in the prevalence of insufficient iodine nutrition status (median UIC <150 µg/L) among pregnant women in Africa following the implementation of national iodization programs and to establish if this has had a positive impact on the iodine nutrition status of pregnant women in Africa.

2.3 Methods

The methods of this systematic reviews and meta-analysis were described in a protocol (17) that was also registered with PROSPERO (CRD42018099434) Observational and intervention studies with data on iodine nutrition status in pregnancy conducted in the various African countries were included in this systematic review. The iodine nutrition status was defined according to the WHO/ICCIDD classification of iodine intake of populations using median urinary iodine concentration (14). All studies reported in the English or French, or Portuguese languages and conducted on human subjects were considered. We excluded studies conducted among populations of African origin but living outside Africa; studies lacking prevalence rates and with absence of data to compute them; and studies not performed in human participants or published in languages other than English, French and Portuguese. This systematic review is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Guidelines (18).

Search strategy for study identification

Electronic searches

We searched PubMed-MEDLINE, Google Scholar, SCOPUS, ISI Web of Science (Science Citation Index), Africa Wide Information, African Index Medicus (AIM) and AFROLIB databases for published studies on iodine deficiency in pregnancy in Africa up to 30th September 2020. This search was conducted using a pre-defined comprehensive and sensitive search strategy combining relevant terms with names of countries in Africa, to obtain the maximum possible number of studies. The search was guided by the African search filter, which has been reported to have good sensitivity (and improved precision) of 74% (1.3–9.4%) and 73% (5–28%) for MEDLINE and EMBASE, respectively (19). This search filter included names of each African country and shortened terms to capture studies from regions. Countries with official names in a language other than English were entered in the official form, and for countries that have changed names over time, both names were included in the search. The search strategy can be found in the published protocol for this review (17). We also searched reference lists of relevant citations for articles of interest.

Grey literature

We also searched for national ministries of Health, international organisations such as the WHO, UNICEF, ICCIDD, IGN, other non-government organisations' reports, conference and workshop proceedings using Google scholar search engine and major relevant websites such as WHO African Index Medicus and African Journals Online (AJOL).

Data management

All identified studies were entered into endnote software for de-duplication of records. Prior to screening of studies, we created standardised questions according to the inclusion criteria which were pre-tested on a sample of eligible studies.

Screening

Two investigators (CBB and HM) independently selected studies that meet inclusion criteria. Citations and abstracts were screened for possible inclusion, and duplicate citations were excluded. Titles and abstracts were then screened following inclusion criteria described above, following which the full texts of potentially eligible articles were obtained. The full texts were then screened using a standardised and pre-tested form to include eligible studies. Disagreements were resolved by consensus, or consultation of a third author (APK). Corresponding authors of potentially eligible studies that did not report the relevant data were contacted. Reasons for exclusion of non-eligible studies were documented. The entire selection process was summarised in a flow chart (Figure 2.1)

Data extraction

Two investigators (CBB and HM) independently extracted data from included studies, using a standardised and pre-tested data extraction form. Any inconsistencies or disagreement resolved by consensus or consultation with the third investigator (APK).

Data items

Data including the year, geographic region and country where the study was conducted, year of publication, study design, setting (rural or urban, health facility or community-based, national or sub-national), sample size, and the criteria used for determination of the iodine intake were extracted. The median (25th-75th percentiles) and or mean (standard deviation) UIC were recorded.

PRISMA Flow Diagram

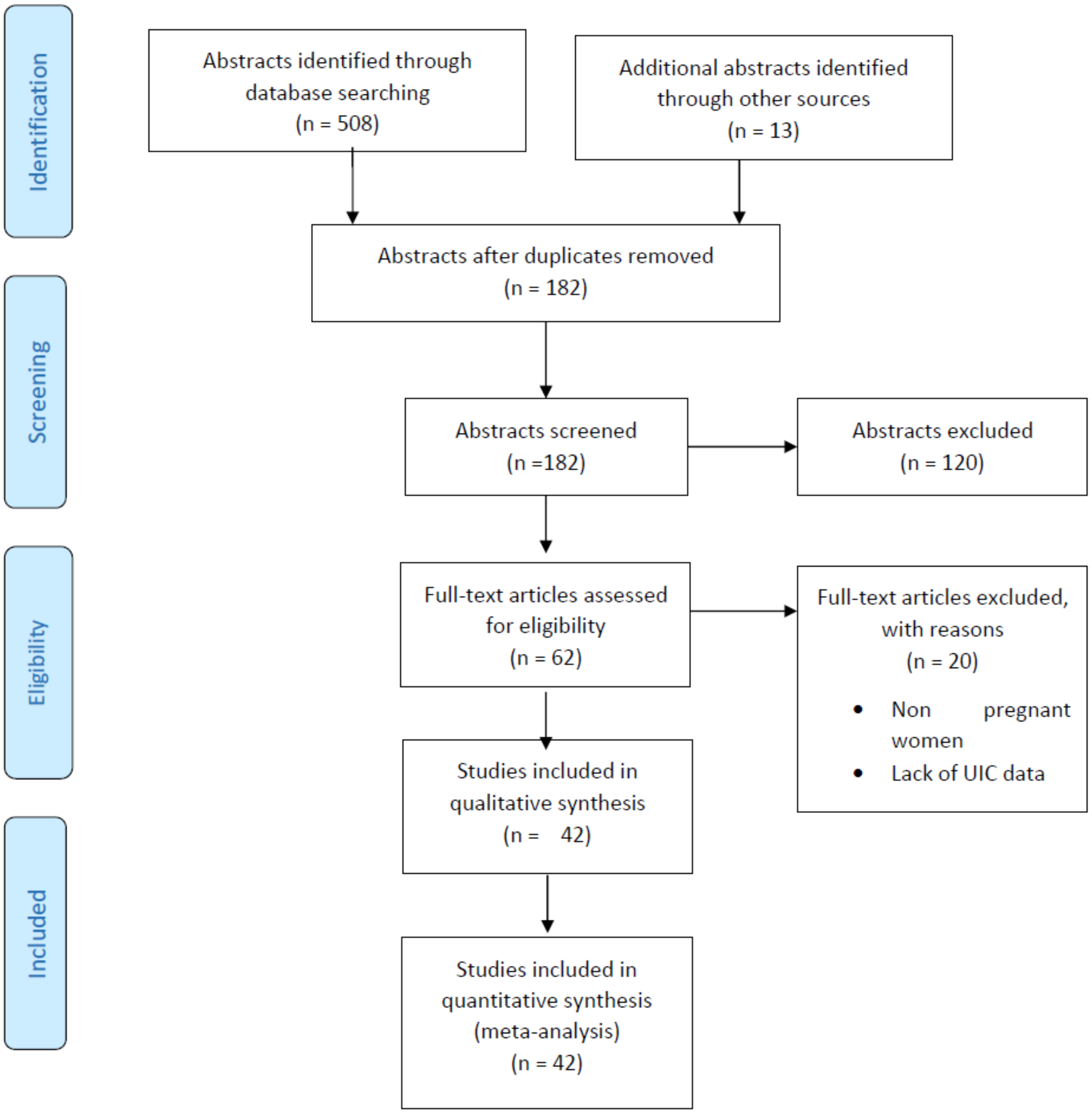


Figure 2. 1: Flow chart showing the processes of selection of studies included in the systematic review

Assessment of methodological quality and risk of bias

Two investigators (CBB and HM) independently scored the quality of included studies. The risk of bias in individual studies was assessed using the Risk of Bias Tool for Prevalence Studies as previously described (17, 20). Discrepancies were resolved by consensus. The risk of bias and quality scores are presented in Table 2.1.

Table 2. 1: Characteristics of included studies

First author, year	**Country, period of data collection**	**Sample type (National / subnational)**	**Sample size**	**UIC Median [IQR] or [Mean** ± SD]	**Risk of Bias:** 0 - 3 low 4 - 6 intermediate 7 - 9 high
	Before 1995				
Chaouki, 1994	Algeria (1994)	Sub-national	982	[17.9 ± 0.1]	2—low
Ngo, 1997	DRC (1991 -92)	National	306	39.3 [31.1, 52.9]	0–low
	1995 -2005				
Hess, 1999	Cote de'Ivore	Sub-national	72	351 [74 - 2241]	2–low
Hess, 1999	Cote de'Ivore	Sub-national	66	136 [12 - 915]	1–low
Ojule 1998	Nigeria, 1998	Sub-national	90	[213.4 ± 9.9]	3–low
Ojule 1998	Nigeria, 1998	Sub-national	105	[149 ± 14.7]	3–low
Dillon, 2000	Senegal 1996-1997	Sub-national	462	[60 ± 39]	1–low
Eltom, 2000	Sudan 1998 - 1999	Sub-national	47	38 [12.7, 50.8]	3–low
Lwenje, 2000	Swaziland	National	165	295 [95% CI 265.5–425.6]	1–low
	2006–2020				
Akdader-Oudahmane, 2020	Algeria 2016 - 2017	Sub-national	173	233 [157, 326]	4–intermediate
Garnier et al., 2016	Burkina Faso	National	946	74 [NA, NA]	1–low
Kavishe et al., 2020	Burundi 2018	National	87	86.7 [NA, NA]	1–low
Habimana et al., 2013	DRC, 2009–2011	Sub-national	225	138 [105, 172]	1–low
IGN, 2017	Djibouti, 2015 (NS)	National	230	265 [170, 445]	0–low
MOHP, 2017	Egypt, 2014–2015	National	1498	135 [NA, NA]	0–low
Hamza, 2007	Egypt, 2006	Sub-national	113	[102.9 ± 31.1]	2–low
Elsayed, 2016	Egypt,2016	Sub-national	400	170 [NA, NA]	1–low
Mohammed, 2019	Ethiopia, 2013–2014	Sub-national	562	120.6 [68.9, 216.4]	1–low
Fereja, 2018	Ethiopia	Sub-national	354	85.7 [45.7, 136]	1–low
Kedir, 2014	Ethiopia, 2012	Sub-national	435	58.1[21.4, 111.1]	1–low
Ersino, 2013	Ethiopia, 2009	Sub-national	172	15 [2.5, 33]	2–low
Takele, 2018	Ethiopia, 2017	Sub-national	403	137 [97, 177]	1–low

Keno, 2017	Ethiopia, 2014	Sub-national	40	88.6 [66.9, 133.5]	3–low
Negeri, 2014	Ethiopia, 2011	Sub-national	423	48 [NA, NA]	2–low
NaNA, 2019	Gambia 2018	National	118	113.5 [50.1, 205.9]	0–low
GHS, 2017	Ghana, 2015	National	102	183.5 [NA, NA]	0–low
Gyamfi, 2018	Ghana, 2016 (ss)	Sub-national	239	159 [NA, NA]	3–low
Adu-Afarwuah, 2018	Ghana, 2009 - 2011	Sub-national	295	137 [78, 221]	2–low
Farebrother et al., 2018	Kenya	Sub-national	162	337 [198, 505]	4–intermediate
Randremanana, 2019	Madagascar, 2014	National	170	53 [9, 89]	0–low
Stinca, 2017	Morocco 2013–2014	Sub-national	245	32 [17, 58]	3–low
Sadou 2013	Niger 2012	Sub-national	240	119 [NA, NA]	2–low
Hess, 2016	Niger, 2014 -2015	Sub-national	662	69 [38.1, 114.3]	1–low
Jibril, 2016	Nigeria, 2014	Sub-national	300	193 [NA, NA]	2–low
Kayode, 2019	Nigeria, 2012	Sub-national	133	135 [NA, NA]	1–low
Ujowundu, 2010	Nigeria, 2009	Sub-national	302	[152.09 ± 41.65]	2–low
Rohner, 2016	Sierra Leone, 2013	National	154	175.8 [NA, NA]	0–low
MOH-FGS, 2020	Somalia, 2018 - 2019	National	236	369 [142.9, 752]	0–low
Mabasa, 2019	South Africa, 2012 -2013	Sub-national	565	164 [92, 291]	3–low
Stinca, 2017	South Africa	Sub-national	207	174 [95.3, 297.6]	3–low
Mtumwa, 2017	Tanzania, 2009 - 2010	National	947	136.8 [58.8, 258]	1–low
Ba, 2020	Tanzania, 2015 -2016	National	266	156.1 [64.6, 260.4]	0–low
Stinca, 2017	Tanzania, 2016	Sub-national	330	422 [270, 609]	3–low
Chinyanga, 2006	Zimbabwe, 2006	Sub-national	94	115.5 [43, 225]	4–intermediate

Data synthesis, analysis and assessment of heterogeneity

Prevalence data was summarised by country and period of study (Table 2.1). Median pregnancy IUC were pooled using R meta-median package. Sub-group analysis was carried out according to the time the studies were conducted that is before 1995, between 199 and 004 and 2005–2020 (Figure 2.2) In order to check for heterogeneity and publication bias, we transformed the median and the interquartile (IQR) UIC data into sample means and standard deviations using the quantile method for estimating the sample mean and the smoothly weighted estimator for the sample standard deviation as described elsewhere (21, 22) (Table 2.3). The derived means were then pooled using metamean R package and degree of heterogeneity between the included studies and the difference in the mean of subgroups estimated. Publication bias was assessed using a funnel plot and an accompanying linear regression test.

2.4 Results

Figure 1 shows the PRISMA flow chart of the study selection process. A total of 521 abstracts were identified from the searches. After removing duplicates, the titles and abstracts of 182 articles were screened for eligibility. Of these, 62 full-text articles were accessed and screened out of which 42 studies met the inclusion criteria and were included in the meta-analysis (23-65).

Characteristics of included studies

Out of the 42 studies, two were carried out before 1995, five between 1995 and 2005, and thirty-five between 2006 and 2020. Only eleven of the forty-two studies had data derived from national representative samples. The internal and external validity of the included studies were determined using a 9-point score (table 2.1). Most of the studies (37/42) had low risk of bias with the rest having intermediate risk (Table 2.1).

The prevalence of insufficient iodine intake (UIC <150 µg/L) among pregnant women on the various African countries before 1995, 1995 - 2005, and 2006 - 2020

Before 1995, available data from two studies revealed moderate countrywide iodine deficiency in pregnancy in the Democratic Republic of Congo at the time and severe iodine deficiency in pregnancy in a subnational sample from North-Eastern Algeria (23, 24). The pooled median UIC across the two studies was 28.6 µg/L (95% CI 7.6–49.5), with considerable heterogeneity (I^2 99.73%, $p < 0.001$, Figure 2.2).
Between 1995 and 2005 four subnational studies from Ivory Coast, Nigeria Sudan and Senegal, and one national survey from Swaziland (25-29) yielded a pooled pregnancy UIC of 174.1 µg/L (95% CI 90.4–257.7, Figure 2.2); with considerable heterogeneity (I^2 99.96%, $p < 0.001$).

Between 2005 and 2020, 35 studies from 18 countries had pregnancy median UIC data. Eleven of the studies were national surveys from 10 countries. These national surveys revealed more than adequate intake in Djibouti and Somalia (30, 31) adequate iodine intake in Ghana, Sierra Leone, and Tanzania (32-34); mild inadequate intake in Egypt, Gambia and Tanzania (35-37), and moderate insufficient iodine intake in Burkina Faso, Burundi and Madagascar (38-40). The remaining studies (41-65) were subnational. The pooled median pMUIC across the 35 studies conducted between 2005 and 2020 was 145 µg/L (95% CI 126–172), with substantial heterogeneity (I^2 99.81%, $p < 0.001$) (Figure 7.2). There was a significant increase in pregnancy median UIC between 1995 and 2020 compared to the period before 1995 (Kendaull's tau correlation co-efficient 0.270, $p = 0.032$).

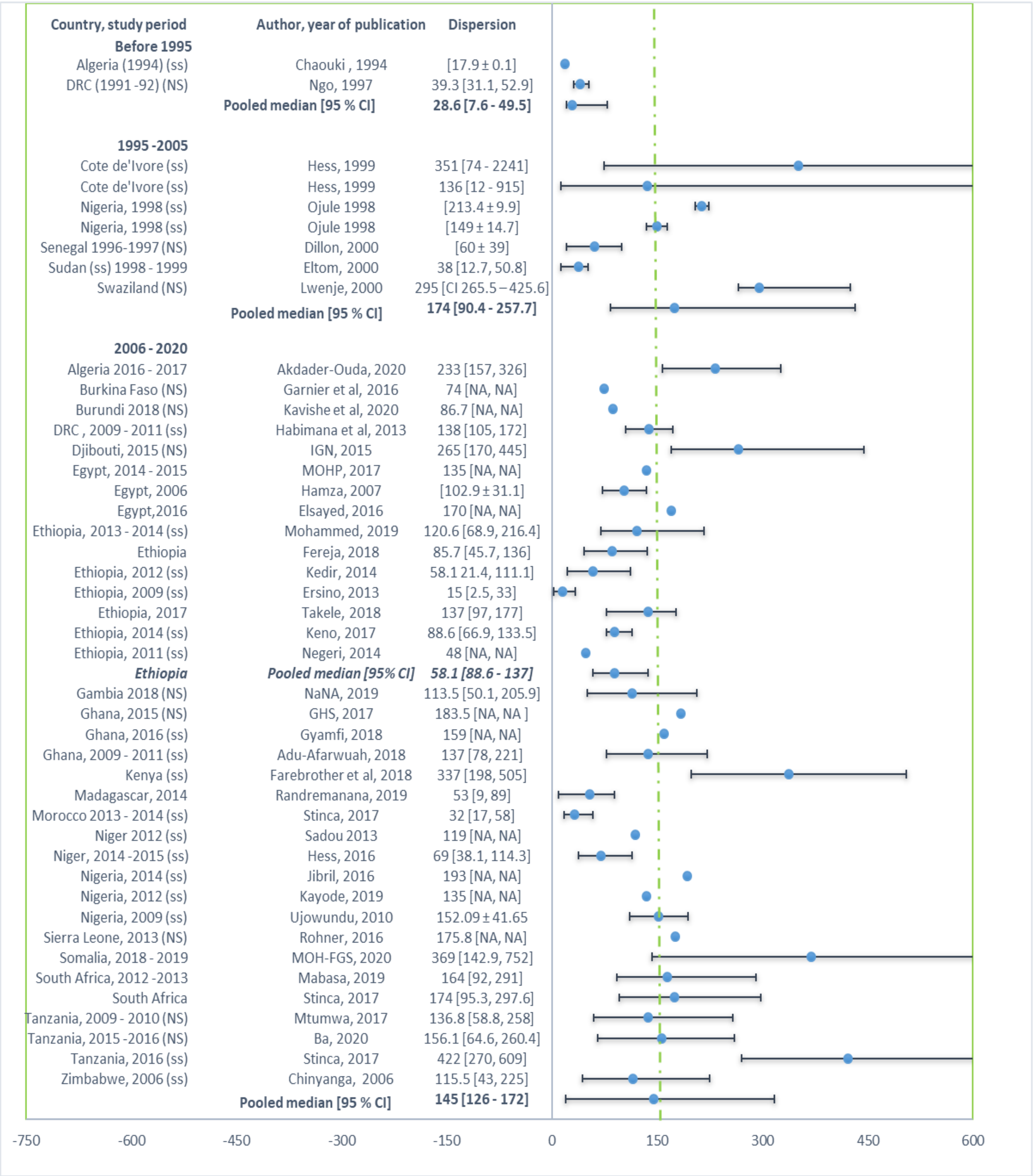


Figure 2. 2: median urinary Iodine concentration (IUC µg/L) of pregnant women for studies conducted before 1995, between 1999 and 2004, and 2005 to 2020 (the dashed vertical line shows adequate median UIC during pregnancy; a [b, c] denotes median with IQR, a [±b] denotes mean and standard deviation, and a [b–c] median with the range). NS = national survey; ss = sub-national survey.

Derived mean UIC by time-period

The pooled derived mean pregnancy UIC (Table 2.2, Figure 2.3) was 27.96 µg/L (95% CI 11.6–67.04, tau 0.630) before 1995; 143.22 µg/L (95% CI 108.65–188.78, tau 0.362) between 1995 and 2005; and 127.99 µg/L (95% CI 108.59–150.85, tau 0.493); with significant difference across time-period (Q = 12.24, d.f. = 2, p = 0.002).

Assessment of publication bias

We assessed publication bias using funnel plots. The funnel plot for the studies in the period 1995–2004 was not suggestive of potential publication bias (Figure 2.4) (R metabias linear regression test t = - 0.36005, p-value = 0.7335). No additional studies were imputed after checking for funnel asymmetry using the Twedie and Duval's trim and fill test. The funnel plot for the studies carried out between 2005 and 2020 was asymmetrical (Figure 2.5). The trim and fill test imputed sixteen potential missing studies suggesting potential publication bias (Figure 2.6). The funnel plot asymmetry was confirmed by the R metabias linear regression test (t = 3.872, p < 0.001).

Table 2. 2: Means and standard deviations (SD) derived from the medians of the included studies

First author, year	Country, period of data collection	Sample size	median	Derived mean	Derived SD
Chaouki, 1994	Algeria (1994)	982	17.9	17.9	0.1
Ngo, 1997	DRC (1991 -92)	306	39.3	43.7	21.5
Hess, 1999	Cote de'Ivore	72	351	351	455
Hess, 1999	Cote de'Ivore	66	136	184.2	192.3
Ojule 1998	Nigeria, 1998	90	213	213	9.9
Ojule 1998	Nigeria, 1998	105	149	149	14.7
Dillon, 2000	Senegal 1996-1997	462	60	60	39
Eltom, 2000	Sudan 1998 - 1999	47	38	33.6	29.1
Lwenje, 2000	Swaziland	165	295	299	30
Akdader-O, 2020	Algeria 2016 - 2017	173	233	234.8	37
Garnier, 2016	Burkina Faso	946	74	74	28.2
Kavishe, 2020	Burundi 2018	87	86.7	87	33.2
Habimana, 2013	DRC, 2009–2011	225	138	138.4	50
IGN, 2017	Djibouti, 2015 (NS)	230	265	294	205
MOHP, 2017	Egypt, 2014–2015	1498	135	135	50.5
Hamza, 2007	Egypt, 2006	113	102	102.9	31.1

Elsayed, 2016	Egypt,2016	400	170	173.5	56.5
Mohammed, 2019	Ethiopia, 2013–2014	562	120.6	136.1	109.6
Fereja, 2018	Ethiopia	354	85.7	89.3	67.2
Kedir, 2014	Ethiopia, 2012	435	58.1	63.8	66.7
Ersino, 2013	Ethiopia, 2009	172	15	16.9	22.8
Takele, 2018	Ethiopia, 2017	403	137	137	59.5
Keno, 2017	Ethiopia, 2014	40	88.6	96.8	51.2
Negeri, 2014	Ethiopia, 2011	423	48	48	17.9
NaNA, 2019	Gambia 2018	118	113.5	123.7	116.7
GHS, 2017	Ghana, 2015	102	183.5	183.5	69.5
Gyamfi, 2018	Ghana, 2016 (ss)	239	159	159	59.7
Adu-Afarwuah, 2018	Ghana, 2009 - 2011	295	137	145.8	106.5
Farebrother, 2018	Kenya	162	337	347.2	229.6
Randremanana, 2019	Madagascar, 2014	170	53	50.2	59.8
Stinca, 2017	Morocco 2013–14	245	32	35.9	30.6
Sadou 2013	Niger 2012	240	119	119	44.8
Hess, 2016	Niger, 2014 -2015	662	69	73.9	56.4
Jibril, 2016	Nigeria, 2014	300	193	193	71.5
Kayode, 2019	Nigeria, 2012	133	135	138.5	58.5
Ujowundu, 2010	Nigeria, 2009	302	151.1	152.1	41.7
Rohner, 2016	Sierra Leone, 2013	154	175.8	176	65.9
MOH-FGS, 2020	Somalia, 2018 - 19	236	269	424	454
Mabasa, 2019	South Africa, 2012 -13	565	164	183.3	147.9
Stinca, 2017	South Africa	207	174	189.8	151
Mtumwa, 2017	Tanzania, 2009 - 2010	947	136.8	151.9	147.9
Ba, 2020	Tanzania, 2015 -2016	266	156.1	160.6	146
Stinca, 2017	Tanzania, 2016	330	422	434.3	252.4
Chinyanga, 2006	Zimbabwe, 2006	94	115.5	128.5	137

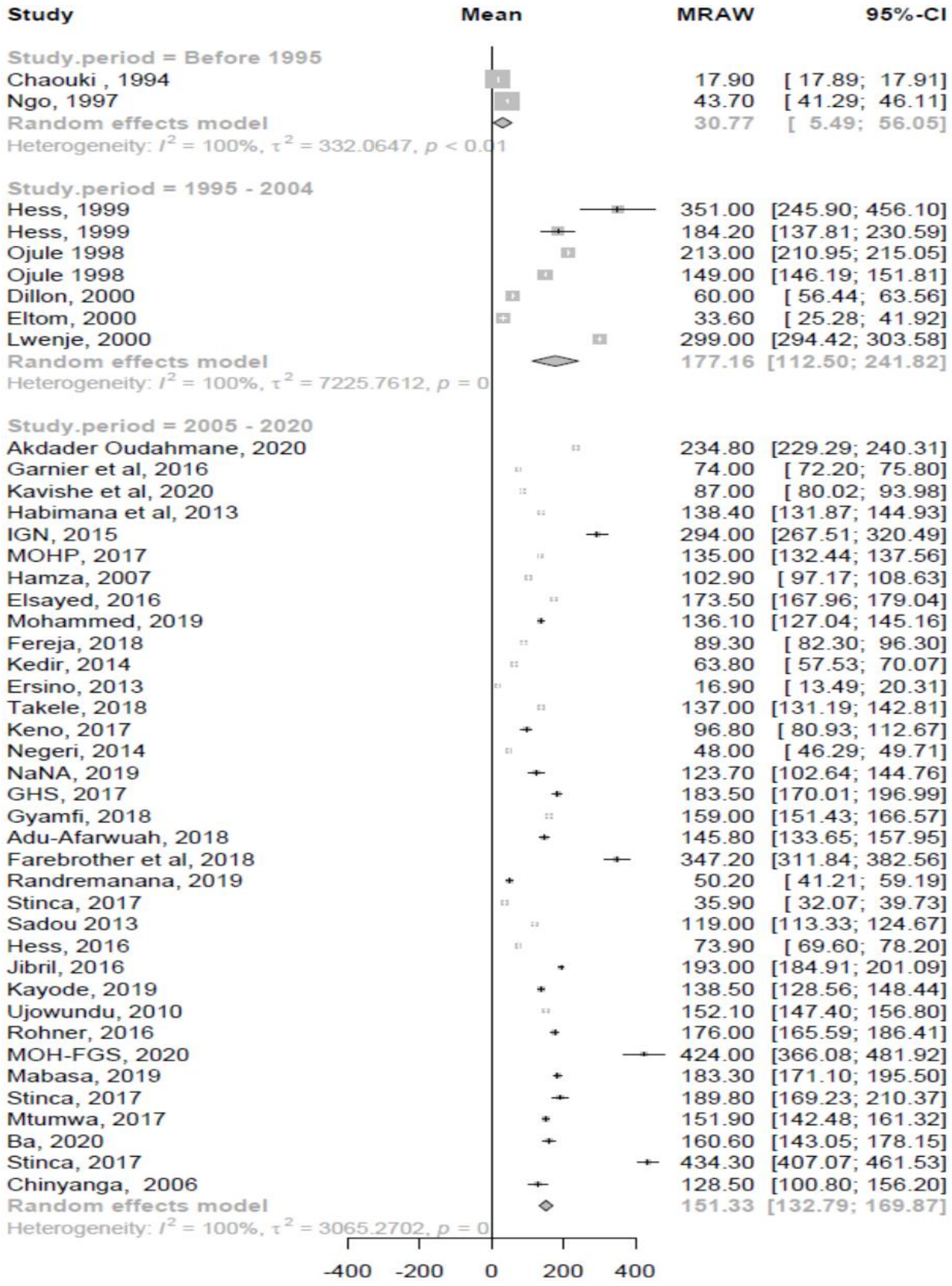


Figure 2. 3: Forest plot showing subgroup analysis of derived mean UIC (µg/L) of the studies conducted before 1995, 1995 to 2005, and 2005 to 2020

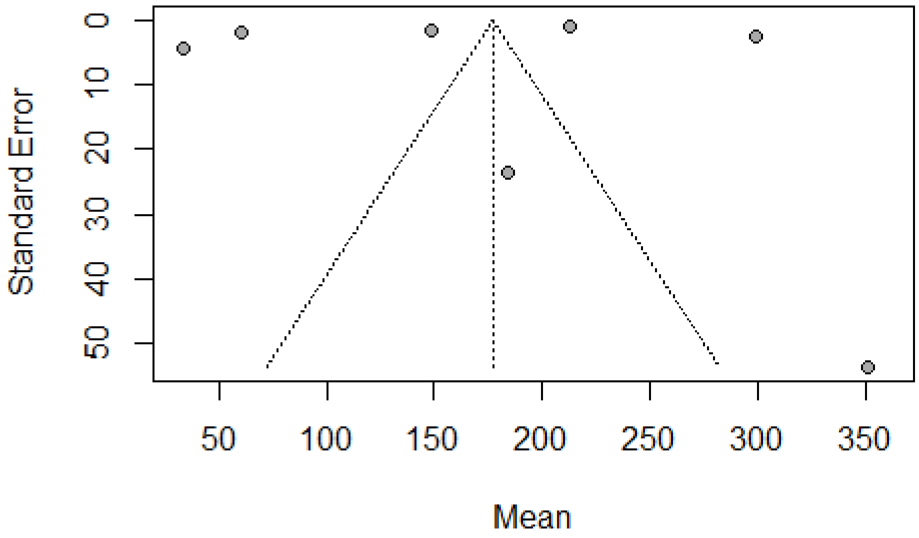


Figure 2. 4: The funnel plot of the studies carried out between 1995 and 2004

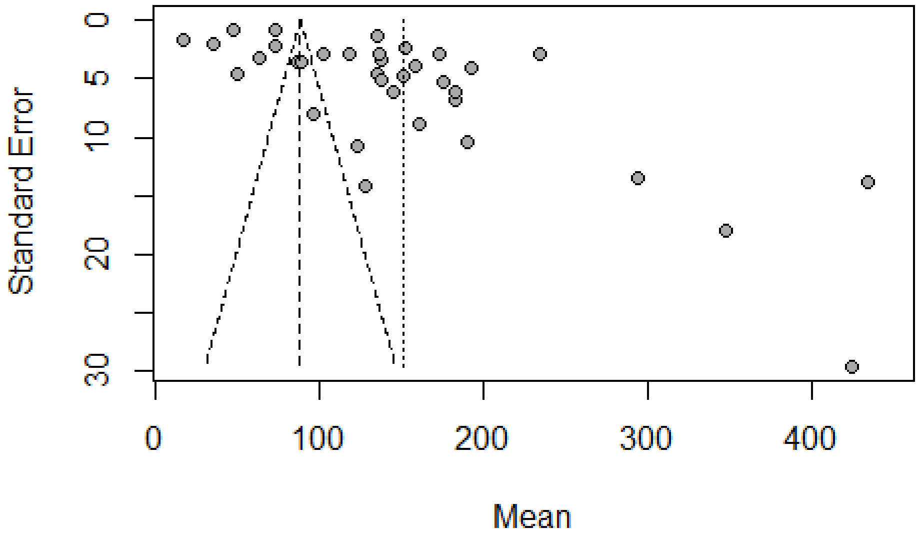


Figure 2. 5: The funnel plot of the studies carried out between 2005 and 2020

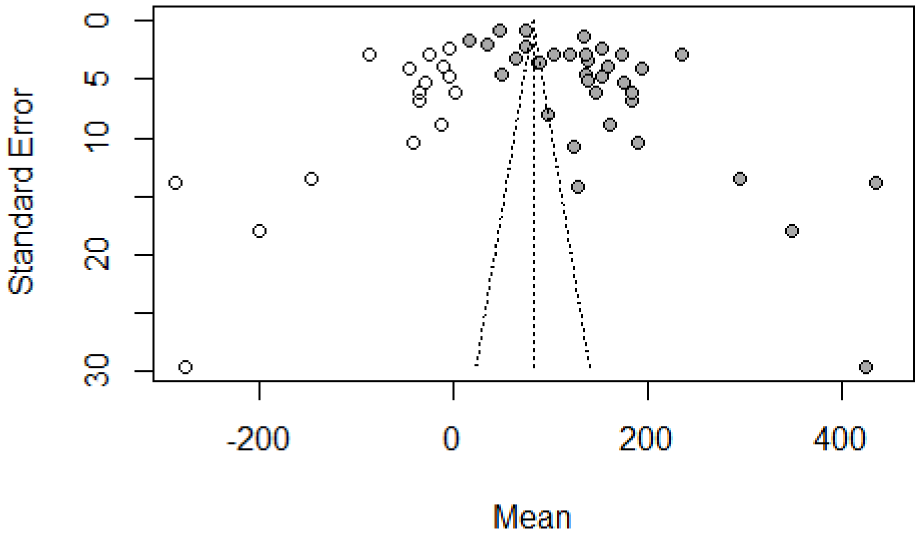


Figure 2. 6: The trim and fill funnel plot of the studies carried out between 2005 and 2020

2.5 Discussion

This review has found that pregnant women in Africa had moderate-to-severe iodine deficiency before the implementation of iodine supplementation in 1995. Mild to moderate iodine deficiency in pregnancy was still prevalent in several regions of various African countries by 2005, the year designated for elimination of iodine deficiency globally. However, there was significant improvement in the iodine nutrition status in pregnancy in Africa between 2005 and 2020 compared to the period before 1995 although this is still insufficient (median pMUIC < 150 µg/L). Overall, there is a paucity of nationwide representative data on iodine nutrition status in pregnancy in Africa. Pregnancy median UIC data was available for about 50% of the African countries with most derived from sub-national samples.

The limited available data before 1995 showed that some African countries had moderate to severe regional or nationwide iodine deficiency in pregnancy (23, 24). This may reflect the continental iodine nutritional status in pregnancy at that time since only about 10% of the general population in Africa had adequate iodine nutrition before 1995 (6). Protracted iodine deficiency predisposes to severe thyroid hyper-stimulation, which together with the prevalent dietary thiocyanates and nitrates in several African countries leads to inflammation, infiltration by immune cells, oxidative damage to thyroid parenchyma and necrosis (66). This is exacerbated by the increased loss of iodine through urine during pregnancy, which could account for the disproportionately higher rates of thyroid diseases among women (4, 7)

Following the initiation of iodine fortification of foodstuffs in most countries in 1995 and thereafter, the World Health Organisation (WHO) earmarked the 2005 as the year for elimination of iodine deficiency globally (14). Although the current study found a pooled UIC of 174.1µg/L from eligible studies conducted between 1995 and 2005, which suggests sufficient iodine intake during pregnancy, the number of studies was small and therefore not representative of all the pregnant women in Africa during this period. The studies also revealed that in several countries there were areas with optimum and others with insufficient iodine nutrition status in pregnancy. This demonstrates lack of equity in implementation of iodine deficiency mitigating strategies within individual countries. This could partly have been due to the dependence on median school age UIC (SAC UIC) as a surrogate measure of national iodine nutrition status. Median SAC UIC does not to accurately estimate iodine nutrition state in pregnancy (11, 67). Hence, in areas with marginally sufficient iodine intake as estimated using median SAC UIC, pregnant women and their unborn babies may still be at high risk of iodine deficiency. However, the level of iodine insufficiency as revealed in studies conducted between 1995 and 2005 was marginal compared to countries with data before 1995 implying a significant positive impact of iodine fortification on the degree of iodine deficiency in pregnancy in Africa.

Between 2005 and 2020, an increased number of national and sub-national surveys were conducted to assess the iodine nutrition status in pregnancy in several African countries. Some regions within individual countries had sufficient while others had various degrees of insufficient iodine intake in pregnancy more than 20 years after implementation of iodine fortification. Some of these subnational surveys revealed a pregnancy median UIC marginally above the sufficient level. This implies that large proportions of pregnant women may still be at risk of iodine deficiency and its attendant adverse effects.

2.6 Strengths and limitations

To our knowledge, this is the first systematic review aiming at assessing the level of iodine deficiency among pregnant women in Africa from the time of initiation of iodine supplementation to September 2020. This review was limited by the small number of studies before 1995 and by the subnational nature of the majority of studies conducted after 1995 most of which were from small geographical locations within the African countries hence not representative of national populations.

2.7 Conclusion

There is still paucity of data on iodine nutrition status in pregnancy from half of the countries in Africa. The available data shows a significant but inadequate improvement in the iodine nutrition status of pregnant women in several African countries after 1995. A few countries still have moderate-to-severe iodine deficiency in pregnancy at national or regional more than two decades after implementation of iodine food fortification.

References

1. Glinoer D. Clinical and biological consequences of iodine deficiency during pregnancy. Endocr Dev. 2007;10:62-85.
2. Zantour B AW, Marmouch H, Chebbi W. Hypothyroidism. In: E P, ed. Pregnancy in Current Topics in Hypothyroidism with Focus on Development. Rijeka, Croatia: In Tech; 2013:29-62.
3. Lazarus J. Thyroid Regulation and Dysfunction in the Pregnant Patient. In: Feingold KR AB, Boyce A, et al, ed. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2000; 2016.
4. Leung A, Pearce E, Braverman L. Iodine Nutrition in Pregnancy and Lactation. Endocrinology and metabolism clinics of North America. 2011;40:765-77.
5. Cheung K, Lafayette R. Renal Physiology of Pregnancy. Advances in chronic kidney disease. 2013;20:209-14.
6. Joy EJ, Ander EL, Young SD, Black CR, Watts MJ, Chilimba AD, et al. Dietary mineral supplies in Africa. Physiol Plant. 2014;151(3):208-29.
7. Tsegaye B, Ergete W. Histopathologic pattern of thyroid disease. East African medical journal. 2003;80:525-8.
8. Kishosha P, Galukande M, Gakwaya A. Selenium Deficiency a Factor in Endemic Goiter Persistence in Sub-Saharan Africa. World journal of surgery. 2011;35:1540-5.
9. Taga I, Oumbe VA, Johns R, Zaidi MA, Yonkeu NJ, Altosaar I. Youth of West Cameroon are at high risk of developing IDD due to low dietary iodine and high dietary thiocyanate. Afr Health Sci. 2008;8(4):227-33.
10. IGN. Global Scorecard of Iodine Nutrition in 2019 in the general population based on school age children (SAC). Zurich, Switzerland: Iodine Global Network; 2019.
11. Gowachirapant S, Winichagoon P, Wyss L, Tong B, Baumgartner J, Melse A, et al. Urinary Iodine Concentrations Indicate Iodine Deficiency in Pregnant Thai Women but Iodine Sufficiency in Their School-Aged Children. The Journal of nutrition. 2009;139:1169-72.
12. Lazarus J. Iodine Status in Europe in 2014. European thyroid journal. 2014;3:3-6.
13. IGN. Global Scorecard of Iodine Nutrition in 2017 in the general population and in pregnant women (PW). Zurich, Switzerland: Iodine Global Network 2017.
14. WHO. Assessment of iodine deficiency disorders and monitoring their elimination : a guide for programme managers. 3rd ed. Geneva: World Health Organization; 2007.
15. WHO. Proceedings of the WHO Technical Consultation on control of iodine deficiency in pregnant women and young children Geneva: World Health Organization; 2005.
16. Harika R, Faber M, Samuel F, Kimiywe J, Mulugeta A, Eilander A. Micronutrient Status and Dietary Intake of Iron, Vitamin A, Iodine, Folate and Zinc in Women of Reproductive Age and Pregnant Women in Ethiopia, Kenya, Nigeria and South Africa: A Systematic Review of Data from 2005 to 2015. Nutrients. 2017;9:1096.
17. Businge CB, Longo-Mbenza B, Kengne AP. The prevalence of insufficient iodine intake in pregnancy in Africa: protocol for a systematic review and meta-analysis. Systematic Reviews. 2019;8(1):209.
18. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.

19. Pienaar E, Grobler L, Busgeeth K, Eisinga A, Siegfried N. Developing a geographic search filter to identify randomised controlled trials in Africa: finding the optimal balance between sensitivity and precision. Health Info Libr J. 2011;28(3):210-5.

20. Hoy D, Brooks P, Woolf A, Blyth F, March L, Bain C, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol. 2012;65(9):934-9.

21. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. Stat Methods Med Res. 2018;27(6):1785-805.

22. Shi J, Luo D, Weng H, Zeng XT, Lin L, Chu H, et al. Optimally estimating the sample standard deviation from the five-number summary. Res Synth Methods. 2020;11(5):641-54.

23. Chaouki ML, Benmiloud M. Prevention of iodine deficiency disorders by oral administration of lipiodol during pregnancy. Eur J Endocrinol. 1994;130(6):547-51.

24. Ngo DB, Dikassa L, Okitolonda W, Kashala TD, Gervy C, Dumont J, et al. Selenium status in pregnant women of a rural population (Zaire) in relationship to iodine deficiency. Trop Med Int Health. 1997;2(6):572-81.

25. Hess SY, Zimmermann MB, Staubli-Asobayire F, Tebi A, Hurrell RF. An evaluation of salt intake and iodine nutrition in a rural and urban area of the Côte d'Ivoire. Eur J Clin Nutr. 1999;53(9):680-6.

26. Ojule AC, Osotimehin BO. Maternal and neonatal thyroid status in Saki, Nigeria. Afr J Med Med Sci. 1998;27(1-2):57-61.

27. Eltom A, Eltom M, Elnagar B, Elbagir M, Gebre-Medhin M. Changes in iodine metabolism during late pregnancy and lactation: a longitudinal study among Sudanese women. Eur J Clin Nutr. 2000;54(5):429-33.

28. Dillon JC, Milliez J. Reproductive failure in women living in iodine deficient areas of West Africa. Bjog. 2000;107(5):631-6.

29. Lwenje S, Mtetwa VSB, Ginindza S. Assessment of Iodine Deficiency in Pregnant Women in Swaziland. UNISWA Research Journal of Agriculture, Science and Technology. 2000;4.

30. IGN. Iodine-rich groundwater, and not iodized salt, provides children and pregnant women in Djibouti with sufficient iodine. Iodine Global Network; 2017.

31. Ministry of Health FGS F, Somaliland, UNICEF, Brandpro, GroundWork. Somalia Micronutrient Survey 2019. Mogadishu, Somalia: MOH-FGS; 2020.

32. GHS. National Iodine Survey Report, Ghana 2015. Accra: Ghana Health Service 2017.

33. Rohner F, Wirth JP, Woodruff BA, Chiwile F, Yankson H, Sesay F, et al. Iodine Status of Women of Reproductive Age in Sierra Leone and Its Association with Household Coverage with Adequately Iodized Salt. Nutrients. 2016;8(2):74.

34. Ba DM, Ssentongo P, Na M, Kjerulff KH, Liu G, Du P, et al. Factors Associated with Urinary Iodine Concentration among Women of Reproductive Age, 20-49 Years Old, in Tanzania: A Population-Based Cross-Sectional Study. Curr Dev Nutr. 2020;4(5):nzaa079.

35. Ministry of Health and Population (Egypt) (MOHP) U. Egypt Iodine Survey 2014 / 2015 Summary Report. Cairo: MOHP; 2017.

36. NaNA-Gambia U, Gambia Bureau of Statistics (GBOS) . Gambia National Micronutrient Survey 2018. Banjul, Gambia: National Nutrition Agency (NaNA)-Gambia; 2019.

37. Mtumwa A, Ntwenya J, Paul E, Huang M, Vuai S**.** Socio-economic and spatial correlates of subclinical iodine deficiency among pregnant women age 15–49 years in Tanzania. BMC Nutrition. 2017;3.

38. Garnier D KS, Kargougou R, Ouaro B, Petry N, Rohner F, Sawadago P, Wirth J, Woodruff BA, Zongo U**.** Iodine status of women of reproductive age and school-age children in Burkina Faso and its association with adequately iodized household salt – Results from a national survey Micronutrient Forum Global Conference. Cancun, Mexico; 2016.

39. Kavishe FP NI, Tom C, Mwai JM, Gorstein J**.** Report on the 2019 consultation on Sustainable Prevention and Control of Iodine Deficiency Disorders in Eastern and Southern Africa held at Sarova Whitesands Hotel 5-7 November 2019, Mombasa, Kenya. Dar es Salaam: Iodine Global Network (IGN); 2020.

