University of Venda

IODINE STATUS OF PREGNANT WOMEN AND CHILDREN AGED 6 TO 12 YEARS FEEDING FROM THE SAME FOOD BASKET IN MOPANI DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

By

MABASA ERIC (11521103)
BSC NUTRITION (UNIVEN)

THE DISSERTATION IS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS OF THEDEGREE OF MSc OF PUBLIC NUTRITION IN THE DEPARTMENT OF NUTRITION IN THE SCHOOL OF HEALTH SCIENCES OF THE UNIVERSITY OF VENDA

SUPERVISORS:
PROFESSOR MBHENYANE XG (UNIVERSITY OF VENDA)
PROFESSOR JOOSTE PL (MEDICAL RESEARCH COUNCIL (MRC) AND INTERNATIONAL COUNCIL FOR CONTROL OF IODINE DEFICIENCY DISORDER (ICCIDD) GLOBAL NETWORK REGIONAL COORDINATOR FOR SOUTHERN AFRICA)

CO-SUPERVISORS:
MR. MABAPA NS (UNIVERSITY OF VENDA)
PROFESSOR AMUSA LO (UNIVERSITY OF VENDA)

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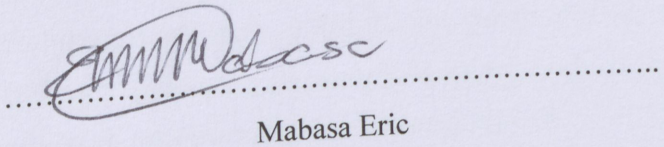
DECLARATION

I, Mabasa Eric hereby declare that this dissertation is for the Masters in Public Nutrition degree at the University of Venda, hereby submitted by me, has not been submitted previously for a degree at this or any other university, this is my own work in design and in execution. All reference material contained therein has been duly acknowledged.



Mabasa Eric

26/07/2014

Date

DEDICATION

This work is dedicated to GOD Almighty for giving me strength and wisdom to complete this project, to my parents, brothers, wife and my two children (Precious Eneto and Nyiko Muyimeri Mabasa) for unconditional love and support.

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ABSTRACT

Introduction: Iodine deficiency (ID) is a worldwide problem that leads to impaired cognitive development, clinical goiter and the syndrome of endemic cretinism. Pregnant women and school age children are the most vulnerable groups for ID. Sufficient iodine is required during pregnancy to ensure adequate maternal thyroid hormones production. ID in utero and early childhood damages the developing brain, leading to the loss of millions of intelligent quotient (IQ) points globally.

Objectives: The aim of the study was to assess iodine status of pregnant women and children aged 6 to 12 years feeding from the same food basket in Mopani district, Limpopo Province, South Africa.

Methods: The study was cross-sectional conducted in five municipalities of Mopani District in Limpopo Province. The total number of clinics selected was 41. A total of 565 pregnant women and 116 children aged 6-12 years were recruited. Urine iodine concentration (UIC) and drinking water iodine concentration were analyzed using the Sandell-Kolthoff reaction. The salt samples were analyzed by means of the iodometric titration method. Dried blood spots on filter paper were analyzed for whole blood thyrotropin /Thyroid Stimulating Hormones (TSH) with an immunoassay.

Results: The findings showed that 52.5% of household salt had iodine concentration level more than 15ppm. Most of household drinking water (41.3%) had iodine concentration level greater than 60µg/L. The median iodine concentration of drinking water in Mopani District was 46.2µg/L. Almost half of pregnant women (44.9%) had UIC level less than 150µg/L. The maternal overall median UIC level was 164µg/L indicating maternal iodine sufficiency. However, median UIC in the first and third trimesters was below 150µg/L, indicating iodine insufficiency. The TSH levels of pregnant women were measured per trimester and majority of study participants had normal TSH levels. Most children (64.3%) had UIC level greater than 300µg/L. The median UIC level of children was 386µg/L indicating excessive iodine status.

Conclusion: Iodine status of children in this study was excessively high. It was twice times higher than the iodine status of pregnant women. It is difficult to explain this significant difference in iodine status of these two groups since they were feeding from the same food basket. It can then be concluded that the median UIC of school aged children may not be an adequate surrogate for monitoring iodine nutrition in pregnant women as was previously assumed.

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DEFINITIONS OF OPERATIONAL TERMS

Goitrogens – Dietary substances that interfere with thyroid metabolism, which aggravate the effects of iodine deficiency.

Iodine – One of the trace elements essential to human and animal health present in uneven and mostly insufficient quantities in the environment around the globe. It is an essential substrate for the synthesis of thyroid hormones, thyroxine (T4) and triiodothyronine (T3).

Iodine deficiency – Lack of dietary iodine intake that results in impaired hormone synthesis leading to a series of functional and developmental abnormalities.