40. Randremanana RV, Bastaraud A, Rabarijaona LP, Piola P, Rakotonirina D, Razafinimanana JO, et al. First national iodine survey in Madagascar demonstrates iodine deficiency. Matern Child Nutr. 2019;15(2):e12717.

41. Adu-Afarwuah S, Young RT, Lartey A, Okronipa H, Ashorn P, Ashorn U, et al. Supplementation during pregnancy with small-quantity lipid-based nutrient supplements or multiple micronutrients, compared with iron and folic acid, increases women's urinary iodine concentration in semiurban Ghana: A randomized controlled trial. Matern Child Nutr. 2018;14(2):e12570.

42. Akdader-Oudahmane S, Hamouli-Saïd Z, Zimmermann MB, Kherrab H, Azzoug S, Meskine D**.** High prevalence of TPO-Abs and subclinical hypothyroidism in iodine-sufficient pregnant women in Northern Algeria. J Trace Elem Med Biol. 2020;61:126533.

43. Chinyanga EA, Chidede O, Machisvo A, Choga T, Malaba L, Sibanda N**.** Urinary iodine excretion in pregnant women as an index of the impact of a national iodization programme. Cent Afr J Med. 2006;52(7-8):78-83.

44. Elsayed NA A-MS, Mohktar SA, Tayel DI. Almost all pregnant women in Alexandria are iodine sufficient. IDD newsletter. 2016.

45. Farebrother J, Zimmermann MB, Abdallah F, Assey V, Fingerhut R, Gichohi-Wainaina WN, et al. Effect of Excess Iodine Intake from Iodized Salt and/or Groundwater Iodine on Thyroid Function in Nonpregnant and Pregnant Women, Infants, and Children: A Multicenter Study in East Africa. Thyroid. 2018;28(9):1198-210.

46. Fereja M, Gebremedhin S, Gebreegziabher T, Girma M, Stoecker BJ**.** Prevalence of iodine deficiency and associated factors among pregnant women in Ada district, Oromia region, Ethiopia: a cross- sectional study. BMC Pregnancy Childbirth. 2018;18(1):257.

47. Negeri Z, Gobena T, Kassim M**.** Determining the Magnitude of Iodine Deficiency and Its Associated Risk Factors among Pregnant Women Visiting Jimma University Specialized Hospital for Antenatal Care. 2014.

48. Keno T, Ahrens C, Lauvai J, Kurabachew H, Biesalski H, Scherbaum V**.** Iodine status in pregnant women and school children of the Aira district in Ethiopia. NFS Journal. 2017;7.

49. Takele WW, Alemayehu M, Derso T, Tariku A**.** Two-thirds of pregnant women attending antenatal care clinic at the University of Gondar Hospital are found with subclinical iodine deficiency, 2017. BMC Res Notes. 2018;11(1):738.

50. Ersino G, Tadele H, Bogale A, Abuye C, Stoecker BJ**.** Clinical assessment of goiter and low urinary iodine concentration depict presence of severe iodine deficiency in pregnant Ethiopian

women: a cross-sectional study in rural Sidama, southern Ethiopia. Ethiop Med J. 2013;51(2):133-41.

51. Mohammed H, Marquis GS, Aboud F, Bougma K, Samuel A. Pre-pregnancy iodized salt improved children's cognitive development in randomized trial in Ethiopia. Matern Child Nutr. 2020;16(3):e12943.

52. Kedir B, Berhane Y, Worku A. Subclinical Iodine Deficiency among Pregnant Women in Haramaya District, Eastern Ethiopia: A Community-Based Study. Journal of nutrition and metabolism. 2014;2014:878926.

53. Gyamfi D, Wiafe Y, Danquah K, Adankwah E, Amissah GA, Odame A. Urinary iodine concentration and thyroid volume of pregnant women attending antenatal care in two selected hospitals in Ashanti Region, Ghana: A comparative cross-sectional study. BMC Pregnancy and Childbirth. 2018;18.

54. Habimana L, Twite KE, Wallemacq P, De Nayer P, Daumerie C, Donnen P, et al. Iodine and iron status of pregnant women in Lubumbashi, Democratic Republic of Congo. Public Health Nutr. 2013;16(8):1362-70.

55. Hamza R YA, Mouharam W, El Danasoury A Maternal and Neonatal Iodine Nutrition in Cairo Internet Journal of Pediatrics and Neonatology. 2007;8(2):1 - 6.

56. Hess S, Ouedraogo C, Young R, Bamba I, Stinca S, Zimmermann M, et al. Urinary iodine concentration identifies pregnant women as iodine deficient yet school-aged children as iodine sufficient in rural Niger. Public health nutrition. 2016;20:1-8.

57. Jibril M-B, Abbiyesuku F, Aliyu IS, Randawa A, Adamu R, Akuyam S, et al. Nutritional iodine status of pregnant women in Zaria, North-Western Nigeria. Sub-Saharan African Journal of Medicine. 2016;3:41.

58. Kassim IA, Moloney G, Busili A, Nur AY, Paron P, Jooste P, et al. Iodine intake in Somalia is excessive and associated with the source of household drinking water. J Nutr. 2014;144(3):375-81.

59. Kayode O, Odeniyi I, Ob O, Iwuala S, Odukoya O, Fasanmade O. Iodine status in pregnant Nigerian women; Does Gestational age matters? Journal of Clinical Sciences. 2019;16:20.

60. Olife I, Okaka A, Dioka C, Meludu S, Orisakwe O. Iodine Status and the Effect of Soil Erosion on Trace Elements in Nanka and Oba Towns of Anambra State, Nigeria. Annali di chimica. 2007;97:895-903.

61. Sadou H, Seyfoulaye A, Alma M, Daouda H. Inadequate status of iodine nutrition among pregnant women residing in three districts of Niamey, the Niger Republic's capital. Maternal & child nutrition. 2013;10.

62. Stinca S, Herter-Aeberli I, Chabaa L, Cherkaoui M, El ansari N, Aboussad A, et al. Moderate-to-Severe Iodine Deficiency in the "First 1000 Days" Causes More Thyroid Hypofunction in Infants Than in Pregnant or Lactating Women. The Journal of Nutrition. 2017;147:jn244665.

63. Stinca S, Hunziker-Weibel S, Herter-Aeberli I, Fingerhut R, Gowachirapant S, Hess S, et al. Dried Blood Spot Thyroglobulin as a Biomarker of Iodine Status in Pregnant Women. The Journal of Clinical Endocrinology & Metabolism. 2016;102:jc.2016-829.

64. Mabasa E, Mabapa S, Jooste PL, Mbhenyane XG. Iodine status of pregnant women and children age 6 to 12 years feeding from the same food basket in Mopani district, Limpopo province, South Africa. South African Journal of Clinical Nutrition. 2018;32:1-7.

65. Cosmas U, Ukoha A, Agha C, Nwachukwu N, Igwe K**.** Assessment of current iodine status of pregnant women in a suburban area of Imo State Nigeria, twelve years after universal salt iodization. African Journal of Biochemistry Research. 2010;4:6-12.

66. Contempré B, Escobar G, Denef J-f, Dumont J, Many M-C**.** Thiocyanate Induces Cell Necrosis and Fibrosis in Selenium- and Iodine-Deficient Rat Thyroids: A Potential Experimental Model for Myxedematous Endemic Cretinism in Central Africa. Endocrinology. 2004;145:994-1002.

67. Andersson M, Karumbunathan V, Zimmermann MB**.** Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744-50.

CHAPTER 3

Iodine nutrition status in Africa: potentially high prevalence of iodine deficiency in pregnancy even in countries classified as iodine sufficient

Businge CB, Longo-Mbenza B, Kengne AP. Iodine nutrition status in Africa: potentially high prevalence of iodine deficiency in pregnancy even in countries classified as iodine sufficient. Public Health Nutr. 2020; 3:1-6. doi: 10.1017/S1368980020002384.

Abstract

Background

Iodine deficiency was endemic in many African countries before the introduction of iodine fortification mainly through universal salt iodization programs about 25 years ago. However, there is a scarcity of data on the level of iodine nutrition in pregnancy in Africa. Women living in settings with pregnancy median urinary iodine concentration (pMUIC) below 150 µg/L are at risk of iodine deficiency related pregnancy complications.

Objective

To assess the burden of iodine deficiency in pregnancy in Africa using estimated pregnancy median urinary iodine concentration.

Design

pMUIC for each African country was estimated using a regression equation derived by correlating the school-age children (SAC) median UIC (mUIC) and the pMUIC from countries around the globe, and the SAC mUIC data for African countries obtained from the Iodine Global Network (IGN) 2017 and 2019 score cards.

Results: A cut-off school age median UIC ≤175 µg/L correlated with insufficient iodine intake in pregnancy (pregnancy median UIC ≤150 µg/L). Twenty-two African countries had median SAC UIC <175 µg/L, which correlated with insufficient iodine intake during pregnancy (pMUIC <150 µg/L). However, nine of these countries had adequate iodine intake based on SAC median UIC.

Conclusion: There is likely a high prevalence of insufficient iodine intake in pregnancy, including in some African countries classified as having adequate iodine intake in the general population. A SAC median UIC ≤175 µg/L predicts insufficient iodine intake among pregnant women in these settings.

Keywords–iodine deficiency, urinary iodine concentration, pregnancy, Africa

3.1 Introduction

Over 89% of the African population was at risk of insufficient iodine intake before the wide scale implementation of population-oriented iodine supplementation through food fortification around 1995 (1, 2). Iodine deficiency to a large extent was a result of low iodine content of soil and groundwater, and diet rich in goitrogen (3-5). As from 1995, nationwide universal salt iodization became the main approach of population-based iodine fortification in most African countries (6). Other vehicles of iodine fortification in some in African countries include bouillon cubes, canned foodstuffs, and other processed foods which are not necessarily regularly consumed (7, 8).

Iodine nutritional status in countries in Africa and elsewhere around the world has been monitored using school-age children (SAC) median urinary iodine concentration (mUIC) (9). However, SAC mUIC does not correctly predict iodine nutritional status in pregnancy (10, 11). This can be attributed to the pregnancy induced physiological changes that predispose the pregnant women to iodine deficiency through increased thyroid hormone production, increased renal perfusion, increased iodine filtration and urinary iodine excretion in addition to increased transfer of iodine to the foetus (12). In the general population, a median urinary iodine concentration (UIC) of 100 - 199 μg/L is considered sufficient nutrition, while in pregnancy a median UIC (pMUIC) below 150 μg/L indicates insufficient iodine intake (12). The prevalence of insufficient iodine intake in pregnancy is likely to be high in African countries where a significant proportion of the population has median UIC below 150 μg/L. In such countries, large proportions of women of childbearing age could therefore be at risk of iodine deficiency in pregnancy and associated adverse outcomes such as miscarriage, foetal and childhood growth restriction, still-births, postpartum thyroiditis, subclinical and overt hypothyroidism, dyslipidemia, neuro-cognitive and psychomotor deficits (13-15). There is a paucity of data on the iodine nutrition status in pregnancy in Africa. Hence this study was carried out to assess the prevalence of iodine deficiency in pregnancy in Africa using estimated pregnancy median UIC.

3.2 Methods

We estimated iodine nutrition in pregnancy using the most recent SAC median UIC from the Iodine Global Network (IGN) 2019 score card (16). The IGN publishes and updates the national iodine nutrition status for most countries every 2 years. In the IGN 2017 score card 65 countries, including 11 in Africa, had data showing both SAC median UIC and pregnancy median UIC (17). We plotted the SAC median UIC against the pregnancy median UIC of these 65 countries to depict the linear relationship between the SAC median UIC and the pregnancy median UIC (Figure 3.1) defined by the following regression equation:

Pregnancy MUIC = 6.8587 + 0.8208*SAC mUIC ($r = 0.8164$, $p < 0.001$; $r^2 = 0.6665$)

(Figure 3.1)

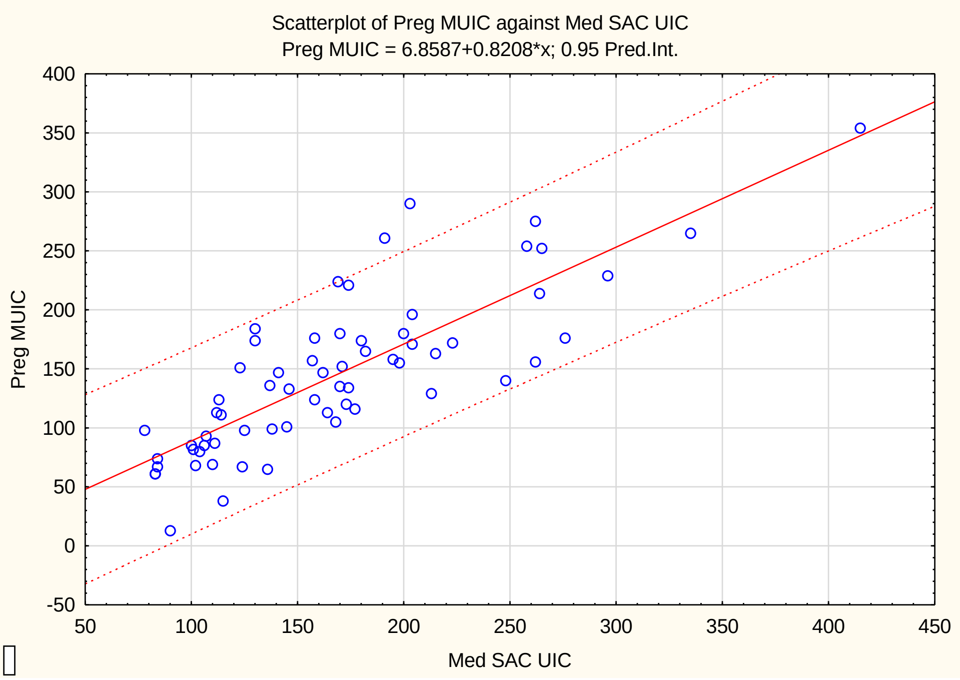


Figure 3. 1: Scatter plot, line of best fit and regression equation of pregnancy MUIC against SAC median UIC

Using this regression equation and the most recent SAC median UIC of African countries obtained from the IGN 2019 Score Card (16), the pregnancy median UIC was estimated for all the African countries with recent iodine nutrition survey data.

3.3 Results

Forty-nine of the 54 countries in Africa had data on the iodine nutrition status in the general population, estimated mainly though SAC UIC surveys (44/49) carried out between 2002 and 2018, except for Botswana, Central African Republic, Rwanda and Swaziland where the most recent SAC UIC surveys were conducted between 1993 and 1999. Eleven of these 44 countries had both SAC median UIC and pregnancy median UIC survey data (16, 17). Three countries had the iodine nutrition status estimated through surveys involving samples of women of reproductive age (Gambia, Madagascar, and Sierra Leone), one from a sample of adults (Mauritius) and another from a sample with broad age (Central African Republic). Five countries (Congo, the Comoros, Libya, Sao Tome and Principe, and Seychelles) had no data on iodine nutrition.

Of the 49 countries with UIC survey data available, three had insufficient iodine intake with moderate deficiency (20–49 µg/L); seven had insufficient iodine intake with mild deficiency (50–99 µg/L); 18 had adequate iodine intake (100–199 µg/L); 17 had more than adequate iodine intake (200–299 µg/L) exposing susceptible population groups to iodine-induced hyperthyroidism; while 5 had excessive iodine intake (≥300 µg/L) which increases risk of autoimmune thyroid disease in addition to iodine induced hyperthyroidism (18) (Table 3.1).

Table 3. 1: SAC median UIC, measured and estimated Pregnancy Median UIC (µg/L) for African Countries

Country	SAC mUIC	*Measured pMIUC	Estimated pMIUC
Northern Africa			
Algeria	241		204.7
Egypt	170	135	146.4
Libya	NA		NA
Morocco	96	31	85.7
Sudan	66		61.0
Tunisia	220		187.4
Western Sahara	NA		NA
Central Africa			
Angola	29		30.7
Cameroon	190		162.8
‡**CAR**	21		24.1
Chad	213		181.7
Congo	NA		NA

DRC	249		211.2
Equatorial-Guinea	564		470
Gabon	196		167.74
Sao Tome & Principe	NA		NA
West Africa			
Benin	318		267.9
Burkina Faso	99	74	88.1
Cape Verde	115		101.3
Cote d'Ivoire	203		173.5
Gambia	143		124.3
Ghana	130	184	113.6
Guinea	139		121.0
Guinea Bissau	110		97.2
Liberia	244	254	218.6
Mali	69		56.64
Mauritania	179		153.8
Niger	101	82	89.8
Nigeria	130		113.6
Senegal	104	80	92.2
Sierra Leone	203	176	173.8
Togo	171		147.2
Eastern Africa			
Burundi	70		64.3
Comoros	NA		NA
Djibouti	335	265	281.8
Eritrea	175		150
Ethiopia	104		92.2
Kenya	208		177.6
Madagascar	46		44.6
Malawi	269		227.7
Mozambique	60		49.3

‡Rwanda	298		251.5
Somalia	417		349
South Sudan	94		84.0
Tanzania	204	171	174.3
Uganda	464		387.7
Southern Africa			
‡Botswana	219		186.6
Lesotho	215		183.3
Namibia	216		177.3
‡Swaziland	120		105.4
South Africa	215	163	183.3
Zambia	245		208.0
Zimbabwe	220		187.4

NA: data not available, ‡data collected before 2002, *Measured pregnancy median UIC (IGN 2017)

The African countries with the lowest iodine intake in the general population (moderate iodine deficiency status) were Angola, Central African Republic and Madagascar. Most countries with mild iodine deficiency were from the Eastern and Western Africa while countries with optimal iodine nutrition in the general population were evenly distributed across northern, eastern and western regions of Africa. All Southern Africa countries apart from Swaziland had more than adequate iodine intake in the general population (SAC mUIC 200 - 299 µg/L). Benin, Djibouti, Equatorial-Guinea, Somalia and Uganda had excessive iodine intake (SAC mUIC > 300 µg/L), likely attributable to enthusiastic implementation of iodine fortification programs without regular evaluation (18); and in the case of Somalia and Djibouti, use of ground water that has a high iodine concentration (5).

Of the 11 African countries with pregnancy median UIC survey data, Burkina Faso, Egypt, Morocco, Niger and Senegal had inadequate iodine intake during pregnancy. Ghana, Sierra Leone, South Africa, and Tanzania had adequate intake, while Djibouti and Liberia had more than enough iodine intake in pregnancy (17).

The pregnancy median UIC values calculated in the current study are comparable to findings from population-based surveys from nine countries. The exceptions include Ghana (with sufficient intake) found to have insufficient iodine intake in pregnancy in the current study and Liberia (more than adequate intake) classified as having adequate iodine intake in pregnancy (Figure 3.2).

After applying the WHO criteria for monitoring progress towards sustainable IDD elimination where pregnancy median UIC <150 μg/L indicates insufficient iodine intake during pregnancy (19), 22 of the 49 countries with median SAC data available, had insufficient iodine nutrition during pregnancy with pregnancy (Figure 8.2). Sixteen had adequate and five had more than adequate iodine intake in pregnancy. None of the African countries with available data had pregnancy median UIC in the range of excessive iodine intake (pMUIC >500 μg/L). The current study also found that a cut-off school age median UIC of ≤175 μg/L correlated with insufficient iodine intake in pregnancy (pMUIC ≤150 μg/L) (Figure 3.1).

Egypt, Gambia, Ghana, Guinea, Guinea-Bissau, Niger, Nigeria, Senegal and Togo are the African countries classified as having sufficient iodine intake in the general population (IGN, 2019) that in the current study have been found to have insufficient iodine intake in pregnancy (estimated pMUIC <150 μg/L).

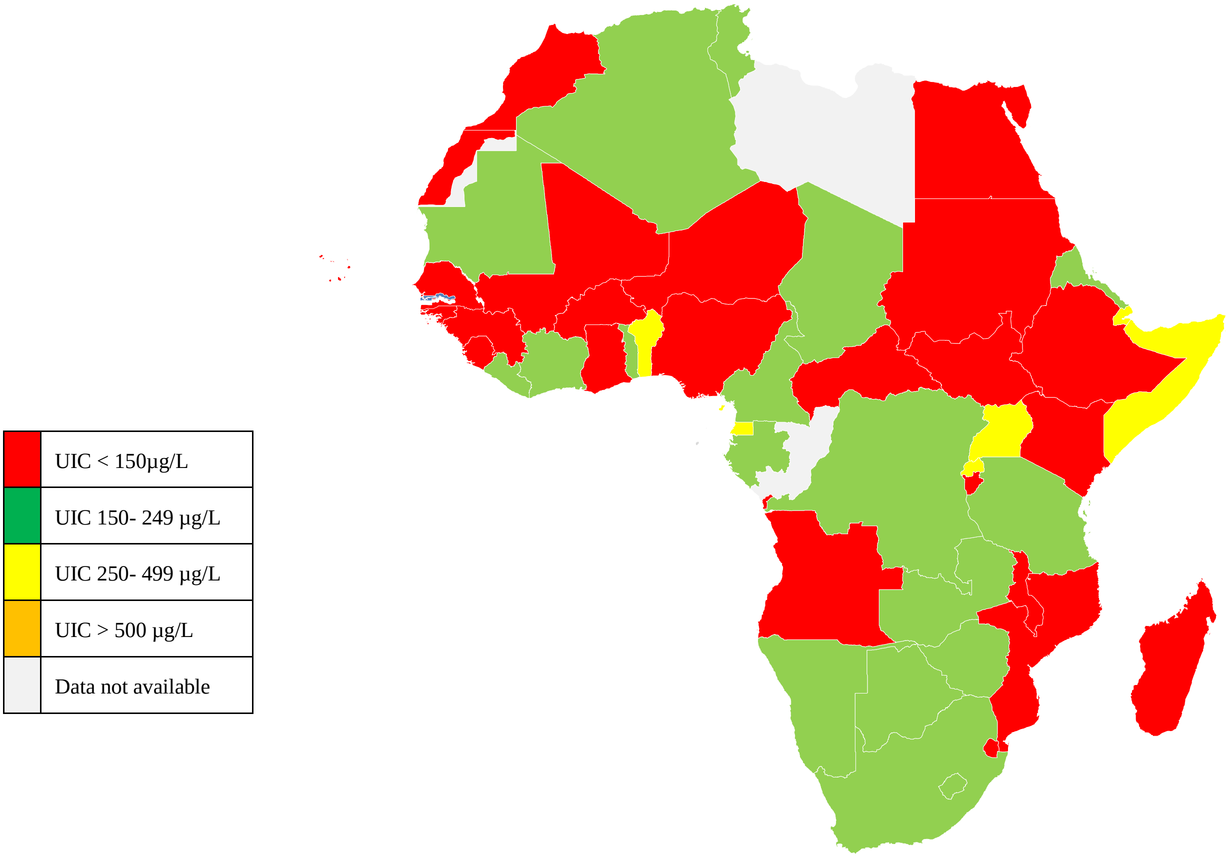


Figure 3. 2: Map of Africa showing country specific iodine nutrition status during pregnancy (pregnancy median UIC) as estimated from median SAC UIC

3.4 Discussion

Despite more than 25 years of iodine fortification in Africa, the prevalence of iodine deficiency in pregnancy in Africa appears to have remained high. This high prevalence seems to be masked by the reliance on SAC mUIC for the monitoring of the iodine nutrition status of populations. Although 40 out 49 African countries are currently classified as having adequate or more than adequate iodine intake based on SAC mUIC (16, 17), the current study estimated that pregnant women in more than half of these countries may be prone to insufficient iodine intake. This finding correlates with results from other studies that have shown SAC median

UIC to inaccurately reflect the state of iodine nutrition of pregnant women within the same study settings (10, 11, 20). The observed discrepancy between the SAC mUIC and pMUIC can be attributed to variations in the access to iodine fortified foodstuffs, differences in food choices between school-age children and pregnant women, and the high depletion of iodine stores in pregnancy secondary to the increased thyroid hormone production, elevated renal filtration and urinary iodine excretion, and trans-placental transfer of iodine to the foetus (10-12).

If future studies confirm the results of the current study, there will be a need to revisit ongoing iodine fortification strategies especially in endemic areas for iodine deficiency. While universal salt iodization is a popular and inexpensive approach suggested by the WHO and ICCIDD, African countries with pregnancy median UIC <150 µg/L could require iodine supplementation during pregnancy, especially those countries with SAC median UIC below 175 µg/L. Oral iodized oil has previously been shown to improve and maintain the level of iodine nutrition within normal limits among adults and children in areas with endemic iodine deficiency, without causing neonatal or maternal hypothyroidism, hyperthyroidism or auto-immune thyroiditis (21-23). This may help mitigate the morbidity which may accrue from widespread insufficient iodine nutrition in pregnancy in Africa.

Since iodine deficiency tends to induce the most adverse effects during pregnancy, infancy and lactation, an appropriate assessment of iodine nutrition would benefit from direct estimation of the pregnancy median UIC as one of the baseline metrics. This can be complemented by assessing the iodine nutrition status of infants although this may be quite challenging. This could however be achieved through the use of the dry blood spot thyroglobulin test (24), if this becomes readily available for public health use in iodine deficient endemic areas and populations.

Although the current study found that five African countries are likely to have more than adequate iodine intake in pregnancy, the thyroid dysfunction associated with high iodine intake tends to be transient and mild especially if the increase in iodine intake in formerly iodine deficient countries was gradual (18). Populations in countries with endemically higher water iodine content such as Djibouti and Somalia tend to be tolerant to high daily iodine intake (5). However, it is recommended that excessive iodine intake should be prevented especially in countries formerly with chronic iodine deficiency (25).

3.5 Strength and limitations

This study has provided an estimate the level of iodine nutrition state in pregnancy for most countries in Africa and the minimum SAC median IUC that can be used to predict adequate iodine nutrition status among pregnant women in these settings. The current study design precluded reliable estimation of the true magnitude of iodine deficiency in pregnancy. However, the data from the current study forms a basis for further research to ascertain the true iodine nutrition status among pregnant women in the various countries in Africa. While deriving the regression equation for estimating the pMIUC, we assumed that SAC mUIC would correlate with pMIUC. However, this may not always be the case given the different vehicles of iodine fortification around the world and the diverse nutritional practices during pregnancy.

3.6 Conclusion

Insufficient iodine nutrition in pregnancy seems to be prevalent in several African countries even in countries thought to have sufficient iodine intake in the general population based on SAC mUIC surveys. The SAC mUIC, the current recommended method by WHO to assess iodine nutrition status, seems to conceal insufficient iodine nutrition in pregnancy. Optimum assessment of iodine nutrition status in pregnant women may not be achieved without direct estimation of the pregnancy mUIC. Oral iodized oil supplementation could be a possible remedy for insufficient iodine nutrition in pregnancy in countries with high proportion of the populations still prone to moderate-to-severe iodine deficiency despite universal salt iodization.

References

1. Kavishe F**.** Can Africa Meet the Goal of Eliminating Iodine-Deficiency Disorders by the Year 2000? Food and Nutrition Bulletin. 1996;17:262-7.
2. Joy E, Ander E, Young S, Black C, Watts M, Chilimba A, et al. Dietary mineral supplies in Africa. Physiologia Plantarum. 2014;151.
3. Taga I, Oumbe V, Johns R, Zaidi M, Yonkeu J, Altosaar I**.** Youth of West Cameroon are at high risk of developing IDD due to low dietary iodine and high dietary thiocyanate. African health sciences. 2008;8:180-5.
4. Kassim IA, Moloney G, Busili A, Nur AY, Paron P, Jooste P, et al. Iodine intake in Somalia is excessive and associated with the source of household drinking water. J Nutr. 2014;144(3):375-81.
5. Farebrother J, Zimmermann M, Abdallah F, Assey V, Fingerhut R, Gichohi-Wainaina W, et al. The effect of excess iodine intake from iodized salt and/or groundwater iodine on thyroid function in non-pregnant and pregnant women, infants and children: a multicenter study in East Africa. Thyroid. 2018;28.
6. de Benoist B, McLean E, Andersson M, Rogers L**.** Iodine deficiency in 2007: global progress since 2003. Food Nutr Bull. 2008;29(3):195-202.
7. Abu B, Wilna O-T, Aryeetey R**.** Risks of excess iodine intake in Ghana: current situation, challenges, and lessons for the future. Annals of the New York Academy of Sciences. 2018.
8. Spohrer R, Knowles J, Jallier V, Ndiaye B, Indorf C, Guinot P, et al. Estimation of population iodine intake from iodized salt consumed through bouillon seasoning in Senegal. Annals of the New York Academy of Sciences. 2015;1357:43-52.
9. WHO**.** Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. 3rd ed. Geneva: World Health Organisation; 2007.
10. Gowachirapant S, Winichagoon P, Wyss L, Tong B, Baumgartner J, Melse A, et al. Urinary Iodine Concentrations Indicate Iodine Deficiency in Pregnant Thai Women but Iodine Sufficiency in Their School-Aged Children. The Journal of nutrition. 2009;139:1169-72.
11. Wong E, Sullivan K, Perrine C, Rogers L, Pena-Rosas J**.** Comparison of Median Urinary Iodine Concentration as an Indicator of Iodine Status among Pregnant Women, School-Age Children, and Nonpregnant Women. Food and nutrition bulletin. 2011;32:206-12.
12. Zantour B AW, Marmouch H, Chebbi W Hypothyroidism in Pregnancy. In: E P, ed. Current Topics in Hypothyroidism with Focus on Development. Rijeka, Croatia: In Tech 2013: 29-62.
13. Zimmermann MB, Boelaert K**.** Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 2015;3(4):286-95.
14. de Escobar GM, Obregón MJ, del Rey FE**.** Iodine deficiency and brain development in the first half of pregnancy. Public Health Nutr. 2007;10(12a):1554-70.
15. Lee KW, Shin D, Song WO**.** Low Urinary Iodine Concentrations Associated with Dyslipidemia in US Adults. Nutrients. 2016;8(3):171.
16. IGN**.** Global Scorecard of Iodine Nutrition in 2019 in the general population based on school age children (SAC). Zurich, Switzerland: Iodine Global Network; 2019.
17. IGN**.** Global Scorecard of Iodine Nutrition in 2017 in the general population and in pregnant women (PW). Zurich, Switzerland: Iodine Global Network; 2017.

18. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744-50.

19. De Benoist B AM, Egli I, Takkouche B, Allen H Iodine status worldwide: WHO Global Database on Iodine Deficiency. Geneva WHO; 2004.

20. Lazarus J. Iodine Status in Europe in 2014. European thyroid journal. 2014;3:3-6.

21. Benmiloud M, Chaouki ML, Gutekunst R, Teichert HM, Wood WG, Dunn JT. Oral iodized oil for correcting iodine deficiency: optimal dosing and outcome indicator selection. J Clin Endocrinol Metab. 1994;79(1):20-4.

22. Furnée CA, Pfann GA, West CE, van der Haar F, van der Heide D, Hautvast JG. New model for describing urinary iodine excretion: its use for comparing different oral preparations of iodized oil. Am J Clin Nutr. 1995;61(6):1257-62.

23. Delange F. Administration of iodized oil during pregnancy: a summary of the published evidence. Bull World Health Organ. 1996;74(1):101-8.

24. Stinca S, Andersson M, Weibel S, Herter-Aeberli I, Fingerhut R, Gowachirapant S, et al. Dried Blood Spot Thyroglobulin as a Biomarker of Iodine Status in Pregnant Women. J Clin Endocrinol Metab. 2017;102(1):23-32.

25. Laurberg P, Cerqueira C, Ovesen L, Rasmussen LB, Perrild H, Andersen S, et al. Iodine intake as a determinant of thyroid disorders in populations. Best Pract Res Clin Endocrinol Metab. 2010;24(1):13-27.

Part 2: Assessment of the relationship between iodine nutrition state in pregnancy, thyroid dysfunction and preeclampsia

This part comprises the following three chapters:

Chapter 4: Insufficient iodine nutrition status and the risk of preeclampsia: a systemic review and meta-analysis

Chapter 5: Iodine deficiency in pregnancy, along a concentration gradient, is associated with increased severity of preeclampsia in rural Eastern Cape, South Africa

Chapter 6: Mildly elevated thyroid-stimulating hormone is associated with endothelial dysfunction and severe preeclampsia among pregnant women with insufficient iodine intake in Eastern Cape Province, South Africa

CHAPTER 4

Insufficient iodine nutrition status and the risk of preeclampsia: a systemic review and meta-analysis

Businge CB, Usenbo A, Longo-Mbenza B, Kengne AP. Insufficient iodine nutrition status and the risk of pre-eclampsia: a systemic review and meta-analysis. BMJ Open. 2021 Feb 10;11(2):e043505. doi: 10.1136/bmjopen-2020-043505.

The protocol for this systematic review was published in the BMJ Open journal:

Businge CB, Madini N, Longo-Mbenza B, Kengne AP. Insufficient iodine nutrition status and the risk of pre-eclampsia: a protocol for systematic review and meta-analysis. BMJ Open. 2019;9(5):e025573.

The full text is included at the end of this thesis as Appendix IVb.

Abstract

Background: Although sub-clinical hypothyroidism (SCH) in pregnancy is one of the established risk factors for preeclampsia, the link between iodine deficiency, the major cause of hypothyroidism, and preeclampsia remains uncertain. We conducted a systematic review to determine the iodine nutrition status of pregnant women with and without preeclampsia and the risk of preeclampsia due to iodine deficiency around the globe.

Methods: MEDLINE, EMBASE, Google Scholar, SCOPUS and Africa Wide Information were searched up to June 30th 2020. Random-effect model meta-analysis was used to pool mean difference UIC between preeclamptic and normotensive controls; and to pool odds ratios and incidence rates of preeclampsia among women with UIC <150 µg/L.

Results: Five eligible studies were included in the meta-analysis. There was a significant difference in the pooled mean UIC of 254 preeclamptic women and 210 normotensive controls enrolled in three eligible case-control studies (mean UIC 164.4 µg/L [95% CI 45.1–283.6, $p < 0.01$, $I^2 > 50$]). The overall proportions of preeclampsia among women with UIC <150 µg/L and UIC >150 µg/L in two cross-sectional studies were 203/214 and 67/247 respectively with a pooled odds ratio of 0.01 (95% CI 0.00–4.23, $p = 0.14$, $I^2 > 50$) for preeclampsia among women with UIC >150 µg/L. The overall incidence of preeclampsia among women with UIC <150 µg/L and UIC >150 µg/L in two cohort studies were 6/1411 and 3/2478 respectively with a pooled risk ratio of 2.85 (95% CI 0.42–20.05, $p = 0.09$, $I^2 < 25$)

Conclusion: Although preeclamptic women seem to have lower UIC than normotensive pregnant women, the available data does not provide a conclusive answer on association of iodine deficiency with preeclampsia risk.

.

4.1 Introduction

Sub-clinical hypothyroidism (SCH) is a risk factor for preeclampsia, which is a prominent cause of maternal and perinatal morbidity and mortality (1-3). Iodine deficiency, which is exacerbated by pregnancy related physiological changes, is a leading cause of hypothyroidism (4, 5). Hence, among women within the reproductive age bracket, insufficient nutrition status prior to the onset of pregnancy, which potentially worsens during pregnancy, could increase the risk of preeclampsia like is the case for foetal neurological complications, particularly in endemic iodine deficiency settings (6, 7).

Over two billion people live in areas with iodine insufficiency (8). Iodine deficiency is on the rise in areas originally thought to be iodine sufficient, despite concerted worldwide efforts to promote iodine fortification. This is partly attributed to high concentration of perchlorate and thiocyanate in water sources and the diet, which impairs the uptake of iodine by the thyroid gland, particularly among individuals with TSH-related thyroid stimulation secondary to iodine deficiency; and to ineffective implementation and monitoring of dairy and bread-based iodine supplementation strategies (9-12).

In this systematic review and meta-analysis, using literature from around the world, we sought to establish if there is a difference in the urinary iodine concentration (UIC) of pregnant women with and without pre-eclampsia; and whether pregnant women with insufficient iodine nutrition status are at increased risk of pre-eclampsia.

The study is reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Guidelines (13), and was based on a protocol that was registered with the International Prospective Register of Systematic Reviews, PROSPERO (registration no. CRD42018099427).

4.2 Methods

Inclusion criteria

The Population, Intervention/exposure, Comparison and Outcome (PICO) protocol guided the selection of studies for inclusion. The target population were pregnant women, and the exposure was insufficient iodine nutrition status before pregnancy for cohort studies, and insufficient iodine nutrition status during pregnancy for case-control studies. The iodine nutrition status was defined according to the World Health Organization / International Council for Control of Iodine Deficiency Disorders (ICCIDD) classification of iodine intake using median urinary iodine concentration (UIC) (14, 15). For pregnant women, a urine iodine concentration (UIC) <150, 150-249, 250-499 and >500µg µg/L is considered an estimate of insufficient, adequate, more than adequate and excessive iodine nutritional status, respectively (15). The comparators were study participants with sufficient iodine nutrition status (UIC ≥150 µg/L) during pregnancy (14, 15). The outcomes were the prevalence and incidence rates of preeclampsia among women with and without adequate iodine nutrition status from which the odds ratios for case control and risk ratios for cohort studies were determined.

Preeclampsia was defined as new onset hypertension after 20 weeks of amenorrhoea characterised by elevated systolic blood pressure (SBP ≥140 mmHg) and/or diastolic blood pressure (DBP ≥90 mmHg), based on two measurements four hours apart; or SBP>160 mmHg and/or DBP>110 mmHg from a single measurement. Elevated BP had to be accompanied by at least one of the following: proteinuria in 24 h-urine ≥ 300 mg or protein/creatinine ratio ≥ 0.3mg/mg or urine protein measured by dipstick ≥ 2+; thrombocytopenia (platelets count less than 150,000/µL), kidney insufficiency (serum creatinine levels above 90 µmol/L), decreased liver function (AST and ALT twice higher than the upper limit of the reference interval), compromised lung function or pulmonary oedema, visual or other symptoms and signs of impaired cerebral function (16). There may be considerable heterogeneity if preeclampsia has been variably defined in different studies that are eligible for inclusion in the current systematic review.

Exclusion criteria

Studies were excluded if they lacked means, medians, odds ratios, incidence and prevalence rates data to compute them even after repeated unsuccessful attempts to contact the authors via email for relevant information. Letters to editors, reviews, commentaries, editorials and any publication without primary data were also excluded.

Patient and public involvement

There was no involvement of the public or patients.

Search Strategy and Selection Criteria

We searched PubMed, Scopus, Web of Science, Academic Search Premier, Africa Wide Information, CINAHL, Cochrane Library, Google Scholar, and Health Source: Nursing/Academic Edition databases for all published studies on iodine deficiency and preeclampsia up to 30th June 2020. This search was conducted using a pre-defined comprehensive and sensitive search strategy combining relevant terms and synonyms which are variably used to denote abnormally high blood pressure in pregnancy and insufficient iodine intake or iodine deficiency as detailed in the published protocol for this review (17).

Study Selection and Data Extraction

Two authors (CBB and AU) independently screened the titles and abstracts of identified studies. Citations and abstracts were initially screened and duplicate citations excluded. Titles and abstracts were then screened following inclusion criteria described in the protocol, after which the full-texts of potentially eligible articles were obtained. These full texts were screened using a standardised and pretested form to include eligible studies. Disagreements were resolved by consensus. For each study, one reviewer (CBB) extracted the data and a second reviewer (AU) checked the accuracy. For the five studies included here, there were no disagreements between the two reviewers. Figure 1 shows the flowchart for the selection process. The following data were extracted from the eligible studies: studies characteristics (authors, years, design, and study regions), study population (age, and sample size), iodine nutrition status of the various study groups, and the methods of outcomes measurement.

Quality Assessment

Two reviewers (CBB and AU) independently scored the risk of bias and the quality of included studies (Tables 4.1 and 4.2) using the Newcastle-Ottawa scale (18). Inter-rater agreement on screening, data abstraction and methodological quality (selection, comparability of groups and ascertainment of exposure/outcome) was assessed using Cohen's κ coefficient (19). The Kappa value for inter-rater agreement for quality assessment was 0.694 ($p < 0.001$). Discrepancies were resolved by consensus.

Data synthesis, analysis and assessment of heterogeneity

The analysis was performed with the Review Manager (RevMan) Software, version 5.4 (The Nordic Cochrane Centre, The Cochrane Collaboration) and the 'meta' and 'metafor' packages of the statistical software R (version 4.0.2, The R Foundation for statistical computing, Vienna, Austria). For the outcomes of interest (means, prevalence and incidence rates), random effects model meta-analyses were used to pool estimates across studies with similar design (20). Heterogeneity across studies was assessed using the Cochrane Q statistic and inconsistency index (I^2) statistic and values ranked as indicating low: $I^2<25\%$; moderate: 25%–50%; high heterogeneity: $I^2>50\%$) (21). The Egger funnel plot was used to check for publication bias (22).

Table 4. 1: Risk of bias assessment (Reviewer AU)

Study	**Study Type**	**Selection**	**Comparability**	**Outcome/ Exposure**	**AHRQ scale good/fair/poor**
Gulaboglu, 2009	Case-control	***	**	***	Good
Cuellar-Rufino, 2017	Case-control	***	**	**	Good
Businge 2017	Case-control	****	**	***	Good
Yang 2018	Cohort study	****	**	***	Good
Xiao 2017	Cohort study	****	**	***	Good

Table 4. 2: Risk of bias assessment (Reviewer CBB)

Study	**Study Type**	**Selection**	**Comparability**	**Outcome/ Exposure**	**AHRQ scale good/fair/poor**
Gulaboglu, 2009	Case control	***	**	***	Good
Cuellar-Rufino, 2017	Case control	****	**	***	Good
Businge, 2017	Case control	****	**	**	Good
Yang, 2018	Cohort	****	**	***	Good
Xiao, 2017	Cohort	****	**	***	Good

4.3 Results

The review process

The process for selecting the relevant studies is summarised in Figure 4.1. We identified a total of 2380 records via database searches. After removing duplicates, we scanned the titles and abstracts of 522 articles, of which 11 full texts were further reviewed. Of these, 5 articles met criteria for inclusion in the current systematic review.

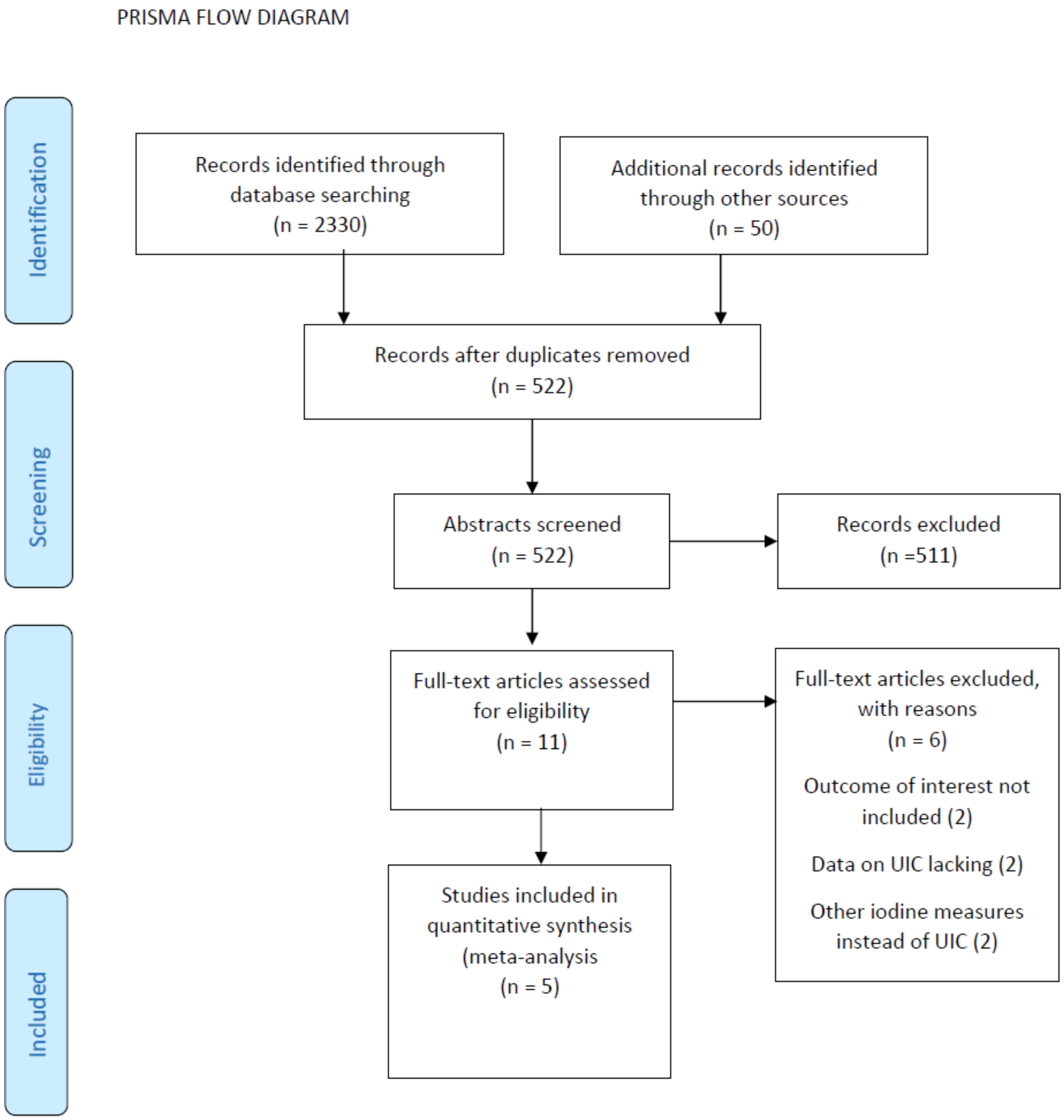


Figure 4. 1: Flow process of study selection

Characteristics of included studies

All the five included studies were categorised as having a low risk of bias. Their characteristics are summarised in Table 4.3. Three were institutional-based case-control studies one from each of the following countries: Turkey, Mexico and the Democratic Republic of Congo while 2 were prospective cohort studies were from two different provinces (Henan and Liaoning) in China (23-27).