LIST OF ABBREVIATIONS

ATA	American Thyroid Association
ANC	Antenatal clinic
ADHD	Attention deficit hyperactivity disorder
CDC	Centers for Disease Control and Prevention
DC	District code
DIT	diiodotyrosine
EQUIP	Ensuring the Quality of Urinary Iodine Procedures
FAO	Food and Agricultural Organization of the United Nations
GSH	Glutathione
H_2O_2	Hydrogen peroxide
ICCIDD	International Council for Control of Iodine Deficiency disorders
ID	Iodine deficiency
IDD	Iodine Deficiency Disorder
IQ	intelligent quotient
KI	Potassium iodide
mg	milligram
ml	milliliter
MIT	monoiodotyrosine
MRC	Medical Research Council
MRC-NIRU	Medical Research Council-Nutritional Intervention Research Unit
MUIC	median urinary iodine concentration
n	Number
NFCS	National Food Consumption Survey
NFCS-FB	National Food Consumption Survey-Fortification Baseline
NRF	National Research Foundation
NSNP	National School Nutrition Programme
PBI	Protein bound iodine
PII	Plasma inorganic iodide
ppm	parts per million
RDA	Recommended dietary allowance
SA	South Africa
SPSS	Statistical package for social sciences
IOM	The Institute of Medicine

TSH	Thyroid-stimulating hormone
Tg	thyroglobin
TRH	thyrotropin releasing hormone
T3	triiodothyronine
T4	thyroxine
UI	Urinary iodine
UIC	Urinary iodine concentration
UNICEF	United Nations Children's Fund
UNIVEN	University of Venda
US	United States
USA	United States of America
USI	Universal salt iodization
WHO	World Health Organization
µg/day	microgram per day
µg/L	microgram per litre
%	Percentage

CHAPTER ONE

Introduction

1.1 Overview

Iodine deficiency (ID) is a worldwide problem that leads to impaired cognitive development, clinical goiter and the syndrome of endemic cretinism (Zimmermann *et al*., 2008, Pharoah and Connolly, 1990). It is estimated that 2 billion people throughout the world are living in iodine-deficient environments and are therefore at risk of iodine deficiency (Zimmermann, 2009). About 200 million people are suffering from goiter, while 20 million have preventable brain damage. In South Africa there are areas where the population exhibits Iodine Deficiency Disorders (IDDs), with increasing evidence that this problem is greater than previously thought (Jooste, 2001). Examples of IDDs include: impaired cognitive development (brain damage), hypothyroidism, endemic goiter, reproductive failure, child mortality, and cretinism. The various effects of IDDs result in human suffering, increased medical expenses, poor educational results and reduced work capacity. The sustained elimination of IDDs could therefore have tremendous developmental and economic positive results. In the human body, iodine is essential for the production of thyroid hormones required for normal mental and physical development. A lack of iodine in the diet results in IDDs, which may appear during the whole life cycle (Hallenren *et al*., 2009).

Iodine (as iodide) is widely but unevenly distributed in the earth's environment. In many regions, leaching from glaciations, flooding, and erosion have depleted surface soils of iodide, and most iodide is found in the oceans. The concentration of iodide in sea water is approximately 50µg/L. Iodide ions in seawater are oxidized to elemental iodine, which volatilises into the atmosphere and is returned to the soil by rain as part of an ecological cycle (Zimmermann, 2009). A lack of iodine in the environment in many geographical areas usually results in insufficient iodine in the food chain and consequently in IDDs. Pregnant women and their young children are the main targets of the effects of iodine deficiency (Delange, 2004). A study done by Cheryl *et al.* (2006) indicated that women need more iodine during pregnancy to maintain normal iodine metabolism, as well as meeting the requirement for the production of thyroxine (T4) and iodine transfer to the fetus. The study further indicated that in areas where iodine deficiency is severe, intelligent quotient (IQ) scores in children are decreased, psychomotor deficits are more common, hearing may be

impaired and there is a marked increase of attention deficit hyperactivity disorder (ADHD) (Delange, 2004).

1.2. Problem statement

South Africa, like most of the countries in the world, lacks iodine in the environment, in many geographical areas. Limpopo is one of the provinces where iodine is deficient, particularly in the Vhembe region of the province (Zimmermann *et al*., 2007, Mabapa, 2005). This iodine deficiency prevails despite the mandatory salt iodization introduced in South Africa in 1995 for elimination of ID. Pregnant women and school age children are the most vulnerable groups for developing ID (Delange, 2004). Sufficient iodine is required during pregnancy to ensure adequate maternal production of thyroid hormones. The fetus is entirely dependent on T4 transferred from the mother and any compromise in the placental transfer prejudices normal central nervous system organisation and maturation (Eastman, 2005). Iodine deficiency in utero and early childhood damages the developing brain, leading to the loss of millions of IQ points globally, making it one of the most important preventable causes of brain damage worldwide (Zimmermann and Andersson, 2013).