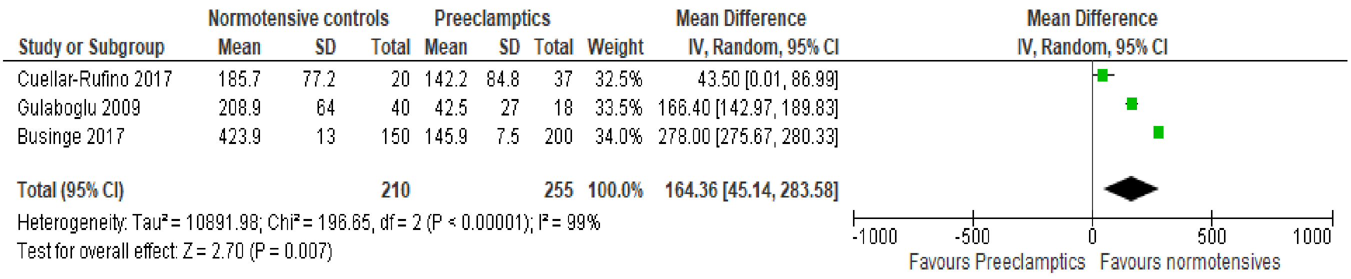
Table 4. 3: Characteristics of included studies

First author, year	Country	Study design	Study period	Cases (n)	Controls (n)	Comparator	Diagnostic criteria	Potential sources of confounding
Gulaboglu, 2009	Turkey	Case-control	Not stated	Severe preeclampsia (40)	Normotensives (18)	Mean IUC	Sandell–Kolthoff reaction	Small sample Serum magnesium and other micronutrients
Cuellar-Rufino, 2017	Mexico	Case-control	Jan–April 2015	Preeclampsia (20)	Normotensives (37)	Mean IUC IUC<150 µg/L	Fast colorimetric method	Small sample size Taking all HDP as one group Inclusion of women with Previous HDP without matching Other trace elements/ dietary antioxidants
Businge, 2017	Congo Democratic Republic	Case-control	Jan 2007 to December 2008	Preeclampsia (68) and Severe preeclampsia/ Eclampsia (182)	Normotensives (150)	Mean IUC IUC<150 µg/L	Sandell–Kolthoff Reaction	Non serial UIC Different gestational ages
Yang, 2018	Henan Province, China	cohort	July to September 2015	Incident preeclampsia 01/718 for women with IUC <150 µg/L Incident gestational HT 17/718 for women with IUC <150 µg/L	Incident preeclampsia 01/1602 for women with IUC >150 µg/L Incident gestational HT 25/1602 for women with IUC >150 µg/L	IUC<150 µg/L	Fast colorimetric method	Differences in BMI UIC>249 µg/L Small sample sizes within comparative strata
Xiao, 2017	Liaoning province, China	Cohort	2012–2014	Incident preeclampsia 05/693 for women with IUC <150 µg/L Incident gestational HT 18/693 for women with IUC <150 µg/L	Incident preeclampsia 02/876 for women with IUC >150 µg/L Incident gestational HT 25/876 for women with IUC >150 µg/L	IUC<150 µg/L	Sandell–Kolthoff Reaction	Small number with iodine IUC<150 GDM Other dietary anti-oxidants Single measurement

Meta-analysis

Mean difference in urinary iodine concentration of preeclamptic and normotensive women

Three studies reported the mean UIC of preeclamptic and normotensive pregnant women (23-25). Overall, there was a significant and positive mean difference in UIC and standardised mean UIC of normotensive pregnant women and preeclamptic women, with substantial heterogeneity across studies (Fig 4.2).



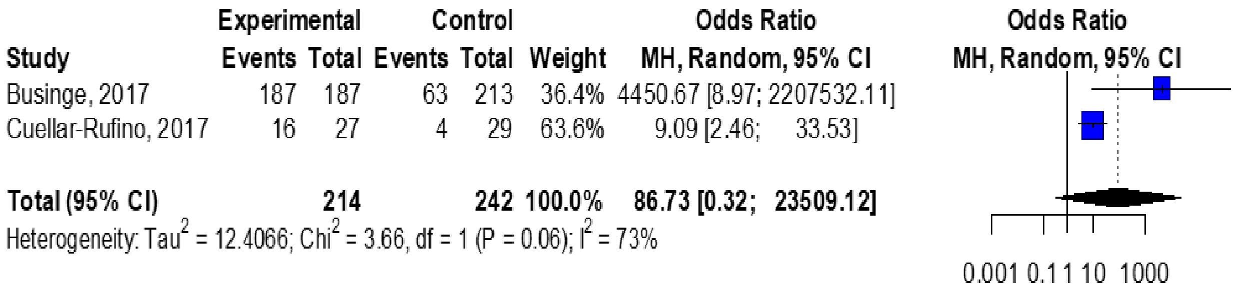
	Normotensive controls			Preeclamptics				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Cuellar-Rufino 2017	185.7	77.2	20	142.2	84.8	37	32.5%	43.50 [0.01, 86.99]
Gulaboglu 2009	208.9	64	40	42.5	27	18	33.5%	166.40 [142.97, 189.83]
Businge 2017	423.9	13	150	145.9	7.5	200	34.0%	278.00 [275.67, 280.33]
Total (95% CI)			**210**			**255**	**100.0%**	**164.36 [45.14, 283.58]**

Heterogeneity: Tau² = 10891.98; Chi² = 196.65, df = 2 (P < 0.00001); I² = 99%
Test for overall effect: Z = 2.70 (P = 0.007)

Figure 4. 2: Forests plot showing the mean difference in UIC of normotensive and preeclamptic mothers

The risk of preeclampsia among women with UIC < 150 µg/L

Two case-control studies had data with proportions of preeclamptic and normotensive participants with UIC above or below <150 µg/L (24, 25). The odds of preeclampsia among women with UIC<150 µg/L was above unity for individual studies but the pooled odds ratio of 86.73 (0.32, 23509.12) was not significant with substantial heterogeneity across studies ($I^2 = 73\%$) (Fig 4.3 and 4.4).



	Experimental		Control			Odds Ratio
Study	**Events**	**Total**	**Events**	**Total**	**Weight**	**MH, Random, 95% CI**
Businge, 2017	187	187	63	213	36.4%	4450.67 [8.97; 2207532.11]
Cuellar-Rufino, 2017	16	27	4	29	63.6%	9.09 [2.46; 33.53]
Total (95% CI)		**214**		**242**	**100.0%**	**86.73 [0.32; 23509.12]**

Heterogeneity: $Tau^2 = 12.4066$; $Chi^2 = 3.66$, df = 1 (P = 0.06); $I^2 = 73\%$

Figure 4. 3: Forest plot showing the odds of preeclampsia among women with UIC < 150 µg/L

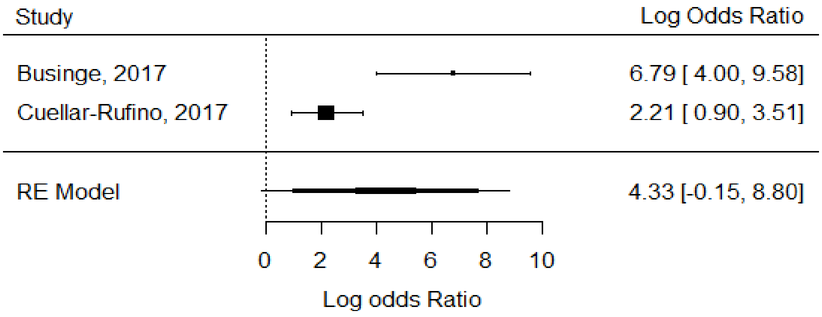


Figure 4. 4Figure 4.4: Forest plot showing the Log odds of preeclampsia among women with UIC > 150 µg/L. p = 0.068, Tau^2 = 9.8, I^2 = 88.94%.

The incidence of preeclampsia in the two cohort studies was 2/2320 and 7/1576 respectively (26, 27). There was no difference in the incidence of preeclampsia among participants with or without low UIC (UIC <150µg/L) as shown in Figure 4.5.

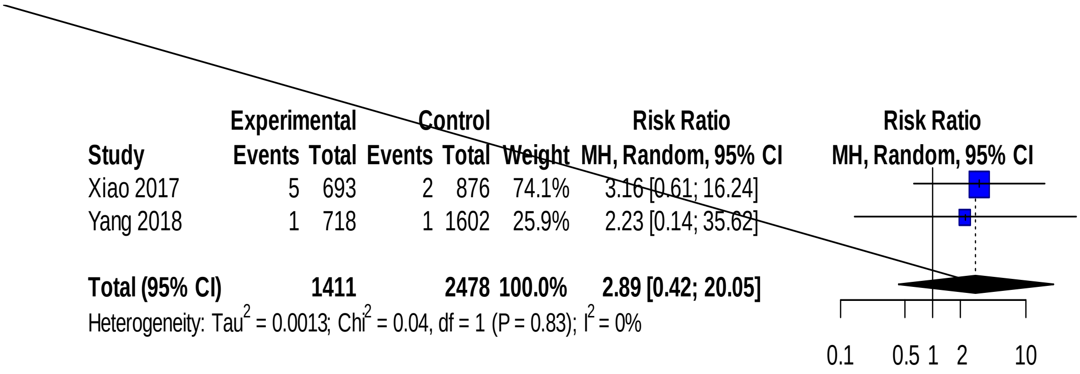


Figure 4. 5: Forest plot showing the risk of preeclampsia among women with <150 µg/L (designated as the experimental group). Pooled RR 2.89 [0.42–20.05], t = 0.091.

Publication Bias

Visual inspection of funnel plot symmetry suggested potential publication bias for the studies included in the meta-analysis of UIC difference preeclamptic and normotensive counterparts as well as the odds of preeclampsia among women with <150 µg/L (Figure 4.6).

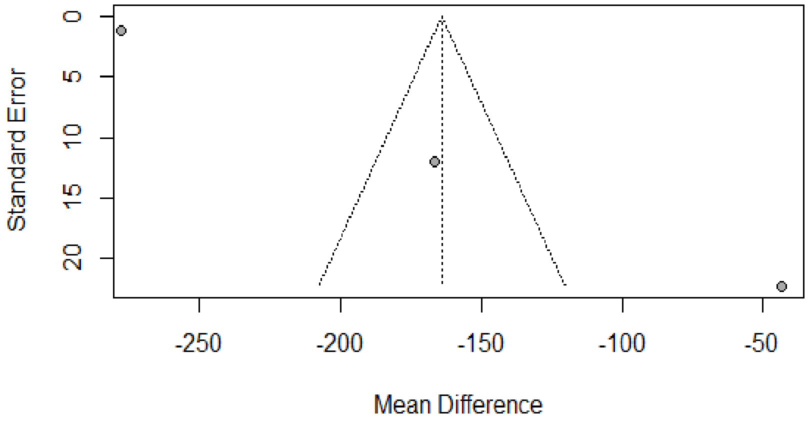


Figure 4. 6: Funnel plot for the studies selected for the analysis of the mean difference UIC of preeclamptic women and normotensive counterparts

After adjustment of the effect size for potential publication bias using the trim-and-fill correction, 2 potentially missing studies (Figure 4.7) were imputed in funnel plot (Mean UIC differences of -389.60 [-413.02; -366.17] and -512.50 [-556.23; -468.78] respectively. With potential inclusion of the missing studies the pooled mean UIC was estimated to be -278.0000 [-438.3025; -117.6975] which differs significantly from the pooled estimate of the three included studies (p <0.001).

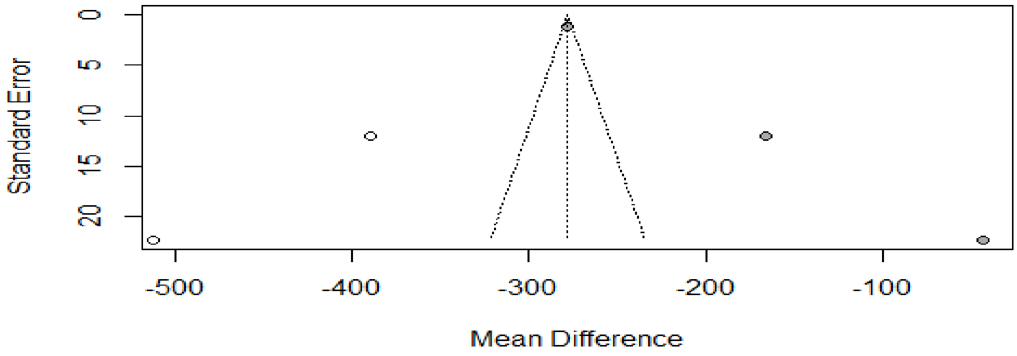


Figure 4. 7: Funnel plots for publication bias in the studies selected for the analysis of the mean difference UIC of preeclamptic women and normotensive counterparts. Empty circles represent the 2 imputed studies.

The funnel plot for the cohort studies included in the assessment of the incidence of preeclampsia among women with UIC <150 µg/L was not suggestive of potential publication bias (Figure 4.8).

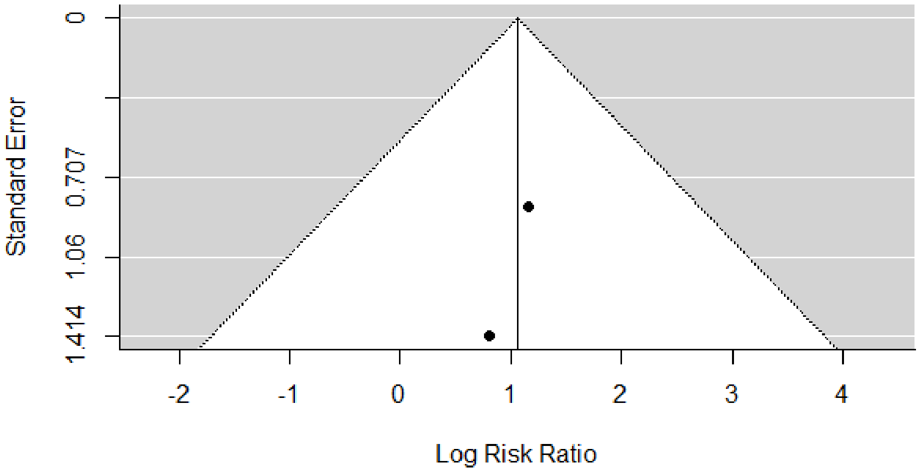


Figure 4. 8: Funnel plot for the studies selected for the analysis of the risk of preeclampsia among women with <150 µg/L

4.4 Discussion

The current review has shown that preeclamptic women have significantly lower mean UIC than their normotensive counterparts. This trend was observed in all the 3 included studies despite being from 3 different continents: Africa, Europe and South America (23-25). This association between low UIC and preeclampsia may reflect inadequate iodine intake predating pregnancy persisting till the third trimester that may increase the risk of preeclampsia among susceptible women. A recent Norwegian study reported that among women with mild-to-moderate deficiency, long-term preconception iodine supplementation was associated with reduced incidence of preeclampsia (7).

Although there was a trend towards a positive association between low UIC (UIC<150µg/L) in the third trimester and preeclampsia for the included case-control and cohort studies, the pooled odds and risk ratios showed a non-significant association. The small number of eligible studies that also had substantially high heterogeneity may partially account for this result.

Hence, the available data does not provide a definitive answer on the risk of preeclampsia associated with low UIC in the third trimester.

Iodine deficiency is thought to predispose to incident preeclampsia through two mechanisms. The first one is the reduction of the anti-oxidant capacity of the placenta, which is one of the organs where the sodium iodine symporter maintains a high concentration of iodine which among other roles, is thought to reduce oxidative stress and lipid peroxide formation which are elevated in patients with preeclampsia (12, 28, 29). The second mechanism is persistent iodine deficiency predisposing to elevated TSH. TSH operating via its endothelial receptors has been shown to diminish endothelial Nitric Oxide and prostacyclin production as well as upregulate endothelin production, which lead to endothelial dysfunction and systemic vasoconstriction (30-32). Since baseline pre-pregnancy and serial pregnancy UIC analyses were not carried out in the two cohort studies, it remains uncertain whether the iodine nutritional status at enrolment truly reflected the iodine nutritional status before pregnancy and for the remaining duration of the pregnancy following enrolment. Iodine nutritional status is likely to change with dietary habits and the progressive physiological changes of pregnancy. This could lead to misclassification of study participants and dilute the association between iodine deficiency and preeclampsia (33). The estimation of maternal intra-thyroid iodine concentration, even though more technical, has been proposed as a more objective measure of pre-pregnancy iodine nutrition status than spot UIC (34). Concurrent measurement of spot UIC and serum thyroglobulin may help identify individuals with long-term exposure to iodine deficiency in studies where it is not possible to measure serial UIC and intra-thyroid iodine concentration (35, 36).

4.5 Study limitations

This review was limited by the small number of eligible studies with small sample sizes and substantial heterogeneity. The varied research designs of the eligible studies precluded the pooling of all the test results.

4.6 Conclusion

Although the UIC of women who present with preeclampsia seems to be lower than that of women who remain normotensive till delivery, the available data is insufficient to reliably draw a conclusion on the association of iodine deficiency with the risk of preeclampsia. More well-designed and adequately powered studies that also include the estimation of pre-pregnancy iodine nutrition status are needed to address this question.

References

1. Abalovich M, Gutierrez S, Alcaraz G, Maccallini G, Garcia A, Levalle O**.** Overt and subclinical hypothyroidism complicating pregnancy. Thyroid. 2002;12(1):63-8.
2. Khanam M, Ilias M**.** Study of Thyroid Hormonal Status in Preeclamptic Patients. Medicine Today. 2014;25.
3. Wilson KL, Casey BM, McIntire DD, Halvorson LM, Cunningham FG**.** Subclinical thyroid disease and the incidence of hypertension in pregnancy. Obstet Gynecol. 2012;119(2 Pt 1):315-20.
4. Lazarus JH**.** Screening for thyroid dysfunction in pregnancy: is it worthwhile? J Thyroid Res. 2011;2011:397012.
5. Jameson LJ MS, Weetman AP Disorders of the Thyroid gland. In: Kasper DL HS, Jameson JL, Fauci AS, Longo DL, Loscalzo J, ed. Principles of Internal Medicine 19th ed. New York: McGraw Hill Education; 2015: 2283 - 308.
6. Pharoah PO, Buttfield IH, Hetzel BS**.** Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. Lancet. 1971;1(7694):308-10.
7. Abel MH, Caspersen IH, Sengpiel V, Jacobsson B, Meltzer HM, Magnus P, et al. Insufficient maternal iodine intake is associated with subfecundity, reduced foetal growth, and adverse pregnancy outcomes in the Norwegian Mother, Father and Child Cohort Study. BMC Med. 2020;18(1):211.
8. Andersson M, Karumbunathan V, Zimmermann MB**.** Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744-50.
9. Zimmermann MB**.** Iodine deficiency in industrialized countries. Clin Endocrinol (Oxf). 2011;75(3):287-8.
10. De Groef B, Decallonne BR, Van der Geyten S, Darras VM, Bouillon R**.** Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. Eur J Endocrinol. 2006;155(1):17-25.
11. Steinmaus C, Miller MD, Howd R**.** Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001-2002 national health and nutrition examination survey. Environ Health Perspect. 2007;115(9):1333-8.
12. Vidal ZE, Rufino SC, Tlaxcalteco EH, Trejo CH, Campos RM, Meza MN, et al. Oxidative stress increased in pregnant women with iodine deficiency. Biol Trace Elem Res. 2014;157(3):211-7.
13. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.
14. WHO U, ICCIDD**.** Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. 3rd ed. Geneva: WHO; 2007.
15. WHO**.** Proceedings of the WHO Technical Consultation on control of iodine deficiency in pregnant women and young children Geneva: World Health Organization; 2005.
16. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4(2):97-104.

17. Businge CB, Madini N, Longo-Mbenza B, Kengne AP. Insufficient iodine nutrition status and the risk of pre-eclampsia: a protocol for systematic review and meta-analysis. BMJ Open. 2019;9(5):e025573.

18. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses.

19. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22(3):276-82.

20. Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. Bmj. 2011;342:d549.

21. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Bmj. 2003;327(7414):557-60.

22. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Bmj. 1997;315(7109):629-34.

23. Gulaboglu M, Borekci B, Delibas I. Urine iodine levels in preeclamptic and normal pregnant women. Biol Trace Elem Res. 2010;136(3):249-57.

24. Cuellar-Rufino S, Navarro-Meza M, García-Solís P, Xochihua-Rosas I, Arroyo-Helguera O. Iodine levels are associated with oxidative stress and antioxidant status in pregnant women with hypertensive disease. Nutr Hosp. 2017;34(3):661-6.

25. Businge C, Longo-Mbenza B, Adeniyi V, Babeki V, Kaboka A, Muaka M, et al. Iodine Deficiency in Pregnancy as a Predictor of Sub-clinical Hypothyroidism, Preeclampsia and Future Cardiovascular Disease. Asian Journal of Clinical Nutrition. 2017;9:118-23.

26. Yang J, Liu Y, Liu H, Zheng H, Li X, Zhu L, et al. Associations of maternal iodine status and thyroid function with adverse pregnancy outcomes in Henan Province of China. J Trace Elem Med Biol. 2018;47:104-10.

27. Xiao Y, Sun H, Li C, Li Y, Peng S, Fan C, et al. Effect of Iodine Nutrition on Pregnancy Outcomes in an Iodine-Sufficient Area in China. Biol Trace Elem Res. 2018;182(2):231-7.

28. Burns R, O'Herlihy C, Smyth PP. The placenta as a compensatory iodine storage organ. Thyroid. 2011;21(5):541-6.

29. Brown SH, Eather SR, Freeman DJ, Meyer BJ, Mitchell TW. A Lipidomic Analysis of Placenta in Preeclampsia: Evidence for Lipid Storage. PLoS One. 2016;11(9):e0163972.

30. Tian L, Zhang L, Liu J, Guo T, Gao C, Ni J. Effects of TSH on the function of human umbilical vein endothelial cells. J Mol Endocrinol. 2014;52(2):215-22.

31. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2006;91(10):4175-8.

32. Lioudaki E, Mavroeidi NG, Mikhailidis DP, Ganotakis ES. Subclinical hypothyroidism and vascular risk: an update. Hormones (Athens). 2013;12(4):495-506.

33. Haine D, Dohoo I, Dufour S. Selection and Misclassification Biases in Longitudinal Studies. Front Vet Sci. 2018;5:99.

34. Dineva M, Fishpool H, Rayman MP, Mendis J, Bath SC. Systematic review and meta-analysis of the effects of iodine supplementation on thyroid function and child neurodevelopment in mildly-to-moderately iodine-deficient pregnant women. Am J Clin Nutr. 2020;112(2):389-412.

35. Du Y, Gao YH, Feng ZY, Meng FG, Fan LJ, Sun DJ**.** Serum Thyroglobulin-A Sensitive Biomarker of Iodine Nutrition Status and Affected by Thyroid Abnormalities and Disease in Adult Populations. Biomed Environ Sci. 2017;30(7):508-16.

36. Bath SC, Pop VJ, Furmidge-Owen VL, Broeren MA, Rayman MP**.** Thyroglobulin as a Functional Biomarker of Iodine Status in a Cohort Study of Pregnant Women in the United Kingdom. Thyroid. 2017;27(3):426-33.

CHAPTER 5

Iodine deficiency in pregnancy along a concentration gradient is associated with increased severity of preeclampsia in rural Eastern Cape, South Africa

Charles Bitamazire Businge, Benjamin Longo-Mbenza, Andre Pascal Kengne

Businge CB, Longo-Mbenza B, Kengne AP. Iodine deficiency in pregnancy along a concentration gradient is associated with increased severity of preeclampsia in rural Eastern Cape, South Africa. BMC Pregnancy Childbirth. 2022;22(1):98. doi: 10.1186/s12884-021-04356-6.

Abstract

Background: Preeclampsia is a leading cause of maternal mortality and morbidity in South Africa. Iodine deficiency in pregnancy, which is amenable to correction through iodine supplementation, has been reported to increase the risk of preeclampsia. However, the association of iodine nutrition status with preeclampsia in South Africa has not been studied.

Methods: Fifty-one randomly selected normotensive pregnant controls at term together with 51 consecutively selected cases of preeclampsia and 51 cases with severe preeclampsia/eclampsia all in the third trimester were enrolled from Mthatha Regional and Nelson Mandela Academic Hospital in the Eastern Cape Province, South Africa. The urinary iodine concentration (UIC), serum thyroid-stimulating hormone (TSH), triiodothyronine (FT3), thyroxine (FT4) and thyroglobulin (Tg) levels were compared between cases and controls.

Results: The median values for normotensive, preeclampsia and severe preeclampsia/eclampsia participants were: age (yrs) 23, 24 and 19 p= 0.001; UIC (µg/L) 217.1,127.7, and 98.8 p=0.005; Tg (µg/L) 19.4, 21.4, and 32.9 p=0.001; FT4 (pmol/L) 14.2, 13.7, and 12.8 p=0.005; FT3 (pmol//L) 4.8, 4.4, and 4.0, p=0.001; TSH (mIU/L) 2.3, 2.3, and 2.5 p=0.661). UIC < 100 µg/L, Tg > 20µg/L and FT4 < 12 pmol/L were independent predictors of preeclampsia.

Conclusion: women with preeclampsia/eclampsia had significantly lower UIC and higher Tg, suggesting protracted inadequate iodine intake that was also associated with lower levels of thyroid hormones. Inadequate iodine intake during pregnancy severe enough to significantly lead to elevated Tg and or diminished FT4 was associated with increased risk of severe preeclampsia.

5.1 Background

Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality around the world, including in South Africa (1, 2). The foetal complications of preeclampsia arise from placenta ischaemia. These include intrauterine growth restriction, intrauterine foetal death, premature birth with attendant perinatal morbidity and mortality, increased risk of metabolic syndrome in adult life (2, 3). The maternal complications of preeclampsia arise from endothelial dysfunction leading to systemic malfunction. The systemic malfunction includes thrombocytopenia, haemolysis, hepatocellular injury, pulmonary oedema, acute kidney failure, cerebral oedema, cerebral haemorrhage, eclamptic fits, future cardiovascular disease and maternal death (2, 3).

The exact cause of preeclampsia is not known with certainty (4). However, several risk factors have now been identified (2). Thyroid dysfunction in pregnancy has also been associated with preeclampsia (5). Particularly, overt and subclinical hypothyroidism are increasingly being recognised as risk factors for preeclampsia (5-7). Hypothyroidism in pregnancy also predisposes to increased risk of maternal-anaemia, caesarean delivery, post-partum haemorrhage, miscarriage, low birthweight, low Apgar score and neonatal intensive care admission (8, 9). Chronic iodine deficiency is associated with increased risk of hypothyroidism (10). An association has been reported between iodine deficiency and preeclampsia (11, 12). Therefore, the increase in renal filtration of iodine secondary to the physiological increase in renal blood flow, and the progressive transfer of iodine to the foetus (13) may predispose pregnant women with low daily iodine to iodine deficiency disorders and incident preeclampsia.

The incidence of preeclampsia in the general population of South Africa and specifically in the Eastern Cape is not known with certainty. In one study among South African primigravida women, Moodley et al. (14) reported that the incidence of preeclampsia-eclampsia syndrome was about 5.8% with a resultant perinatal mortality rate of 5.9% compared to 2.2% in the general population. Panday et al. (15), in a community-based study in KwaZulu-Natal Province, South Africa, reported an incidence of 12.5% of hypertensive disease in pregnancy at the community level and 14.5% at the referral tertiary hospital. Hypertensive disease in pregnancy accounts for about 22% of all preventable maternal deaths in South Africa (16).

Before universal iodization of salt South Africa had endemic iodine deficiency and this was the underlying cause of wide spread goitre in the population (17, 18). Despite the initiation of universal salt iodization in 1995 about 30% of the population do not have regular access to adequately iodized salt especially rural communities (17, 18). Since pregnancy is associated with increased physiological iodine loss, undiagnosed iodine deficiency in pregnancy may be prevalent in South Africa. Although the association between iodine deficiency and preeclampsia has been reported in some studies (11, 12), such an association has not yet been investigated in South Africa. If iodine deficiency in pregnancy, which can be mitigated with iodine supplementation, is found to be a risk factor of preeclampsia in this population, efforts to improve the iodine nutrition state in reproductive years may help reduce on the burden of preeclampsia.

Therefore, this study was carried out to ascertain the iodine nutrition status of normotensive pregnant women and counterparts with hypertensive disease in pregnancy; whether iodine deficiency is associated with increased risk and severity of preeclampsia; and whether women with preeclampsia/eclampsia had significant elevation of thyroid-stimulating hormone (TSH) one of the pathological mechanisms of preeclampsia.

5.2 Materials and methods

Study site

The study was conducted at two hospitals in OR Tambo District Municipality (ORTDM), Eastern Cape, South Africa. Mthatha Regional Hospital (MRH) is a 500-bed referral hospital for the district hospitals and Health centres located in the King Sabata Dalindyebo Health sub district (KSD) within ORTDM. It has a maternity unit of 80 beds that mainly conducts normal deliveries as well as emergency and elective caesarean sections for low-risk antenatal mothers and refers high-risk obstetrics patients to Nelson Mandela Academic Hospital (NMAH). NMAH is an 800-bed teaching/tertiary hospital with 100 maternity beds also located in KSD. It is also a tertiary and referral hospital for districts situated in the former Transkei region. This area of the Eastern Cape is largely composed of rural and peri-urban human settlements with a population of about 1.8 million people. About 4,500 births are conducted at the NMAH labour ward, out of which 40% are mothers whose pregnancies are complicated with hypertensive diseases in pregnancy.

Study design

This was a case-control study to ascertain the relationship between iodine deficiency, SCH secondary to iodine deficiency and preeclampsia among women attending NMAH and MRH labour wards between August 2018 and March 2020.

Study setting and population

The study population comprised of women referred to NMAH with preeclampsia/eclampsia during the study period and normotensive women without chronic medical diseases who have come for delivery at Mthatha Regional Hospital and Nelson Mandela Academic Hospital, Mthatha. Both hospitals care for patients referred from district hospitals and Community Health Centres in the northeastern part of the Eastern Cape Province of South Africa, a region with a high incidence of preeclampsia/eclampsia. This is also an area formally with endemic goitre before iodization of salt was implemented in 1995 (17). Seventy-five percent of the population are not gainfully employed hence considered to live under the poverty line.

Although iodization of salt was implemented in South Africa since 1995, the consumption of iodized salt for some individuals including women in reproductive years could be inadequate given that some rural communities have sometimes been prone to use of cheaper non-iodised salt (18). Secondly, the peri-urban communities are more likely to be dependent on foodstuffs from the food chain stores given the level of globalization (19). These have a high probability of being processed without use of iodized salt, and may have high content of perchlorate a common ingredient of fertilizers and an inhibitor of thyroid iodine uptake rendering the population at risk of iodine deficiency that becomes more apparent during pregnancy (20).

Sample size

The sample size for the study will be determined as follows (21):

$N = [(1/q_1 + 1/q_2)\ SD^2\ (z_\alpha + z_\beta)^2] / E^2$

Where:

z_α is the standard normal deviate of α (0.05) = 1.96

z_β is the standard normal deviate of β (0.20) = 0.84

q1 = ½ (the proportion of participants in study group 1)

q2 = ½ (the proportion of participants in study group 2)

SD = the standard deviation

E = the effect size (mean UIC of controls cases minus mean UIC of cases)

N = total number of study participants

Cuéllar-Rufino (9) found that the UIC of normotensive pregnant women was 185.7 ± 77.16 µg/L and that of women with hypertensive disease in pregnancy of 142.15 ± 84.8 µg/L. The difference in the two means (E) was 42.3 µg/L.
Assuming a difference in mean UIC (E) of cases with preeclampsia and normotensive pregnant controls in our study population of 45 µg/L and a standard deviation of 80 µg/L
The sample size for the current study will be:

$N = [(1/q_1 + 1/q_2)\ SD^2\ (z_\alpha + z_\beta)^2] / E^2$

$N = [(1/0.5 + 1/0.5)\ (80)^2\ (1.96 + 0.84)^2] / (45)^2]$

$N = [4 \times 6400 \times 7.84] / (45 \times 45)]$

$N = 99.1$

Each group of cases or controls should comprise at least 50 participants.

We enrolled 51 normotensive pregnant controls, and 51 newly diagnosed participants in each group of cases of preeclampsia without severe features, and preeclampsia with severe features/eclampsia. The cases were assigned the diagnosis of eclampsia, preeclampsia with or without severe features according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines (22). Normotensive pregnant women admitted at term for elective caesarean section and counterparts in latent labour at term admitted for vaginal delivery were enrolled as controls.

Sampling

The participants with hypertensive disease in pregnancy (preeclampsia and eclampsia) were enrolled by consecutive sampling while eligible normotensive controls were enrolled after matching their chronological age with those of the cases and obtaining written informed consent. All controls were enrolled at term just before delivery in order to avoid potential misclassification of cases and controls.

Inclusion and exclusion criteria

All consenting women who fulfilled the definition of cases or controls were eligible to participate in the study. Women with multiple pregnancy, those with a history of thyroid disease, chronic hypertension or a history of diabetes mellitus were excluded.

Ethics approval and consent to participate

This study was granted ethical approval by Human Research Ethics Review Committee of the University of Cape Town (ref no. 135/2018) and Walter Sisulu University (ref no. 066/2017). Informed consent was obtained from all the mothers that were enrolled in the study. The study was conducted as stipulated in the Helsinki declaration.

Data collection

After obtaining written informed consent, we used a structured questionnaire to collect the following data: participants' age, parity, gestational age, past obstetric history of adverse pregnancy outcomes, to rule out past medical history of thyroid disease or type 1 diabetes, thyroidectomy, radioactive iodine therapy, and external radiotherapy of the head and neck.

The participants' weight was measured using a portable electronic scale and height with a portable height measuring board according to standard procedures (23).

The Blood pressure was determined by taking the average of the two measurements obtained with an electronic sphygmomanometer taken at intervals ≥ 2 minutes part according to the American Heart Association guidelines (24).
Nitric oxide levels, TSH, free thyroxine (FT4), free triiodothyronine (FT3), and thyroglobulin (Tg) were assayed from venous blood collected at enrolment that was immediately centrifuged, and the serum aliquoted and stored at -20 0C until the time of analysis. The Roche/Hitachi cobas-c systems electrochemiluminescence immunoassay was used for determining the levels of serum TSH, FT4 and FT3. The inductively coupled plasma (ICP) Mass Spectrometry method was used to determine the urinary iodine concentration (UIC) (25). The estimated daily iodine intake was determined from the UIC as described by the United States Institute of Medicine (26). Serum nitric oxide levels were determined using the Cayman Chemical Nitrite/Nitrate Colorimetric Assay kit (Cayman Chemical company, 1180 E. Ellsworth RD Ann Arbor, MI, USA) a two-step process where nitrate is first converted to nitrite using nitrate

reductase followed by the addition of Griess Reagent 2 [N- (1-Naphthyl)ethylenediamine] that converts nitrite into a deep purple azo compound whose concentration is then determined using a colorimeter.

Statistical analysis

Data analysis was performed using soft-ware package IBM SPSS® STATISTICS version 22 for Windows (IBM Inc., Chicago IL, USA). We used the Shapiro–Wilk's test to check if the data followed the normal distribution. The data were summarized as proportions (%) for categorical variables, means ± standard deviation (SD) for normally distributed, and as median (p25, p75) for skewed continuous variables, respectively. The Chi-square test was used to compare the distribution of categorical variables by status for preeclampsia. The Jonckheere-Terpstra test for trend, the Student's t-test, Mann-Whitney U and Kruskal- Wallis tests were used as appropriate for continuous variable comparisons across groups. Spearman correlation was used to determine relations between variables in the groups. Univariable and multivariable logistic regressions were used to investigate the correlates of preeclampsia. Thyroid function parameters, categorised according to cut-off limits of normal ranges, and they included potential confounders in the regression models. A p-value <0.05 was considered significant.

5.3 Results

General characteristics of the participants

There was no statistical difference in BMI, and gestational age at booking between the normotensive controls and cases with hypertensive diseases in pregnancy (preeclampsia and severe preeclampsia/eclampsia) (p > 0.05, Table 5.1). Participants with hypertensive diseases in pregnancy tended to present before term (median gestational age of 34 (30, 39) and 35 (32, 38) weeks) compared to controls whose median gestation age at enrolment was 38 (37, 40) weeks. Participants with severe preeclampsia/eclampsia were significantly younger than their normotensive counterparts (p = 0.004), they also had significantly lower number of pregnancies (gravidity) (p = 0.030). The highest recorded blood pressure levels during the antenatal period and the blood pressure levels at enrolment were as expected significantly higher among cases than controls (p <0.001, Table 5.1).

Table 5. 1: The general characteristics of normotensive pregnant controls and cases of preeclampsia and severe preeclampsia/eclampsia [median (25th and 75th percentiles)]

Variable	**Normotensive (n = 51)**	**Preeclampsia (n = 51)**	***P value**	**Severe preeclampsia/ eclampsia (n = 51)**	****P value**
Age (years)	23 (17, 28)	24 (20, 29)	0.341	19 (18, 23)	0.004
BMI (kg/m^2)	27.7 (25.5, 30.9)	29.3(26.5, 35.3)	0.060	27.1 (23.2, 36.7)	0.250
Gravidity	1 (1, 2)	1 (1, 2)	0.941	1 (1, 1)	0.030
GA at booking (WOA)	20.1 (17.5, 23.3)	22.0 (20.0, 25.8)	0.171	22.0 (19.0, 24.0)	0.274
GA at enrolment (WOA)	38 (37, 40)	34 (30, 39)	<0.001	35 (32, 38)	<0.001
Highest SBP (mmHg)	121 (114, 127)	151 (145, 160)	<0.001	162 (154, 174)	<0.001
Highest DBP (mmHg)	74 (60, 78)	100 (90, 109)	<0.001	102 (94, 114)	<0.001
SBPe (mmHg)	123 (113, 130)	139 (126, 146)	<0.001	144 (130, 151)	<0.001
DBPe (mmHg)	77 (70, 84)	90 (81, 99)	<0.001	92 (81, 99)	<0.001
NO (µmol/L)	5.2 (4.0, 7.5)	4.2 (4.0, 5.1)	0.001	3.4 (1.8, 4.6)	<0.001

*P value: Mann-Whitney test normotensive vs preeclampsia; **P value: Mann-Whitney test normotensive vs severe preeclampsia/eclampsia; GA gestational age; WOA weeks of amenorrhoea; BMI body mass index; SBP systolic blood pressure; DBP diastolic blood pressure; SBPe systolic blood pressure at enrolment; DBPe diastolic blood pressure at enrolment; NO nitric oxide; (p25, p75) 25th and 75th percentiles.

Both cases of preeclampsia and those with severe preeclampsia/eclampsia had significantly lower levels of nitric oxide than normotensive pregnant controls (Table 5.1).

Renal, thyroid function and iodine nutritional status of cases and controls

There was no difference in the median serum creatinine of normotensive pregnant controls and participants with uncomplicated preeclampsia (56.0 and 58 µmol/L respectively, p= 0.507) Although the median serum creatinine of women with severe preeclampsia was significantly higher than that of normotensive pregnant controls (68.0 µmol/L, p = 0.001, Table 5.2), it was still within the normal third trimester range (35–80 µmol/L) with the 75th percentile in the range of mildly elevated serum creatinine in pregnancy. Participants with preeclampsia and severe preeclampsia/eclampsia had significantly lower median UIC (respectively 127.7 and 98.8 µg/L) than controls (217.1 µg/L) (Table 2). However, it is only the participants with severe preeclampsia/eclampsia when compared to controls who had a significantly lower median estimated daily iodine intake (respectively 178.2 and 362.2 µg/day, p = 0.004), urine iodine/urine creatinine ratio (respectively 12.5 and 30.7 g/mol, p = 0.017), serum FT4 (12.8 and 14.2 pmol/L respectively, p <0.001) and FT3 (4.0 and 4.8 pmol/L respectively, p <0.001) but significantly higher median serum thyroglobulin (32.9 and 19.4 µg/L respectively, p <0.001) (Table 5.2).

There was no significant difference in the median serum TSH levels of both groups of cases and the controls, (Table 5.2).

Using second and third trimester upper serum TSH limit of 3.0 IU/L (27) and the 10th FT4 percentile of 11.3 pmol/L (28) to determine the thyroid function status, the prevalence of subclinical hypothyroidism (SCH) was 23.5%, 25.5% and 27.5%; and overt hypothyroidism (OH) 3.9%, 5.9.0% and 7.8% respectively for normotensive pregnant women, severe preeclampsia and eclamptic participants (p = 0.18) hence no significant difference between the groups (Table 5.3). There were no participants with overt or subclinical hyperthyroidism.

Table 5. 2: Comparison of median (p25, p75) of thyroid function parameters TSH, FT3, FT4, Tg, UIC estimated daily iodine intake (EDII) of normotensive pregnant controls and cases of preeclampsia and severe preeclampsia/eclampsia

Variable	**Normotensive (n=51)**	**Preeclampsia (n=51)**	**⁑P value**	**Severe preeclampsia/ eclampsia (n = 51)**	**⁑⁑P value**
Ser Cr (µmol/L)	56.0 (42.0, 65.0)	58.0 (45.0, 115.8)	0.507	68.0 (53.0, 99.6)	0.001*
UIC (µg/L)	217.1 (110.3, 374.5)	127.7 (75.7, 365.0)	0.046*	98.8 (39.9, 312.8)	0.005*
EDII (µg/day)	362.2 (171.5, 662.8)	240.0 (128.5, 767.0)	0.144	178.2 (68.5, 508.2)	0.004
UI/UCr (g/mol)	30.7 (18.9, 88.5)	24.6 (9.9, 144.4)	0.321	12.5 (5.1, 72.9)	0.017*
FT4 (pmol/L)	14.2 (13.0, 16.1)	13.7 (11.4, 16.0)	0.117	12.8 (11.5, 14.6)	0.001*
FT3 (pmol//L)	4.8 (4.2, 5.0)	4.4 (4.0, 5.0)	0.087	4.0 (3.3, 4.7)	<0.001*
Tg (µg/L)	19.4 (12.5, 31.2)	21.4 (13.2, 36.3)	0.405	32.9 (18.8, 50.9)	<0.001*
TSH (mIU/L)	2.3 (1.7, 3.1)	2.3 (1.9, 3.3)	0.443	2.5 (1.6, 3.7)	0.424

⁑P value: Mann-Whitney U Test normotensive vs preeclampsia; ⁑⁑P value: Mann-Whitney U Test normotensive vs severe preeclampsia/eclampsia; * p <0.05; Ser Cr serum creatinine; UIC urinary iodine concentration; EDII estimated daily iodine intake; UI/UCr urine iodine-creatinine ratio; FT4 free thyroxine; FT3 free Triiodothyronine; Tg Thyroglobulin; TSH thyroid-stimulating hormone;

Table 5. 3: Thyroid function status of normotensive, preeclamptic, severe preeclampsia/ eclamptic participants

Thyroid status	**Normotensive n (%)**	**Preeclampsia n (%)**	**Severe preeclampsia/eclampsia n (%)**	**Chi square**	**P value**
Euthyroid	36 (70.6)	27 (52.9)	25 (49.0)	8.926	0.18
SCH	12 (23.5)	13 (25.5)	14 (27.5)		
Hypothyroxinaemia	1 (2.0)	8 (15.7)	8 (15.7)		
Overt Hypothyroidism	2 (3.9)	3 (5.9)	4 (7.8)		
Total	51 (100)	51 (100)	51 (100)		

SCH: subclinical hypothyroidism

The trend of thyroid function parameters and other assays with increasing severity of preeclampsia

The levels of serum FT3, FT4, and urinary iodine and the urine iodine/creatinine ratio showed a significant diminishing trend and serum thyroglobulin an increasing trend with the severity of hypertensive disease in pregnancy. Serum TSH levels showed a modest non-significant increase along the gradient of severity of hypertensive disease in pregnancy (Table 5.2 and 5.4).