A study done by Zimmermann *et al*. (2007) among primary school children in Limpopo province of South Africa found that median urinary iodine concentration (UIC) of children was 74µg/L, which indicates mild ID. Further 31% of the children had UI less than 50µg/L, and 27% were goitrous. The iodine status of pregnant women has never been studied in South Africa. Despite the mandatory iodization of table salt in South Africa and the remarkable progress achieved in the elimination of ID in the country, pockets of ID still remain in Limpopo (Jooste and Zimmermann, 2008). Therefore, a study focusing on the current iodine status of pregnant women and primary school children in Limpopo province was required to establish the iodine status of these vulnerable groups.

1.3. Aim

The aim of the study was to assess iodine status of pregnant women and children aged 6 to 12 years from the same households and feeding from the same food basket in Mopani district of the Limpopo province in South Africa.

1.4. Objectives

- To determine urinary iodine concentration (UIC) of pregnant women in Mopani District of Limpopo Province, South Africa.
- To determine the concentration of Thyroid Stimulating Hormone (TSH) of these pregnant women.
- To determine UIC of children aged 6 to 12 years living in the same households as that of pregnant women.
- To determine iodine concentrations in the table salt in these households.
- To determine iodine content of drinking water in these households.
- To determine dietary factors influencing iodine status of pregnant women.
- To establish the correlation between process factors (coverage of adequately iodized salt, iodine content of drinking water) and impact factors (UIC and TSH of pregnant women, as well as UIC of children).

1.5. Structure of the dissertation

- Chapter 1 introduces the background information on iodine nutrition, particularly for pregnant women and children. It also presents the problem statement, aim and objectives of the study.
- Chapter 2 discusses the background literature on iodine nutrition worldwide and also the situation in South Africa.
- Chapter 3 describes the research methodology.
- Chapter 4 presents the results of the study which include demographic data, process and impact factors of iodine nutrition.
- Chapter 5 deals with the discussion of the results.
- Chapter 6 gives the conclusion and recommendations.

CHAPTER TWO

Literature review

2.1. Overview

Iodine is an essential element for the production of thyroid hormones, triiodothyronine (T3) and T4. These hormones are essential for normal physical and mental development (Cheryl *et al.*, 2006). According to Delange (2004), pregnant women require more iodine for fetal brain development than non-pregnant women. Dietary iodine requirements of pregnant women have been the subject of considerable recent attention. Iodine deficiency during pregnancy and infancy may impair growth and neurodevelopment of the offspring and increase infant mortality. Deficiency during childhood reduces somatic growth, cognitive and motor function (Zimmermann, 2012). There is consensus on the need to take action to eliminate iodine deficiency in those countries where endemic goiter or even cretinism is prevalent. In regions where dietary iodine intake is borderline (50-100µg/day), it may be necessary to focus on increasing dietary supply during pregnancy (Smyth, 1997).

2.2. Function of iodine in the body

The function of iodine in the human body is that it is the essential substrate for the synthesis of the thyroid hormones T3 and T4 (Guthrine and Picciano, 1995). The thyroid hormones L-thyroxine and 3,5,3′-triiodo-L-thyronine are among the fundamental factors that contribute to normal development of the central nervous system through genomic and nongenomic actions in neurons and glial cells. Thyroid hormones are iodinated amino acids, released in the thyroid gland by TSH induced proteolysis of the iodinated thyroglobulin. Therefore, iodine is an essential component of thyroid hormones. The amount of iodine intake per day, which varies according to the age and physiological state of individuals, is therefore crucial for the thyroid gland to produce adequate amounts of thyroid hormones. In particular, iodine intake is fundamental during gestation and lactation, because in these developmental periods the mother is the only source of TSH, T4 and iodine for the fetus and iodine for the breastfed neonate (Berbel *et al.*, 2007).

Once taken up from the blood system by the thyroid cells, iodine is released in colloid of the thyroid gland where it is oxidized by hydrogen peroxide derived from the thyroid peroxidase system. Iodine is then incorporated into tyrosine of thyroglobin (Tg) to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). When DIT molecules are coupled with

another DIT, tetraiodotyrosine or T4 is formed, and when MIT and DIT are coupled, T3 is formed. The Tg is then taken up by the process of proteolysis. The secretion of T4 and T3 from the thyroid gland into the blood stream is under the influence of TSH, which is stimulated by thyrotropin releasing hormone (TRH) from the hypothalamus (Guthrine and Pacciano, 1995).

The fetus depends on maternal thyroid hormones for its development. This reliance is mainly on maternal T4 as only very small amounts of maternal T3 reach fetal tissues and almost all T3 found in the developing cerebral cortex is generated through local deiodination of T4, ultimately derived from the maternal circulating T4. Thus, during these earliest periods of human life, it is essential for the mother to produce sufficient amounts of thyroid hormones for herself and her progeny. To achieve this, pregnant women need to double the recommended normal daily intake of iodine to at least 250µg/day. Furthermore, to meet neonatal requirements, iodine intake should remain increased during lactation if the infant is solely breastfed. Very frequently, these increased needs are not met, and thus maternal hypothyroxinemia during pregnancy is receiving increased attention as a cause of neurodevelopmental disorders. In addition to maternal thyroid hormones, the fetus also depends on the mother for its iodine supply to the fetal thyroid, as does the neonatal thyroid during lactation (Berbel *et al.,* 2007).

2.3. Dietary requirements for iodine in the body

The Food and Nutrition Board of the United States (US) National Academy of Science has deemed that a daily iodine intake of 1µg/kg of body weight is adequate for most adults. Growing children, especially adolescent females, may require more than this amount (Guthrine and Pacciano, 1995). Mahan and Stump (2012) indicated that an iodine intake of 150µg/day has been suggested as sufficient for all adult and adolescents. The recommended dietary allowance (RDA) for pregnant and lactating women increased to 250µg/day. The RDA for preschool and school children is 90µg/day and 120µg/day respectively, 150µg/day for adults, and pregnant and lactating women should get 250µg/day (World Health Organization (WHO)/United Nations Children Emergency Fund (UNICEF)/International Council for Control of Iodine Deficiency Disorders (ICCIDD), 2007).

Iodine requirements increase by approximately 50% starting in early pregnancy. The requirement for iodine is increased during pregnancy because of at least three factors: 1) there

is an increased requirement for T4 in order to maintain maternal euthyroidism and transfer thyroid hormones to the fetus early in the first trimester, before the fetal thyroid is functioning, 2) there is a transfer of T4 and iodide from the mother to the fetus and 3) there is supposed to be an increased loss of iodide through the kidney due to an increase in the renal clearance of iodide. Because of these three factors, the recommended dietary intake of iodine during pregnancy is higher than the value of 150µg recommended for non pregnant adults and adolescents (Delange, 2004, Zimmermann, 2012). Dietary requirements for iodine are summarised in table 2.1.

Table 2.1 Dietary requirements for iodine in the body (Recommended minimum daily iodine intake)

Group	Daily iodine requirements
Preschool children (0 to 59 months)	90µg
School Children (6 to 12 years)	120µg
Adolescents (above 12 years) and adults	150µg
Pregnant and lactation women	250µg

As recommended by, WHO, UNICEF and ICCIDD (2007)

2.4. Indicators of iodine status

Four methods are generally recommended for assessment of iodine nutrition in populations: median urinary iodine concentration (UIC), serum TSH, serum Tg and goiter rate. These indicators are complementary, in that UI is a sensitive indicator of recent iodine intake (days) and Tg shows an intermediate response (weeks to months), whereas changes in the goiter rate reflect long-term iodine nutrition (months to years) (Zimmermann, 2009, Zimmermann *et al.*, 2013, WHO/UNICEF/ICCIDD, 2007).

2.4.1. Urinary Iodine

Considering that most (above 90 %) of the iodine absorbed in the body eventually appears in the urine, urinary iodine excretion is a good marker of a very recent dietary iodine intake. Therefore, median urinary iodine in the general population varying from 100 to 199µg/L is considered an indicator of an adequate iodine intake and an optimal status of iodine nutrition. Currently, it is internationally being considered to expand this category to a median of 100 to 299µg/L as an indicator of adequate iodine intake. WHO/UNICEF/ICCIDD (2007)

assessment criteria show that during pregnancy, a median UI of less than150µg/L indicates iodine deficiency, 150 to 249µg/L reflects iodine sufficiency, 250 to 499µg/L implies that the intake is above requirements and median UI intakes of 500µg/L or higher are excessive and there is no added health benefit expected. Median UI of 100 to 199µg/L in school children is considered iodine sufficient (WHO/UNICEF/ICCIDD, 2007). It should be noted that the diagnosis of ID, iodine sufficiency and iodine excess is made not in individuals but in groups of children or pregnant women. Furthermore, this diagnosis is made using median UIC of the group and Table 2.2 illustrates the diagnosis of iodine status of a particular group of individuals.