Table 5.4: Jonckheere-Terpstra test for trends of Table4: Jonckheere-Terpstra test for trend of various variables along the gradient of severity of preeclampsia (normotensive, preeclampsia and severe preeclampsia/eclampsia)

Variable	Standard J-T statistic	P value for trend
Highest SBP	10.19	<0.001
Highest DBP	8.64	<0.001
TSH	0.836	0.403
Tg	3.60	<0.001
FT4	-3.17	0.002
FT3	-4.26	<0.001
UIC	-2.97	0.003
Nitric oxide	-4.76	<0.001
Serum creatinine	3.26	0.001
Urine iodine-creatinine ratio	-2.43	0.015

SBP systolic blood pressure; DBP diastolic blood pressure; TSH thyroid-stimulating hormone; Tg Thyroglobulin; FT3 free Triiodothyronine; UIC urinary iodine concentration; FT4 free thyroxine

Correlates of preeclampsia

Participants with preeclampsia/severe preeclampsia/eclampsia were taken together as one group in comparison with normotensive pregnant controls to ascertain if UIC was an independent predictor of preeclampsia-eclampsia syndrome. In regression models that included UIC and potential confounders, urinary iodine concentration, serum thyroxine and thyroglobulin and were significantly associated with increased odds of preeclampsia-eclampsia syndrome after adjustment for age, gravidity, BMI, TSH, FT3 (Table 5.5)

Table 5. 5: Univariable and multivariable odds ratios of urinary iodine concentration, thyroid hormones and other factors that are associated with preeclampsia-eclampsia syndrome

Variable	**Univariable OR (95% CI)**	**P value**	**Multivariable OR (95% CI)**	**P value**
Age < 20 yrs	1.51 (0.75–3.01)	0.248	1.14 (0.41–3.25)	0.792
Gravidity ≤ 1	1.70 (0.86–3.38)	0.128	1.96 (0.73–5.26)	0.184
BMI >30 Kg/m^2	1.04 (0.52–2.12)	0.905	1.53 (0.62–3.74)	0.355
TSH ≥ 3.0 IU/L	1.32 (0.63–2.77)	0.460	1.14 (0.48–2.73)	0.765
FT3 < 4.3 pmol/L	2.34 (1.16–4.72)	0.017	2.07 (0.94–4.54)	0.071
FT4 < 12 pmol/L	4.21 (1.53–11.59)	0.005	5.07 (1.57–16.39)	0.007
Tg ≥ 20 µg/L	2.79 (1.39–5.58)	0.004	3.11 (1.41–6.85)	0.005
UIC ≤ 100 µg/L	3.37 (1.52–7.46)	0.003	2.65 (1.12–6.25)	0.026

BMI: Body mass index; TSH: Thyroid-stimulating hormone; FT3: Triiodothyronine; FT4: Thyroxine; Tg: Thyroglobulin; UIC: urine iodine concentration; OR odds ratio; CI confidence interval.

Coefficients of variation

Apart from FT4 and FT3 the rest of the biomarkers had high co-efficients of variation (Table 6)

Table 5. 6: The ranges and co-efficient of variation of various assays

Assay	**Range**	**Co-efficient of variation**
Thyroid-stimulating hormone (IU/L)	0.9–11	0.60
Free thyroxine (pmol/L)	4.0–20.6	0.20
Free triiodothyronine (pmol/L)	1.9–6.9	0.20
Thyroglobulin (µg/L)	1.3–147.2	0.78
Urinary iodine concentration (µg/L)	00–1247.7	1.173
Nitric oxide (µmol/L)	0.2–12.5	0.52
Serum creatinine (µmol/L)	27.0–402.0	0.62
Urine iodine-creatinine ratio (g/mol)	00–2024.5	2.31

5.4 Discussion

This study finds women with preeclampsia had insufficient iodine intake (median urinary iodine concentration < 150 µg/L) while normotensive pregnant women from the same setting had adequate iodine intake in pregnancy. The degree of insufficient iodine nutrition increased while serum nitric oxide levels decreased with the severity of preeclampsia. In addition, low urinary iodine concentration, low serum thyroxine and high serum thyroglobulin were independent predictors of hypertensive disease in pregnancy in the study population. These findings correlate with previous case-control studies that have found a positive association between insufficient iodine intake and preeclampsia (12, 29, 30). A recent systematic review found a significant difference in the mean UIC of preeclamptic and normotensive pregnant controls but no increased risk of preeclampsia among pregnant women with UIC < 150 µg/L in the included cohort studies (31). The study was however limited by the very few numbers of included studies and high heterogeneity across studies. Reische et al. (32) in a recent case control study using nationally representative data from Finland reported no difference in serum iodine, TSH and other thyroid parameters of preeclamptic and normotensive pregnant women.

Despite these seemingly conflicting studies, the results our study are in line with findings of Abel et al. (11) who in a recent Norwegian Cohort study reported the risk of preeclampsia to increase with the degree of inadequate iodine intake. In the same study, supplementation with iodine before the onset of pregnancy reduced the risk of preeclampsia (odds ratio 0.85 (95% CI 0.74, 0.98). It seems plausible that iodine deficiency predisposes to increased risk and severity of preeclampsia at higher degrees of iodine deficiency. Our results reveal that even though participants with uncomplicated preeclampsia had lower UIC and higher Tg than normotensive controls, these were not significantly different and tended to be in the range of mild clinical derangement. However, women with preeclampsia complicated with severe features had thyroid function parameters suggestive of moderate iodine deficiency with significantly elevated Tg that suggests longer exposure to iodine deficiency. Considering the results of Abel et al. (11), the exposure to iodine deficiency among women with severe preeclampsia in our study may have predated the current pregnancy.

Iodine is one of the exogenous scavengers of oxidative molecules produced during various metabolic processes (33, 34). Compared to the first two trimesters of pregnancy, women in the third trimester have a higher metabolic rate and are predisposed to greater iodine loss due to increased renal filtration (35, 36). Hence, women with low thyroid iodine content at the inception of pregnancy will be at risk of developing oxidative imbalance with resultant reduction in Nitric Oxide the principal mediator of endothelial relaxation (37, 38). This may in part explain the findings in the current study where the levels of systolic and diastolic blood pressure increased while the urinary iodine concentration and serum nitric oxide reduced along the gradient of severity of preeclampsia.

Although spot UIC is not a reliable predictor of prolonged exposure to inadequate iodine nutrition (39), the significantly higher serum thyroglobulin levels among women with severe preeclampsia/eclampsia compared to normotensive pregnant controls in the current study seems to suggest prolonged iodine deficiency. During the acute phase of iodine deficiency, the thyroid gland undergoes autoregulation that is independent of TSH in which there is increased vascularity and iodide uptake and maintenance of physiological levels of T3 and T4 (40). If mild-to-moderate iodine deficiency persists, apart from preferential T3 production, there is increased production of less iodinated thyroglobulin, which easily leaks into the blood stream (40, 41). This may account for the higher serum thyroglobulin observed among preeclamptic participants in the current study who also had UIC levels suggestive of moderate iodine deficiency.

The significantly lower FT3 and FT4 in the background of low UIC among women with preeclampsia when compared to normotensive women suggests inadequate iodination of thyroglobulin secondary to iodine deficiency (40, 42). However, the levels of serum FT3 and FT4 of the women with preeclampsia were still within physiological ranges, hence the mild but non-significant elevation in TSH. Previous research has revealed that high serum TSH is associated with endothelial dysfunction (43). The proposed mechanism is the protracted stimulation of endothelial TSH receptors that is associated with diminished endothelial NO synthase activity ultimately leading to vasoconstriction and reduced flow-mediated dilatation (44, 45).

Overt and subclinical hypothyroidism were more prevalent among women with hypertensive disease in pregnancy. However, they did not emerge as independent predictors of hypertensive disease in pregnancy in the study population.

5.5 Strengths and limitations

The inclusion of three comparison groups of varying severity of preeclampsia in the current study and the concurrent measurement of urinary iodine concentration and serum thyroglobulin has enabled an adequate assessment of the influence the level of iodine deficiency on the severity of preeclampsia. The study is however limited by our inability to measure levels of selenium another micronutrient whose deficiency is associated with thyroid dysfunction as well as vitamin C and Vitamin E, known dietary antioxidants, whose deficiency has variably been associated with preeclampsia. In addition, we could not collect some clinical data such as foetal weight that would have enabled us to detect foetal growth restriction, a criterion for diagnosis of preeclampsia. Resource constraints precluded the histological assessment of the placentae that would have further assessed the vascular changes of the cases and controls. Other limitations include the significant difference in gestation age at enrolment and inability to assess the influence of iodine status on postpartum preeclampsia. While the high coefficient of variation for various variables could indicate substantial variability that can affect internal validity, this may have arisen from the heterogenous nature of the 3 study groups that had varied levels of pathology.

5.6 Conclusion

Iodine deficiency, diagnosed in the current study through low UIC, resultant hypothyroxinaemia and elevated thyroglobulin, are independent predictors of preeclampsia. Specifically, iodine deficiency that is severe enough to lead to hypothyroxinaemia and elevated thyroglobulin was associated with increased risk of severe preeclampsia/eclampsia in the study population.

References

1. DOH. Saving Mothers 2014 - 2016: Seventh triennial report on confidential. Pretoria: Department of Health 2018.
2. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. Bmj. 2019;366:l2381.
3. Khalil G HA. Preeclampsia: Pathophysiology and the Maternal-Fetal Risk Journal of Hypertension and Management 2017;3:024.
4. Tallarek AC, Huppertz B, Stepan H. Preeclampsia - Aetiology, Current Diagnostics and Clinical Management, New Therapy Options and Future Perspectives. Geburtshilfe Frauenheilkd. 2012;72(12):1107-16.
5. Nazarpour S, Ramezani Tehrani F, Simbar M, Azizi F. Thyroid dysfunction and pregnancy outcomes. Iran J Reprod Med. 2015;13(7):387-96.
6. Manjunatha S, Basavraja G, Patil R. Thyroid Dysfunction in Pregnancy and Preeclampsia. Scholars Journal of Applied Medical Sciences. 2014;2:3297-99.
7. Banik P, Devi R, Bidya Devi A, Tamphasana A, Agalya M, Singh L. Thyroid dysfunction in preeclampsia and related fetomaternal outcomes. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019;8:1928.
8. Kiran Z, Sheikh A, Malik S, Meraj A, Masood M, Ismail S, et al. Maternal characteristics and outcomes affected by hypothyroidism during pregnancy (maternal hypothyroidism on pregnancy outcomes, MHPO-1). BMC Pregnancy and Childbirth. 2019;19(1):476.
9. Mahadik K, Choudhary P, Roy PK. Study of thyroid function in pregnancy, its feto-maternal outcome; a prospective observational study. BMC Pregnancy and Childbirth. 2020;20(1):769.
10. Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 2015;3(4):286-95.
11. Abel MH, Caspersen IH, Sengpiel V, Jacobsson B, Meltzer HM, Magnus P, et al. Insufficient maternal iodine intake is associated with subfecundity, reduced foetal growth, and adverse pregnancy outcomes in the Norwegian Mother, Father and Child Cohort Study. BMC Med. 2020;18(1):211.
12. Cuellar-Rufino S, Navarro-Meza M, García-Solís P, Xochihua-Rosas I, Arroyo-Helguera O. Iodine levels are associated with oxidative stress and antioxidant status in pregnant women with hypertensive disease. Nutr Hosp. 2017;34(3):661-6.
13. Zantour B AW, Marmouch H, Chebbi W Hypothyroidism in Pregnancy. In: E P, ed. Current Topics in Hypothyroidism with Focus on Development. Rijeka, Croatia: In Tech 2013: 29-62.
14. Moodley J, Soma-Pillay P, Buchmann E, Pattinson RC. Hypertensive disorders in pregnancy: 2019 National guideline; 2019.
15. Panday M, Mantel GD, Moodley J. Audit of severe acute morbidity in hypertensive pregnancies in a developing country. J Obstet Gynaecol. 2004;24(4):387-91.
16. Moodley J, Kalane G. A review of the management of eclampsia: practical issues. Hypertens Pregnancy. 2006;25(2):47-62.
17. Kalk WJ, Paiker J, van Arb MG, Pick W. Dietary iodine deficiency in South Africa. Surveys before the introduction of universal salt iodisation. S Afr Med J. 1998;88(3 Endocrinology):357-8.

18. Jooste P, Zimmermann M. Progress towards eliminating iodine deficiency in South Africa. South African Journal of Clinical Nutrition. 2008;21(1):8-14.

19. Stern R, Puoane T, Tsolekile L. An exploration into the determinants of noncommunicable diseases among rural-to-urban migrants in periurban South Africa. Prev Chronic Dis. 2010;7(6):A131.

20. Friel S, Chopra M, Satcher D. Unequal weight: equity oriented policy responses to the global obesity epidemic. Bmj. 2007;335(7632):1241-3.

21. Hulley S, Cummings S, Browner W, Grady D, Newman T. Designing Clinical Research. 4th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2013.

22. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4(2):97-104.

23. WHO. The WHO approach to noncommunicable disease risk factor surveillance Geneva: World Health Organisation; 2017.

24. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves JW, Hill MN, et al. Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. J Clin Hypertens (Greenwich). 2005;7(2):102-9.

25. Yu S, Yin Y, Cheng Q, Han J, Cheng X, Guo Y, et al. Validation of a simple inductively coupled plasma mass spectrometry method for detecting urine and serum iodine and evaluation of iodine status of pregnant women in Beijing. Scand J Clin Lab Invest. 2018;78(6):501-7.

26. IOM. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington (DC): National Academies Press (US); 2001.

27. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid. 2011;21(10):1081-125.

28. Moncayo R, Zanon B, Heim K, Ortner K, Moncayo H. Thyroid function parameters in normal pregnancies in an iodine sufficient population. BBA Clinical. 2015;3:90-5.

29. Gulaboglu M, Borekci B, Delibas I. Urine iodine levels in preeclamptic and normal pregnant women. Biol Trace Elem Res. 2010;136(3):249-57.

30. Businge C, Longo-Mbenza B, Adeniyi V, Babeka V, Kitambala A, Muaka M, et al. Iodine Deficiency in Pregnancy as a Predictor of Sub-clinical Hypothyroidism, Preeclampsia and Future Cardiovascular Disease. Asian Journal of Clinical Nutrition. 2017;9:118-23.

31. Businge CB, Usenbo A, Longo-Mbenza B, Kengne AP. Insufficient iodine nutrition status and the risk of pre-eclampsia: a systemic review and meta-analysis. BMJ Open. 2021;11(2):e043505.

32. Reische EC, Männistö T, Purdue-Smithe A, Kannan K, Kim U-J, Suvanto E, et al. The Joint Role of Iodine Status and Thyroid Function on Risk for Preeclampsia in Finnish Women: a Population-Based Nested Case-Control Study. Biological Trace Element Research. 2021;199(6):2131-7.

33. Winkler R, Griebenow S, Wonisch W. Effect of iodide on total antioxidant status of human serum. Cell Biochem Funct. 2000;18(2):143-6.

34. Agbor G, Taga I, Nguindex D, Zaidi M, Lehman LG, Altosaar I, et al. Effect of Iodine Supplementation on Antioxidant Status of Normal and Alloxan Monohydrate in Toxicated Rats. International Journal of Pharmacology. 2011;7:726-31.

35. Lof M, Olausson H, Bostrom K, Janerot-Sjöberg B, Sohlstrom A, Forsum E. Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. Am J Clin Nutr. 2005;81(3):678-85.

36. Dafnis E, Sabatini S. The effect of pregnancy on renal function: physiology and pathophysiology. Am J Med Sci. 1992;303(3):184-205.

37. Smyth PP. Role of iodine in antioxidant defence in thyroid and breast disease. Biofactors. 2003;19(3-4):121-30.

38. Vidal ZE, Rufino SC, Tlaxcalteco EH, Trejo CH, Campos RM, Meza MN, et al. Oxidative stress increased in pregnant women with iodine deficiency. Biol Trace Elem Res. 2014;157(3):211-7.

39. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744-50.

40. Obregon MJ, Escobar del Rey F, Morreale de Escobar G. The effects of iodine deficiency on thyroid hormone deiodination. Thyroid. 2005;15(8):917-29.

41. Santisteban P, Obregon MJ, Rodriguez-Peña A, Lamas L, Del Rey FE, De Escobar GM. Are iodine-deficient rats euthyroid? Endocrinology. 1982;110(5):1780-9.

42. Lazarus J. Thyroid Regulation and Dysfunction in the Pregnant Patient. In: Feingold KR AB, Boyce A, et al, ed. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2000; 2016.

43. Lu M, Yang CB, Gao L, Zhao JJ. Mechanism of subclinical hypothyroidism accelerating endothelial dysfunction (Review). Exp Ther Med. 2015;9(1):3-10.

44. Tian L, Zhang L, Liu J, Guo T, Gao C, Ni J. Effects of TSH on the function of human umbilical vein endothelial cells. J Mol Endocrinol. 2014;52(2):215-22.

45. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2006;91(10):4175-8.

CHAPTER 6

Mildly elevated thyroid-stimulating hormone is associated with endothelial dysfunction and severe preeclampsia among pregnant women with insufficient iodine intake in Eastern Cape Province, South Africa

Businge CB, Longo-Mbenza B, Kengne AP. Mildly elevated thyroid-stimulating hormone is associated with endothelial dysfunction and severe preeclampsia among pregnant women with insufficient iodine intake in Eastern Cape province, South Africa. Ann Med. 2021;53(1):1083-1089.

Abstract

Background: Preeclampsia and hypothyroidism are associated with endothelial dysfunction. Iodine deficiency is a risk factor for subclinical hypothyroidism in pregnancy. However, there is a paucity of data on the relationship between iodine nutrition state in pregnancy, the degree of endothelial dysfunction and the risk of preeclampsia.

Methods: Ninety-five normotensive pregnant women, 50 women with preeclampsia with no severe features, and 50 women with severe preeclampsia were enrolled into the current study from the maternity units of Nelson Mandela Academic Hospital and Mthatha Regional Hospitals in Eastern Cape Province, South Africa. Urinary iodine concentration (UIC), serum markers of thyroid function, aortic augmentation index and pulse wave velocity (PWV) were compared.

Results: Median UIC was 167.5 µg/L, 127.7 µg/L and 88.5 µg/L respectively for normotensive pregnant women, those with preeclampsia and severe preeclampsia ($p=0.150$). Participants with severe preeclampsia had significantly higher median thyroid-stimulating hormone (TSH) and oxidised LDL than normotensive and preeclamptic women without severe features (respectively 3.0, 2.3 and 2.3 IU/L; 1.2, 1.0 and 1.0 IU/L, $p < 0.05$). The median Aortic augmentation index was 7.5, 19.0 and 21.0 ($p < 0.001$) and the pulse wave velocity 5.1, 5.7 and 6.3 respectively for normotensive, preeclampsia and severe preeclampsia participants (both $p<0.001$). In linear regressions, TSH, age and hypertensive disease were independent predictors of elevated PWV.

Conclusion: Upper normal-range TSH levels in women with severe preeclampsia were associated with markers of endothelial dysfunction. The iodine deficiency and trend towards elevation of thyroglobulin suggest that inadequate iodine intake likely increase TSH levels and indirectly cause endothelial dysfunction.

Keywords:

Preeclampsia, iodine deficiency, elevated thyroid-stimulating hormone, Pulse Wave Velocity, endothelial dysfunction

6.1 Background

Endothelial dysfunction, which is characterised by atherosis and vasoconstriction, is a precursor of cardiovascular disease (1, 2). Both preeclampsia and hypothyroidism are risk factors for cardiovascular disease among women (3-5). High serum thyroid-stimulating hormone (TSH) levels above the physiological range, a common feature of subclinical (SCH) and overt hypothyroidism (OH), predispose to endothelial dysfunction through inhibition of endothelial Nitric oxide (NO) synthase (6-8). This leads to diminished flow mediated dilation that is modulated by local endothelial NO synthesis in the lumen of the blood vessels (7). The resultant arterial stiffness is an early marker of incident cardiovascular disease and a pathological feature of preeclampsia (1). Due to the transfer of iodine across the placenta to the growing foetus and the increased physiological renal filtration and loss of iodine in urine during pregnancy, pregnant women in populations with insufficient iodine intake are at increased risk of worsening iodine deficiency (9, 10). This is now thought to lead to subclinical or overt hypothyroidism, endothelial dysfunction, preeclampsia and other adverse pregnancy outcomes such as miscarriage, preterm delivery and intrauterine growth restriction (11-13).

We carried out this case-control study to find out how the iodine nutrition status and serum TSH levels varied with the degree of endothelial dysfunction and severity of preeclampsia among women in the Eastern Cape South Africa.

6.2 Methods

Study setting

The participants were enrolled at the maternity units of Nelson Mandela Academic Hospital and Mthatha Regional Hospitals situated in OR Tambo municipality one of the rural districts in the Eastern Cape Province, South Africa. South Africa is one of the sub-Saharan countries with iodine deficient soils (14). Universal salt iodization has been implemented in South Africa for about 25 years however, 30% of the population especially in rural settings do not have access to adequately iodised salt (14).

Sample size calculation

Namugowa and Meeme (15) found the mean pulse wave velocity (PWV) of preeclamptic and normotensive pregnant women of 6.7 ± 1.5 and 5.1 ± 0.7 m/s respectively (a difference of 1.6

between the two means). Assuming a standard deviation for PWV of 1.5 in our study population, a sample size of 126 (42 preeclamptic and 84 normotensive pregnant women) has power of 0.80 at an alpha of 0.05 to detect a difference of 0.8 between the mean PWV of preeclamptic and normotensive pregnant women.

Enrolment of participants

Cases were women diagnosed with preeclampsia according to the criteria stipulated by the International Society for the Study of Hypertension in Pregnancy (ISSHP) (16). Controls were pregnant women who remained normotensive and presented at term either in latent labour or for delivery by elective caesarean section.

We enrolled 195 participants who voluntarily accepted to participate in the current study (50) women with preeclampsia with no severe features, and 50 women with severe preeclampsia who were matched for chronological age with 95 normotensive pregnant women. The participants with preeclampsia were consecutively enrolled soon after diagnosis while the normotensive pregnant women were selected by simple random sampling of women with similar chronological age who remained normotensive till they presented at term in early labour or for elective caesarean section.

Inclusion and exclusion criteria

All women who fulfilled the criteria for cases or controls were eligible for inclusion in the study after providing informed consent. Potential participants who had a history of thyroid disease, those currently with multiple pregnancy, those who had conceived after artificial reproductive technique procedure, and those with chronic hypertension or a history of diabetes mellitus were excluded.

Data collection

Data on demographic and past obstetric history was obtained in addition to the participants' weight and height that were measured using standard procedures. The Blood pressure was measured according to the American Heart Association guidelines, with the patient's elbow flexed at the heart level. The average of the two measurements with a standard mercury sphygmomanometer taken at intervals ≥ 2 minutes after the participants had been sitting for at least 30 minutes was used (17). The degree of endothelial dysfunction was determined by calculating the pulse wave velocity and the augmentation index using the SphygmoCor system (version 7.01, Atcor Medical, Sydney, Australia). Venous blood was collected, centrifuged, and the serum aliquoted and stored at -20 0C until analysed for thyroid function tests (TSH, free thyroxine [FT4], free triiodothyronine [FT3], and thyroglobulin). These were compared with the trimester specific ranges (18, 19). The TSH, FT4 and FT3 levels were determined using the Roche/Hitachi cobas-c systems, an electrochemiluminescence immunoassay (ECLIA) technology. Mid-stream urine was collected and the Urinary Iodine concentrations determined using the inductively coupled plasma (ICP) Mass Spectrometry method according to the manufacturer's instructions (Quadrupole Inductively Coupled Plasma Mass Spectrometry (X-Series 2 ICP-MS–Thermo- Fisher Scientific, Bremen, Germany) as previously described (20). Median urinary iodine concentrations of < 150, 150-249, 250-499 and >500µg respectively are a measure of insufficient, adequate, more than adequate and excessive iodine intake during pregnancy (21).

Statistical analysis

The IBM SPSS STATISTICS version 22 for windows (IBM Inc., Chicago IL, USA) soft-ware package was used for data analysis. Data were checked to identify variables that were normally distributed using the Shapiro–Wilk's test. The data were then summarized into proportions (%) for categorical variables, means ± standard deviation (SD) for normally distributed, and as median (p25, p75) for non-normally distributed variables, respectively. The Chi-square test was used to compare the distribution of categorical variables by status for preeclampsia. The Student's t-test, Kruskal-Wallis and Mann-Whitney U tests were used as appropriate for continuous variable comparisons across groups. Univariable and multivariable linear regression were used to investigate the correlates of preeclampsia. A p-value <0.05 was considered significant.

Ethical considerations

The Human Research Ethics Review Committee of Walter Sisulu University and the University of Cape Town approved this study (reference number 066/2017 and 135/2018 respectively). Participation in the study was voluntary with the participants having the freedom to withdraw from the study at any time. All participants that were enrolment into the study provided informed consent.

6.3 Results

The normotensive pregnant women, women with preeclampsia and severe preeclampsia had comparable age (median of 23, 24 and 23 years respectively) and BMI (median 28, 29.3 and 30.2 Kg/m^2), $p > 0.05$ (Table 6.1). The normotensive pregnant women had a higher gestational age at enrolment (median of 39.0 WOA) than women with preeclampsia (34.0 WOA) and severe preeclampsia (32.5 WOA) $p < 0.001$. The peripheral systolic and diastolic pressure as well as the aortic systolic and diastolic pressure of normotensive pregnant women were significantly lower than that of women with preeclampsia and severe preeclampsia $p < 0.05$, Table 6.1).

Table 6. 1: general characteristics, endothelial, and thyroid function features of cases and controls

Variable	**Normotensive Median (p25, p75)**	**Preeclampsia Median (p25, p75)**	**Severe preeclampsia Median (p25, p75)**	**P-value**
Age (years)	23 (20.0, 29.0)	24.0 (20.0, 29.8)	23.0 (20.0, 29.0)	0.839
BMI (Kg/m^2)	28.0 (25.9, 31.6)	29.3 (27.0, 38.8)	30.2 (26.2, 34.6)	0.180
GA at enrolment (WOA)	39.0 (37.0, 40.0)	34.0 (29.3, 38.8)	32.5 (30.0, 36.3)	< 0.001
Peripheral systolic BP (mmHg)	121 (114.0, 127)	139.5 (128.3, 145.5)	143.0 (130.8, 158.0)	< 0.001
Peripheral diastolic BP (mmHg)	76.5 (70.0, 82.0)	90.0 (81.0, 99.0)	95.0 (84.5, 102.3)	< 0.001
Aortic systolic BP (mmHg)	106.0 (98.5, 115.0)	128.0 (117.0, 138.5)	126.0 (120.0, 144.0)	<0.001
Aortic diastolic BP (mmHg)	79.5 (72.0, 84.0)	94.0 (85.8, 94.0)	98.0 (93.0, 124.0)	< 0.001
Aortic Augmentation BP (mmHg)	2.0 (0.0, 5.0)	5.0 (1.0, 10.0)	7.0 (2.0, 13.0)	< 0.001
Aortic Augmentation index	7.5 (-1.0, 18.3.0)	19.0 (6.0, 32.0)	21.0 (8.0, 39.0)	< 0.001
Pulse wave velocity (m/s)	5.1 (4.7, 5.7)	5.7 (4.9, 6.5)	6.3 (5.7, 7.0)	< 0.001
OxLDL (IU/L)	1.0 (0.9, 1.2)	1.0 (0.8, 1.2)	1.2 (1.0, 1.3)	0.003
TSH (IU/L)	2.3 (1.8, 3.1)	2.3 (1.8, 3.3)	3.0 (2.2, 4.2)	0.005
UIC (µg/L)	167.5 (88.5, 295.5)	127.7 (75.8, 364.3)	88.5 (52.6, 550.5)	0.150
Thyroglobulin (µg/L)	19.7 (13.0, 34.8)	21.4 (13.2, 36.2)	22.4 (15.0, 38.5)	0.785

BMI: body mass index; GA: gestational age; WOA: weeks of amenorrhoea; BP: blood pressure; OxLDL: oxidised low-density lipoprotein; TSH: thyroid-stimulating hormone; UIC: urinary iodine concentration

The median aortic augmentation pressure was 2.0, 5.0, and 7.0 mmHg respectively for normotensive, preeclampsia and severe preeclampsia participants ($p < 0.001$). A similar pattern was also observed for the Aortic augmentation index (medians of 7.5, 19.0 and 21.0, $p < 0.001$) and the pulse wave velocity (m/s) (medians of 5.1, 5.7 and 6.3 respectively for normotensive, preeclampsia and severe preeclampsia participants ($p < 0.001$, Table 6.1). Participants with severe preeclampsia had significantly higher TSH and oxidised low-density lipoproteins than the counterparts with preeclampsia or normotensive pregnant controls ($p < 0.001$, Table 6.1). There was no significant difference in the UIC and thyroglobulin of the three groups ($p = 0.785$). However, the median UIC of normotensive pregnant women, those with preeclampsia and severe preeclampsia were 167.5 µg/L, 127.7 µg/L, 88.5 µg/L respectively demonstrating adequate, mild and moderate insufficient iodine intake in pregnancy that is clinically significant.

We used the second and third trimester upper serum TSH limit of 3.0 IU/L (18) and the 10th FT4 percentile of 11.3 pmol/L (19) to determine the thyroid function status. The prevalence of subclinical hypothyroidism (SCH) and overt hypothyroidisms (OH) were SCH: 22.9%, 24% and 35.6%; and OH: 3.5%, 6.0% and 11.1% for normotensive pregnant women, preeclampsia and severe preeclampsia participants respectively (Table 6.2).

Table 6.2: Thyroid function status of normotensive, preeclampsia and severe preeclampsia participants

Thyroid status	**Normotensive n (%)**	**Preeclampsia n (%)**	**Severe preeclampsia n (%)**	**Chi square**	**P value**
Euthyroid	59 (67.8)	27 (54.0)	17 (37.8)	13.788	0.032
SCH	20 (22.9)	12 (24.0)	16 (35.6)		
Hypothyroxinaemia	5 (5.8)	8 (16.0)	7 (15.6)		
Overt Hypothyroidism	3 (3.5)	3 (6.0)	5 (11.1)		
Total	87 (100)	50 (100)	45 (100)		

SCH: subclinical hypothyroidism

Of the three markers of iodine nutrition status (UIC, thyroglobulin and TSH), TSH was positively correlated with PWV, aortic systolic and diastolic pressure, as well as peripheral diastolic and systolic pressure (Tables 6.3 and 6.4). UIC was significantly correlated with oxidised LDL (Pearson correlation coefficient 0.205, $p = 0.008$).

Table 6. 3: parametric correlation matrix of iodine nutrition biomarkers and biomarkers of endothelial dysfunction

	Pearson	TSH	UIC	Tg	PWV	ASBp	ADBp	PSBp	PDBp	AABp	AAI
TSH	correlation		.002	.182*	.194*	.250**	.208**	.108	.161*	.099	.108
	p value		.978	.022	.012	.001	.007	.162	.037	.205	.166
UIC	correlation	.002		.062	.010	-.036	-.060	.006	.059	-.079	-.040
	p value	.978		.460	.895	.647	.444	.934	.453	.314	.610
Tg	correlation	.182*	.062		-.076	.086	.096	.061	-.004	.071	.007
	p value	.022	.460		.357	.295	.245	.459	.957	.387	.930

TSH: thyroid-stimulating hormone; UIC: urinary iodine concentration; Tg: thyroglobulin; ASBp: aortic systolic blood pressure; ADBp: aortic diastolic blood pressure; PSBp: peripheral systolic blood pressure; PDBp: peripheral diastolic blood pressure; AABp: aortic augmentation blood pressure; AAI: aortic augmentation index. * p<0.05; **p<0.01.

Table 6. 4: non-parametric correlation matrix of iodine nutrition biomarkers and biomarkers of endothelial dysfunction

	Spearman's	TSH	UIC	Tg	PWV	ASBp	ADBp	PSBp	PDBp	AABp	AAI
TSH	rho		-.079	.182*	.113	.223**	.255**	.175*	.188*	.058	.052
	p value		.319	.022	.147	.004	.001	.023	.015	.456	.502
UIC	rho	-.079		-.042	.012	-.093	-.038	-.052	.004	-.134	-.131
	p value	.319		.616	.878	.235	.629	.509	.959	.087	.095
Tg	rho	.182*	-.042		-.061	.127	.082	.067	-.031	.025	.007
	p value	.022	.616		.459	.124	.319	.417	.705	.766	.930

TSH: thyroid-stimulating hormone; UIC: urinary iodine concentration; Tg: thyroglobulin; ASBp: aortic systolic blood pressure; ADBp: aortic diastolic blood pressure; PSBp: peripheral systolic blood pressure; PDBp: peripheral diastolic blood pressure; AABp: aortic augmentation blood pressure; AAI: aortic augmentation index. * p<0.05; **p<0.01.

Participants with TSH ≥ 4 IU/L, in comparison with those with TSH < 4 IU/L, had higher median pulse wave velocity, and borderline higher aortic systolic and diastolic pressure, as well as peripheral diastolic pressure (5.4 vs 6.0, 115 vs 122 mmHg, 84 vs 91 mmHg and 82 vs 88 mmHg respectively) (Table 6.5).

Table 6. 5: Median pulse wave velocity, aortic systolic and diastolic pressure, and peripheral diastolic pressure of participants with Thyroid-stimulating hormone levels < 4 IU/L or ≥ 4 IU/L

Variables	**TSH < 4 IU/L Median (p25, p75)**	**TSH ≥ 4 IU/L Median (p25, p75)**	***P value**
Pulse wave velocity (m/s)	5.4 (4.7, 6.3)	6.0 (5.1, 7.1)	0.025
Aortic systolic (BP mmHg)	115.0 (104.0, 126.0)	122.0 (105.8, 135.8)	0.057
Aortic diastolic (BP mmHg)	84 (77.0, 95.0)	91.0 (81.5, 107.0)	0.055
Peripheral diastolic (BP mmHg)	82.0 (75.0, 92.5)	88.0 (77.3, 95.2)	0.252

*Mann-Whitney U test

In multivariable linear regression models that included maternal age and hypertensive disease status, two known independent predictors of endothelial dysfunction as well as gestational age, a potential confounder that was statistically significant on univariable analysis, TSH together with maternal age and hypertensive disease status remained independent predictors of PWV (Table 6.6).

Table 6. 6: Linear regression univariable and multivariable Beta coefficients of the factors associated with pulse wave velocity

Variable	**Univariable Beta coefficient (95% CI)**	**P value**	**Multivariable Beta coefficient (95% CI)**	**P value**
Age (years)	0.036 (0.011–0.061)	0.005	0.037 (0.013–0.061)	0.003
HDP	0.469 (0.282–0.656)	< 0.001	0.353 (0.116–0.590)	< 0.004
TSH (UI/L)	0.142 (0.031–0.253)	0.012	0.109 (0.001–0.217)	0.048
GA (weeks)	-0.062 (-0.095–-0.030)	< 0.001	-0.029 (-0.067–0.100)	0.140
Parity	0.106 (-0.05–0.216)	0.060		
BMI (Kg/m^2)	0.008 (-0.20–0.035)	0.583		

TSH: Thyroid-stimulating hormone; HDP: hypertensive disease in pregnancy; GA: gestational age

6.4 Discussion

In the current study, the pulse wave velocity (PWV), aortic augmentation index and augmentation pressure, which are measures of arterial stiffness (22) were significantly increased among women with preeclampsia when compared with normotensive pregnant controls. This is consistent with other studies and implies structural changes in lamina media of the aorta among women with elevated values (23, 24). PWV, an objective measure of endothelial dysfunction and arterial stiffness, becomes elevated secondary to changes in vascular wall especially a higher collagen-elastin ratio that decreases dispensability (25).

The current study also found that elevated TSH is an independent predictor of PWV after adjusting for maternal age, gestational age and preeclampsia status. Elevated TSH level, consistent with SCH, was associated with significantly higher median PWV. This is similar to findings by others who reported that even mildly elevated TSH was associated with endothelial dysfunction and structural vascular changes associated with arterial stiffness (26, 27) although the study was not conducted among pregnant women. The mildly elevated TSH observed in the current study was not associated with atherogenic lipid profile that has been reported in some previous studies (28).

Although there was no significant correlation between UIC and TSH, participants with severe preeclampsia had lower median UIC but higher median TSH, which suggests prolonged exposure to insufficient iodine intake. This finding correlates with that of Abel et al. (13) who reported that women with insufficient iodine intake at the inception of pregnancy are at increases risk of preeclampsia and other adverse pregnancy outcomes. Screening for iodine deficiency and supplementation is not yet part of the routine maternity care in South Africa (29). Of recent iodine intake in pregnancy in South Africa was reported to be adequate (30, 31). However, these surveys did not use nationally presentative samples hence could have masked inadequate iodine intake in pregnancy in some local geographical settings.

The degree of iodine deficiency and the levels of oxidized LDL both increased with the severity of preeclampsia. Oxidized LDL is a known precursor of atherosis, endothelial dysfunction and arterial stiffness (28). Hence, the observed relationship between PWV and TSH could partially be mediated by the underlying exposure to iodine deficiency causing reduced anti-oxidant status (32) and the resultant increase in oxidized LDL.

Others have found elevated TSH to be associated with increased carotid media thickness, which like PWV, is an early marker of atherosclerosis and future cardiovascular disease (33). This is mediated through stimulation of endothelial TSH receptors, inhibition of endothelial nitric oxide synthase, NO depletion resulting in reduced flow mediated dilation and endothelial activation (6-8, 33).

The increase in arterial stiffness among women with preeclampsia has been found to persist up to ten years post-delivery and may in part be responsible for the increased risk of future cardiovascular disease and end-stage renal failure (3, 34-36). If the observed relationship in the current study between iodine deficiency and TSH that seems to increase the risk and severity of endothelial dysfunction and preeclampsia is confirmed by future studies, it will be worthwhile to screen and treat iodine deficiency among pregnant women who live in geographical locations and populations at risk of insufficient iodine intake.

6.5 Strengths and limitations

This study has found a probable association between iodine deficiency-mediated TSH elevation, endothelial dysfunction and preeclampsia which if confirmed by further research can be prevented with iodine supplementation. However, this study limited by the indirect measurement of endothelial dysfunction as well as non-measurement of potential confounders of the association between preeclampsia and endothelial dysfunction such as endothelin. Several women with preeclampsia had spent some days admitted in hospital where they were exposed to meals prepared with adequately iodized salt that may have significantly affected their urinary iodine concentration. Although median spot urinary iodine concentration is recommended as a measure of iodine nutrition status at population level, it is not a robust measure of the iodine nutritional status at individual level (21, 37).

6.6 Conclusion

Preeclampsia is characterized by increased PWV, aortic augmentation index and aortic augmentation pressure, which are markers of arterial stiffness and predictors of future cardiovascular disease. Elevated TSH increases the risk of endothelial dysfunction in pregnancy even when it is within levels suggestive of mild subclinical hypothyroidism as observed in the current study.

References

1. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A**.** Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000;101(9):948-54.
2. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T**.** Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation. 2001;104(22):2673-8.
3. Christen M KC, Knudsen UB Preeclampsia and arterial stiffness – A 10-year follow up of previous preeclamptic women. . Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 2015:72 – 3.
4. Floriani C, Gencer B, Collet TH, Rodondi N**.** Subclinical thyroid dysfunction and cardiovascular diseases: 2016 update. Eur Heart J. 2018;39(7):503-7.
5. Wu R, Wang T, Gu R, Xing D, Ye C, Chen Y, et al. Hypertensive Disorders of Pregnancy and Risk of Cardiovascular Disease-Related Morbidity and Mortality: A Systematic Review and Meta-Analysis. Cardiology. 2020;145(10):633-47.
6. Tian L, Zhang L, Liu J, Guo T, Gao C, Ni J**.** Effects of TSH on the function of human umbilical vein endothelial cells. J Mol Endocrinol. 2014;52(2):215-22.
7. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2006;91(10):4175-8.
8. Lioudaki E, Mavroeidi NG, Mikhailidis DP, Ganotakis ES**.** Subclinical hypothyroidism and vascular risk: an update. Hormones (Athens). 2013;12(4):495-506.
9. Lazarus J**.** Thyroid Regulation and Dysfunction in the Pregnant Patient. In: Feingold KR AB, Boyce A, et al, ed. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2000; 2016.
10. Zantour B AW, Marmouch H, Chebbi W**.** Hypothyroidism. In: E P, ed. Pregnancy in Current Topics in Hypothyroidism with Focus on Development

Rijeka, Croatia: In Tech; 2013:29-62.

11. Carare Candido A, Azevedo F, Machamba A, Pinto C, Lopes S, Macedo M, et al. Implications of iodine deficiency by gestational trimester: a systematic review. Archives of Endocrinology and Metabolism. 2020;64.
12. Nazarpour S, Ramezani Tehrani F, Simbar M, Azizi F**.** Thyroid dysfunction and pregnancy outcomes. Iran J Reprod Med. 2015;13(7):387-96.
13. Abel MH, Caspersen IH, Sengpiel V, Jacobsson B, Meltzer HM, Magnus P, et al. Insufficient maternal iodine intake is associated with subfecundity, reduced foetal growth, and adverse pregnancy outcomes in the Norwegian Mother, Father and Child Cohort Study. BMC Med. 2020;18(1):211.
14. Jooste P, Zimmermann M**.** Progress towards eliminating iodine deficiency in South Africa. South African Journal of Clinical Nutrition. 2008;21(1):8-14.
15. Namugowa AV, Meeme A**.** PP017 Comparison of vascular function in preeclamptic and normotensive pregnant women in the rural eastern Cape province of South Africa. Pregnancy Hypertens. 2012;2(3):250-1.

16. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4(2):97-104.

17. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation. 2005;111(5):697-716.

18. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid. 2011;21(10):1081-125.

19. Moncayo R, Zanon B, Heim K, Ortner K, Moncayo H**.** Thyroid function parameters in normal pregnancies in an iodine sufficient population. BBA Clinical. 2015;3:90-5.

20. Yu S, Yin Y, Cheng Q, Han J, Cheng X, Guo Y, et al. Validation of a simple inductively coupled plasma mass spectrometry method for detecting urine and serum iodine and evaluation of iodine status of pregnant women in Beijing. Scand J Clin Lab Invest. 2018;78(6):501-7.

21. WHO**.** Proceedings of the WHO Technical Consultation on control of iodine deficiency in pregnant women and young children Geneva: World Health Organization; 2005.

22. Foo FL, McEniery CM, Lees C, Khalil A**.** Assessment of arterial function in pregnancy: recommendations of the International Working Group on Maternal Hemodynamics. Ultrasound Obstet Gynecol. 2017;50(3):324-31.