Table 2.2 WHO/UNICEF/ICCIDD (2007) criteria for assessing iodine nutrition based on median urinary iodine concentration of school age children and pregnant women

Iodine Status	**Median UIC in school children ≥ 6 years old**
Severe Iodine Deficiency	<20µg/L
Moderate Iodine Deficiency	20-49µg/L
Mild Iodine Deficiency	50-99µg/L
Iodine Sufficient	100-199µg/L
Above requirements	200-299µg/L
Excessive	≥300µg/L
Iodine Status (median in UIC)	**Pregnant and lactating women**
Iodine insufficient	<150µg/L
Iodine sufficient	150-249µg/L
Above requirements	250-499µg/L
Excessive	≥500µg/L

2.4.2. Serum TSH

The pituitary gland secretes TSH in the response to circulating level of T4. Serum TSH rises when serum T4 concentrations are low and falls when they are high. Iodine deficiency lowers circulating T4 and raises the serum TSH, so iodine deficient populations generally have higher serum TSH concentration than iodine sufficient population (WHO/UNICEF/ICCIDD,

2007). However, TSH and thyroid hormones concentrations are generally poor indicators of iodine status in iodine sufficient populations because of the reserve of iodine available in the thyroid gland. In iodine deficient populations, serum TSH rise and T3 remains unchanged whereas serum T4 usually falls. These changes often fall within the normal range and the overlap with iodine sufficient populations is large enough to make thyroid hormone and TSH concentrations an insensitive measure of iodine deficiency in school age children and adults (Zimmermann *et al.*, 2008, WHO/UNICEF/ICCIDD, 2007).

In contrast, TSH and T4 concentrations in neonates are valuable indicators for iodine deficiency. Compared with adults, the thyroids in newborn babies contain less iodine but has higher rate of iodine turnover. When iodine supply is low, maintaining high iodine turnover needs an enhanced TSH stimulation and thus TSH concentrations increase in iodine deficient infants for the first week of life. This condition is termed transient newborn hyperthyrotropinaemia and newborn TSH obtained 3 to 4 days after birth is a sensitive indicator of iodine deficiency (Zimmermann *et al.*, 2008, WHO/UNICEF/ICCIDD, 2007).

According to Green *et al.* (2011), there is strong evidence that the reference range for TSH is lower throughout pregnancy i.e. both the lower normal limit and upper normal limit of serum TSH are decreased by about 0.1 – 0.2 mIU/L and 1.0 mIU/L, respectively compared with the customary TSH reference interval of 0.4 – 4.0 mIU/L in non pregnant women. The trimester specific reference range for TSH as defined in the population with optimal iodine intake should be applied. The kit specific normal ranges for adults for the Perkin Elmer Kit are, TSH: 0.1 – 3.7 mIU/L and T4: 65 – 165 mIU/L. See tables 2.3 and 2.4 for the summary of TSH and T4 reference ranges.

Table 2.3 American Thyroid Association's (ATA) specific reference range for TSH:

Stage of pregnancy	Reference range
First trimester	0.1 – 2.5 mIU/L
Second trimester	0.2 – 3.0 mIU/L
Third trimester	0.3 – 3.0 mIU/L

(Green (2011) ATA reference range)

Table 2.4 Thyroid hormones and TSH levels in various stages

Group	Serum T4(µg/dL) range	Serum T3(ng/dL) range	Serum TSH (mIU/L) range
Euthyroid individuals	4.5 – 11.5	60 – 180	0.6 – 4.1
Infants(<2 wk)	8.0 – 15.0	-	0.6 – 4.1
Children(prepubertal)	6.5 – 11.5	80 – 220	0.6 – 4.1
Hyperthyroid individuals	>11.5	-	0.0 – 0.5
Hypothyroid individuals	< 4.5	-	>4.1

(Copstead, 1995and Ujowundu *et al.*, 2010)

2.5.3. Serum Tg

Tg is synthesised only in the thyroid and is the most abundant intrathyroidal protein. Transcytosis of Tg-containing endosomes across the thyrocyte results in the release of small amounts of Tg into the circulation. Serum Tg is elevated in iodine-deficient areas due to TSH hyperstimulation and thyroid hyperplasia. In intervention studies in adults, serum Tg is a more sensitive indicator than TSH or T4 in measuring response to iodized oil, potassium iodide and iodized salt (Zimmermann *et al.*, 2013).

WHO/UNICEF/ICCIDD now recommends Tg for the monitoring of iodine status in school-aged children. The study conducted by Zimmerman *et al.* (2013) found that, over a range of iodine intake from severely deficient to excessive, Tg concentrations show a clear U-shaped curve. Compared to children with UIC in the adequate and more than adequate range (100–299µg/L), there was a higher prevalence of elevated Tg values in children with iodine excess (300µg/L) and iodine deficiency (100µg/L), and mean Tg values were significantly higher in children with UIC indicating moderate to severe deficiency and iodine excess. It can then be concluded that Tg could be used as a sensitive indicator of iodine status in children not only of low iodine intake but also of excessive intake.

2.5.4. Goiter

The term goiter refers to a thyroid that is enlarged. The size of thyroid gland changes inversely in response to alteration in iodine intake, with time interval that varies from a few

months to several years depending on several factors. These include the severity and duration of iodine deficiency, the type and effectiveness of iodine supplementation, age, sex and goitrogenic factors (WHO/UNICEF/ICCIDD, 2007).

The traditional method for determining thyroid size is inspection and palpation. Ultrasonography on the other hand provides a more precise and objective method. When palpation is used, goiter is present when each thyroid lobe has a volume greater than a terminal phalanx of the thumb of the patient being examined. Grade 0 is a normal sized thyroid which is neither palpabale nor visible. Grade 1 goiter is palpable but not visible when the neck is in a normal position and grade 2 goiter is clearly visible when the neck is in a normal position (Zimmermann *et al.*, 2008, WHO/UNICEF/ICCIDD, 2007).

In areas with mild to moderate ID, the sensitivity and specificity of palpation is poor and measurements of thyroid size using ultrasound is preferable. Ultrasonography is a safe, specialised technique that can be quickly done (2-3 minutes per subject). This becomes more significant when the prevalence of visible goiter is small, and in monitoring iodine control programme where thyroid volumes are expected to decrease over time (WHO/UNICEF/ICCIDD, 2007).

2.5. Sources of iodine

Iodine is found in the soil and makes its way to humans and livestock through food grown in these areas. Unfortunately, the iodine is not distributed evenly across the Earth's crust. According to WHO/UNICEF/ICCIDD (2007) at least 29% of the world's population lives in areas of iodine deficient soils. These include mountainous regions, areas prone to flooding and those subject to soil erosion and deforestation. The oceans contain adequate iodine and those eating seafood are less likely to suffer from IDD. However, in some coastal communities this does not necessarily hold true (Jooste, 2001).

In countries in which salt is iodized, it is generally the main dietary source of iodine. In settings in which foods are mainly prepared at home, household iodized salt is the major iodine source. In contrast, in industrialised countries, salt used in processed foods contributes approximately 60 to 80% of total salt intake (Zimmerman and Anderson, 2012). Dietary iodide from sources such as iodized salt or sea foods is rapidly and nearly completely absorbed (>90%) in the stomach and duodenum (Zimmermann, 2011).

Mahan and Stump (2012) indicated that iodine exists in variable amounts in the food and drinking water. Sea foods such as clams, lobsters, oysters, sardines and other salt water fish is the richest natural source of iodine. Salt water fish contain 300 to 3000µg/kg, fresh water fish contain 20 to 40 µg, but they are still good sources of iodine. The iodine content of cow milk and eggs is determined by iodine availability in the diet of the animals, iodine content of vegetables varies according to iodine content of soil in which they are grown but the iodine content of vegetables is generally low and therefore not a significant source of dietary iodine. Iodine also enters the food chain through iodophors, which are used as disinfectants in dairy processing, coloring agent and dough conditioners. The use of iodized salt should still be advocated to prevent IDD. The best way to obtain an adequate intake of iodine is to use iodized salt in food preparation.

2.6. Iodine deficiency

Iodine is an essential micronutrient for the synthesis of the thyroid hormones. Lack of dietary iodine intake therefore results in impaired hormone synthesis leading to a series of functional and developmental abnormalities collectively referred to as the IDD (Zimmermann, 2012 and Jooste *et al.,* 2005). Iodine deficiency remains the most frequent cause worldwide, after starvation, of preventable mental retardation in children. It causes maternal hypothyroxinemia, which affects pregnant women even in apparently iodine-sufficient areas, and often goes unnoticed because T4 levels remain within the normal range, and TSH is not increased. Even a mild hypothyroxinemia during pregnancy increases the risk of neurodevelopmental abnormalities, and experimental data clearly demonstrate that it damages the cortical cytoarchitecture of the fetal brain (Berbel *et al.,* 2007).

According to Zimmermann (2012), ID during pregnancy impairs the neurological development of fetus. In the area of severe chronic ID, maternal and fetal hypothyroxinemia can occur from early gestation onwards. The devastating effects of ID on the mental and neurological impairment leading to lower IQ, poor school performance and reduced work capacity is of great public importance (Jooste *et al.,* 2005).

In the case of mild deficiency (i.e. median urinary iodine in school children of between 50 and 100µg/L) to moderate (i.e. median urinary iodine in school children of between 20 and 49µg/L), the thyroid gland responds rapidly to iodine deficiency through autoregulatory mechanisms (independent of changes in circulating TSH) by decreasing the synthesis and

secretion of T4 in favor of T3 which is the active hormone that binds to specific nuclear receptors (Berbel *et al.,* 2007).

An estimated 2 billion people worldwide are at risk of ID, with those in South Asia and Sub-Saharan Africa particularly affected (Zimmermann *et al.,* 2008). These individuals may have a moderate iodine deficiency, even when obvious goiter is not evident. Iodized salt is the most effective public health intervention to prevent ID. However, in areas where iodized salt is not implemented or not effective, pregnant women should be supplemented with iodine in the form of potassium iodide tablets or iodine-containing prenatal multivitamin preparations. Another option is oral iodized oil; it is safe and its single dose is easier to administer (Zimmermann and Delange, 2004).