23. Torrado J, Farro I, Zócalo Y, Farro F, Sosa C, Scasso S, et al. Preeclampsia Is Associated with Increased Central Aortic Pressure, Elastic Arteries Stiffness and Wave Reflections, and Resting and Recruitable Endothelial Dysfunction. Int J Hypertens. 2015;2015:720683.

24. Osman MW, Nath M, Breslin E, Khalil A, Webb DR, Robinson TG, et al. Association between arterial stiffness and wave reflection with subsequent development of placental-mediated diseases during pregnancy: findings of a systematic review and meta-analysis. J Hypertens. 2018;36(5):1005-14.

25. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588-605.

26. Dagre AG, Lekakis JP, Papaioannou TG, Papamichael CM, Koutras DA, Stamatelopoulos SF, et al. Arterial stiffness is increased in subjects with hypothyroidism. Int J Cardiol. 2005;103(1):1-6.

27. Lambrinoudaki I, Armeni E, Rizos D, Georgiopoulos G, Kazani M, Alexandrou A, et al. High normal thyroid-stimulating hormone is associated with arterial stiffness in healthy postmenopausal women. J Hypertens. 2012;30(3):592-9.

28. Li D, Mehta JL**.** Oxidized LDL, a critical factor in atherogenesis. Cardiovasc Res. 2005;68(3):353-4.

29. DOH**.** Guidelines for maternity care in South Africa. Pretoria, South Africa: Department of Health; 2016.

30. Mabasa E, Mabapa S, Jooste PL, Mbhenyane XG**.** Iodine status of pregnant women and children age 6 to 12 years feeding from the same food basket in Mopani district, Limpopo province, South Africa. South African Journal of Clinical Nutrition. 2018;32:1-7.

31. IGN**.** Global Scorecard of Iodine Nutrition in 2017 in general population and in pregnant women (PW) Zurich, Switzerland: Iodine Global Network; 2017.

32. Winkler R, Griebenow S, Wonisch W**.** Effect of iodide on total antioxidant status of human serum. Cell Biochem Funct. 2000;18(2):143-6.

33. O'Leary DH, Polak JF**.** Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol. 2002;90(10c):18l-21l.

34. Vlachopoulos C, Aznaouridis K, Stefanadis C**.** Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27.

35. Estensen ME, Remme EW, Grindheim G, Smiseth OA, Segers P, Henriksen T, et al. Increased arterial stiffness in pre-eclamptic pregnancy at term and early and late postpartum: a combined echocardiographic and tonometric study. Am J Hypertens. 2013;26(4):549-56.

36. Orabona R, Sciatti E, Vizzardi E, Bonadei I, Valcamonico A, Metra M, et al. Endothelial dysfunction and vascular stiffness in women with previous pregnancy complicated by early or late pre-eclampsia. Ultrasound Obstet Gynecol. 2017;49(1):116-23.

37. König F, Andersson M, Hotz K, Aeberli I, Zimmermann MB**.** Ten repeat collections for urinary iodine from spot samples or 24-hour samples are needed to reliably estimate individual iodine status in women. J Nutr. 2011;141(11):2049-54.

Part 3: Investigating possible mechanisms through which iodine deficiency predisposes to various degrees of preeclampsia and eclampsia

This part comprises of the following two chapters:

Chapter 7: Exploration of the underlying nutritional, inflammatory and oxidative stress pathological mechanisms in Preeclampsia using principal component analysis

Chapter 8: Low serum triiodothyronine and potassium levels are associated with increased risk of Eclampsia among women in the Eastern Cape Province of South Africa

CHAPTER 7

Exploration of the underlying nutritional, inflammatory and oxidative stress pathological mechanisms in Preeclampsia using principal component analysis

Charles Bitamazire Businge, Benjamin Longo-Mbenza, Andre Pascal Kengne

Businge CB, Longo-Mbenza B, Kengne AP. Exploration of the underlying inflammatory and oxidative stress pathological mechanisms in preeclampsia using principal component analysis. [Poster presentation]. 8th Annual Conference on Clinical Research & Biomarkers July 19 - 20, 2018 Prague, Czech Republic. DOI: 10.21767/2472-1646-C1-003.

*Businge CB, Longo-Mbenza B, Kengne AP. Exploration of the Underlying Nutritional, Inflammatory and Oxidative Stress Pathological Mechanisms in Preeclampsia Using Principal Component Analysis. EJMED. 2021; 3: 19-24.***

Abstract

Background: Normal pregnancy is characterized by a mild systemic inflammatory response and progressive increase in serum inflammatory cytokines that peak in the third trimester. During pregnancy pre-existing inflammatory conditions, acquired oxidative stress arising from the placenta malfunction and nutritional deficiencies can trigger intense systemic responses that lead to endothelial activation, dysfunction and preeclampsia. We investigated the principal nutritional, oxidative and inflammatory pathways that trigger the clinical manifestation of preeclampsia.

Methods: This case-control study included 250 women with preeclampsia and 150 normotensive pregnant women. Urinary Iodine concentration (IUC) and serum levels of Ferritin, Thyroid-stimulating Hormone (TSH), selenium, Nitric Oxide (NO) gamma glutamyl transferase (GGT), Rheumatoid factor, and high sense C-reactive protein (hs-CRP) of cases and controls were compared using the student's t and the Mann-Whitney U tests. Principal component analysis was carried out to delineate the patterns of association between nutritional, inflammatory and oxidative markers and preeclampsia.

Results: The main pathophysiological pathways identified were the interactions between selenium/iodine deficiency, and elevated serum TSH (endothelial dysfunction); serum ferritin, GGT, CRP and low urinary iodine excretion (inflammatory oxidative stress); elevated serum hs-CRP and Rheumatoid factor subclinical inflammation and immune cell activation) and high T3/T4 ratio (acute TSH stimulation of thyroid with low thyroid iodine stores)

Conclusion: Combined selenium and iodine deficiency resulting into elevated TSH, low NO and preferential T3 secretion; acute inflammatory conditions associated with elevated serum GGT, CRP, and Ferritin; and subclinical inflammatory conditions characterized by autoimmunity are some of the major oxidant and inflammatory pathways associated with increased risk of preeclampsia.

Keywords: preeclampsia, oxidative stress, inflammation, selenium, iodine, TSH, GGT, CRP

7.1 Introduction

The normal physiological changes in pregnancy include mild systemic inflammatory response (1). This is characterized by an elaboration of inflammatory leukocytes, endothelial activation, the acute phase response, and metabolic features of systemic inflammation, a decrease in plasma albumin levels and increased plasma fibrinogen levels. There is a progressive increase in serum inflammatory cytokines which peak in the third trimester (2, 3). Redman et al. hypothesized that systemic inflammatory response of preeclampsia is just a more extreme part of the spectrum common to all pregnancies, with preeclampsia developing when the systemic inflammatory process causes maternal systems to decompensate (3). It is hypothesized that an oxidatively stressed placenta in a previously normal woman, or a normal systemic inflammatory response in a woman with a chronic inflammatory response due to pre-existing chronic infection such as Helicobacter pylori, obesity, essential hypertension, diabetes or other stimulus, can trigger an intense systemic response that leads to endothelial activation, dysfunction and preeclampsia (1).

The aim of the study was to investigate the principal oxidative and inflammatory pathways that are associated with the clinical manifestation of preeclampsia in a peri-urban population of Kinshasa Province, Democratic Republic of Congo.

7.2 Materials and Methods

Study design

This case-control study was carried out as a secondary analysis data of expectant mothers who enrolled as participants of the Communicable Disease, Nutritional, Environmental Epidemiology and Cardio-metabolic Risk Study (CDNECR). Cases were women with preeclampsia, while controls were age-matched pregnant women without preeclampsia. All cases were managed at Maternity Unit of Lomo Medical Centre, Kinshasa, DRC. The controls were women with normal pregnancy that delivered at term at the maternity units of hospitals that referred patients to LMC for the CDNECR study.

Ethical clearance

The primary study was approved by the Lomo Medical Centre Institutional Review Board (Reference no. LMDE031LMB02). All participants gave informed consent and study procedures complied with the Helsinki Declaration. Further clearance was obtained from the

University of Cape Town Human Research Ethics Review Committee (reference number 135/2018) for secondary data analysis and inclusion of the findings in the current thesis.

Study setting

The CDNER study was carried out in Kinshasa Province, Democratic Republic of Congo between 2007 and 2008 and coordinated at Lomo Medical Centre. Lomo Medical Centre (LMC) is a tertiary private healthcare centre in the Kinshasa province of the Democratic Republic of Congo (DRC). LMC has a High Dependency Unit with 12 beds that care for patients most of whom are referred from Bondeko clinic, St Joseph Hospital and Ndjili Hospital in Kinshasa Province. The catchment population is estimated to be about 3 million people. The staff complement is composed of four cardiologists, two Obstetricians and Gynaecologists, three surgeons, two anaesthesiologists, one ophthalmologist, two radiologists, two clinical pathologists and one paediatrician. On average, LMC cares for about 400 complicated maternity cases annually, 80% of whom are preeclamptic mothers referred for tertiary care.

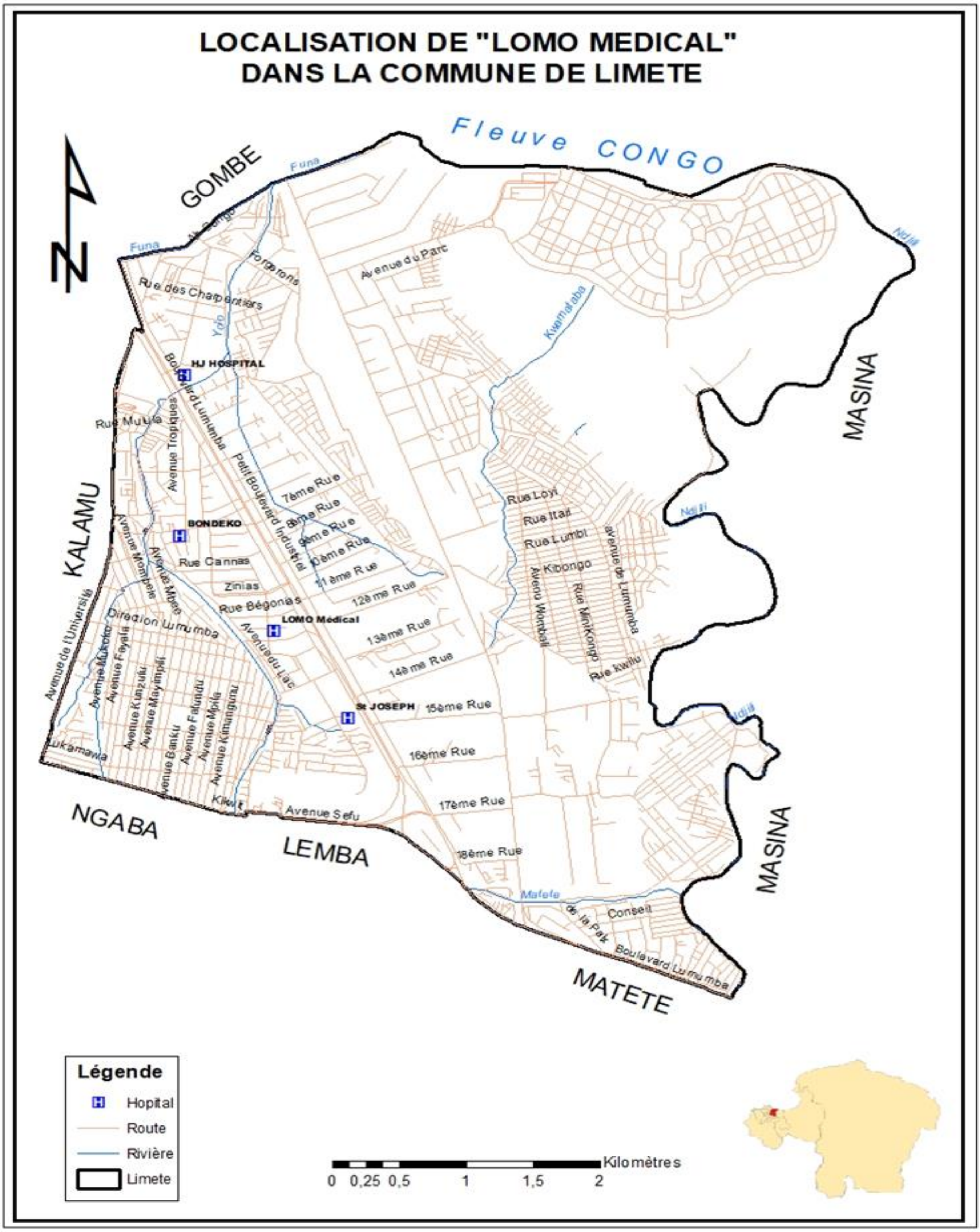


Figure 7. 1: Map showing the location and catchment area of LOMO Medical Centre and referring Hospitals within Kinshasa Province, Democratic Republic of Congo.

Sample size and derivation of the analytic sample

During the study period, about 2100 antenatal women patients were monitored. One hundred and fifty (150) who delivered at term without developing preeclampsia (normotensive controls) were matched for age with three hundred of the women who developed preeclampsia (cases). However, only 250 cases with complete data were included in the analysis.

Data collection

Preeclampsia was defined according the International Society for the Study of Hypertension in Pregnancy (4). Participants were diagnosed with pre-eclampsia when they presented with new onset of hypertension [systolic blood pressure (SBP) >140 mmHg and or diastolic (DBP) blood pressure >90 mmHg] after 20 weeks gestation with proteinuria (spot urine protein/creatinine >30 mg/ mmol, or >300 mg/day or 2 + on dipstick testing) or other maternal organ dysfunction: renal insufficiency (creatinine >90 µmol/L; 1.02 mg/dL); liver involvement (elevated transaminases at least twice upper limit of normal ± right upper quadrant or epigastric abdominal pain), neurological complications (altered mental status, blindness, stroke, hyperreflexia, severe headaches, and persistent visual scotomata), haematological complications (thrombocytopenia–platelet count below 150,000/dL, disseminated intravascular coagulation or haemolysis) and uteroplacental dysfunction (foetal growth restriction, abruptio placentae or intrauterine foetal death). Participants were diagnosed with severe pre-eclampsia when they presented with SBP >160 mmHg or DBP >110 mmHg with or without systemic organ involvement. Participants were diagnosed with eclampsia when they presented with SBP >140 mmHg or DBP >90 mmHg and convulsions after 20 weeks' gestation.

The height and weight were measured according to standardized procedures. Blood pressure was measured according to the American Heart Association guidelines, with the patient's elbow flexed at the heart level. An average of the two measurements with a standard sphygmomanometer taken at intervals of 2 minutes after the participants had been sitting for at least 30 minutes was used (5).

Blood sample collection and analysis

Overnight fasting venous blood was drawn between 7:00 and 9:00 a.m. Fasting serum and plasma samples preserved with ethylenediaminetetraacetic acid (EDTA) and sodium fluoride (NaF) were collected from the cubital fossa. Blood samples were assayed immediately to measure the concentrations serum levels of highly sensitive C-reactive protein (hs-CRP), Ferritin, gamma glutamyl transferase (GGT), Rheumatoid factor (RF), C-reactive protein (CRP), thyroid-stimulating hormone (TSH), selenium and nitric oxide (NO). At the same sitting, a mid-stream urine sample was collected to determine urinary Iodine excretion. Serum levels were obtained using calibrated spectrometers and standard routine procedures and specific protocols of manufacturers. TSH were measured by enzyme-linked immunosorbent assay. NO was measured using Cayman kits (Cayman Chemical company Ann Arbot, MI). Urinary iodine concentration was measured using the Sandell-Kolthof method.

Statistical analysis

Categorical variables were compared using chi-square test, while continuous variables were compared using Student's t-test for normally distributed data and the Mann-Whitney U test for skewed data. A p-value < 0.05 was considered as statistically significant. All analyses were performed using the Statistical Package for Social Sciences (SPSS) for windows version 23.0 (SPSS Inc) Chicago, Il, USA. Principal component analysis was carried out to delineate the patterns of association between selected inflammatory and oxidative markers which had significant association with preeclampsia. This statistical tool groups together variables that are strongly correlated with one another and weakly with all the variables in other groups/components. Eigen-values = 1 were used to identify the key components accounting for the bulk of the observed variance in the manifestation of preeclampsia in the study population. This was to help disaggregate different pathological pathways that may trigger the intense systemic response that leads to endothelial activation, dysfunction and clinical manifestation of preeclampsia.

7.3 Results

General characteristics of the study population

There were 250 cases of preeclampsia with a mean age 32.4 ± 6 years and 150 controls cases with a mean age of 33.5 ± 5.2, p = 0.072. The mean gestational age at recruitment and sample collection was 37.7 ± 4.0 weeks of amenorrhoea (WOA) for controls and 31.0 ± 7.9 WOA for cases p <0.0001. The mean BMI and IgG anti H pylori for cases and controls respectively were: 25.2 ± 6.0 and 22.2 ± 5.5 Kg/m2; 98.6 ± 68.0 and 73.6 ± 68.1, p <0.0001.

Median values and main biomarker components

Participants with preeclampsia had significantly higher median serum levels of hs-CRP, GGT, Rheumatoid factor, TSH and a significantly higher mean T3/T4 but significantly lower Selenium, NO and urinary Iodine excretion than controls (Table 5.1). Serum Ferritin and CRP were elevated in the lower half of the cases with no significant difference when compared among all cases and controls.

Table 7. 1: Median/mean values of select oxidative stress and inflammatory Biomarkers measured in participants with and without preeclampsia

Variable	**Cases (n=250)**	**Controls (n=150)**	**P value**
	Median (p25, p75) or Mean ± SD	**Median (p25, p75) or Mean ± SD**	
Selenium µg/L	9.0 (9.0, 17.3)	44.0 (21.0, 102.8)	<0.0001
hs - CRP mg/L	8.0 (3.3, 9.0)	3.1 (2.9, 5.0)	<0.0001
NO µmol/L	2.0 (1.0, 6.0)	20.8 (4.0, 43.3)	<0.0001
Serum Ferritin µg/L	213.0 (180.0, 345.0)	199.0 (167.0, 344.0)	0.114
GGT	99.0 (88.0, 113.0)	33.0 (11.0, 99.0)	<0.0001
Rheumatoid Factor	56.0 (9.0, 88.0)	8.5 (7.0, 21.5)	<0.0001
CRP mg/dL	58.5 (39.0, 66.0)	57.0 (13.0, 88.0)	0.437
TSH mIU/L	6.3 (4.1, 8.0)	2.5 (0.13, 4.4)	<0.0001
Urinary Iodine excretion µg/L	90.0 (78.0, 157.2)	351.0 (299.0, 555.0)	<0.0001
T3/T4 ratio	0.14 ± 0.05	0.12 ± 0.00	<0.0001

(p25, p75) = 25th and 75th percentiles; SD = Standard deviation

Among the cases, the principal components (suggestive of pathological pathways) (Table 7.2 and

Figure 7.2), with Eigen-values above 1, accountings for over 70 percent of the manifestation of preeclampsia were:

Component 1: Low serum Selenium, low NO, low urinary iodine excretion and elevated serum

TSH which are features of iodine deficiency, sub-clinical hypothyroidism and selenium-mediated endothelial dysfunction

Component 2: Elevated serum Ferritin, GGT, CRP and low urinary iodine excretion which are features of inflammation and oxidative stress

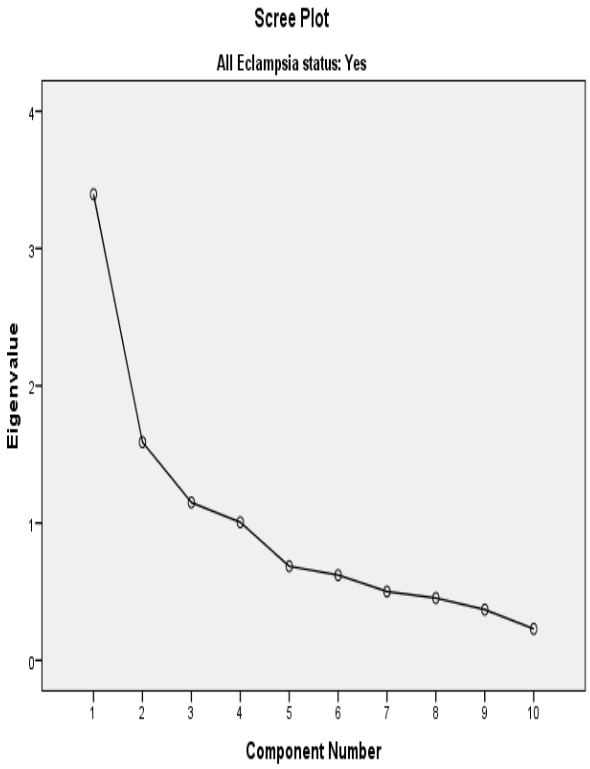
Component 3: Elevated serum hs-CRP and Rheumatoid factor which are features of inflammation and immune cell activation

Component 4: high T3/T4 ratio reflecting the increased TSH stimulation of the thyroid in states of chronic iodine insufficiency and low thyroid iodine stores

Table 7. 2: Rotated component matrix for cases

Biomarker	**Component**			
	1	**2**	**3**	**4**
Selenium µg/L	**0.834**	-0.184	-.197	-.077
NO µmol/L	**0.870**	-0.081	-.077	-.055
TSH mIU/L	**-0.789**	0.097	-.128	-.077
Urinary Iodine excretion µg/L	**0.541**	**-0.454**	-.157	.061
Serum Ferritin µg/L	-0.050	**0.777**	-.247	-.054
GGT	-.239	**0.703**	.336	.040
CRP mg/dL	-.188	**0.696**	.359	.119
hs - CRP mg/L	-0.056	0.017	**0.861**	-.003
Rheumatoid Factor	-.047	0.159	**0.812**	-.032
T3/T4 ratio	-.005	0.028	-.031	**0.988**

The main variables in each component are highlighted



Components, Eigen-values and variances of cases

Component	Eigen Values	% of variance	Cumulative %
1	**3.395**	**33.950**	**33.950**
2	**1.589**	**15.894**	**49.844**
3	**1.150**	**11.499**	**61.342**
4	**1.006**	**10.058**	**71.401**
5	.686	6.856	78.257
6	.621	6.211	84.467
7	.501	5.009	89.476
8	.454	4.538	94.015
9	.370	3.695	97.710
10	.229	2.290	100.000

Figure 7. 2: Scree plot showing Eigenvalues for various components of oxidative and inflammatory biomarkers for cases. The Eigenvalues for the 4 major components are shown in bold on the adjacent table.

There were 3 major components (Table 7.3 and Figure 7.3), with Eigen-values above 1, accounting for over 58 percent variance for a normal pregnancy without manifestation of preeclampsia (the control group) were:

Component 1: high serum Selenium, NO and low TSH which are features of normal thyroid function without thyroid risk of increased thyroid derived superoxide and hydrogen peroxide

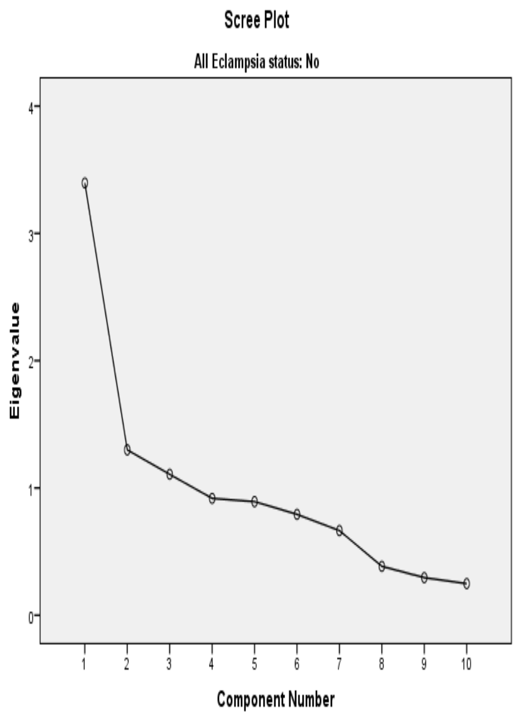
Component 2: normal serum GGT, hs-CRP, CRP and Ferritin depicting absence of systemic inflammation

Component 3: Normal T3/T4 ratio and normal urinary iodine excretion depicting adequate iodine reserves for normal thyroid function

Table 7. 3: Rotated component matrix for controls

Biomarker	**Component**		
	1	**2**	**3**
Selenium µg/L	**-0.834**	-0.116	0.062
NO µmol/L	**-0.824**	-0.249	0.038
Baseline TSH mIU/L	**0.883**	0.096	0.048
hs - CRP mg/L	0.128	**0.631**	0.074
GGT	0.148	**0.802**	-0.147
CRP mg/dL	0.379	**0.711**	-0.207
Serum Ferritin µg/L	0.087	**0.589**	0.151
Urinary Iodine excretion µg/L	-0.040	0.229	**0.757**
T3/T4 ratio	0.000	0.181	**-0.672**
Rheumatoid Factor	0.411	0.318	-0.006

The main variables in each component are highlighted



Eigen-values and variance of various components for controls

Component	Eigen values	% of variance	Cumulative %
1	**3.396**	**33.962**	**33.962**
2	**1.299**	**12.993**	**46.955**
3	**1.107**	**11.073**	**58.029**
4	.918	9.184	67.213
5	.893	8.928	76.141
6	.793	7.926	84.068
7	.665	6.650	90.717
8	.384	3.842	94.560
9	.296	2.956	97.515
10	.248	2.485	100.000

Figure 7. 3: Scree plot showing Eigenvalues of various components of oxidative and inflammatory biomarkers for controls. Eigenvalues for the three major components are shown in bold

7.4 Discussion

Using the principal component analysis in the current study ten possible mechanisms through which maternal micronutrient, oxidant and inflammatory state increases the risk of preeclampsia were identified. Out of the ten, there were four major contributory pathways in the study population associated with more than 70% of the cases of preeclampsia. The four were:

Iodine and selenium deficiency acting together with elevated TSH and low serum NO, accounting for almost 34% of the observed variance among participants with preeclampsia. This matches perfectly with the pathophysiology of thyroid gland in states of combined iodine and selenium deficiency, which is endemic in DRC: iodine deficiency exacerbated by the physiological changes of pregnancy predisposing to low production of thyroxine (T4) and tri-iodothyronine (T3), diminished negative feedback on the pituitary and elevated TSH (6-8). In the thyroid gland, TSH induces Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which oxidizes NADPH to NADP+, liberating superoxide radicals which are then converted to the less potent hydrogen peroxide by Superoxide dismutase, a selenium-dependent enzyme. The hydrogen peroxide not used up in this process, is neutralized by selenium-dependent glutathione peroxidase (9). Therefore, elevated TSH with simultaneous selenium deficiency will result in excessive production of serum superoxide and hydrogen peroxide by the thyroid gland (9). The increased circulation of superoxide and hydrogen peroxide radicals which react with Nitric Oxide (NO) forming peroxynitrite which is a more potent oxidant and inducer of inflammation and endothelial dysfunction (9). This will further diminish NO, accentuating oxidative stress, endothelial dysfunction (9, 10) (hence increasing the risk of preeclampsia. Elevated TSH is also a known stimulant of endothelial dysfunction by inhibition of endothelial NO synthase(11). This is associated with reduced serum NO and reduction in flow mediated dilatation and endothelial activation, which are well-known features of preeclampsia (12). TSH by acting on its extra-thyroidal receptors (TSHr) predisposes to endothelial dysfunction and increased carotid media-intima thickness (cIMT) (13, 14). The increased cIMT is an early marker of atherosclerosis and future cardiovascular disease (14).

The second component was made of elevated serum Ferritin, GGT, CRP and low urinary iodine excretion which are features of inflammation and oxidative stress. Pre-pregnancy states such as chronic infections and obesity are associated with elevated serum CRP (15-17). CRP is associated with increased hepcidin production from the liver. Hepcidin is a negative regulator of intracellular iron storage: it leads to reduced iron cellular uptake and increased cellular ferritin secretion by ferroportin, the net effect being an increased serum iron concentration (18-20). GGT is a cell membrane bound enzyme with its active site in the plasma, whose main role is to metabolize reduced extracellular glutathione (GSH) into its precursor amino acids glutamate and cysteine facilitating their re-absorption and intracellular synthesis of GSH (21) GGT by hydrolysing the glutamyl bond between glutamate and cysteine, produces cysteinyl-glycine which is subsequently cleaved by dipeptidase into cysteine and glycine. Cysteinyl-glycine and cysteine have been shown to initiate reductive release of iron from its carrier proteins transferrin and ferritin (iron which is physiologically transported in the bound state as Fe3+ is instead released in its bioactive form of Fe2+) leading to the Fenton and Harber–Weiss reactions with the release of potent hydroxyl (OH) oxidant radicals (21, 22). This pathway is further enhanced by low dietary and serum iodine which is exacerbated due physiological changes of pregnancy. Iodine is a potent exogenous scavenger of oxidant radicals, with up to three times the activity of Vitamin C, whose deficiency is likely to significantly alter the oxidation-redox balance, cause endothelial activation and has been found to be associated with increased risk of preeclampsia (23, 24). Indeed, the placenta is not only a highly metabolic organ with potential for production of oxidant radicals, lipid peroxidation in addition to release of soluble endoglin and soluble fms-like tyrosine kinase 1 in hypoxic states (25-28). It is also one of the organs in the body that concentrates iodine, whose function has hitherto not yet been fully established but could be among others used for scavenging the reactive oxidant radicals (23, 29).

The third principal component encompassed elevated serum hs-CRP and Rheumatoid factor which are features of inflammation and immune cell activation. While CRP is an acute phase protein produced by the liver secondary to any cause of inflammation and has low sensitivity when levels are within normal ranges, hs-CRP has been found to be a sensitive marker of early atherosclerosis and coronary heart disease even when CRP levels are within normal ranges (30). Thus, the association of hs-CRP and Rheumatoid factor suggests low level chronic inflammation secondary to immune activation. Rheumatoid factors (RF) are a group of antibodies (IgM, IgG or IgA) against the Fc portion of IgG (31). Although present in 70% of patients with rheumatoid arthritis, they are not diagnostic for they are also found patients with chronic infections, older people and many auto-immune disorders like systemic lupus erythematosus (SLE) (31). In the current study, low grade inflammation depicted by elevated hs-CRP and chronic infections depicted by elevated serum Rheumatoid factor may be a result of concurrent central obesity and Helicobacter pylori infection. This may explain the increased risk of preeclampsia among women in the upper normal range of BMI observed in the current study.

The last significant component was characterised by higher T3/T4 ratio among the cases but a lower T3/T4 among controls coupled with normal high urinary iodine excretion reflecting the increased TSH stimulation of the thyroid in states of chronic iodine insufficiency and low thyroid iodine stores among the cases. Among women with insufficient iodine intake during pregnancy, the transient rise in total T4 secondary to increased thyroid binding globulin (TBG) is relatively low, and this is accompanied by relative reduction in free T4 and T3 of about 30% after 20 weeks of gestation (32). If iodine deficiency persists, there is preferential secretion of T3 instead of T4 from the thyroid, resulting in a rise in T3/T4 molar ratio above normal range of 10–22 x 10^{-3}. This has been observed in states of persistent thyroid stimulation such as pregnancy and Graves' disease in the presence of depleted intra-thyroidal iodine stores depicting chronic iodine insufficiency (31). In the current study, this pathway may represent women in the study population with suboptimal iodine intake but adequate selenium intake when compared to those in component one. In these women, the extra-thyroidal stimulation of TSH on TSHr receptors on the endothelium that inhibit NO synthesis and the reduced exogenous anti-oxidant capacity due to iodine deficiency may account for the increased risk of preeclampsia (12, 13). Our hypothesis is reinforced by the observation in the current study of normal urinary iodine and T3/T4 among controls, which also were grouped together in the resultant principal component analysis.

Although the trigger for clinical manifestation of preeclampsia are placenta derived factors such as soluble Fms-like tyrosine kinase 1 and endoglin which are produced by ischaemic placentae following failure of secondary trophoblastic invasion of myometrial spiral arteries, the severity of preeclampsia seems to depend to some extent on pre-pregnancy maternal oxidative stress and systemic inflammatory state (26, 27). This inflammatory and oxidative state is upregulated as part of normal physiology of pregnancy which results into early onset preeclampsia in women with high placenta derived factors or late onset preeclampsia, when the normal physiological changes of pregnancy in the third trimester greatly exacerbate the pre-existing oxidant and inflammatory state leading to endothelial activation and dysfunction (1-3). The current study used principal component analysis to investigate how different inflammatory and oxidant pathways interact to increase the risk of preeclampsia. The identified prominent role of selenium and iodine deficiency in the process, suggests an opportunity for screening and nutritional intervention in populations with high incidence of preeclampsia. Similarly, our findings of differential role of elevated CRP and hs-CRP, suggest the need to control conditions such as obesity and chronic infections, which are associated with acute and chronic inflammation in women of reproductive age.

7.5 Strength and limitations

This study shows how some micronutrient deficiencies, oxidative stress, acute and chronic inflammatory conditions interact to increase the risk of preeclampsia providing an opportunity for designing prevention measures that are targeted at the identified pathological pathways.

Although the principal component analysis can be used for delineating how various factors relate to each other during a pathological process, it is not the most appropriate for studying causation. Therefore, some of the observed associations may accrue from preeclampsia other than being factors on the causal pathway to the development of preeclampsia (i.e., reverse causality). Hence, a cohort study, or a cluster randomised trial where ethically feasible, would have been a better design to help establish the temporal relationships between various exposures and the eventual clinical manifestation of preeclampsia. This was not feasible in terms of time and resources available for my research project.

7.6 Conclusion

Low dietary intake of iodine and selenium that diminish NO and lead to increased TSH and preferential T3 secretion, acute inflammatory conditions associated with elevated CRP, serum Ferritin and GGT, and subclinical inflammatory conditions characterised by autoimmunity are some of the major oxidant and inflammatory pathways associated with preeclampsia.

References

1. Redman CW, Sargent IL**.** Preeclampsia and the systemic inflammatory response. Semin Nephrol. 2004;24(6):565-70.
2. Sacks GP, Studena K, Sargent K, Redman CW**.** Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol. 1998;179(1):80-6.
3. Redman CW, Sacks GP, Sargent IL**.** Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999;180(2 Pt 1):499-506.
4. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4(2):97-104.
5. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves JW, Hill MN, et al. Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. J Clin Hypertens (Greenwich). 2005;7(2):102-9.
6. Ngo DB, Dikassa L, Okitolonda W, Kashala TD, Gervy C, Dumont J, et al. Selenium status in pregnant women of a rural population (Zaire) in relationship to iodine deficiency. Trop Med Int Health. 1997;2(6):572-81.
7. Glinoer D**.** The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. Best Pract Res Clin Endocrinol Metab. 2004;18(2):133-52.
8. Zimmermann M**.** The Effects of Iodine Deficiency in Pregnancy and Infancy. Paediatric and perinatal epidemiology. 2012;26 Suppl 1:108-17.
9. Smyth PP**.** Role of iodine in antioxidant defence in thyroid and breast disease. Biofactors. 2003;19(3-4):121-30.
10. Johansen JS, Harris AK, Rychly DJ, Ergul A**.** Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol. 2005;4:5.
11. Tian L, Zhang L, Liu J, Guo T, Gao C, Ni J**.** Effects of TSH on the function of human umbilical vein endothelial cells. J Mol Endocrinol. 2014;52(2):215-22.
12. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2006;91(10):4175-8.
13. Lioudaki E, Mavroeidi NG, Mikhailidis DP, Ganotakis ES**.** Subclinical hypothyroidism and vascular risk: an update. Hormones (Athens). 2013;12(4):495-506.
14. O'Leary DH, Polak JF**.** Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol. 2002;90(10c):18l-21l.
15. Lapice E, Maione S, Patti L, Cipriano P, Rivellese AA, Riccardi G, et al. Abdominal adiposity is associated with elevated C-reactive protein independent of BMI in healthy nonobese people. Diabetes Care. 2009;32(9):1734-6.
16. Anty R, Bekri S, Luciani N, Saint-Paul MC, Dahman M, Iannelli A, et al. The inflammatory C-reactive protein is increased in both liver and adipose tissue in severely obese patients

independently from metabolic syndrome, Type 2 diabetes, and NASH. Am J Gastroenterol. 2006;101(8):1824-33.

17. WHO**.** C-reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. Geneva: World Health Organization; 2014.

18. Dao MC, Sen S, Iyer C, Klebenov D, Meydani SN**.** Obesity during pregnancy and fetal iron status: is Hepcidin the link? J Perinatol. 2013;33(3):177-81.

19. Manolov V, Marinov B, Velizarova M, Atanasova B, Vasilev V, Tzatchev K, et al. Anemia in pregnancy and serum hepcidin levels. 2015;3:758-61.

20. Toldi G, Stenczer B, Molvarec A, Takáts Z, Beko G, Rigó J, Jr., et al. Hepcidin concentrations and iron homeostasis in preeclampsia. Clin Chem Lab Med. 2010;48(10):1423-6.

21. Drozdz R, Parmentier C, Hachad H, Leroy P, Siest G, Wellman M**.** gamma-Glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transferrin system. Free Radic Biol Med. 1998;25(7):786-92.

22. Enoiu M, Aberkane H, Salazar JF, Leroy P, Groffen J, Siest G, et al. Evidence for the pro-oxidant effect of gamma-glutamyltranspeptidase-related enzyme. Free Radic Biol Med. 2000;29(9):825-33.

23. Winkler R, Griebenow S, Wonisch W**.** Effect of iodide on total antioxidant status of human serum. Cell Biochem Funct. 2000;18(2):143-6.

24. Gulaboglu M, Borekci B, Delibas I**.** Urine iodine levels in preeclamptic and normal pregnant women. Biol Trace Elem Res. 2010;136(3):249-57.

25. Chen CY, Chen CP, Lin KH**.** Biological functions of thyroid hormone in placenta. Int J Mol Sci. 2015;16(2):4161-79.

26. Redman CW, Sargent IL**.** Placental debris, oxidative stress and pre-eclampsia. Placenta. 2000;21(7):597-602.

27. De Vivo A, Baviera G, Giordano D, Todarello G, Corrado F, D'Anna R**.** Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia. Acta Obstet Gynecol Scand. 2008;87(8):837-42.

28. Burns R, Azizi F, Hedayati M, Mirmiran P, O'Herlihy C, Smyth PP**.** Is placental iodine content related to dietary iodine intake? Clin Endocrinol (Oxf). 2011;75(2):261-4.

29. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12(6):642-9.

30. Pearson TA, Mensah GA, Hong Y, Smith SC**.** CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease. 2004;110(25):e543-e4.

31. Shipley M, Rahman A, O’Gradaigh D, JE C**.** Rheumatology and bone disease. In: Kumar P, Clark M, eds. Kumar and Clark’s Clinical Medicine. 7th ed. London Elsevier Saunders; 2009:499 – 568.

32. Glinoer D**.** The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev. 1997;18(3):404-33.

Chapter 8

Low serum triiodothyronine and potassium levels are associated with increased risk of Eclampsia among women in the Eastern Cape Province of South Africa.

*Businge CB, Longo-Mbenza B, Kengne AP. Low serum triiodothyronine and potassium levels are associated with increased risk of eclampsia among women in the Eastern Cape Province of South Africa**.** Clin Exp Obstet Gynecol. 2022; 49(2): 032*
*http://doi.org/10.31083/j.ceog4902032**.***

Abstract

Background*:* There is paucity of data on the relationship between thyroid hormones, potassium and eclampsia. Moderate-to-severe iodine deficiency that worsens during pregnancy leads to decreased thyroid hormone output and bioavailability to the brain. Apart from metabolic functions, T3 and T4 are essential as fast acting cytosolic and synaptosomal neural transmitters that also regulate neuronal excitatory-inhibitory mechanisms. T3 also regulates the Na+-K+-ATPase pump that maintains the membrane ionic gradient. Hence altered serum potassium, thyroxine and triiodothyronine levels could increase the risk of eclamptic seizures.

Methods*:* Forty-five women with eclampsia, 45 severe preeclampsia and 90 normotensive pregnant controls were enrolled into this study. Levels of thyroid hormones, thyroglobulin and urine iodine concentration (UIC) were measured and compared between the three groups.

Results*:* Eclamptic participants had significantly lower median serum potassium (K), triiodothyronine (T3), urinary iodine concentration (UIC) but higher serum thyroglobulin (Tg) (K= 3.7 mmol/L; T3 = 3.8 pmol/L; UIC = 69.5 µg/L; Tg = 39.0 µg/L) than normotensive pregnant controls (K = 4.3 mmol/L; T3 = 4.7 pmol/L; UIC = 169.5 µg/L; Tg = 19.5 µg/L) and participants with severe preeclampsia (K = 4.2 mmol/L; T3 = 4.4 pmol/L; UIC = 95.7 µg/L; Tg = 22.4 µg/L), $p < 0.05$. Low UIC, low serum T3 and potassium and elevated Tg were independent predictors of eclampsia.

Conclusion: Women with iodine deficiency in pregnancy may be at increased risk of eclampsia secondary to the ensuing rapid peripheral turnover of thyroid hormones leading to hypothyroxinaemia and reduced triiodothyronine bioavailability to the central nervous system.

Keywords

Low serum triiodothyronine, low serum potassium, pregnancy, eclampsia

8.1 Background

Although preeclampsia complicates 5–10% of all pregnancies worldwide, eclampsia, one of the most severe complications of preeclampsia, is associated with 8-fold maternal mortality compared to preeclampsia (1, 2). Thyroid dysfunction, iodine and potassium deficiency in pregnancy have all been associated with preeclampsia (3-6). However, there is a paucity of data on the thyroid function status, iodine nutrition status, as well as the serum potassium levels of women with eclampsia. The pathways through which iodine and potassium deficiency and thyroid dysfunction may interact to increase the risk of eclampsia have not yet been described.

The thyroid gland responds to iodine deficiency by preferential secretion of triiodothyronine (T3) instead of T4 leading to low serum thyroxine (T4) (7). However, only a minute amount of T3 is directly transported from the blood stream to the central nervous system (CNS). The brain derives most of its T3 by deiodination of T4 by type 2 deiodinase (D2) (8, 9). Apart from regulation of metabolism, T3 and T4 are essential in the central nervous system for modulation of fast acting cytosolic physiological action and neurotransmitter activity at synaptic junctions (8, 9). T3 also regulates the Na+-K+-ATPase pump that maintains the membrane ionic gradient of the neurones by pumping sodium out and potassium into the cells (8). Hence among pregnant women with iodine deficiency, the preferential production of T3 could result into low serum T4 leading to T3 deficiency in the central nervous system (CNS). This could adversely affect the synaptosomal neurotransmitter function of T3 and the stability of the cell membrane ionic gradients potentially increasing the risk of eclampsia.

The pathophysiology of eclampsia is yet to be fully understood. It is still not yet certain why eclampsia complicates both women with mild and severe hypertension in pregnancy. We carried out this case-control study to compare the serum levels of thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4) and potassium of women with eclampsia, preeclampsia with severe features (from now on wards termed as ‘severe preeclampsia’) and normotensive pregnant controls; and to find out if serum T3, T4 and potassium levels were associated with the risk of eclampsia.

8.2 Materials and methods

This prospective case control study enrolled eligible participants who received maternity care from Nelson Mandela Academic Hospital, Mthatha and Mthatha Regional Hospital between August 2018 and March 2020. Forty-five women with eclampsia referred to Nelson Mandela Academic Hospital were consecutively recruited into the study as cases. Forty-five women with severe preeclampsia and ninety counterparts who remained normotensive until delivery were randomly selected as controls. Participants with eclampsia and severe preeclampsia were diagnosed and managed according to International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines (10). The patients with hypertensive disease in pregnancy receive aldomet as the first-line drug for the control of hypertension if they have mild hypertension, and nifedipine, hydralazine and labetalol as recommended by the ISSHP for those with severe hypertension. Once the blood pressure is within the range of 110–140/ 85 mmHg they are we maintained on aldomet alone or in combination with oral hydralazine and or amlodipine when necessary. Magnesium sulphate is administered according to the guidelines ISSHP for the prevention and attenuation of eclamptic seizures.

The levels of TSH, FT4 and FT3 were determined using electrochemiluminescence immunoassay (Roche/Hitachi cobas c systems). Serum obtained by centrifuging venous blood samples was aliquoted and stored at -20 ^{0}C until analysis for thyroid function tests (TSH, T4, T3, and thyroglobulin) at the Mthatha National Laboratories Services (NHLS) Chemical Pathology Laboratory. Urinary Iodine concentrations was determined from urine samples stored at -20 ^{0}C using the inductively coupled plasma (ICP) Mass Spectrometry method according to the manufacturer's instructions (Quadrupole Inductively Coupled Plasma Mass Spectrometry) (X-Series 2 ICP-MS–Thermo- Fisher Scientific, Bremen, Germany).

Statistical analysis

Data analysis was performed using IBM SPSS STATISTICS version 22 for Windows (IBM Inc., Chicago IL, USA). We used the Shapiro–Wilk's test to check if the data followed the normal distribution. I summarize the data as proportions (%) for categorical variables, means ± standard deviation (SD) for normally distributed, and as median (p25, p75) for non-normally distributed continuous variables, respectively. The Chi-square test was used to compare the distribution of categorical variables by status for hypertensive disease in pregnancy. The Kruskal-Wallis test, ANOVA and equivalents were used as appropriate for continuous variable comparisons across groups. Univariable and multivariable logistic regressions were used to investigate the correlates of eclampsia. A p-value <0.05 was considered significant.

Ethical considerations

Ethical clearance was obtained from Walter Sisulu University and the University of Cape Town Human Research Ethics Review Committee (reference number 066/2017 and 135/2018 respectively). The study conducted according to the Helsinki declaration.

8.3 Results

The median chronological age was 23 years for normotensive pregnant women, 23 years for women with severe preeclampsia and 18 years for eclamptic women. Eclamptic participants had lower median BMI (25.6 Kg/m^2) compared to women with severe preeclampsia (27.2 Kg/m^2) and normotensive pregnant women (28.1 Kg/m^2) $p < 0.05$ (Table 8.1). The median gestational age at delivery was lower for women with eclampsia (34 WOA) and severe preeclampsia (33 WOA) than normotensive pregnant counterparts (38 WOA) $p < 0.001$.

As expected, the median systolic BP (137.0 and 143.0 mmHg) and diastolic BP (88.0 and 95.0, mmHg respectively) of eclamptic and severe preeclampsia participants were higher than that of normotensive counterparts (systolic BP 123.0 and diastolic BP 76 mmHg, both $p < 0.001$). Eclamptic participants had significantly lower median UIC, FT3 and serum potassium but higher median thyroglobulin ($p < 0.05$) than normotensive and severe preeclamptic controls. Eclamptic participants had a lower median TSH than severe preeclampsia controls (1.9 vs 3.0 IU/L, $p > 0.05$, Table 8.1).

Table 8. 1: Comparative profile of women by status of hypertension in pregnancy

Variable	**Normotensive** **(n = 90)** **Median (p25, p75)**	**Severe preeclampsia** **(n = 45)** **Median (p25, p75)**	**Eclampsia** **(n = 45)** **Median (p25, p75)**	**P value**
Age (yrs)	23.0 (20.0, 29.0)	23.0 (18.0, 29.0)	18.0 (17.0, 21.0)	<0.001
Gravidity	2.0 (1.0, 2.0)	1.0 (1.0, 3.0)	1.0 (1.0, 1.0)	0.001
GA at booking (WOA)	21.5 (18.0, 24.0)	20.0 (18.0, 23.0)	22.0 (17.5, 26.0)	0.601
GA at delivery (WOA)	39.0 (37, 40)	33.5 (30.0, 37.0)	34.0 (32.0, 38.0)	<0.001
BMI (Kg/m^2)	27.9 (25.9, 31.6)	28.1 (24.9, 34.5)	25.6 (22.5, 28.7)	0.002
SBP (mm Hg)	121.5 (114.0, 127.3)	143.0 (131.0, 151.0)	137.0 (125.5, 150.0)	<0.001
DBP (mm Hg)	77.0 (70.0, 82.0)	95.0 (85.3, 99.8)	88.0 (73.0, 96.5)	<0.001
K$^+$ (mmol/L)	4.3 (3.9, 4.7)	4.2 (3.8, 4.7)	3.7 (3.2, 4.1)	<0.001
Creatinine (mmol/L)	55.5 (44.0, 62.0)	60.0 (48.5, 78.5)	72.5 (58.0, 85.5)	<0.001
HDL (mmol/L)	1.7 (1.3, 2.0)	1.9 (1.7, 2.7)	2.1 (1.5, 2.5)	0.002
LDL (mmol/L)	1.2 (0.82, 2.1)	1.4 (1.1, 1.6)	1.4 (1.1, 1.7)	0.349
TSH (IU/L)	2.3 (1.8, 3.0)	3.0 (2.1, 3.1)	1.9 (1.3, 3.4)	0.001
FT4 (pmol//L)	14.0 (12.4, 16.0)	12.9 (11.2, 14.6)	13.2 (11.5, 14.9)	0.010
FT3 (pmol//L)	4.7 (4.2, 5.1)	4.4 (3.7, 4.8)	3.8 (3.1, 4.2)	<0.001
Tg (µg/L)	19.5 (13.0, 33.9)	22.4 (14.9, 38.5)	39.0 (27.1, 54.2)	<0.001
UIC (µg/L)	169.5 (89.1, 288.9)	95.7 (53.2, 579.8)	69.5 (14.2, 238.1)	<0.001

Values, medians and 25th-75th percentiles.

BMI: Body mass index; DBP: diastolic blood pressure; GA: gestational age; HDL: high density lipoprotein; K$^+$: Serum Potassium; LDL: low density lipoprotein; TSH: Thyroid-stimulating hormone; FT4: Thyroxine; FT3: Triiodothyronine; Tg: Thyroglobulin, UIC: urine iodine concentration; SBP: systolic blood pressure

Correlates of eclampsia

Using normotensive pregnant women as the reference group, age below 20 years, primigravida status, BMI > 30 Kg/m^2, UIC ≤ 100 µg/L, FT3 < 4.3 pmol//L, thyroglobulin ≥ 20 µg/L and K$^+$ < 3.3 mmol/L were significantly associated with eclampsia in univariable analyses. However, only primigravida status, UIC ≤ 100 µg/L, FT3 < 4.3 pmol//L, thyroglobulin ≥ 20 µg/L and K$^+$ < 3.3 mmol/L remained significantly associated with eclampsia in a multivariable logistic regression model that included all the 9 variables (Table 8.2).

Table 8. 2: Univariable and multivariable odds ratios of the factors associated with eclampsia

Variable	**Univariable** OR (95% CI)	**P value**	**Multivariable** OR (95% CI)	**P value**
Age < 20 (yrs)	4.90 (2.11–12.05)	<0.001	2.02 (0.45–9.17)	0.362
***Primigravida**	5.05 (2.11–12.05)	<0.001	8.33 (1.34–52.63)	0.023
BMI > 30 (Kg/m^2)	3.58 (1.37–9.35)	0.009	2.39 (0.53–10.64)	0.254
TSH ≥ 3.0 (IU/L)	1.0 (0.46–2.16)	1.000	1.02 (0.29–3.66)	0.970
FT4 < 12 (pmol//L)	2.36 (0.96–5.8)	0.060	2.34 (0.52–10.62)	0.269
***FT3 < 4.3 (pmol/L)**	6.67 (2.92–15.15)	<0.001	6.41 (1.80–22.73)	0.004
***Tg ≥ 20 (µg/L)**	4.54 (1.74–11.81)	0.002	6.42 (1.23–32.80)	0.025
***UIC ≤ 100 (µg/L)**	3.62 (1.61–8.13)	0.002	3.36 (1.02–11.0)	0.045
***K$^+$ < 3.3 (mmol/L)**	20 (4.27–90.91)	<0.001	32.25 (3.86–250.00)	0.001

BMI: Body mass index; K$^+$: Serum Potassium; TSH: Thyroid-stimulating hormone; FT3: Triiodothyronine; FT4: Thyroxine; Tg: Thyroglobulin; UIC: urine iodine concentration; *: $p < 0.05$

8.4 Discussion

The current study found that participants with eclampsia had significantly lower median UIC, FT3, serum potassium but higher median thyroglobulin than both normotensive and severe preeclampsia participants. This suggests iodine deficiency as the possible underlying cause of the low thyroid hormone output among eclamptic women. Secondly, primigravida status, low urinary iodine concentration, low serum FT3, low serum potassium and high serum thyroglobulin were independently associated with eclampsia. Women with eclampsia and severe preeclampsia had lower FT4 than normotensive counterparts. The major difference between women with eclampsia and severe preeclampsia was that participants with severe preeclampsia had mild iodine deficiency, relative hypothyroxinaemia, and raised TSH but no significant reduction in T3 and serum potassium. Chasalow et al. (11) reported that some preeclamptic patients have low spiral steroids, a type of potassium sparing endogenous steroid. This observation deserves further investigation in our study population.

Previous studies have shown that persistent mild-to-moderate iodine deficiency with intact thyroid parenchyma is associated with preferential T3 secretion from the thyroid, lower serum T4 and raised serum thyroglobulin as observed in the current study among eclamptic respondents (12, 13). Low serum T4 is associated with reduced bioavailability of T3 to brain tissue (12). In adults, the CNS contains a high concentration of thyroid hormones with the T3/T4 ratio higher than that in the serum (14). The brain derives about 75% of T3 by deiodination of T4 (15, 16). The thyroid hormones (T3 and T4) not only stimulate metabolic functions through nuclear receptors with subsequent transcription and production of effector proteins (17), but also have been found to be cytosolic and synaptic neurotransmitters with the latter non-genomic action being relatively rapid compared to the genomically mediated metabolic function (18).

Hence, the current study seems to suggest that women at risk of eclampsia may not only present with moderate iodine deficiency and lower serum FT4 than normotensive counterparts but could also be prone to rapid peripheral turnover of T3 that is not matched by adequate replacement from the thyroid gland (7). Alternatively, there may be defective peripheral deiodination of FT4 with resultant generalised FT3 deficiency in serum, as well as in the central nervous system and other organs. This can occur because of selenium deficiency and other circumstances associated with inadequate function of the enzyme deiodinase 2 that is involved in peripheral conversion of FT4 to FT3 (19, 20). On the other hand, the pattern of thyroid function observed in the current study among women with severe preeclampsia is due to prolonged exposure to mild iodine deficiency in pregnancy, resultant thyroid adaptation with preferential T3 production and attenuated T4 negative feedback on the pituitary gland and resultant increase in TSH (7).

Early in the 20th century, use of large doses of thyroid extract was one of the interventions for the management and prevention of eclampsia (21). However, the probable mode of action and the pathophysiology of preeclampsia and eclampsia was still elusive (21). Given that the thyroid gland has iodine stores as well as T3 and T4 hormones, it was not stated which one or a combination of these compounds was of therapeutic value. It is now known that T4 and T3 stimulate fast and non-genomic effects in the central nervous system that are mediated through modulatory effect on Glutamate and GABA that respectively are the main excitatory and inhibitory neurotransmitters in the nervous system (22). Glutamate binds to its ionotropic and metabotropic receptors eliciting excitatory postsynaptic potential and a cascade of intracellular reactions that are crucial for normal and balanced cognitive and motor function (22).

Both T3 and T4 potentiate glutamate excitatory post-synaptic responses mediated mainly through the ionotropic alphaamino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and Kainate receptors (23). However, it is only T3 that negatively modulates the glutamate post-synaptic response on the N-methyl-D-aspartate (NMDA) receptors, decreasing the excitability of post-synaptic neurone (24). Therefore, low T3 may cause net motor neurone excitability resulting in the tonic-clonic seizures characteristic of eclampsia. The alteration in the physiological brain T3/T4 ratio has potential to alter normal neuronal function (25) and may partially explain the findings in the current study where women with eclampsia had lower FT3 and FT4 than women that remained normotensive until term.

The NMDA glutamate receptors, whose inhibitory effect is accentuated by T3, have high permeability for sodium and calcium ions that at resting membrane potential are blocked by magnesium ions (26). Previous studies have found that magnesium sulphate attenuates eclamptic convulsions through blockage of these glutamate NMDA receptors (27). Several authors have reported that women with eclampsia and preeclampsia had low serum magnesium (28-30). These observations together with the finding of low FT3 as independent predictor of eclampsia in the current study seems to imply that low T3 increases the risk of eclamptic seizures among women with hypomagnesaemia through decreased inhibition of post-synaptic glutamate NMDA receptors.

GABA inhibitory responses are mediated via ionotropic GABA-A and metabotropic GABA-B receptors (31). Stimulation of post-synaptic GABA$_A$ receptors elicits transient large amplitude phasic inhibitory currents whose frequency and amplitude can be attenuated by both T3 and T4 (23). Stimulation of extra-synaptic GABA$_A$ receptors stimulates lower amplitude but longer lasting tonic inhibitory currents that cause hyperpolarisation of the post-synaptic neurones (31-33) GABA$_B$ receptors on presynaptic membranes are sensitive to high concentration of GABA within the synaptic cleft hence inhibit further/excessive release of GABA from the synaptic vesicles (34). Therefore, derangement in the physiological brain T3/T4 ratio may alter the GABA inhibitory responses with resultant clinical manifestations, one of which could be eclamptic seizures.

The stimulation of post-synaptic GABA$_B$ receptors results in activation of inward potassium channels and inhibition voltage gated sodium channels leading to hyperpolarization and increased action potential threshold (35). In the current study, the observed association between low serum potassium and the increased risk of eclampsia may be secondary to the diminished effectiveness of this GABA$_B$ receptor mediated inhibition further predisposing pregnant women with low FT3 to the tonic-clonic convulsions that are characteristic of eclamptic fits.

The lower age and Body Mass Index for eclamptic participants compared to normotensive and women with severe preeclampsia in the current study may be accounted for by teenage pregnancy a known risk factor for eclampsia, which may predispose them to diet low on vegetables and fruits that are sources of potassium (36-38). The lower serum potassium levels observed among participants with eclampsia in the current study may be partially attributed to increased risk of hypokalaemia among young mothers in the study area that tend to depend on diet deficient in fresh vegetables and meat (39). Yang et al. (40) attributed the higher prevalence of hypokalaemia among women with eclampsia and preeclampsia in their study population to possible higher potassium excretion secondary to over expression of the angiotensin genes among women at risk of hypertensive disease in pregnancy. These two possible causes of hypokalaemia in pregnancy require further investigation.

8.5 Strength and limitations

To our knowledge, this study is the first to report the potential role of combined triiodothyronine and potassium deficiency in the aetiology of eclampsia. Although median UIC at population level is good predictor of iodine intake, considerable diurnal variation affects spot UIC hence is it not a reliable measure of iodine intake at individual level. However, serum thyroglobulin, a reliable measure of prolonged insufficient iodine intake, was significantly high among women with eclampsia suggesting that the observed thyroid dysfunction in the current study may be secondary to iodine deficiency. This study is limited by the inability to determine serum magnesium levels before routine administration of prophylactic magnesium sulphate since magnesium deficiency is a potential confounder. The non-thyroidal illness syndrome (Euthyroid sick syndrome) is another potential confounder of the observed thyroid dysfunction attributed to iodine deficiency among participants with severe preeclampsia and eclampsia. The wide confidence interval for some of the predictors of eclampsia obtained on multivariable analysis may have been due to a small sample size or presence of outliers or a result of collinearity between explanatory variables such as potassium and triiodothyronine. Studies with bigger sample sizes will be needed to confirm the observations in the current study.

8.6 Conclusion

Low serum T4 and T3 may predispose to reduced bioavailability of T3 in the CNS and alteration in the physiological T3/T4 ratio. This may attenuate the inhibitory effects of GABA, while the excitatory function of glutamate remains intact, resulting in net motor neurone stimulation. The net motor stimulation may predispose to the involuntary tonic-clonic convulsions observed in eclampsia. This may further be exacerbated by low serum potassium that attenuates $GABA_B$ receptor mediated tonic extra-synaptic inhibition and magnesium deficiency, which potentiates the NMDA receptor mediated activation of post-synaptic neurones.

References

1. Vest AR, Cho LS. Hypertension in pregnancy. Curr Atheroscler Rep. 2014;16(3):395.
2. Nakimuli A, Nakubulwa S, Kakaire O, Osinde MO, Mbalinda SN, Kakande N, et al. The burden of maternal morbidity and mortality attributable to hypertensive disorders in pregnancy: a prospective cohort study from Uganda. BMC Pregnancy Childbirth. 2016;16:205.
3. Männistö T, Mendola P, Grewal J, Xie Y, Chen Z, Laughon SK. Thyroid diseases and adverse pregnancy outcomes in a contemporary US cohort. J Clin Endocrinol Metab. 2013;98(7):2725-33.
4. Paulino-Morente JMA, Cacas-David IG, VVL P. Association of hypokalaemia and preeclampsia and correlation of serum potassium to blood pressure severity in preeclampsia. Philippine Journal of Obstetrics and Gynecology. 2018;42(2):9 - 16.
5. Sayyed AK SA. Electrolyte status in preeclampsia. Online International Interdisciplinary Research Journal. 2013;3(3):30 - 3.
6. Candido AC, Azevedo FM, Machamba AAL, Pinto CA, Lopes SO, Macedo MdS, et al. Implications of iodine deficiency by gestational trimester: a systematic review %J Archives of Endocrinology and Metabolism. 2020;64:507-13.
7. Glinoer D. Clinical and biological consequences of iodine deficiency during pregnancy. Endocr Dev. 2007;10:62-85.
8. Kundu S RK. Thyroid Hormone Homeostasis in Adult Mammalian Brain: A Novel Mechanism for Functional Preservation of Cerebral T3 Content During Initial Peripheral Hypothyroidism. Al Ameen Journal of Medical Sciences. 2010;3(1):5 - 20.
9. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. Eur J Endocrinol. 2004;151 Suppl 3:U25-37.
10. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension. 2018;72(1):24-43.
11. Chasalow F, John CM, Bochner R. Spiral steroids as potential markers for pre-eclampsia: A pilot study. Steroids. 2019;151:108466.
12. Obregon MJ, Escobar del Rey F, Morreale de Escobar G. The effects of iodine deficiency on thyroid hormone deiodination. Thyroid. 2005;15(8):917-29.
13. Lazarus J. Thyroid Regulation and Dysfunction in the Pregnant Patient. In: Feingold KR AB, Boyce A, et al, ed. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2000; 2016.
14. Dratman MB, Crutchfield FL, Gordon JT, Jennings AS. Iodothyronine homeostasis in rat brain during hypo- and hyperthyroidism. Am J Physiol. 1983;245(2):E185-93.
15. Leonard J, Köhrle J. Intracellular pathways of iodothyronine metabolism. In: Braverman L, and Utiger R, eds. Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text. 8th ed. Philadelphia: Lippincott-Raven; 2000:136 -73.
16. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev. 2002;23(1):38-89.

17. Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81(3):1097-142.

18. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev. 2010;31(2):139-70.

19. Ventura M, Melo M, Carrilho F. Selenium and Thyroid Disease: From Pathophysiology to Treatment. Int J Endocrinol. 2017;2017:1297658.

20. Olivieri O, Girelli D, Azzini M, Stanzial AM, Russo C, Ferroni M, et al. Low selenium status in the elderly influences thyroid hormones. Clin Sci (Lond). 1995;89(6):637-42.

21. Nicholson HO. Further Notes on the Use of Thyroid Extract in Puerperal Eclampsia and the Pre-Eclampic State. Trans Edinb Obstet Soc. 1902;27:160-74.

22. Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. Biochem Pharmacol. 2008;75(1):218-65.

23. Puia G, Losi G. Thyroid hormones modulate GABA(A) receptor-mediated currents in hippocampal neurons. Neuropharmacology. 2011;60(7-8):1254-61.

24. Losi G, Garzon G, Puia G. Nongenomic regulation of glutamatergic neurotransmission in hippocampus by thyroid hormones. Neuroscience. 2008;151:155-63.

25. Puia G. Non Genomic Modulation of Ligand Gated Ionotropic Receptors by Thyroid Hormones. Immunology Endocrine & Metabolic Agents - Medicinal Chemistry (Formerly Current Medicinal Chemistry - Immunology Endocrine & Metabolic Agents). 2011;11:179-87.

26. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev. 2010;62(3):405-96.

27. Cotton DB, Hallak M, Janusz C, Irtenkauf SM, Berman RF. Central anticonvulsant effects of magnesium sulfate on N-methyl-D-aspartate-induced seizures. Am J Obstet Gynecol. 1993;168(3 Pt 1):974-8.

28. Patel N, Shah N. Serum magnesium level in eclampsia at tertiary care center, Gujarat, India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019;8.

29. Tavana Z, Hosseinmirzaei S. Comparison of Maternal Serum Magnesium Level in Pre-eclampsia and Normal Pregnant Women. Iran Red Crescent Med J. 2013;15(12):e10394.

30. Patil VP, Choudhari NA. A study of serum magnesium in pre-eclampsia and eclampsia. Indian Journal of Clinical Biochemistry. 1991;6(2):69-72.

31. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev. 2008;60(3):243-60.

32. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron. 2012;73(1):23-34.

33. Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev. 2004;84(3):835-67.

34. Davies CH, Starkey SJ, Pozza MF, Collingridge GL. GABA autoreceptors regulate the induction of LTP. Nature. 1991;349(6310):609-11.

35. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active GABA A receptors: modulating gain and maintaining the tone. Trends Neurosci. 2004;27(5):262-9.

36. Brantsaeter AL, Haugen M, Samuelsen SO, Torjusen H, Trogstad L, Alexander J, et al. A dietary pattern characterized by high intake of vegetables, fruits, and vegetable oils is associated with

reduced risk of preeclampsia in nulliparous pregnant Norwegian women. J Nutr. 2009;139(6):1162-8.

37. Moodley J**.** Lessons learnt from the Saving Mothers 2014 - 2016 report. South African Medical Journal. 2018;108:1012.

38. Ndaboine EM, Kihunrwa A, Rumanyika R, Im HB, Massinde AN**.** Maternal and perinatal outcomes among eclamptic patients admitted to Bugando Medical Centre, Mwanza, Tanzania. Afr J Reprod Health. 2012;16(1):35-41.

CHAPTER 9

Summary of study methods and findings, discussion, conclusions, limitations and recommendations

9.1 Introduction

This chapter provides a summary of the key findings in this thesis, discussion, limitations, conclusion, clinical and public health implications, and the recommendations.

Aims

The aims of the thesis were: 1) To determine the burden of iodine deficiency in pregnancy in Africa as well as to find out the trends in the prevalence of iodine deficiency in pregnancy in Africa following the implementation of national iodine fortification initiatives; 2) To find out if there is an association between iodine deficiency in pregnancy, subclinical hypothyroidism, endothelial dysfunction and preeclampsia; 3) To investigate possible mechanisms through which iodine deficiency in pregnancy may predispose to preeclampsia, eclampsia, endothelial dysfunction and early cardiovascular pathological changes.

Summary of methods

The thesis comprises published manuscripts and manuscripts currently under peer review arising from the PhD study protocol with the following study designs: systematic reviews and meta-analyses and case-control studies.

In Part I, a systematic review and meta-analysis was carried to assess the trend and current prevalence of iodine deficiency in pregnancy in Africa following iodine fortification of salt and other food staffs that commenced in 1995. An adjunct study to this systematic review was carried to estimate the level of iodine intake in pregnancy from all African countries derived from the more readily available school age median Urinary Iodine Concentration (UIC) data through a regression equation. The findings of the two studies were synthesized in chapters 2 and 3, respectively. A second systematic review and meta-analysis was carried to establish the association of iodine deficiency in pregnancy and preeclampsia, and the data synthesized in chapter 4.

In Part II, case-control studies comparing the iodine nutrition status and other relevant attributes of normotensive pregnant women at term and counterparts with hypertensive disease in pregnancy of various degrees of severity (preeclampsia, severe preeclampsia and eclampsia) were carried out at Nelson Mandela Academic and Mthatha Regional Hospitals and the data synthesised in chapters 5 and 6.

In Part III, two studies were carried out to delineate the possible mechanisms through which iodine deficiency interacts with other nutritional and environmental factors to increase the risk of preeclampsia (secondary analysis of data from Kinshasa province, Democratic Republic of Congo) and eclampsia (using data collected through a prospective case control study at Nelson Mandela Academic and Mthatha Regional Hospitals in Eastern Cape, South Africa). The findings were synthesized into chapters 7 and 8.

9.2 Summary of the key findings

Part I: *To determine the burden of iodine deficiency in pregnancy in Africa as well as to ascertain the trends in the prevalence of iodine deficiency in pregnancy in Africa following the implementation of national iodine fortification initiatives.*

Chapter 2: The aim of this systematic review and meta-analysis was to determine the degree and trend in iodine nutrition status in pregnancy in Africa between 1995 and September 2020. Only 42% of all countries in Africa had data on iodine nutrition in pregnancy most of which was from subnational samples. Before 1995 very scanty data showed that severe iodine deficiency was prevalent in pregnancy with a pooled pregnancy median UIC of 28.6 µg/L (95% CI 7.6–49.5). By 2005, there was a trend towards improvement in iodine nutrition state in pregnancy with a pooled pregnancy median UIC of 174.1 µg/L (95% CI 90.4–257.7). Between 2006 and 2020 increased numbers of national and subnational studies from 18 African countries revealed that some entire countries in Africa, and some specific regions within other countries, had attained sufficient iodine intake in pregnancy (pregnancy median UIC of ≥150 µg/L). Most of the rest had mildly inadequate iodine intake, and some moderate inadequate iodine intake in pregnancy. The pooled pregnancy median UIC was 145 µg/L (95% CI 126–172) which is in the category of mildly inadequate iodine intake in pregnancy.

Chapter 3: The objective of study in this chapter, an adjunct study to the systematic review in chapter 2, was to assess the level of insufficient iodine intake in pregnancy in all African countries using pregnancy median urinary iodine concentration (pMUIC) estimated from the frequently available school-age children (SAC) median urinary iodine concentration (mUIC). Relevant raw data was available for 49/54 African countries from the Iodine Global Network score cards of 2017 and 2019. This study established that a cut-off school age median UIC ≤175 µg/L correlated with insufficient iodine intake in pregnancy (median pregnancy UIC ≤150 µg/L). Using this cut-off, 82% (9/11) of the countries with both SAC mUIC and pMUIC were correctly classified. Further analysis showed that 45% (22/49) of all African countries with relevant data had insufficient iodine intake in pregnancy (pMUIC <150 µg/L).

Part II:

To ascertain the relationship between iodine nutrition status in pregnancy and preeclampsia as well as determine and correlate the degree of iodine deficiency and thyroid function with the severity endothelial dysfunction and preeclampsia.

Chapter 4: In this chapter, a systematic review and meta-analysis to establish the association of insufficient iodine nutrition status with risk of preeclampsia is presented. Five eligible studies were included in the meta-analysis. Three eligible studies, each from a different continent, showed that preeclamptic participants had significantly lower mean UIC than the normotensive counterparts (pooled mean UIC difference between normotensive pregnant women and preeclamptic participants of 164.4 µg/L [95% CI 45.1–283.6, $p < 0.01$, $I^2 > 50$]). There was a non-significant risk of preeclampsia for women with UIC <150 µg/L in two eligible cohort studies with a pooled risk ratio of 2.85 (95% CI 0.42–20.05, $p = 0.09$, $I^2 < 25$).

Chapter 5: In this chapter, we present a case control study conducted to ascertain the relationship between iodine nutrition status in pregnancy and preeclampsia. This was carried out from August 2018 to May 2020 at the maternity units of Nelson Mandela Academic Hospital (NMAH) and Mthatha Regional Hospital (MRH) in Eastern Cape South Africa. Participants with severe preeclampsia/eclampsia and those with uncomplicated preeclampsia had lower iodine intake than normotensive pregnant controls: median UIC (µg/L) 98.8, 127. 7 and 217.1 respectively ($p=0.005$). The respective serum levels of thyroglobulin a marker of prolonged iodine deficiency were: Tg (µg/L) 32.9, 21.4, and 19.4 $p=0.001$. The urinary iodine concentration showed a significant decreasing trend and the serum thyroglobulin a significant increase along the gradient of severity of hypertension (UIC: Standard J-T statistic–2.97, p for trend 0.003; Tg: Standard J-T statistic 3.60, p for trend < 0.001 respectively). In a multivariable regression model UIC < 100 µg/L, Tg > 20µg/L were independent predictors of hypertensive disease in pregnancy (HDP).

Chapter 6: The objective of the study in this chapter was to find out how the iodine nutrition status and serum thyroid-stimulating hormone (TSH) levels varied with the degree of endothelial dysfunction among normotensive pregnant controls and cases with uncomplicated or severe preeclampsia. Participants with uncomplicated preeclampsia and severe preeclampsia had mild and moderate insufficient iodine intake in pregnancy (median urinary iodine concentration (UIC) 127.7 µg/L and 88.5 µg/L respectively). The normotensive pregnant controls had adequate iodine intake in pregnancy (median UIC 167.5 µg/L) with significant difference only between women with severe preeclampsia and normotensive controls ($p < 0.05$). Participants with severe preeclampsia had significantly higher median TSH and oxidised low-density lipoprotein (LDL) than normotensive and preeclamptic women without severe features (respectively ***TSH***: 3.0, 2.3 and 2.3 IU/L; ***LDL***: 1.2, 1.0 and 1.0 IU/L, $p < 0.05$). The median Aortic augmentation index was 7.5, 19.0 and 21.0 ($p < 0.001$) and the pulse wave velocity (PWV) 5.1, 5.7 and 6.3 respectively for normotensive, preeclampsia and severe preeclampsia participants (both $p<0.001$). In a linear regression model, TSH, age and hypertensive disease were independent predictors of elevated PWV which is a biomarker of endothelial dysfunction.

Part III: *To investigate the possible pathways through which iodine deficiency in pregnancy may increase the risk and severity of preeclampsia and preeclampsia*

Chapter 7: The objective of the study in this chapter was to investigate the main nutritional, oxidative and inflammatory pathways that may be associated with the clinical manifestation of preeclampsia in a peri-urban population of Kinshasa Province, Democratic Republic of Congo through Principal Component Analysis of data from 250 cases of preeclampsia and 150 normotensive controls who had complete data. The main pathophysiological pathways identified were the interactions between selenium/iodine deficiency and elevated serum TSH (suggestive of TSH mediated endothelial dysfunction); serum ferritin, γ-Glutamate transferase (GGT), C-reactive protein (CRP), and low urinary iodine excretion (inflammatory oxidative stress). The others were elevated serum high sense-C-Reactive protein (hs-CRP) and Rheumatoid factor (subclinical inflammation and immune cell activation) and high T3/T4 ratio (prolonged TSH stimulation of thyroid with low thyroid iodine stores and reduced anti-oxidant capacity secondary to prolonged insufficient iodine intake).

Chapter 8: The objective the study in this chapter was to compare the serum levels of thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4) and potassium of women with eclampsia, severe preeclampsia and normotensive pregnant controls; and to ascertain if serum T3, T4 and potassium levels were associated with the risk of eclampsia. Eclamptic participants had significantly lower median serum potassium (K), triiodothyronine (T3), urinary iodine concentration (UIC) but higher serum thyroglobulin (Tg) than both participants with severe preeclampsia and normotensive pregnant controls. Their respective values were K (mmol/L) 3.7, 4.4 and 4.7; T3 (pmol/L) 3.8, 4.4 and 4.7; UIC (µg/L) 69.5, 95.7 and 169.5; Tg (µg/L) 39.0, 22.4 and 19.5, $p < 0.05$. In a multivariable model low UIC, low serum T3 and potassium and elevated Tg were independent predictors of eclampsia.

9.3 Discussion

9.3.1 Trends in the prevalence of insufficient iodine deficiency in pregnancy in Africa between 1995 and 2020

This study found that there was paucity of data on iodine deficiency in pregnant women from more than half of the countries on the African continent. The limited data showed a positive trend from severe insufficient iodine intake in pregnancy before implementing fortification of salt and other foodstuffs with iodine in 1995 towards sufficient iodine intake by 2020. Although few countries had more than adequate iodine intake in pregnancy from either highly iodinated ground water or overzealous food fortification with iodine, several African countries still have moderate insufficient iodine intake in pregnancy at national or subnational level more than 20 years after the initiation national iodine fortification programs.

Our estimate that 45% of the African countries still have insufficient iodine intake in pregnancy reveals an immense burden since iodine deficiency in pregnancy and infancy is associated with defective neuro-cognitive and psychomotor development (1). From the current study and findings of other studies, iodine deficiency in pregnancy, the resultant thyroid hyper-stimulation and reduced antioxidant capacity increase the risk of cardiovascular disease, thyroid dysfunction in the women, and epigenetic changes in the foetus in utero whose consequences are yet to be elucidated (1, 2). This risk will depend on other environmental factors such as high dietary thiocyanate and low selenium intake that is prevalent in some African countries in central and Southern Africa (3).

9.3.2 Insufficient iodine intake and the risk of preeclampsia

This study has demonstrated that the degree of severity of preeclampsia increases with the severity of iodine deficiency. In addition, insufficient iodine intake in pregnancy was found to be a predictor of preeclampsia independent of elevated thyroid-stimulating hormone, that occurs in subclinical and overt hypothyroidism, that are known factors of preeclampsia. This is consistent with earlier studies although they did not report the relationship between the degree of severity of preeclampsia and that of iodine deficiency (4, 5).

The rise in serum Thyroglobulin (Tag) and decline in urinary iodine concentration (UIC) along a clinical gradient of preeclampsia may be due to prolonged exposure to insufficient iodine intake which may even predate the onset of pregnancy (6). Unlike women with severe preeclampsia, women with uncomplicated preeclampsia had no significant difference in both UIC and Tg from normotensive pregnant controls. This seems to imply that there are other underlying risk factors of preeclampsia apart from insufficient iodine intake; and that insufficient iodine intake may exacerbate the severity of preeclampsia in individuals already at risk of incident preeclampsia. The inconsistent association between insufficient iodine intake and preeclampsia found in the systemic review and meta-analysis may partially be due to brief exposure insufficient iodine intake in pregnancy.

9.3.3 Iodine deficiency in pregnancy and endothelial dysfunction

This study found that nitric oxide a biomarker of normal endothelial function and UIC were lower but oxidised LDL and PWV biomarkers of endothelial dysfunction as well as serum TSH were elevated with more extreme values among cases with severe preeclampsia when compared to normotensive pregnant controls. Iodine deficiency seems to be central to the observed phenomenon as it can lead to reduced serum antioxidant capacity, low thyroid hormone output from the thyroid gland and elevated TSH (7, 8). Prolonged elevation of TSH is associated with inhibition of nitric oxide synthase, low nitric oxide and oxidation of LDL (9, 10). This leads to reduction in flow mediated vascular dilatation and structural changes that are consistent with high PWV (9-12). Elevated PWV like intima media thickness is a robust measure of endothelial dysfunction and atherosclerosis that herald the onset of cardiovascular disease (13). Hence, moderate-to-severe iodine deficiency in pregnancy may not only predispose to endothelial dysfunction, elevated PWV and severe preeclampsia but also to future cardiovascular disease.

9.3.4 Mechanisms through which iodine deficiency, other micronutrient deficiencies and inflammatory conditions may predispose to preeclampsia and eclampsia.

Secondary analysis of data from the Democratic Republic of Congo established that the major oxidant and inflammatory pathways associated with increased risk of preeclampsia were concurrent selenium and iodine deficiency resulting into elevated TSH, low nitric oxide (endothelial dysfunction) and preferential T3 secretion accounting for the greatest percentage. Others were acute inflammatory conditions associated with elevated serum GGT, CRP, and Ferritin leading to excessive release of oxidant hydroxyl radicals; and subclinical inflammatory conditions secondary to autoimmunity. This study has revealed some of the background risk factors and mechanisms that operate in concert with iodine deficiency to precipitate and exacerbate the severity of preeclampsia.

Some women present with very severe hypertension and organ failure but without eclampsia or signs of imminent eclampsia while others with modest increase of blood pressure above normal present with eclamptic fits. In a case control study comprised of eclamptic cases, severe preeclampsia, and normotensive pregnant controls from the Eastern Cape, South Africa, we identified low serum potassium levels and prolonged iodine deficiency characterised by elevated serum thyroglobulin and low serum T3 as independent risk factors for eclampsia.

Apart from the well-known metabolic functions, T3 and T4 are fast acting cytosolic and synaptosomal neural transmitters that also regulate neuronal excitatory-inhibitory mechanisms (14). T3 also regulates the Na+-K+-ATPase pump that maintains the membrane ionic gradient (15). The reduction in thyroid hormone output from the thyroid gland following prolonged insufficient iodine intake in pregnancy and depletion of iodine stores may increase the risk of eclampsia through rapid peripheral turnover of thyroid hormones leading to hypothyroxinaemia and reduced triiodothyronine bioavailability to the central nervous and alteration in the physiological T3/T4 ratio (1). This may lead to malfunction of the inhibitory GABA and excitatory glutamate mechanisms hence initiating involuntary tonic-clonic eclamptic fits (16, 17). This can further be accentuated by low potassium levels that attenuate $GABA_B$ receptor mediated extra-synaptic inhibition hence explaining our findings in the current study (18).

9.4 Strength and limitations

9.4.1 Study strengths

This thesis seems to be among the first studies to demonstrate the increased risk and severity of preeclampsia with the degree of iodine deficiency in pregnancy. The study has also suggested the possible mechanisms through which prolonged exposure to iodine deficiency in pregnancy on its own or operating through resultant elevation in TSH and reduction in T3 and T4 production can predispose to preeclampsia and eclampsia through interaction with other micronutrient deficiencies like selenium and potassium, toxic agents such as thiocyanates autoimmune and infective conditions.

9.4.2 Study limitations

We used cases control study design, regression and the principal component analysis to explore the pathological pathways through which iodine deficiency interacts with nutritional, demographic and environmental factors to predispose to preeclampsia. A cohort study design, with sequential data collection in each trimester of pregnancy, is a more appropriate method for studying causation, as temporal relationships can be determined with more certainty. We attempted to control for this pitfall by measuring serum thyroglobulin that provides a better marker of prolonged exposure to iodine deficiency than UIC or serum TSH. So, future studies with a cohort design are needed to further explore the associations found in the current study.

Secondly, in the study carried out at Mthatha Regional and Nelson Mandela Academic Hospital, we could not determine serum magnesium, as all referred patients are routinely loaded with magnesium sulphate at the referring health units. Because of limited resources, it was not possible assay serum selenium levels and vaso-active markers such as endothelin that are also associated with preeclampsia and are potential confounders of the observed association in the various studies that comprise this thesis. The systematic reviews were limited by the small number of eligible studies with small sample sizes and substantial heterogeneity.

The case control studies at Mthatha Regional and Academic Hospitals are further limited by lack of data on thyroid autoimmune antibody status, other nutritional deficiencies such vitamin C and E that in some studies have been associated with the risk of preeclampsia. Furthermore, the wide confidence intervals of some independent predictors of preeclampsia or eclampsia may be due to the small sample sizes or some confounding factors that may not have been considered in the current study.

9.5 Conclusion

Insufficient iodine intake in pregnancy is an independent risk factor of preeclampsia whose severity increases with the degree of iodine deficiency. Iodine deficiency predisposes to various degrees of severity and complications of preeclampsia through reduced serum antioxidant capacity, elevation in serum TSH, and low serum FT3 and FT4. Prolonged iodine deficiency in pregnancy that causes elevated TSH predisposes severe preeclampsia through endothelial dysfunction which causes severe hypertension and organ malfunction. The risk of eclampsia is increased among women with iodine deficiency in pregnancy who develop T3 deficiency and accentuated in those with concurrent low serum potassium. Alternative measures need to be considered to mitigate against the persistently high prevalence of iodine deficiency in pregnancy in Africa despite national salt iodization programs and other iodine fortification efforts that have been in place since 1995.

9.6 Clinical and public health implications

Optimizing iodine intake during pregnancy may reduce the incidence and severity of preeclampsia. Maternal and perinatal outcomes of women with early onset preeclampsia who have iodine deficiency could be improved by supplementation with both iodine and LT4. Previous intervention studies did not find enough evidence that LT4 supplementation improved pregnancy outcomes among women with elevated TSH secondary to subclinical hypothyroidism apart from those with underlying autoimmune thyroid disease. However, these intervention studies did not consider supplementation with iodine whose deficiency is an alternative and more prevalent cause of elevated serum TSH and hypothyroidism than autoimmune thyroid disease. In the current study, we have suggested possible mechanisms, through which iodine deficiency operates both independent of elevated TSH and through TSH to predispose to preeclampsia and vascular changes characteristic of early cardiovascular disease.

Iodization of salt and other methods of fortification of foodstuffs have improved the iodine intake in the general population in Africa with reduction in cretinism and goitre. However, the persistent high prevalence of inadequate iodine intake in pregnancy that is worsened by the increased renal iodine filtration during pregnancy predisposes large proportions of foetuses in utero to iodine deficiency hence inadequate psychomotor and neurological development and reduced physical and mental capacity in later life. This could also be accompanied by epigenetic DNA changes that some have proposed increase the risk of chronic non-communicable diseases.

Ensuring adequate iodine nutrition during pregnancy will prevent thyroid hyper stimulation hence preventing excessive uptake of thiocyanates and nitrates that increase the risk of chronic thyroid disease (2).

9.7 Recommendations

Regular assessment of iodine nutrition status in pregnancy using pregnancy median UIC is necessary in all African countries preferably as part of National Demographic and health surveys as already being implemented in Tanzania (19, 20).

Countries with regions having moderate-to-severe iodine deficiency in pregnancy should consider iodine supplementation for women in reproduction or women early in the first trimester with lipoid oil, which is safe and effective at correcting iodine deficiency (21-23).

Intervention studies are necessary to assess the iodine nutrition state, thyroid function and the impact of iodine and thyroxine supplementation on maternal and fetal outcomes of women with very early onset preeclampsia.

Studies are needed to assess epigenetic changes associated with in utero exposure to iodine deficiency for babies born to normotensive women and women who develop complicated or uncomplicated preeclampsia or eclampsia. Follow-up studies can assess the risk of psychomotor, neurocognitive deficiency, and future thyroid, cardiovascular risk, and end organ damage among these women and their progeny.

9.8 Contribution of this thesis

This study demonstrated that prolonged insufficient iodine intake in pregnancy, which was confirmed by concurrent low UIC and elevated serum thyroglobulin, is an independent risk factor for preeclampsia. These results were corroborated by Abel et al. (6) in a recent Norwegian cohort study who found that iodine deficiency increased the risk of preeclampsia and other adverse perinatal outcomes but supplementation with iodine before pregnancy reduced the risk of preeclampsia among women with low dietary iodine.

This study therefore will help add to the emerging body of literature that insufficient iodine intake in pregnancy, which has not yet been officially recognised by scientific bodies such as the World Health Organisation (WHO) and the International Federation of Obstetrics and Gynaecology Organisations (FIGO), is a preventable risk factor for preeclampsia and other adverse maternal and foetal outcomes that is worth screening in areas and populations with iodine-deficient diet or diet rich in goitriens.

This study has also shown that the severity of preeclampsia increases with the degree of iodine deficiency and the resultant pattern of thyroid dysfunction. Mild iodine deficiency predisposes to preeclampsia without complications. Moderate to severe iodine deficiency associated with elevated thyroid-stimulating hormone (TSH), even in the upper range of normal, predisposes to more severe endothelial dysfunction and clinical features such as severe hypertension and organ malfunction that define the diagnosis of preeclampsia with severe features. Participants with preeclampsia accompanied with severe features were found to have elevated pulse wave velocity which suggests structural changes that herald future cardiovascular disease implying that prolonged exposure to elevated TSH in pregnancy may increase the risk of future cardiovascular disease.

This study also found that irrespective of the severity of the blood pressure, women with moderate-to-severe iodine deficiency in pregnancy that results in low serum free triiodothyronine (FT3) are at increased risk of Eclampsia especially if they have concurrent low serum potassium level.

The results of this study also suggest that the primary mechanisms through which iodine deficiency predisposes to preeclampsia is the reduction in the antioxidant capacity and initial endothelial dysfunction. In addition, prolonged insufficient iodine intake in pregnancy that results in depletion of thyroid iodine stores, reduced thyroxine and triiodothyronine production and elevated TSH further exacerbating the degree of endothelial dysfunction and preeclampsia.