2.7. Possible causes of iodine deficiency

The main cause of ID in soil is glaciations, floods or high rain fall and therefore the erosion of iodine from the soil over thousands of years. Mountainous region have some of the highest prevalence of ID. In areas of endemic ID, water and foods have low iodine content. Some staple foods that are consumed in developing nations contain cyanogenic glucosides that can liberate the cyanide. Cyanide is converted to thiocyanate in the body. This is a goitrogen as it blocks the uptake of iodine by the thyroid gland (Maberly *et al.,* 1980).

Selenium is an essential component of enzyme type 1 deiodinase, which catalyses the conversion of T4 to T3. Combined iodine and selenium deficiency are thought to be the cause of myxoedematous form of cretinism. Selenium deficiency influences thyroid function and in turn brain development in the fetus. Selenium deficiency results in glutathione (GSH) peroxidase deficit and consequently in the accumulation of hydrogen peroxides (H_2O_2). Excess H_2O_2 could induce thyroid cell destruction and finally thyroid fibrosis, resulting in thyroid failure (Kohrle, 1999).

On the other hand, deficiency in iodothyronine-5-deiodinase in pregnant women induced by selenium deficiency causes decreased catabolism of T4 to T3 and thus increases the availability of maternal TSH to the foetus and its brain. Iron deficiency impairs thyroid hormones metabolism because the two steps in thyroid hormone synthesis are catalyzed by thyroperoxidases which are iron requiring enzymes (Guthrine and Pacciano, 1995).

2.8. Consequences of iodine deficiency during pregnancy

Iodine deficiency before and during pregnancy can result in cretinism in the infants. Thyroid dysfunction in pregnant women can influence the outcome for mother and fetus at all stages of pregnancy as well as interfere with ovulation and fertility. Maternal hypothyroidism during early pregnancy is associated with impaired neuropsychological development of children and other adverse outcomes, including premature birth, preeclampsia, breech delivery, and increased fetal mortality. These complications are seen in overt hypothyroidism, which occurs in about 0.2% of pregnancies, as well as subclinical hypothyroidism, found in about 2.3% of pregnancies. Maternal overt hyperthyroidism is associated with fetal loss, fetal growth restriction, whereas subclinical hyperthyroidism has not been specifically associated with adverse pregnancy outcomes. As the fetus is entirely dependent on maternal thyroid hormones for its development until about 13 weeks of gestation, it is important to ensure adequate T4 supply in pregnant women during the first trimester (Hallengren *et al.*, 2009, Zimmermann, 2003).

There is also evidence that maternal iodine deficiencies that are not severe enough to cause cretinism can cause impaired motor and cognitive function in children. Intakes of iodine that is sufficient to prevent goiter under normal circumstances frequently prove inadequate during pregnancy, leading to goiter in the mother especially if she is also adolescent due to the increased requirement for iodine during pregnancy (Guthrine and Pacciano, 1995).

2.9. Strategies for prevention and management of iodine deficiency during pregnancy

2.9.1. Salt iodization

Salt iodization remains the most affordable, safe and cost effective method of prevention and elimination of IDD. The South African Medical Research Council (MRC) believes that a multi-pronged approach needs to be adopted to strengthen the positive trend in order to achieve a situation where at least 90% of all households use adequately iodized salt containing at least 15ppm of iodine (Jooste, 2001).The average cost of prevention of IDD through Universal salt iodization (USI) is small. It is at least 10 times less expensive than use of pharmaceuticals and food supplements that are currently aggressively advertised and promoted (Gerasimov, 2008).

WHO (2007) estimates that less than one fifth of households in the developing world were using iodized salt at the time of the World Summit for Children in 1990. Some experts

believe universal salt iodization may be the most successful public health intervention of the past two decades. The proportion of households consuming adequately iodized salt has increased to some degree in every region of the world, yet large geographical differences in levels of consumption remain. Two regions are close to achieving the goal: Latin America and the Caribbean, with 85 per cent of households consuming adequately iodized salt, and East Asia and the Pacific, with 84 percent. In China, the household coverage of adequately iodized salt exceeded 95% and was less than 80% in only 33 countries of China's 2831 countries (Wu *et al.*, 2012).

Since the 2002 United Nations Special Session on Children, many countries have reported continued progress towards the goal of eliminating iodine deficiency through universal salt iodization. Others face severe challenges. In 2007, WHO identified 16 countries in need of special efforts and extra support. If these countries achieve universal salt iodization, about 85 per cent of households worldwide will be consuming adequately iodized salt. Progress goes beyond numbers. Another mark of achievement is programme maturation, which has been reflected in wide spread agreement on the techniques for solving the problem, government responsibility for financing, improved political and regulatory environments, strengthened monitoring systems, stronger partnerships, and realisation of the key role of advocacy and communication (Jooste *et al.*, 2001).