References

1. Glinoer D. Clinical and biological consequences of iodine deficiency during pregnancy. Endocr Dev. 2007;10:62-85.
2. Contempré B, de Escobar GM, Denef JF, Dumont JE, Many MC. Thiocyanate induces cell necrosis and fibrosis in selenium- and iodine-deficient rat thyroids: a potential experimental model for myxedematous endemic cretinism in central Africa. Endocrinology. 2004;145(2):994-1002.
3. Ogbera AO, Kuku SF. Epidemiology of thyroid diseases in Africa. Indian J Endocrinol Metab. 2011;15(Suppl 2):S82-8.
4. Cuellar-Rufino S, Navarro-Meza M, García-Solís P, Xochihua-Rosas I, Arroyo-Helguera O. Iodine levels are associated with oxidative stress and antioxidant status in pregnant women with hypertensive disease. Nutr Hosp. 2017;34(3):661-6.
5. Gulaboglu M, Borekci B, Delibas I. Urine iodine levels in preeclamptic and normal pregnant women. Biol Trace Elem Res. 2010;136(3):249-57.
6. Abel MH, Caspersen IH, Sengpiel V, Jacobsson B, Meltzer HM, Magnus P, et al. Insufficient maternal iodine intake is associated with subfecundity, reduced foetal growth, and adverse pregnancy outcomes in the Norwegian Mother, Father and Child Cohort Study. BMC Med. 2020;18(1):211.
7. Winkler R, Griebenow S, Wonisch W. Effect of iodide on total antioxidant status of human serum. Cell Biochem Funct. 2000;18(2):143-6.
8. Zantour B AW, Marmouch H, Chebbi W. Hypothyroidism. In: E P, ed. Pregnancy in Current Topics in Hypothyroidism with Focus on Development

Rijeka, Croatia: In Tech; 2013:29-62.

9. Tian L, Zhang L, Liu J, Guo T, Gao C, Ni J. Effects of TSH on the function of human umbilical vein endothelial cells. J Mol Endocrinol. 2014;52(2):215-22.
10. Oge A, Sozmen E, Karaoglu AO. Effect of thyroid function on LDL oxidation in hypothyroidism and hyperthyroidism. Endocr Res. 2004;30(3):481-9.
11. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2006;91(10):4175-8.
12. Lioudaki E, Mavroeidi NG, Mikhailidis DP, Ganotakis ES. Subclinical hypothyroidism and vascular risk: an update. Hormones (Athens). 2013;12(4):495-506.
13. Bruno RM, Bianchini E, Faita F, Taddei S, Ghiadoni L. Intima media thickness, pulse wave velocity, and flow mediated dilation. Cardiovasc Ultrasound. 2014;12:34.
14. Kundu S RK. Thyroid Hormone Homeostasis in Adult Mammalian Brain: A Novel Mechanism for Functional Preservation of Cerebral T3 Content During Initial Peripheral Hypothyroidism. Al Ameen Journal of Medical Sciences. 2010;3(1):5 - 20.
15. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. Eur J Endocrinol. 2004;151 Suppl 3:U25-37.
16. Puia G, Losi G. Thyroid hormones modulate GABA(A) receptor-mediated currents in hippocampal neurons. Neuropharmacology. 2011;60(7-8):1254-61.

17. Losi G, Garzon G, Puia G**.** Nongenomic regulation of glutamatergic neurotransmission in hippocampus by thyroid hormones. Neuroscience. 2008;151:155-63.

18. Semyanov A, Walker MC, Kullmann DM, Silver RA**.** Tonically active GABA A receptors: modulating gain and maintaining the tone. Trends Neurosci. 2004;27(5):262-9.

19. Ba DM, Ssentongo P, Na M, Kjerulff KH, Liu G, Du P, et al. Factors Associated with Urinary Iodine Concentration among Women of Reproductive Age, 20-49 Years Old, in Tanzania: A Population-Based Cross-Sectional Study. Curr Dev Nutr. 2020;4(5):nzaa079.

20. Mtumwa A, Ntwenya J, Paul E, Huang M, Vuai S**.** Socio-economic and spatial correlates of subclinical iodine deficiency among pregnant women age 15–49 years in Tanzania. BMC Nutrition. 2017;3.

21. Anees M, Anis RA, Yousaf S, Murtaza I, Sultan A, Arslan M, et al. Effect of maternal iodine supplementation on thyroid function and birth outcome in goiter endemic areas. Curr Med Res Opin. 2015;31(4):667-74.

22. WHO**.** Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation. Public Health Nutr. 2007;10(12a):1606 -11.

23. WHO**.** Safe use of iodized oil to prevent iodine deficiency in pregnant women. Bulletin of the World Health Organization 1996 ; 74(1) : 1-3. 1996.

APPENDICES

This part comprises of:

Appendix I: Questionnaire

Appendix II: Participant information sheet and consent form

Appendix III: Ethics clearance certificates

Appendix IV: Published systematic review protocols

APPENDIX I: Questionnaire

Questionnaire for the risk and severity of preeclampsia secondary to Iodine deficiency and iodine deficiency-mediated subclinical hypothyroidism: mechanisms and early cardiovascular consequences

STUDY SERIAL ☐☐☐ ☐☐☐ NUMBER

DATE: ___/___ /_____

SECTION 1			
101	Parity	Gravida ___+____	
102	LNMP	_____/____/201_____	
	EDD and WOA	_____/____/201_____ WOA ______	
103	Date of HIV serology	_____/____/201_____	
104	Outcome of HIV serology	Non reactive--------1 Reactive-------------2	
105	Working diagnosis		

Section 2: Socio-demographic characteristics

	Questions and Filters	Coding	
201	Age	_________	
202	Highest level education E.g. grade 6, Matrix, college, University	________________	
203	Marital status	Never in union--------------------------1 Married---------------------------------2 Widowed--------------------------------3 Divorced---------------------------------4 Separated---------------------------------5 Re-married-------------------------------6	
204	Occupation	Scholar ------------------1 Unemployed------------2 Businesswoman-------3 Educator ----------------4 Farmer -------------------5 Clerk----------------------6 Homemaker---------------7 Other specify_______________	
205	If you are employed, on average, how much do you earn per month?	a. R _______________________	

		b. un employed	
206	Religion	Methodist-------------------1 Anglican---------------------2 Pentecostal/born again--3 Catholic----------------------4 Muslim-----------------------5 Traditional--------------------6 Other(specify)--------------206oth ____ _________________	
207	Ethnic/cultural group	Xhosa--------1 Zulu-----------2 Tshwane-----3 Sotho---------4 Pedi ----------5 Africans-------6 Coloured------7 Indian----------8 Other----------9 ________	
	Residential address: **Ward**	____________________________________	
	Village	_____________________________________	
208	Residential area	On a farm------------------------------1 In city/town-----------------------------2 In township formal settlement-----3	

		Township informal settlement-----4 In rural area (not a farm)------------5 Other, Specify______________		
211	Type of house where the respondent currently lives	Brick house/flat--------------1 Wendy house-----------------2 Shack in the yard------------3 Shack in a squatter area---4 Other, specify ___________________		
212	Do you have electricity in the house where you stay?	Yes-------1 No--------2		
300	**Parity and previous pregnancy outcomes**			
301	Parity	Para _________ + _____________		
302	**Outcomes of previous pregnancies**	**sex**	**Mode of delivery and outcome**	
	1			
	2			
	3			
	4			
	5			
	6			
	7			
400	**Antenatal care**			
401	Antenatal Care booking status	Booked --------1 Not booked----2		

402	Gestational age at booking	WOA________	
403	Number of ANC visits	______________	
404	Date of last ANC Visit before admission	______/_____/201__	
500	**Previous history of pregnancy complications:**		
501	Miscarriage	Yes ----1 No------2	
502	IUFD	Yes ----1 No------2	
503	Abruptio placentae	Yes ----1 No------2	
504	Preeclampsia/eclampsia/HT in pregnancy	Yes ----1 No------2	
505	Gestational DM	Yes ----1 No------2	
506	Other		
600	**History suggestive of risk of thyroid disease:**		
601	Thyroid disease	Yes ----1 No------2	
602	Radioactive iodine therapy	Yes ----1 No------2	
603	Thyrodectomy	Yes ----1 No------2	
604	External radiation to the neck	Yes ----1 No------2	
700	**Previous history of DM/HT:**		
701	Insulin dependent DM	Yes ----1 No------2	
702	Type 2 DM	Yes ----1 No------2	
703	Chronic hypertension	Yes ----1 No------2	
800	**Physical findings**		

801	Maternal height (cm)		
802	Maternal weight (kg)		
803	BMI		
804	Pulse rate		
805	Highest recorded Blood pressure mm Hg		
806	Current blood pressure		
807	FHR		
900	**Obstetric Ultrasound findings**		
901	Estimated gestational age		
902	Estimated foetal weight		
903	Placental grade		
904	AFI (amniotic fluid index)		
905			
1000	**Laboratory tests**		
1001	TSH		
1002	T4		
1003	T3		
	Thyroid function profile		
1004	TPOAbs (anti-thyroid peroxidase antibodies)		
1005	Thyroglobulin		

1006	Urine Iodine Concentration		
1007	Urine creatinine		
	Urine Potassium Concentration		
	Urine magnesium Concentration		
	Serum superoxide dismutase activity (SOD)		
	Serum Selenium levels		
	Serum glutathione levels		
	Serum Malondialdehyde levels		
	Placenta growth factor (PlGF)		
	sFLT1		
	Serrum ferritin		
	Transferrin		
	Placenta growth factor (PlGF)		
	U & E profile		
1009	Serum potassium		
	Serum Magnesium		
	Serum sodium levels (Na+)		
1010	Serum Creatinine		
	Serum urea		
	AST		

	ALT		
	HB		
	FBC WBC total and differential counts		
	CRP quantitative		
	CRP-ultra sensitive		
	C-Pepetide		
	Insulin		
	Lipogram		
	Total Cholesterol		
	HDL cholesterol		
	LDL cholesterol		
	Oxidised LDL		
	Triglycerides		
	Serum Albumin		

APPENDIX II: PARTICIPANT INFORMATION SHEET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

The risk and severity of preeclampsia secondary to Iodine deficiency and iodine deficiency-mediated subclinical hypothyroidism: mechanisms and early cardiovascular consequences

REFERENCE NUMBER:

INVESTIGATOR: **Charles Bitamazire Businge**

ADDRESS: **Department of Obstetrics and Gynaecology, Faculty of Health Sciences, Walter Sisulu University, Private Bag X1, WSU, 5117, Mthatha.**

CONTACT NUMBER: **+27727243520**

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. You are free to say yes or to say no. If you say no, you will still be given the same quality of treatment, and it will not be held against you. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Committee for Human Research at Walter Sisulu University and the University of Cape Town. In addition, permission has been granted by the Eastern Cape Department of Health and the Heads of Clinical Governance at Mthatha Regional and Nelson Mandela Academic Hospital to conduct this research. This research will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

This study aims to determine if iodine deficiency in pregnancy increases the risk and severity of preeclampsia among pregnant women in South Africa and other African countries. Preeclampsia is one of the serious conditions that lead to severe illness among pregnant women and their babies worldwide and in South Africa. The exact cause is not known but several risk factors that are associated with preeclampsia have been identified. This study will help to establish if iodine deficiency, which increases in pregnancy and is likely to be common in mothers not taking adequately iodized salt in South Africa, is associated with increased risk and severity of preeclampsia.

If this study results finds show iodine deficiency and the associated under-functioning of the thyroid gland (subclinical hypothyroidism) increase the risk of preeclampsia, it will help the Department of Health to plan screening and treatment of affected women in our area with iodine or thyroxine to improve on the successful outcome of the future pregnancies of the affected women.

Why have you been invited to participate?

You have been chosen to participate because the results will help in establishing whether the women with preeclampsia, a common complication of pregnancy in this region, have an underlying iodine deficiency or subclinical hypothyroidism whose treatment or prevention can improve the pregnancy outcome of many women in this setting. So your participation is precious.

What will your responsibilities be?

You will be required to allow us to use the information in your maternity record like results of blood tests and examination findings and sometimes give an extra sample of blood and urine for tests to the level of thyroid hormones and associated factors in your blood and iodine in the urine. We will also ask you a few other questions your past health, life history and lifestyle. In addition, we shall use a specialized machine SphygmaCor to measure your blood pressure and wave pattern of your radio and femoral arteries. This helps us to know if your blood vessels can expand and contract normally and if this function is associated with iodine deficiency of thyroid gland function.

Will you benefit from taking part in this research?

Your participation will be valuable as it will help establish whether iodine deficiency and abnormal thyroid function increase the risk of preeclampsia among pregnant women in this area. This will help the department of health and the hospital in planning better care for the affected population. Some results may help identify whether your iodine intake is adequate and if your thyroid gland is normal or if there is need for further assessment of your thyroid gland or treatment. Otherwise, you will receive the same standard care given to women with your condition presenting at Nelson Mandela Academic Hospital.

Are there in risks involved in your taking part in this research?

There is no known risk involved in your participation in this study.

If you do not agree to take part, what alternatives do you have?

Whether you decide to participate in this study or not will not affect your access maternity or neonatal care.

Who will have access to your medical records?

All personal information collected will be treated as confidential and access to it will be strictly controlled and limited to the investigators. All identifying information will be anonymised at the earliest possible time point. All patient specimens will be assigned study numbers for identification when used in a publication or thesis.

What will happen in the unlikely event of some form injury occurring because of your taking part in this research study?

We do not expect any extra risk as a result from participation in this study as no other products are going to be offered other than the standard treatment for patients with preeclampsia or a normal pregnancy. If any complications arise, you will be given the same standard approach to care given to patients at Nelson Mandela Academic Hospital according to their specific condition.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to take part in the study, and you will not incur any costs either.

Is there anything else that you should know or do?

- You can contact Prof M L Mdaka (Telephone: 0824690644)
- and Dr C B Businge (Tel: 0727243520) if you have any further queries or encounter any problems.
- You will receive a copy of this information and consent form for your own records.

Participants consent form

By signing below, I……………………………...…………. agree to take part in a research study entitled:

The increased risk and severity of preeclampsia secondary to Iodine deficiency and iodine deficiency-mediated subclinical hypothyroidism at Nelson Mandela and Mthatha Regional Hospitals

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have asked questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way. If I choose to leave the study a month after I have donated a sample with no sample, I may ask that my sample be discarded.

Signed at (*place*) ....…........…………….. on (*date*)…………...……….. 20...

....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)*……………………………………………..……… declare that:

- I explained the information in this document to…………………………………..
- I encouraged her to ask questions and took adequate time to answer them.
- I am satisfied that she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used, then the interpreter must sign the declaration below).*

Signed at (*place*) ....…......……………….. on (*date*)…………...……….. 20...

....

Signature of investigator   **Signature of witness**

Declaration by interpreter

I *(name)*……………………………………………..……… declare that:

- I assisted the investigator (*name*)……................…………………………………. to explain the information in this document to (*name of participant*) ....……………..…………………………….. Using the language medium of Afrikaans/Xhosa/ Other Specify:___________________________
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ....…......……………….. on (*date*)…………...……….. 20..

....

Signature of interpreter   **Signature of witness**

INTATHO NXAXHEBA KWEZEMFUNALWAZI KUXWEBHU NESIVUMELWANO KUXWEBHU

Isihloko semfunalwazi kwiprojekti:

ingaba ukuncipha kwe Iodine nokuhamba amaziko oncedo lokuqala i Hypothyroidism izala ipreeclampsia phakathi kwabafazi abakhulelweyo kwisibhedlele iNelson Mandela kunye nezibhedlele sesixeko saseMtata?

Oyena uphambili kuphando: Charles Bitamazire Businge

iAdress:

Isebe lezentsana nokubeleka

Imfundo yempilo nenzululwazi

Walter Sisulu Dyunivesiti, private bag x 15117, Mthatha

Inombolo zomxeba: 0727243520

Uyamenywa ukuzothabatha inxaxheba kwimfunalwazi yaleprojekti. Nceda uthathe ixesha ufunde ulwazi oluvezwe apha, izakuchaza ngokweminininingwane yaleprojekti. Nceda ubuze kwiqela labaphandi okanye kuGqirha nawuphina umbuzo nandawoni kule projekti ongayiqondiyo. Kubalulekile ukuba wonele yinkcazelo kulemfunalwazi ekuqaleni nakwimeko yokuba ungazibandakanya njani. Ukhululekile ukuba uvume okanye wale, ukuba uthi hayi uyakunikwa into efanayo ngokubaluqilima ngempatho. Ayizukuba yinto ezakuchasana nawe. Ukhululekile ukuba urhoxe ekufundeni phantsi kweyiphi imeko, nokuba awuvumi ukuthabatha inxaxheba. Lemfunalwazi ipasiswe yikomiti yophando ngomntu eWalter Sisulu Dyunivesiti izakukhokhelwa ngokwemigaqo yalondawo, yase Helsinki, neyaseMzantsi weafrika imigaqonkqubo ephucukileyo yezonyango nophando lwezamachiza (MRC) imigaqophando.

YINTONI EPHATHELENE NOLUPHANDO?

Oluphando lujolise ekwahlululeleni iIodine ukhulelo olugqibeleleyo yandisa ingcuphe nentlungu yepreeclampsia phakathi kwabafazi abakhulelweyo eMzantsi weAfrika. IPreeclampsia yenye yezifo ezibuthathaka imeko ekhokhelela kwingulo engamandla kubafazi abakhulelweyo neentsana zabo emhlabeni jikelele nase Mzantsi weafrika. Oyena nobangela akaziwa kodwa ezonampawu ezinxulumene nepreeclampsia zifumaneki olulwazi lunceda ukuveza ukuba iiodine eshokoxekileyo, eyandayo kukhulelo yinto exhaphakileyo kumanina ngokuba akukho okwaneleyo nge Iodizede ityuwa eMzantsi Afrika, inxulumene nengcuphe eyandayo nongxunguphalo lwePreeclampsia. Ukuba oluphando lungafumana iodine engonelanga kunye nonxulumano locwaningo lwezempilo hypothyroidism eyandayo kwiPreeclampsia, izakunceda icandelo lezempilo ukucwangcisa ukuphonononga nonyango lwenina elichaphazelekileyo kwindawo yethu kwiIodine okanye iThyroxine ukuphucula kwimpumelelo yeziphumo kwixesha elizayo kukhulelo kwinina elichaphazelekileyo.

KUTHENI UMENYIWE UKUBA UTHATHE IXAXHEBA?

Ukhethiwe ukuba ubengumthathi nxaxheba kuba iziphuma zakunceda ukuveza ipreeclampsia eqhelekileyo kwiinzima zokukhulelwa kulengingqi ukuze ufumane iIodine ikunqongophala okanye kuncedo lokuqala kwezempilo iHyporoidism enyangwa kukuthintelwa ingaphucula amaninzi akhoyo kulondawo, ke intatho nxaxheba ibalulekile.

ZINTONI OMAWUZIGADE

Kuzofuneka usivumele sisebenzise ulwazi kwincwadana yakho yokuqala kwezempilo neziphumo zovavanyo gazi nihlahlubo olufumeneyo ngamanye amaxesha unikezele ngamagazi akho nokuvavanywa komchamo kwinqanaba lwencindi yeThyroid nonxulumano lwezinye izinto egazini neIodine emchameni, Imbhali ngobom nendlela oliphethe ngayo kungezelelo sizakusebenzisa umatshini okhethekileyo iSphygmacor ukukala unxinizelelo lwegazi ukuvavanya ipateni yexilongo neFemoral arteries. Inceda ukwazi ukuba imithambo luvo ikwazi ukukhukhumala kunye nokunciphisa okuvumelekileyo ukuba lento esebenza ngayo inxulumene noshokoxeko lweIodine Thyroid gland iyasebenza.

IKHONA INZUZO OZAKUYIFUMANA NGOKUTHATHA INXAXHEBA?

Ibalulekile Inxaxheba yakho njengoko inceda ekuveliseni ukuba unqongophalo lweIodine nokungaqheleki kweThyroid ngongasebenzi yandisa ingcuphe yepreeclampsia ukucwangcisa abo bachaphazelekileyo. Iyakunceda icandelo lwezempilo nezibhedlele ukucwangcisa abo bachaphazelekileyo. Ezinye zeziphumo zinganceda ukuba iIodine esetyenziswa emzimbeni yonele ukuba ithyroid gland ukuba ilungile okanye ifunwa ingqwalasela ngokuthechatha ithyroid inyangwe, ngapha koko azakuzuza unakekelo olufanayo ngokokubonakala imeko okuyo kwisibhedlele sasNelson Mandela Academic Hospital.

INGABA KUKHONA UNXUNGUPHALO NGOKUTHATHA INXAXHEBA?

Akukho ngcuphe eyaziwayo ekhona kwintatho nxaxheba yakho koluphando.

UKUBA AWUVUMI KUTHATHA NXAXHEBA, YEYIPHI INTO ONGEZA NAYO EYOHLUKILEYO.

Nokuba ugqiba ukuthabatha inxaxheba okanye hayi asiyikukuphazamisa kwinkululeko okanye ukuthathela ineonatal. Ngubani ozokuba nendlela yokujonga imeko yakho yonyango. Kuyo yonke ingqokelela yolwazi iyakubayimfihlo. Nokuvela kwayo iyakubanendlela yokulawula ngabaphandi. Yonke into eyakhuthi ifunyaniswe iyakungavezwa iyeyomntu kwixesha elikhawulezileyo. Yonke into efumanekileyo kwisigulane kuyakuphawulwa ukwenzela ukuba kubonwe lowo ufunwayo ukuze isebenze kubantu abaliqela.

KUYAKWENZEKANI KWINTO ENGAFUNIYO KWIZINTO EZIFANA NENGOZI XA ISENZEKA UKWIPHULO LWEMFUNA LWAZI?

Asikwazi ukucingela nayiphina ingcuphe ngokweziphumo njengoko kungekho chiza elinikezwa ngaphandle kwechiza eliqhelekileyo ngokwezonyango kwiziguli ezinePreeclampsia okanye ukhulelo oluqhelekileyo. Ukuba kuvela ingxaki uyakunikwa into efanayo naleyo esetyenziswa esiBhedlele ngokwemeko ababone ngayo kuwe.

INGABA UZAKUBHATALWA NGOKUTHATHA INXAXHEBA KWEZIZIFUNDO INGABE KUKHONA OKUHLAWULWAYO?

Awuzukubhatalwa ngokuthatha inxaxheba kwezemfundo, awunakuzibona sele usengxakini yohlawula.

INGABE IKHONA ENYE INTO OFUNA ULWAZI LWAYO?

Ungadibana noProf ML Mdaka (umnxeba 0824690644) nogqirha CB Businge (umxeba 0727243520) ukuba unengxaki okanye udibene nento ongayiqondiyo. Uzakufumana ushicilelo yolulwazi noxwebhu lwemvumelwano ukuze unike ingxelo yakho.

ABATHATHI NXAXHEBA KWIXWEBHU LWESIVUMELWANO.

Ngokutyikitya ngezantsi, mna.. ndiyavuma ukuthabatha inxaxheba kwizifundo zophando ezidingekayo.

Ukwanda kwengcuphe yepreeclampsia elandela ukunqongophala kweiodine enqongopheleyo ithi ilanyulwe zinkonzo zempilo ihypothyroidism kwisibhedlele saseNelson Mandela nakwisixeko saseMthatha.

Ndiyavuma ukuba ndifundile ngolulwazi noxwebhu oluvuma lubhalwe ngolwimi endikwaziyo ukuluthetha nokulonwabela.

Ndibenethuba lokubuza imibuzo yonke imibuzo yam iphenduleke ngokwanelisayo.

Ndiyaqonda ukuba ukuthabatha inxaxheba kwezizifunda kungokuzithandela kanjalo andinyanzelwangaa ukuba yinxalenye.

Ndingakwazi ukukhetha ukusiyeka esisifundo nangaliphina ixesha kananjalo andinokohlwaywa okanye ndiphazanyiswe nangaluphi na uhlobo. Ukuba ndikhethe ukuyeka kwesosiifundo emvakwenyanga ndikhuphe isampula okanye kungesampula ndingacela ukuba isampula sisuswe.

Kutyikitywe (indawo)............................ phi(usuku)..............................20.........

ISIBHENGEZO SOMPHANDI

Mna(igama)... ndibhengeza ukuthi:

-Ndicacisile ngolwazi koluxwebhu ku... ndathabatha ixesha eloneleyo ukuyiphendula imibuzo.

-Ndonelisekile ukuba ukwazile ukuqonda ngokwaneleyo zonke iinkalo zophando njengoko bezixoxiwe ngasentla.

-Ndithe/andisebenzisanga toliki, ukuba kusetyenziswe itoliki, itoliki leyo mayityikitye isibhengezo ngezantsi

Ityikityiwe (Indawo)..................…………….. kwi(usuku)…………....……….. 20.........

Utyikityo lwetoliki...

ISIBHENGEZO SETOLIKI

Ndi(igama).. ndibhengeza ukuthi.

-Ndincedise umphandi (igama)...ukucacissa ulwazi ku (igama lomthathi nxaxheba)..ndisebenzisa ulwimi lwesibhulu/Xhosa nezinye ilwimi..

-Sikhuthaze usisi okanye ubhuti ukubuza imibuzo sathatha ixesha eloneleyo ukubaphendula.

-ndiye ndaqwalasela olona vuthondaba olululo olunxulumene nam.

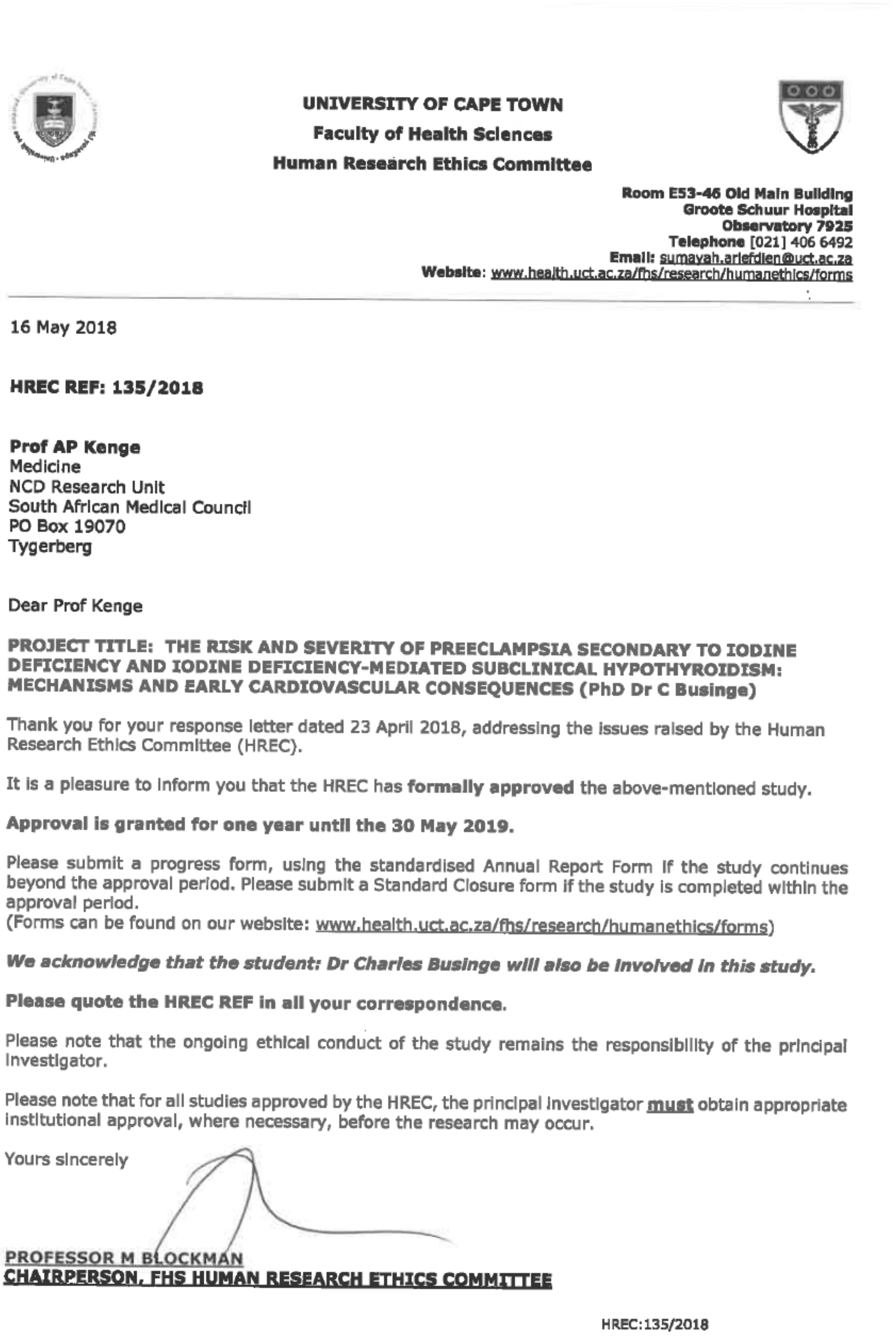
-Ndonelisekile ukuba umthathi nxaxheba uyaqonda ngokwanelisayo uvuthondaba loxwebhu lwesivumelwano kuvakele ukuba yonke imibuzo iphenduleke ngokwanelisayo.

Ityikitywe e(indawo)....................................kwi(usuku)..........................20...

....

Utyikityo lwetoliki Utyikityo lwengqina

APPENDIX III: ETHICS CLEARANCE CERTIFICATES



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

16 May 2018

HREC REF: 135/2018

Prof AP Kenge
Medicine
NCD Research Unit
South African Medical Council
PO Box 19070
Tygerberg

Dear Prof Kenge

PROJECT TITLE: THE RISK AND SEVERITY OF PREECLAMPSIA SECONDARY TO IODINE DEFICIENCY AND IODINE DEFICIENCY-MEDIATED SUBCLINICAL HYPOTHYROIDISM: MECHANISMS AND EARLY CARDIOVASCULAR CONSEQUENCES (PhD Dr C Businge)

Thank you for your response letter dated 23 April 2018, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 May 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Dr Charles Businge will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

HREC:135/2018

FACULTY OF HEALTH SCIENCES
POSTGRADUATE EDUCATION, TRAINING, RESEARCH AND ETHICS UNIT

HUMAN RESEARCH COMMITTEE
CLEARANCE CERTIFICATE

PROTOCOL NUMBER : 066/ 2017

PROJECT : THE RISK AND SEVERITY OF PREECLAMPSIA SECONDARY TO IODINE DEFICIENCY AND SUBCLINICAL HYPOTHYROIDISM

INVESTIGATOR(S) : DR CB BUSINGE

DEPARTMENT : OBSTETRICS & GYNAECOLOGY

DATE CONSIDERED : 25 OCTOBER 2017

DECISION OF THE COMMITTEE : APPROVED

N.B You are required to provide the committee with a progress or outcome report of the research after every 6 months. The committee expects a report on any changes in the protocol as well as any untoward events that may occur at any time during the study as soon as they occur.

WALTER SISULU UNIVERSITY
ACADEMIC HEALTH SERVICE COMPLEX OF THE EASTERN CAPE
POSTGRADUATE EDUCATION AND TRAINING
FACULTY OF HEALTH SCIENCES
WALTER SISULU UNIVERSITY
P/BAG X 1, WSU, 5117, E.C
TEL: (047) 502 2100 / FAX: (047) 502 2101

....
DR Z VUNDLE
CHAIRPERSON

30/10/2017
....
DATE

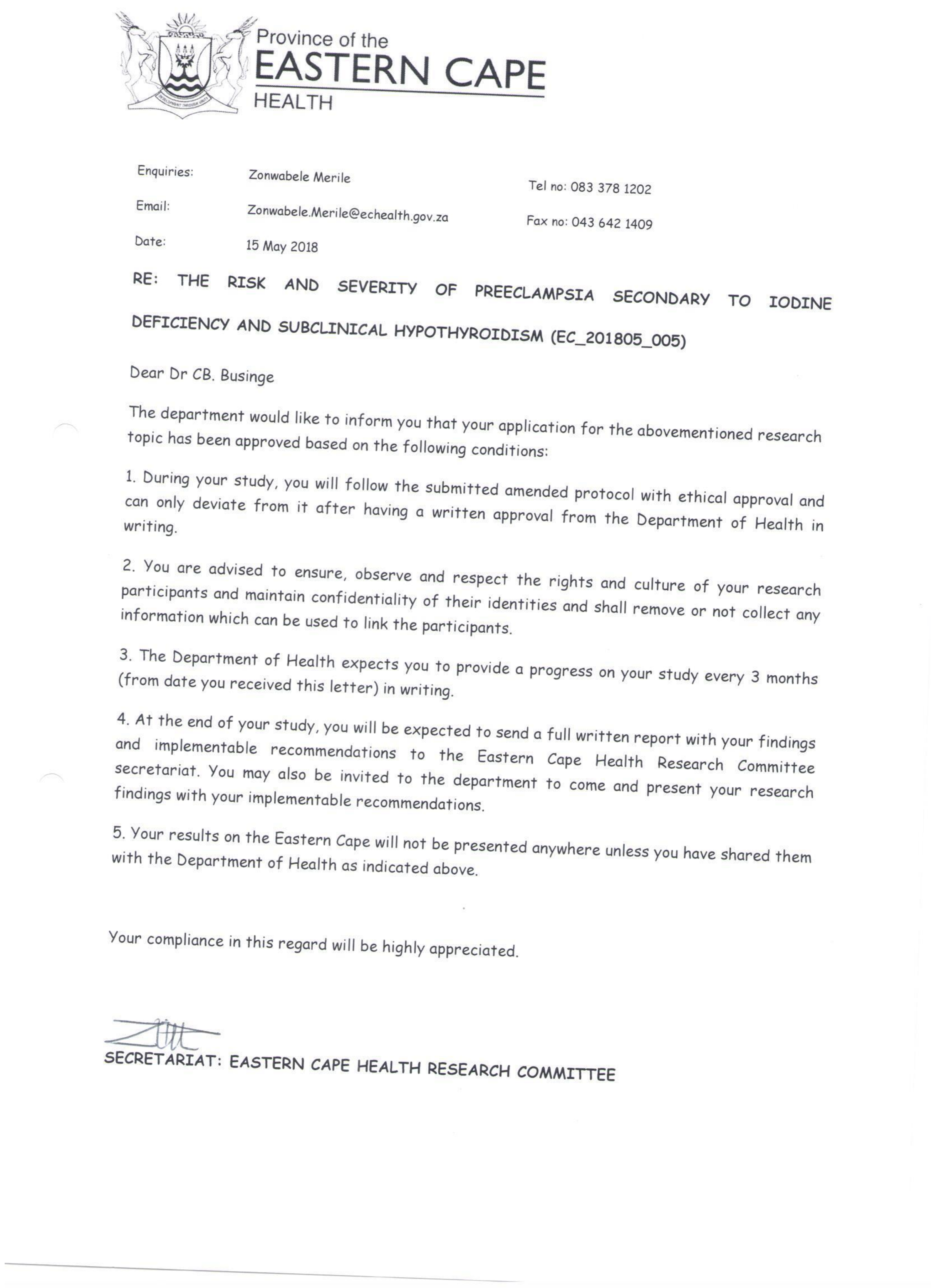
DECLARATION OF INVESTIGATOR(S)

(To be completed in duplicate and one copy returned to the Research Officer at Office L311, 3rd Floor, Old Library Building, NMD Campus, WSU)

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Research Ethics Committee. I/We agree to a completion of a yearly progress report.

....

N. B. Please quote the protocol number in all enquiries.



Province of the
EASTERN CAPE
HEALTH

Enquiries: Zonwabele Merile
Email: Zonwabele.Merile@echealth.gov.za
Date: 15 May 2018

Tel no: 083 378 1202
Fax no: 043 642 1409

RE: THE RISK AND SEVERITY OF PREECLAMPSIA SECONDARY TO IODINE DEFICIENCY AND SUBCLINICAL HYPOTHYROIDISM (EC_201805_005)

Dear Dr CB. Businge

The department would like to inform you that your application for the abovementioned research topic has been approved based on the following conditions:

1. During your study, you will follow the submitted amended protocol with ethical approval and can only deviate from it after having a written approval from the Department of Health in writing.

2. You are advised to ensure, observe and respect the rights and culture of your research participants and maintain confidentiality of their identities and shall remove or not collect any information which can be used to link the participants.

3. The Department of Health expects you to provide a progress on your study every 3 months (from date you received this letter) in writing.

4. At the end of your study, you will be expected to send a full written report with your findings and implementable recommendations to the Eastern Cape Health Research Committee secretariat. You may also be invited to the department to come and present your research findings with your implementable recommendations.

5. Your results on the Eastern Cape will not be presented anywhere unless you have shared them with the Department of Health as indicated above.

Your compliance in this regard will be highly appreciated.

SECRETARIAT: EASTERN CAPE HEALTH RESEARCH COMMITTEE

APPENDIX IV: PUBLISHED SYSTEMATIC REVIEW PROTOCOLS

This part comprises of:

Appendix IVa:

Businge CB, Longo-Mbenza B, Kengne AP. The prevalence of insufficient iodine intake in pregnancy in Africa: protocol for a systematic review and meta-analysis. Systematic Reviews. 2019;8(1):209.

Appendix IVb:

Businge CB, Madini N, Longo-Mbenza B, Kengne AP. Insufficient iodine nutrition status and the risk of pre-eclampsia: a protocol for systematic review and meta-analysis. BMJ Open. 2019;9(5):e025573.

APPENDIX IVa

Businge *et al. Systematic Reviews* _####################_
https://doi.org/10.1186/s13643-019-1092-7

Systematic Reviews

PROTOCOL **Open Access**

The prevalence of insufficient iodine intake in pregnancy in Africa: protocol for a systematic review and meta-analysis

Charles Bitamazire Businge[1,2*], Benjamin Longo-Mbenza[3] and Andre Pascal Kengne[1,4]

Abstract

Background: Insufficient iodine intake in pregnancy is associated with many adverse pregnancy outcomes. About 90% of African countries are at risk of iodine deficiency due to poor soils and dietary goitrogens. Pregnancy predisposes to insufficient iodine nutrition secondary to increased physiological demand and increased renal loss. Iodine deficiency is re-emerging in countries thought to be replete with pregnant women being the most affected. This review seeks to identify the degree of iodine nutrition in pregnancy on the entire African continent before and after the implementation of national iodization programmes.

Methods: A systematic search of published literature will be conducted for observational studies that directly determined the prevalence of insufficient iodine intake among pregnant women in Africa. Electronic databases and grey literature will be searched for baseline data before the implementation of population-based iodine supplementation and for follow-up data up to December 2018. Screening of identified articles and data extraction will be conducted independently by two investigators. Risk of bias and methodological quality of the included studies will be assessed using a risk of bias tool. Appropriate meta-analytic techniques will be used to pool prevalence estimates from studies with similar features, overall and by major characteristics including the region of the study, time period (before and after implementation of iodization programmes), sample size and age. Heterogeneity of the estimates across studies will be quantified and publication bias investigated. This protocol is reported according to Preferred Reporting Items for Systematic reviews and Meta-Analysis protocols (PRISMA-P) 2015 guidelines.

Discussion: This review will help ascertain the impact of national iodization programmes on the iodine nutrition status in pregnancy in Africa and advise policy on the necessity for monitoring and mitigating iodine deficiency in pregnancy in Africa. This review is part of a thesis that will be submitted to the Faculty of Health Sciences, University of Cape Town, for the award of a PhD in Medicine whose protocol has been granted ethics approval (UCT HREC 135/2018). In addition, the results will be published in a peer-reviewed journal.

Systematic review registration: PROSPERO CRD42018099434

Keywords: Iodine, Insufficiency, Pregnancy, Africa

---

* Correspondence: bsnbit001@myuct.ac.za; cbusingae@gmail.com; cbusinge@wsu.ac.za
[1] Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
[2] Department of Obstetrics and Gynaecology, Faculty of Health Sciences, Walter Sisulu University, Private Bag x1 WSU, 5117, Mthatha, South Africa
Full list of author information is available at the end of the article

Strengths and limitations of this study

- This will be the first systematic review and meta-analysis aiming to estimate the level of iodine deficiency among pregnant women in Africa.
- Methodological and statistical procedures that will be used to derive accurate estimates are robust and reliable.
- This review may be limited by the degree of accuracy of the various methods used to measure urine iodine concentration.
- Since urine iodine concentration varies according to the time day when the sample was collected, this may introduce some degree of heterogeneity
- Further heterogeneity may be introduced by studies with small sample sizes.

Background

Although iodine deficiency affects over 2 billion people worldwide and is re-emerging in formerly iodine-replete industrialised countries, pregnant women, lactating mothers and their offspring are more susceptible to the adverse effects of iodine deficiency [1]. These include stillbirths, miscarriages, intrauterine growth restriction, postpartum thyroiditis, subclinical and overt hypothyroidism, dyslipidemia, neuro-cognitive and psychomotor deficits [2–4].

In endemic areas, chronic iodine deficiency among women in reproductive age will be exacerbated by the increased renal clearance and loss in urine which will predispose the foetus to defective neuronal migration, myelination and glial differentiation which are key features of brain central nervous system development [4, 5]. This is the underlying cause of cretinism in severe cases and neurocognitive and psychomotor deficits. Hence, whole generations will be at risk of chronic thyroid, metabolic and mental diseases, leading to low socio-economic productivity in areas without sustained adequate nutrition [2].

There is a trend towards the re-emergence of iodine deficiency in iodine-replete countries such as the USA, the UK, New Zealand and Australia [6–9]. This has partially been attributed to inadequate use of iodized salt and voluntary instead of universal iodisation of salt used in commercial and household food production. As a result, the median urine iodine concentration (UIC) in the USA declined from 320 μg/l to 144 μg/l between 1971 and 2010 while pregnancy median UIC fell from 153 μg/l between 2001 and 2006 to insufficient levels <150 μg/l between 2007 and 2010 [6–8]. Data from a survey of 21 European countries in 2014 revealed that despite the iodine status of some countries being adequate across all age groups, 13/21 countries had inadequate iodine intake during pregnancy due to poor access to iodized foodstuffs and inadequate of monitoring of iodine nutrition status [9].

Iodine deficiency is widespread in Africa such that without iodine supplementation, almost 90% of the population will be at risk of iodine deficiency [10–12]. This is mainly due to iodine-deficient soils and goitrogens of which the most significant being poorly detoxified cassava which is rich in thiocyanate [13].

By early 1996, iodine deficiency disorder control programmes using iodised salt as the long-term strategy had been initiated in almost all of the 50 countries in Africa where WHO estimated that iodine deficiency disorder was of public health significance. As a result, more than 50% of the salt consumed in Africa was iodised [14]. Although universal iodisation of salt is the main source of dietary iodine in most African countries, other major sources of dietary iodine in some African countries include groundwater in Somalia and Djibouti and bouillon cubes and canned and processed foods such as in Senegal and Ghana [1, 15–18].

By 2017, 85% of the African countries had achieved sufficient iodine nutrition in the general population [19]. However, only four of the eleven African countries that had median pregnancy UIC survey data (South Africa, Tanzania, Sierra Leone and Ghana) had adequate iodine intake during pregnancy; five (Burkina Faso, Egypt, Niger, Morocco, and Senegal) had insufficient intake during pregnancy, while Djibouti and Liberia had more than enough iodine intake during pregnancy.

Since about 90% of dietary iodine intake is excreted in the urine, the World Health Organization (WHO) recommended that urinary iodine concentration (UIC) is a good marker of recent iodine intake. Hence, median UIC has been used to map out populations at increased risk of thyroid disorders due to iodine deficiency [20]. The median school-age children (SAC) UIC is commonly used to estimate the iodine nutrition status of the most population, but this may underestimate the degree of iodine deficiency in pregnancy due to differing dietary habits of SAC and pregnant women, in addition to specific physiological changes of pregnancy [9, 21, 22].

During pregnancy, the urinary iodine excretion increases by about 30–50% secondary to the increased blood volume, hyperdynamic state and the increased renal blood flow and glomerular filtration [23, 24]. Furthermore, serum iodide concentration is progressively reduced in the second and third trimesters by increased trans-placental transfer to the growing foetus which begins production of thyroid hormones from about 20 weeks' gestation [25]. In addition, there is extra iodine demand due to the physiological increase in maternal thyroid hormone output. This is as a result of the oestrogen mediated increase in thyroid-binding globulin that progressively decreases the free T4 in the serum, transfer of iodine to the foetus and increased renal iodine clearance [26]. Therefore, women with mild-to-moderate iodine deficiency may develop severe iodine deficiency in pregnancy with resultant subclinical hypothyroidism (SCH), overt hypothyroidism (OH) or isolated T4 deficiency with resultant maternal and offspring short term and long-term complications [25–27].

Hence, the WHO recommends that the average iodine intake to maintain normal thyroid clearance and cater for renal losses in pregnancy should be at least 200 μg daily for pregnant women compared to 100–150 μg per day for non-pregnant women [1]. Among pregnant women, a median UIC < 150, 150–249, 250–499 and > 500 μg is considered an estimate of, respectively, insufficient, adequate, more than adequate and excessive iodine nutritional status [28].

However, there is a paucity of data on the magnitude of iodine deficiency among pregnant women on the continent of Africa [29] and around the globe [19]. We intend to conduct a systematic review and meta-analysis of observational studies carried out to establish the trend in the state of iodine nutrition among pregnant women in Africa following the implementation of national iodization programmes.

Rationale

Although much gain in access to iodised salt has been achieved in most African countries since the early 1990s, of recent, the implementation of universal salt iodization, the major method of iodine supplementation, seems to be slowing down [7]. Due to challenges with monitoring [30], it is not clear if iodine deficiency may be re-emerging in African countries as in industrialised nations. Like elsewhere around the globe, women in reproductive age, pregnant women and their children will be the most affected, yet the degree of iodine deficiency in pregnancy in Africa is not well documented.

Although the most recent Iodine Global Network (IGN) data suggests that 85% of the African countries have sufficient iodine nutrition in the general population [19], further evaluation of these statistics using the method described by De Benoist et al [30] reveals that 30% of these countries have more than > 50% of the general population with a median UIC < 100 μg/L. This implies a high risk of insufficient iodine intake at the inception of pregnancy among women in reproductive age given that adequate iodine nutrition status in pregnancy is defined by median UIC of 150–249 μg/L [1]. It is recommended that pregnant and lactating women, who make up the most vulnerable portion of the general population, should be considered for supplementation with iodine until the population-based iodization programme is scaled up. Not only is it necessary to achieve sufficient iodine nutrition status, but it is also equally important to sustain adequate iodine nutrition status of the entire population especially the most vulnerable portions. Hence, the impact of iodization should be monitored at a regional or national level at least every 5 years.

Objectives

The aim of this systematic review and meta-analysis is to ascertain the trend in the prevalence of insufficient iodine nutrition status (median UIC < 150 μg/L) among pregnant women in Africa following the implementation of national iodization programmes and to establish if this has had a sustainable positive impact on the iodine nutrition status of pregnant women in Africa.

Review questions

The purpose of this review is to address the following questions:

1. What was the prevalence of insufficient iodine intake (UIC < 150 μg/L) among pregnant women on the various African countries before the implementation of national iodine deficiency disorder control programmes?
2. How has the iodine nutrition status during pregnancy changed in the various African countries following the implementation of national iodine deficiency disorder control programmes between 1994 and 31 December 2018?
3. What was the iodine nutrition status of pregnant women in various African countries between 2005 (the year designated by the WHO for the elimination of iodine deficiency through national iodisation programmes [1]) and 2018?

Methods

Eligibility criteria

Inclusion criteria

The selection of studies for inclusion in the review will be guided by the Population, Intervention/exposure, Comparison and Outcome protocol as stipulated below.

The population comprises of pregnant women on the African continent, the exposure is the period during the implementation of iodine deficiency disorder control programmes from 1994 to 2018, and the comparison is the period before the implementation of iodine deficiency disorder control programmes in 1994. The outcome is the iodine nutrition status during pregnancy in Africa.

Cross-sectional, case-control and cohort studies conducted on iodine deficiency among pregnant women in Africa with data available on mean or median urine iodine concentration will be included in this systematic review. The iodine nutrition status will be defined according to the WHO/ICCIDD classification of iodine intake of populations using median urinary iodine concentration [1]. All studies reported in the English, French or Portuguese languages and conducted on human subjects will be considered.

Exclusion criteria

Studies with the following characteristics will be excluded: studies conducted among populations of African origin but residing outside Africa, studies lacking prevalence rates and with the absence of data to compute them, case series with small sample sizes (sample less than 30 participants), and studies not performed in human participants or published in languages other than English, French and Portuguese.

Source of information

The methods of this systematic review are reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis protocols (PRISMA-P) 2015 Guidelines [31] (Additional file 1: Table S1).

Search strategy for study identification

Electronic searches

We will search PubMed-MEDLINE, Google Scholar, SCOPUS, ISI Web of Science (Science Citation Index), Africa Wide Information, African Index Medicus (AIM) and AFROLIB databases for published studies on iodine deficiency in pregnancy in Africa up to 31 December 2018. This search shall be conducted using a predefined comprehensive and sensitive search strategy combining relevant terms with names of countries in Africa, to obtain the maximum possible number of studies. This search will be guided by the African search filter, which has been reported to have good sensitivity (and improved precision) of 74% (1.3–9.4%) and 73% (5–28%) for MEDLINE and EMBASE, respectively [32]. This search filter includes names of each African country and shortened terms to capture studies from regions. Countries with official names in a language other than English will also be entered in the official form, and for countries that have changed names over time, both names shall be included in the search. Table 1 depicts the main search strategy to be employed. We will search reference lists of relevant citations for articles of interest.

Grey literature

We will search for national ministries of health, international organisations such as the WHO, UNICEF, ICCIDD and IGN, other non-government organisations' reports, conference and workshop proceedings using Google Scholar search engine and major relevant websites such as WHO African Index Medicus and African Journals Online (AJOL). Key experts in the field will be contacted for any unpublished study.

Table 1 Search strategy for MEDLINE and adaptability to regional databases

Search	Search items	Hits
1	iodine deficiency [tw] OR iodine insufficiency [tw] OR insufficient iodine intake [tw] OR insufficiency iodine nutrition [tw] OR iodine	
2	urine iodine excretion [tw] OR urine iodine concentration [tw] OR urinary iodine excretion [tw] OR urinary iodine concentration [tw] OR urine iodine	
3	Pregnancy [tw] OR Pregnant women [tw] OR expectant mothers [tw] first trimester [tw] [tw] OR second trimester [tw] third trimester [tw]	
4	#1 AND #3	
5	#2 AND #3	
6	African filter((((Angola[tw] OR Benin[tw] OR Botswana[tw] OR "Burkina Faso"[tw] OR Burundi[tw] OR Cameroon[tw] OR "Cape Verde"[tw] OR "Central African Republic"[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR "Democratic Republic of Congo"[tw] OR Djibouti[tw] OR "Equatorial Guinea"[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR "Guinea Bissau"[tw] OR "Ivory Coast"[tw] OR "Cote d'Ivoire"[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mozambique[tw] OR Namibia[tw] OR Niger [tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR "Sao Tome"[tw] OR Senegal[tw] OR Seychelles[tw] OR "Sierra Leone"[tw] OR Somalia[tw] OR "South Africa"[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Uganda[tw] OR "Western Sahara"[tw] OR Zambia[tw] OR Zimbabwe[tw] OR "Central Africa"[tw] OR "Central African"[tw] OR "West Africa"[tw] OR "West African"[tw] OR "Western Africa"[tw] OR "Western African"[tw] OR "East Africa"[tw] OR "East African"[tw] OR "Eastern Africa"[tw] OR "Eastern African"[tw] OR "South African"[tw] OR "Southern Africa"[tw] OR "Southern African"[tw] OR "sub Saharan Africa"[tw] OR "sub Saharan African"[tw] OR "subSaharan Africa"[tw] OR "subSaharan African"[tw] NOT "guinea pig" [tw] NOT "guinea pigs" [tw] NOT "aspergillus niger" [tw]))))	
7	# 4 AND # 6 Limits: 01/01/1990 to 31/12/2018 in English, French, and Portuguese on humans	
8	# 5 AND # 6 Limits: 0109/1990 to 31/12/2018 in English, French, and Portuguese on humans	

Study records

Data management

All identified entries will be entered into endnote software for de-duplication of records. Prior to the screening of studies, investigators shall create standardised questions according to the inclusion criteria which will then be pre-tested on a sample of eligible studies.

Screening

Two investigators will independently select studies that meet inclusion criteria. Citations and abstracts will be screened for possible inclusion, and duplicate citations will be excluded. Titles and abstracts will then be screened following inclusion criteria described above,

Table 2 Risk of bias assessment tool

	Risk of bias item	Yes = 1 No = 0
External validity		
1	Was the study target population a close representation of the national population in relation to relevant variables?	
2	Was the sampling frame a true or close representation of the target population?	
3	Was some form of random selection used to select the sample, OR, was a census undertaken?	
4	Was the likelihood of non-participation bias minimal?	
Internal validity		
5	Were data collected directly from the participants (as opposed to medical records)?	
6	Were acceptable case definitions of iodine deficiency in pregnancy used?	
7	Were reliable and accepted diagnostic methods for iodine intake utilised?	
8	Was the same mode of data collection used for all participants?	
9	Were the numerator(s) and denominator(s) for the calculation of the iodine intake appropriate?	
Summary of the overall risk of bias	Low risk	0–3
	Intermediate risk	4–6
	High risk	7–9

following which the full texts of potentially eligible articles will be obtained. These full texts will be screened using a standardised and pre-tested form to include eligible studies. Disagreements will be resolved by consensus or consultation of a third author. Corresponding authors of potentially eligible studies that did not report data that are relevant to our study analysis will be contacted. Reasons for exclusion of non-eligible studies will be documented. The whole selection process will be summarised in a flow chart.

Data extraction

Two investigators will independently extract data from included studies, using a standardised and pre-tested data extraction form. Any inconsistencies or disagreement shall be resolved by consensus or consultation with the third investigator.

Data items

Data will include the geographic region and country where study was conducted, the year study was carried out and year of publication, the language of publication, demographic characteristics of participants (such as mean age), study design, setting (rural or urban, health-facility or community-based), sample size, and the criteria used for determination of the iodine intake. The median (25th–75th percentiles) and or mean (standard deviation) UIC will be recorded.

Assessment of methodological quality and risk of bias

Two reviewers will independently score the quality of included studies. The STROBE checklist [33] will be used to evaluate reporting methodology in each paper while risk of bias in individual studies will be assessed using the risk of bias tool for prevalence studies [34] (Table 2) and the Cochrane guidelines available in Review Manager V.5.3 (http://tech.cochrane.org/revman).

Discrepancies will be resolved by consensus or by consulting the third investigator. Inter-rater agreement on screening, data abstraction and methodological quality will be assessed using Cohen's κ coefficient [35]. We intend to present the risk of bias and quality scores in a table.

Data synthesis, analysis and assessment of heterogeneity

Prevalence data will be summarised by country and country-specific geographic regions where applicable (Table 3). For studies with sufficient data, meta-analysis using random effects models will be conducted overall, that is, across all possible eligible studies. In addition, we will conduct subgroup analysis according to major study-level characteristics such as by country, regions within Africa (as defined by the United Nations); the time period of data collection: before 2005 and after 2005 (the target year for elimination of iodine deficiency through national iodization programmes); the period defined as before and after the implementation of national iodization programmes; and the sample size (below vs. at or above median sample size across included studies) and by age group (below vs. at or above median mean age across included studies). Other criteria for subgroup analyses will include urinary iodine assessment methods and study design. Data will be presented as forest plots showing estimates of mean UIC in pregnancy. For data unsuitable for meta-analysis, we will provide a narrative description of major study characteristics and trends over time.

Study-specific estimates will be pooled after stabilising the variance of individual studies with the use of Freeman-Tukey double arc-sine transformation [36]. This transformation will help reduce the effect of extremely high or extremely low prevalence rates on the pooled estimate. Heterogeneity will be evaluated by the Cochrane's Q statistic and I^2. I^2 values of 25%, 50% and 75% will respectively be deemed to represent low, medium and high heterogeneity, respectively. Funnel plots together with the Egger test of bias will be used to investigate the publication bias [37].

Table 3 Data synthesis template

First author, year	Country language	Setting/geographical region	Study design, period	Population characteristics	Sample size	Diagnostic criteria	Magnitude of iodine intake

Sensitivity analysis

Subgroup analysis using the variables mentioned above and further analysis according to the quality of the studies will be carried out in order to identify possible sources of the heterogeneity. If subgroup differences are identified, they will be described, and the data will be interpreted in light of these differences.

The Duval and Tweedie trim-and-fill will be used to adjust estimates for the effects of potential publication bias. Data analyses will use the *'meta'* package of the statistical software R (version 3.3.3 [2017-03-06], The R Foundation for statistical computing, Vienna, Austria), and the 'meta' package.

Reporting of this review

The proposed systematic review will be reported following the PRISMA guidelines [38]. We intend to publish a PRISMA checklist alongside the final report.

Potential amendments

We do not intend to make any amendments to the protocol, to avoid the possibility of outcome reporting bias. However, any amendments that do prove necessary will be documented and reflected online on the PROSPERO website where the protocol has been registered [PROSPERO CRD42018099434].

Discussion

The degree of iodine nutrition during pregnancy all over the African continent following the implementation of USI and other methods of iodization is not known with certainty. It is not certain whether the trend towards the re-emergence of iodine deficiency among pregnant women in several developed countries around the world is also affecting pregnant women in Africa. A high prevalence of iodine deficiency among pregnant women in Africa would imply an enormous but probably unrecognised predisposition to iodine deficiency disorders affecting not only pregnant women but also lactating mothers and their offspring. The association of iodine deficiency in pregnancy with various adverse pregnancy outcomes and chronic neurocognitive, psychomotor thyroid and cardiovascular diseases among mothers and their offspring requires concerted attention. This review seeks to address the knowledge gap on the magnitude of insufficient iodine intake among pregnant women on the African continent. The data will help shed light on the magnitude of iodine deficiency in pregnancy in Africa which can help inform policy makers on the degree and desirable methods for intervention and the appropriate frequency of monitoring of iodine nutrition status in pregnancy.

Possible limitations of this study would include a predominance of poor quality studies and significant heterogeneity of studies precluding further analysis.

Additional file

Additional file 1: Table S1. PRISMA-P 2015 checklist. (DOCX 30 kb)

Abbreviations

ICCIDD: International Council for Control of Iodine Deficiency Disorders; IGN: Iodine Global Network; PRISMA-P: Preferred Reporting Items for Systematic reviews and Meta-Analysis protocols; SAC: School-age children; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology; UIC: Urine iodine concentration; WHO: World Health Organization; UNICEF: United Nations Children's Education Fund; USI: Universal salt iodization

Acknowledgements

Not applicable

Authors' contributions

CBB and APK conceived and designed the protocol. CBB was responsible for manuscript drafting. APK and LMB took part in the critical revision for methodological and intellectual content. CBB is the guarantor of this review. All the authors read and approved the final version of the manuscript.

Funding

This study is part of a research project supported by Discovery Foundation Rural Fellowship Grant number 038372. APK is employed by the South African Medical Research Council. The Sponsors have no role in designing and conducting of this study.

Ethics approval and consent to participate

The current study is based on published data and hence does not require ethical approval. This review is part of a thesis that will be submitted to the Faculty of Health Sciences, University of Cape Town, for the award of a PhD in Medicine whose protocol has been granted ethics approval by the University of Cape Town Human Research Ethics committee- IRB0001938 (UCT HREC REF:135/2018). In addition, the results will be published in a peer-reviewed journal. The final report of this review in the form of a scientific paper will be published in a peer-reviewed journal. Findings will also be presented at conferences and submitted to relevant health and policy authorities. We also plan to update the review in the future to monitor any progressive changes on the subject.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. [2]Department of Obstetrics and Gynaecology, Faculty of Health Sciences, Walter Sisulu University, Private Bag x1 WSU, 5117, Mthatha, South Africa. [3]Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo. [4]Non-Communicable Disease Research Unit, South African Medical Research Council, Cape Town, South Africa.

Received: 9 August 2018 Accepted: 2 July 2019
[redacted]

References

1. WHO (World Health Organization). Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. Geneva: World Health Organization; 2007.
2. Zimmerman MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet. 2015;3(4):286–95.
3. De Escobar GM, Obregon MJ, del Rey FE. Iodine deficiency and brain development in the first half of pregnancy. *Public Health Nutr*. 2007;10:1554–70.
4. Lee KW, Shin D, Song WO. Low Urinary iodine concentrations associated with dyslipidemia in US adults. Nutrients. 2016;8(171). https://doi.org/10.3390/nu8030171.
5. Auso E, Lavado-Autric R, Cuevas E, et al. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology. 2004;4(145):4037–47.
6. Caldwell KL, Pan Y, Mortinsen ME, et al. Iodine, status in pregnant women in the National Children's Study and in U.S. women (15–44 years), NHANES 2005–2010. Thyroid. 2013;23(8):927–37.
7. Pearce EN, Andersson M, Zimmermann MB. Global iodine status where do we stand in 2013? Thyroid. 2013;23(5). https://doi.org/10.1089/thy.2013.0128.
8. Sullivan KM, Perrine C, Pearce EN, et al. Monitoring the iodine status of pregnant women in the United States. Thyroid. 2013;23:520–1.
9. Lazarus JH. Iodine status in Europe in 2014. *Eur Thyroid J*. 2014;3:3–6.
10. Joy EJM, Ander EL, Young SD, et al. Dietary mineral supplies in Africa. *Physiologia Plantarum*. 2014;151:208–29.
11. Tsegaye B, Ergete W. Histopathologic pattern of thyroid disease. East Afr Med. 2003;80(10):525–8.
12. Kishosha PA, Galukande M, Gakwaya AM. Selenium deficiency a factor in endemic Goiter persistence in Sub-Saharan Africa. World J Surg. 2011;35(7):1540–5.
13. Taga I, Oumbe VA, Johns R, et al. Youth of west-Cameroon are at high risk of developing IDD due to low dietary iodine and high dietary thiocyanate. *Afr Health Sci*. 2008;8(3):180–5.
14. Kavishe FP. Can Africa meet the goal of eliminating iodine-deficiency disorders by the year 2000? Food Nutr Bull. 1996;17:262–7.
15. Kassim IAR, Moloney G, Busili A, Nur AY, Paron P, Jooste P, Gadain H, Seal AJ. Iodine intake in Somalia is excessive and associated with the source of household drinking water. J Nutr. 2014;144(3):375–81.
16. Spohrer R, Knowles J, Jallier V, Ndiaye B, Indorf C, Guinot P, Kupka R. Estimation of population iodine intake from iodized salt consumed through bouillon seasoning in SenegalAnn. N.Y. Acad. Sci. 2015;1357:43–52.
17. Farebrother J, Zimmermann MB, Abdallah F, Assey V, Fingerhut R, Gichohi-Wainaina WN, Hussein I, Makokha A, Sagno K, Untoro J, Watts M, Andersson M. Effect of excess iodine intake from iodized salt and/or groundwater iodine on thyroid function in nonpregnant and pregnant women, infants, and children: a multicenter study in East Africa. Thyroid. 2018;28(9):1198–210.
18. Abu BAZ, Oldewage-Theron W, Aryeetey RNO. Risks of excess iodine intake in Ghana: current situation, challenges, and lessons for the future. Ann N Y Acad Sci. 2018. https://doi.org/10.1111/nyas.13988.
19. Iodine Global Network (IGN). Global Scorecard of Iodine Nutrition in 2017 in the general population and in pregnant women (PW). Zurich, Switzerland: IGN; 2017.
20. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. *Journal of Nutrition*. 2012;142(4):744–50.
21. Gowachirapant S, Winichagoon P, Wyss L, et al. Urinary iodine concentrations indicate iodine deficiency in pregnant Thai women but iodine sufficiency in their school-aged children. *J Nutr*. 2009;139:1169–72.
22. Wang W, Teng W, Shan Z, et al. The prevalence of thyroid disorders during early pregnancy in China: the benefits of universal screening in the first trimester of pregnancy. *Eur J Endocrinol*. 2011;164(2):263–8.
23. Leung AM, Pearce EN, Braverman EL. Iodine nutrition in pregnancy and lactation. Endocrinol Metab Clin North Am. 2011;40(4):765–77.
24. Cheung KL. Lafayette RA. Ren Physiol Pregnancy. 2013;20(3):209–14.
25. Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev. 1997;18(3):403–32.
26. Zantour B, Alaya W, Marmouch H, et al. Hypothyroidism in pregnancy. In: Potluková E, editor. Current Topics in Hypothyroidism with Focus on Development. Rijeka, Croatia: In Tech; 2013. p. 29–62.
27. Lazarus HJ. Ch 14: Thyroid regulation and dysfunction in the pregnant patient. [Online]. 2016. Available: https://www.ncbi.nlm.nih.gov/books/NBK279059/.
28. WHO (World Health Organization). Proceedings of the WHO Technical Consultation on control of iodine deficiency in pregnant women and young children. Geneva: World Health Organization; 2005.
29. Harika R, Faber M, Samuel F, et al. Micronutrient status and dietary intake of iron, vitamin A, iodine, folate and zinc in women of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa: A Systematic Review of Data from 2005 to 2015. Nutrients. 2017. https://doi.org/10.3390/nu9101096.
30. De Benoist B, Andersson M, Egli I, et al. Iodine status worldwide: WHO Global Database on Iodine Deficiency. Geneva: World Health Organisation; 2004.
31. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4:1.
32. Pienaar E, Grobler L, Busgeeth K, et al. Developing a geographic search filter to identify randomised controlled trials in Africa: finding the optimal balance between sensitivity and precision. Health Inf Libr J. 2011;28:210–5.
33. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Bull World Health Organ*. 2007;85:867–72.
34. Hoy D, Brooks P, Woolf A, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. *J Clin Epidemiol*. 2012;65:934–9.
35. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. 2012;22(3):276–82.
36. Barendregt JJ, Doi SA, Lee YY, et al. Meta-analysis of prevalence. *J Epidemiol Community Health*. 2013;67:974–8.
37. Egger M, Davey SG, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–34.
38. Moher D, Liberati A, Tetzlaff J, et al. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med. 2009;151:264–9.

Publisher's Note

APPENDIX IVb

Open access Protocol

BMJ Open

Insufficient iodine nutrition status and the risk of pre-eclampsia: a protocol for systematic review and meta-analysis

Charles Bitamazire Businge,[1,2] Namhla Madini,[3] Benjamin Longo-Mbenza,[4,5] A P Kengne[6,7]

To cite: Businge CB, Madini N, Longo-Mbenza B, *et al*. Insufficient iodine nutrition status and the risk of pre-eclampsia: a protocol for systematic review and meta-analysis. *BMJ Open* 2019;**9**:e025573. doi:10.1136/bmjopen-2018-025573

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-025573).

Received 22 July 2018
Revised 4 February 2019
Accepted 7 February 2019

Check for updates

For numbered affiliations see end of article.

Correspondence to
Dr Charles Bitamazire Businge; cbusingae@gmail.com

ABSTRACT

Introduction Pre-eclampsia is one of the leading causes of maternal and perinatal morbidity and mortality worldwide. Although subclinical hypothyroidism (SCH) in pregnancy is one of the established risk factors for pre-eclampsia, the link between iodine deficiency, the main cause of hypothyroidism and pre-eclampsia remains uncertain. About two billion people live in areas with iodine insufficiency. The increased renal blood flow during pregnancy leading to increased renal iodine clearance together with the increased placental transfer of iodine to the fetus leads to further iodine deficiency in pregnancy. Iodine is one of the most potent exogenous antioxidants whose deficiency is associated with oxidant imbalance and endothelial dysfunction, one of the mechanisms associated with increased risk of pre-eclampsia.

Methods and analysis A systematic search of published literature will be conducted for case–control studies that directly determined the iodine nutrition status of women with pre-eclampsia and appropriate normotensive controls. A similar search will be conducted for cohort studies in which the incidence of pre-eclampsia among pregnant women with adequate and inadequate iodine nutrition status was reported. Databases including MEDLINE, EMBASE, Google Scholar, SCOPUS and Africa Wide Information will be searched up to 31 December 2018. Screening of identified articles and data extraction will be conducted independently by two investigators. Risk of bias of the included studies will be assessed using a Newcastle-Ottawa Scale. Appropriate meta-analytic techniques will be used to pool prevalence and incidence rates, odds and relative risk of pre-eclampsia from studies with similar features, overall and by geographical regions. Heterogeneity of the estimates across studies will be assessed and quantified and publication bias investigated. This protocol is reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis protocols (PRISMA-P) 2015 guidelines.

Ethics and dissemination Since the proposed study will use published data, there is no requirement for ethical approval. This review seeks to identify the risk of pre-eclampsia associated with insufficient iodine nutrition in pregnancy. This will help to ascertain whether insufficient iodine intake may be an independent risk factor for pre-eclampsia. This will advise policy makers on the possibility of maximising iodine nutrition in pregnancy and reproductive age as one of the remedies for prevention of pre-eclampsia among populations at risk of inadequate iodine intake. This review is part of the thesis that will be submitted for the award of a PhD in Medicine to the Faculty of Health Sciences of the University of Cape Town. In addition the results will be published in a peer-reviewed journal.

PROSPERO registration number CRD42018099427.

Strengths and limitations

- To our knowledge, this is the first systematic review and meta-analysis that is aimed at ascertaining the relationship between insufficient iodine nutrition status and pre-eclampsia.
- This review may however be limited by the small number of eligible studies and small sample sizes that may make it liable to a considerable degree of heterogeneity.
- The eligible studies may have varied research designs that may potentially preclude the pooling of the test results.

INTRODUCTION

Pre-eclampsia is one of the leading causes of maternal and perinatal morbidity and mortality worldwide.[1] Although the actual cause of pre-eclampsia remains unknown, the risk factors of pre-eclampsia are multifactorial.[2–4]Subclinical hypothyroidism (SCH) in pregnancy is one of the established risk factors for pre-eclampsia.[5–7] Given that iodine deficiency is the leading cause of hypothyroidism,[8 9] iodine deficiency could be an independent risk factor of pre-eclampsia especially in settings with endemic iodine deficiency. Although some studies have reported an association between iodine nutrition status and pre-eclampsia,[10 11] it is not yet certain whether this association is consistent across different settings around the world.

The WHO estimates that about two billion people live in areas with iodine insufficiency.[12] In addition, iodine deficiency is on the rise in areas originally thought to be iodine sufficient.[13] This is due to inadequate iodisation in the background of increased amount of perchlorate and thiocyanate in water sources and the diet.[14–16] Both perchlorate and thiocyanate significantly diminish the uptake of iodine by the thyroid gland especially in states of increased thyroid stimulation by thyroid stimulating hormone (TSH) secondary to iodine deficiency.[15 16]

Rationale

Iodine deficiency has been shown to be associated with defective trophoblast proliferation and migration, which are some of the mechanisms proposed in the aetiology of pre-eclampsia.[17 18] The placenta is a highly metabolic organ with potential for production of reactive oxygen species. It is also one of the organs with high physiological concentrations of iodine whose antioxidant effect reduces lipid peroxide formation and may ensure normal placentation and function.[19–21]

During pregnancy, there is increased renal perfusion with increased iodine filtration and urinary iodine excretion in addition to increased transfer of iodine to the fetus.[22] Hence women with inadequate iodine intake are at risk of developing iodine deficiency in pregnancy and possibly at increased risk of developing subclinical or overt hypothyroidism and pre-eclampsia.[21–23]

Furthermore, the elevation in serum TSH that occurs in overt or SCH, is associated with hyperstimulation of the thyroid gland excessive superoxide production among individuals with iodine deficiency, which when released into the circulation, causes endothelial dysfunction and atherosclerosis.[24 25] These are known pathological pathways of pre-eclampsia.[26]

Objectives

This systematic review and meta-analysis is intended to ascertain whether insufficient iodine nutrition status is associated with increased risk of pre-eclampsia.

Review questions

The purpose of this review is to address the following questions:

1. Do pregnant women with insufficient iodine nutrition status have an increased risk of pre-eclampsia compared with pregnant women with adequate iodine nutrition status?
2. Is there a difference in the urinary iodine concentration (UIC) of pregnant women with pre-eclampsia versus that of normotensive pregnant women?

METHODS

This protocol is developed following the Preferred Reporting Items for Systematic reviews and Meta-Analysis protocols (PRISMA-P) 2015 Guidelines.[27]

Eligibility criteria

Inclusion criteria

The selection of studies for inclusion in the review will be guided by the Population, Intervention/exposure, Comparison and Outcome protocol as stipulated below:

1. Population: pregnant women.
2. Exposure: the exposure is insufficient iodine nutrition status during pregnancy for both case–control and cohort studies. In this systematic review this will be defined according to the WHO/International Council for Control of Iodine Deficiency Disorders (ICCIDD) classification of iodine intake of populations using median UIC.[28] For pregnant women, a urine iodine concentration (UIC) <150, 150–249, 250–499 and >500 µg/L is considered an estimate of, respectively, insufficient, adequate, more than adequate and excessive iodine nutritional status.
3. Comparator: for both case–control and cohort studies the comparator will be the participants with sufficient iodine nutrition status (UIC >150 µg/L) during pregnancy.
4. Outcome: the outcome is the prevalence (for case–control studies) and the incidence (for cohort studies) of pre-eclampsia among women with and without adequate iodine nutrition status in pregnancy from which the ORs will be determined.

   Pre-eclampsia has been defined as new onset hypertension after 20 weeks of amenorrhoea characterised by elevated systolic blood pressure of 140 mm Hg or diastolic blood pressure of 90 mm Hg or more or both, measured twice with a gap of 4 hour or one measurement of systolic blood pressure of ≥160 mm Hg or diastolic blood pressure of ≥110 mm Hg or both accompanied by one of the following: proteinuria in 24 hour-urine ≥300 mg or protein/creatinine ratio ≥0.3 or urine protein measured by dipstick ≥1+; or thrombocytopenia (platelets less than 150 000/µL), kidney insufficiency (concentration of creatinine in serum above 97 µmol/L), decreased liver function (enzyme activity of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) twice higher than the upper limit of the referential interval), compromised pulmonary function or pulmonary oedema or visual or other symptoms and signs of deficient cerebral function.[29] There may be considerable heterogeneity if pre-eclampsia has been variably defined in different studies that are eligible for inclusion in the current systematic review.

Exclusion criteria

- Studies in which none of the following parameters was computed: means, medians, ORs, incidence and prevalence rates and with absence of data to compute them.
- Eligible studies that are missing some critical data where after repeated attempts to contact an author via email for relevant information, no response is gotten.
- Letters to editors, reviews, commentaries, editorials and any publication without primary data.
- Duplicate publications from the same study. For studies published in more than one journal/conference, the most recent and comprehensive publication will be used.

Table 1 Search strategy for MEDLINE

Population: Pregnant women with Pre-eclampsia		
#1	MeSH terms	Pregnant Women [Mesh] OR Pregnancy [Mesh] OR Pregnancy Trimesters [Mesh]
#2	Free text	Pregnancy OR Pregnant women OR expectant mothers
#3	#1 OR #2	
#4	MeSH terms	Pre-Eclampsia [Mesh] OR Eclampsia [Mesh] OR Hypertension [Mesh]
#5	Free text	Preeclampsia OR Pre-eclampsia OR Eclampsia OR Hypertension OR Hypertensive OR High blood pressure
#6	#4 OR #5	
Exposure: Iodine deficiency		
#7	MeSH terms	Iodine [Mesh]
#8	Free text	Iodine
#9	#7 OR #8	
#10	#3 AND #6 AND #9	

- Studies not performed in human participants.
- No language restriction will be applied.

Patient and public involvement

The public or patients were not involved in the development of this protocol.

Search strategy for study identification

Electronic searches

We will search PubMed MEDLINE, Google Scholar, SCOPUS, ISI Web of Science (Science Citation Index) databases for all published studies on iodine deficiency and pre-eclampsia up to 31 December 2018. This search shall be conducted using a predefined comprehensive and sensitive search strategy combining relevant terms and synonyms which are variably used to denote abnormally high blood pressure in pregnancy and insufficient iodine intake or iodine deficiency. Table 1 depicts the main search strategy to be employed for MEDLINE database that will also be adapted for searches in other electronic databases.

We will search reference lists of relevant citations for articles of interest.

Grey literature

We will contact experts in the field, research organisations, conference websites and conference proceedings that dealt with micronutrient deficiency and pre-eclampsia, for any relevant data.

Study records

Data management

We will use an appropriate citation management software to remove duplicates from the references articles that will have been gathered. Prior to screening of studies, we will create a set of standardised questions according to the inclusion criteria which will then be pretested on a sample of eligible studies.

Screening

Two investigators will independently select studies that meet inclusion criteria. Citations and abstracts will be screened for possible inclusion, and duplicate citations will be excluded. Titles and abstracts will then be screened following inclusion criteria described above, following which the full texts of potentially eligible articles will be obtained. These full texts will be screened using a standardised and pretested form to include eligible studies. Disagreements will be resolved by consensus, with consultation of a third author (when resolution cannot be achieved). Corresponding authors of potentially eligible studies that did not report data that are relevant to our study analysis will be contacted. Reasons for exclusion of non-eligible studies will be documented. The whole review process will be summarised in a flowchart.

Data extraction

Two investigators will independently extract data from included studies, using a standardised and pretested data extraction form. Any inconsistencies or disagreement shall be resolved by consensus or consultation with the third investigator.

Data items

Data will include the geographic region and country where study was conducted, the year study was carried out and year of publication, the language of publication, demographic characteristics of participants, time of measurement of exposure (such as first, second or third trimester of pregnancy or preconception), study design, setting (rural or urban, health-facility or community-based), sample size and the criteria used for determination of the iodine intake. For cross-sectional and case–control studies, the median (25th–75th percentiles) and or mean (SD) UIC will be extracted. The overall mean UIC for cases and controls together with the mean UIC difference will be determined. The proportions of women with insufficient iodine nutrition among cases and controls will be extracted or determined based on the standard definition by WHO and the ICCIDD. Then the odds or data required to compute the odds of insufficient iodine nutrition among the cases will be extracted. Where applicable adjusted ORs will be extracted or computed so account for traditional risk factors for pre-eclampsia such as obesity, primigravida, advanced maternal age or diabetes. For cohort studies, the incidence rates and relative risk of pre-eclampsia according to baseline status for iodine nutrition will be extracted.

Assessment of methodological quality and risk of bias

Two reviewers will independently score the quality of included studies. The risk of bias in individual studies will be assessed using the Newcastle-Ottawa scale[30] and the

Cochrane guidelines available in Review Manager V.5.3 (http://tech.cochrane.org/revman). Discrepancies will be resolved by consensus or by consulting the third investigator. Inter-rater agreement on screening, data abstraction and methodological quality (selection, comparability of groups and ascertainment of exposure/outcome) will be assessed using Cohen's κ coefficient.[31]

Data synthesis, analysis and assessment of heterogeneity

For data unsuitable for meta-analysis, we will provide a narrative description of major study characteristics and findings. For outcomes of interest consistently reported across studies, random effects model meta-analyses will be used to pool estimates across those studies.[32] In process, the Freeman-Tukey double Arcsine and square-root transformation will be applied, respectively, to stabilise the variances of prevalence and incidence rates prior to meta-analysis, and estimates back-transformed for reporting. The degree of heterogeneity across studies will be assessed using the Cochrane Q statistic and inconsistency index (I^2) statistic and will be classified respectively as low: I^2 <25%; moderate: 25%–50%; high: I^2 >50%).[33] For studies with high heterogeneity subgroup and metaregression analyses will be performed to investigate the sources of heterogeneity. The following grouping variables will be used where appropriate: time of measurement of exposure (first, second or third trimester), study setting (rural vs urban, health-facility vs community based), geographical region (continental and or endemicity of iodine deficiency), high income versus low or middle-income status, and study quality. The Begg test and Egger funnel plot will be used to check for the publication bias.[34 35]

Sensitivity analysis

Sensitivity analysis will be carried out to check the effect of every study on pooled estimates by removing one study at a time and assessing the effect on pooled estimates and heterogeneity statistics. The Duval and Tweedie trim-and-fill will be used to adjust estimates for the effects of potential publication bias. Data analyses will use the 'meta' package of the statistical software R (V.3.3.3 [2017-03-06], The R Foundation for statistical computing, Vienna, Austria), and the 'meta' package.

Confidence in cumulative evidence

We will assess the strength of evidence provided by studies included in the review, using the Grading of Recommendations Assessment, Development and Evaluation approach. This assessment of the quality of evidence would include risk of bias, consistency and publication bias. Studies in which further research is unlikely to change effect estimates, or likely to have a considerable impact on effect estimates, or capable of changing the effect estimates, or those in which there is uncertainty in effect estimates, will be respectively described as 'high', 'moderate', 'low' or 'very low' qualities.

Reporting of this review

The proposed systematic review will be reported following the PRISMA guidelines.[36] We intend to publish a PRISMA checklist alongside the final report.

Potential amendments

We do not intend to make any amendments to the protocol, to avoid the possibility of outcome reporting bias. However, any amendments that do prove necessary will be documented and reported transparently.

CONCLUSION

Iodine deficiency whose degree is exacerbated by pregnancy has recently been associated with oxidative imbalance and endothelial dysfunction which are some of the pathophysiological mechanisms that precede the clinical manifestation of pre-eclampsia. Although iodine deficiency is the the most common cause of hypothyroidism, one of the established risk factors of pre-eclampsia, the association between iodine deficiency in pregnancy and pre-eclampsia is still uncertain. This review aims to decipher if iodine deficiency in pregnancy, which can be corrected through supplementation, increases the risk of pre-eclampsia in various settings worldwide.

Possible limitations of this study would include very few studies with small sample sizes and uncertain quality that may generate significant heterogeneity precluding further analysis.

Ethics and dissemination

The current study is based on published data, and hence does not require ethical approval. This review is part of the thesis that will be submitted for the award of a PhD in Medicine to the Faculty of Health Sciences of the University of Cape Town. The final report of this review in the form of a scientific paper will be published in a peer-reviewed journal. Findings will also be presented at conferences and submitted to relevant health and policy authorities. We also plan to update the review in the future to monitor any progressive changes on the subject.

Author affiliations
[1]Department of Medicine, University of Cape Town, Cape Town, Western Cape, South Africa
[2]Department of Obstetrics and Gynaecology, Walter Sisulu University Faculty of Health Sciences, Mthatha, Eastern Cape, South Africa
[3]Health Sciences Library, University of Cape Town Faculty of Health Sciences, Cape Town, Western Cape, South Africa
[4]Universite de Kinshasa Faculte de Medecine, Kinshasa, The Democratic Republic of the Congo
[5]LOMO Medical Centre, Kinshasa, The Democratic Republic of the Congo
[6]Non-Communicable Disease Research Unit, South African Medical Research Council, Tygerberg, South Africa
[7]Department of Medicine, University of Cape Town Faculty of Health Sciences, Cape Town, Western Cape, South Africa

Contributors CBB and APK conceived and designed the protocol. CBB was responsible for manuscript drafting. APK, NM and BL-M took part in critical revision for methodological and intellectual content. CBB is the guarantor of this review. All the authors read and approved the final version of the manuscript.

Funding This study is part of a research project supported by Discovery Foundation Rural Fellowship Grant number 038372. APK is employed by the South African Medical Research Council.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Dekker G, Hypertension. In: Steers PJ, Weiner CP, Gonlk B, Crowther CA, Robson SC, *et al*. eds. *In high risk pregnancy: management options*. 4th edn. St Louis: Elsevier Saunders, 2011:599–626.
2. English FA, Kenny LC, McCarthy FP. Risk factors and effective management of preeclampsia. *Integr Blood Press Control* 2015;8:7–12.
3. Armaly Z, Jadaon JE, Jabbour A, *et al*. Preeclampsia: Novel Mechanisms and Potential Therapeutic Approaches. *Front Physiol* 2018;9:973.
4. Cunningham FG, Leveno KJ, Bloom SL, *et al*. Hypertensive disorders. *Williams Obstetrics*. 24th edn. New York: McGraw-Hill Education, 2014:728–79.
5. Abalovich M, Gutierrez S, Alcaraz G, *et al*. Overt and subclinical hypothyroidism complicating pregnancy. *Thyroid* 2002;12:63–8.
6. Khanam M, Ilias M. Study of thyroid hormonal status in preeclamptic patients. *Med Today* 2013;25:63–6.
7. Wilson KL, Casey BM, McIntire DD, *et al*. Subclinical thyroid disease and the incidence of hypertension in pregnancy. *Obstet Gynecol* 2012;119(2 Pt 1):315–20.
8. Lazarus JH. Screening for thyroid dysfunction in pregnancy: is it worthwhile? *J Thyroid Res* 2011;2011:1–4.
9. Jameson LJ, Mandel SJ, Weetman AP. Ch 405- Disorders of the thyroid gland. In: Kasper DL, Hauser SL, Jameson JL, Fauci AS, Longo DL, Loscalzo J, *et al*. eds. *Principles of internal medicine*. 19th edn. New York: McGraw Hill Education, 2015:2283–308.
10. Gulaboglu M, Borekci B, Delibas I. Urine iodine levels in preeclamptic and normal pregnant women. *Biol Trace Elem Res* 2010;136:249–57.
11. Cuellar-Rufino S, Navarro-Meza M, García-Solís P, *et al*. Iodine levels are associated with oxidative stress and antioxidant status in pregnant women with hypertensive disease. *Nutr Hosp* 2017;34:661–6.
12. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. *J Nutr* 2012;142:744–50.
13. Zimmermann MB. Iodine deficiency in industrialized countries. *Clin Endocrinol* 2011;75:287–8.
14. De Groef B, Decallonne BR, Van der Geyten S, *et al*. Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. *Eur J Endocrinol* 2006;155:17–25.
15. Steinmaus C, Miller MD, Howd R. Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001-2002 national health and nutrition examination survey. *Environ Health Perspect* 2007;115:1333–8.
16. Vidal ZE, Rufino SC, Tlaxcalteco EH, *et al*. Oxidative stress increased in pregnant women with iodine deficiency. *Biol Trace Elem Res* 2014;157:211–7.
17. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005;308:1592–4.
18. Chen CY, Chen CP, Lin KH. Biological functions of thyroid hormone in placenta. *Int J Mol Sci* 2015;16:4161–79.
19. Miller DW. Extrathyroidal benefits of iodine. *Journal of American Physicians and Surgeons* 2006;11:106–10.
20. Aceves C, Anguiano B, Delgado G. The extrathyronine actions of iodine as antioxidant, apoptotic, and differentiation factor in various tissues. *Thyroid* 2013;23:938–46.
21. Zantour B, Alaya W, Marmouch H, *et al*. Hypothyroidism in pregnancy. Potluková E, ed. *Current topics in hypothyroidism with focus on development*. Rijeka, Croatia: In Tech, 2013:29–62.
22. Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. *Endocr Rev* 1997;18:404–33.
23. Lazarus HJ. Thyroid regulation and dysfunction in the pregnant patient. *Endotext [Online]*, 2016.
24. Smyth PP. Role of iodine in antioxidant defence in thyroid and breast disease. *Biofactors* 2003;19:121–30.
25. Lioudaki E, Mavroeidi NG, Mikhailidis DP, *et al*. Subclinical hypothyroidism and vascular risk: an update. *Hormones* 2013;12:495–506.
26. Redman CW, Sargent IL. Immunology of pre-eclampsia. *Am J Reprod Immunol* 2010;63:534–43.
27. Moher D, Shamseer L, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1.
28. WHO. UN Children's Fund, International council for the control of iodine deficiency disorders. *Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers*. 3rd edn. Geneva: World Health Organization, 2007.
29. American College of Obstetricians and GynecologistsTask Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* 2013;122:1122–31.
30. Wells GA, Shea B, O'Connell D, *et al*. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.evidencebasedpublichealth.de/download/Newcastle_Ottawa (cited 2018 Oct 06).
31. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med* 2012;22:276–82.
32. Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. *BMJ* 2011;342:d549.
33. Higgins JP, Thompson SG, Deeks JJ, *et al*. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–60.
34. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50:1088–101.
35. Egger M, Davey Smith G, Schneider M, *et al*. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629–34.
36. Moher D, Liberati A, Tetzlaff J, *et al*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;151:264–9.