The efforts towards universal salt iodization have resulted in three guiding principles that are crucial to sustained success. 1) Secure political commitment. Robust continuous government commitment and industry motivation are essential. This commitment needs to be maintained through regular advocacy, form partnerships and coalitions. 2) Partnerships between governments and donors, between governments and salt producers, and among all those supporting ID elimination efforts need to be strengthened at all levels. Ensure availability of adequately iodized salt. 3) The salt industry must recognise iodization as a fundamental responsibility; governments must work with salt producers to improve their capacity; and producers must maintain and improve this capacity. This will require collaboration between governments, manufacturers and traders (ICCIDD, 2008).

South Africa introduced compulsory iodization of table salt at the end of 1995 to comply with one of the nutrition goals of the 1990 World Summit for Children that aimed to virtually eradicate IDD by the year 2000. At the same time, the level of iodization in salt was

increased to 40- 60ppm. In the following years the success of eliminating IDD in South Africa through compulsory iodization of salt at an elevated iodine concentration was evaluated in a series of MRC studies and in a national IDD survey commissioned by the Department of Health. Compulsory iodization of table salt, as a public health intervention to eliminate iodine deficiency, resulted in dramatic improvements in the short term, both for process (or intermediate) and outcome (or impact) indicators of iodine deficiency and endemic goiter. Improvements in the process indicators were determined. Within one year the iodine content of table salt available in shops in three of the nine provinces more than doubled, from an average of 14ppm to 33ppm. This average further increased to 42ppm over the next two years. However, 19% of the salt packages on retailers' shelves still had an iodine content of less than 20ppm (Jooste *et al.*, 2001).

In a national study on the iodine content of household salt, the Medical Research Council's Nutritional Intervention Research Unit (MRC-NIRU) found that the coverage of iodized salt also improved remarkably, and the average and median iodine content of household salt appeared sufficient to eliminate IDD. The coverage of iodized salt improved from a situation before compulsory iodization where only 30% of table salt was iodized and unequal access to iodized salt existed, to a situation where 62% of households in the country were using adequately iodized salt containing at least 15ppm in 1998 and 77% in 2005 (Jooste and Zimmermann, 2008). Unfortunately, vulnerable groups in the population are still exposed to under- or non-iodized salt. These groups include people of the three northern provinces of the country, rural people, households using predominantly poorly iodized coarse salt, and low socio-economic households (Jooste *et al.*, 2001).

A significant percentage, up to 20% of households in some of the provinces, uses non-iodized agricultural salt in the preparation of their food. Therefore, the significant progress achieved in improving the coverage of adequately iodized salt is accompanied by factors and practices weakening the national iodization programme. Outcome indicators reflect the impact of the national salt iodization programme on the iodine and goiter status of the population.

Due to their vulnerability and accessibility, primary school children had been used worldwide as a proxy group to assess IDD in the community. However, because it was realised that the iodine status of children and pregnant women may differ considerably, the current approach is to study the iodine status of both these groups. Evidence of the success of the salt

iodization programme is given by an improvement in indicators of iodine deficiency status across time. The iodine status of primary school children of four communities in the Langkloof area in the southern Cape was evaluated one year after the introduction of mandatory salt iodization at a higher level than before. Baseline results showed median urinary iodine concentrations ranging from 5 to 65µg/L (overall 22µg/L), and goiter prevalence from 14% to 30% (overall goiter rate 25.6%). With programme implementation, the median urinary iodine concentration in the four communities increased dramatically within one year (ICCIDD, 2008).

In 1998, the South African Institute for Medical Research conducted a national IDD survey commissioned by the Department of Health. The results showed an adequate iodine status in most areas. This represents a major improvement in iodine status compared to the iodine deficiency and endemic goiter observed in several isolated studies in the early 1990s (Jooste *et al.*, 2001).

To strengthen this trend, a multi-pronged approach needs to be adopted to eliminate the barriers preventing the country from achieving coverage of 90% of households using adequately iodized salt of at least 15ppm. Important policy approaches should be included in which the salt suppliers are seen as the primary role-players in implementing the salt regulation. It is in their hands to increase the accuracy of salt iodization and to reduce the variation observed in iodine concentration. To assist the producers in this role, effective liaison among the salt producers, the health authorities and scientists should be strengthened to enhance the mutual flow of information in a concerted effort to achieve the international goal of 90% household coverage of adequately iodized salt (Jooste, 2001).

With the introduction of mandatory iodization in South Africa, an important public health responsibility was placed on the shoulders of the salt producers. To meet the demands of this responsibility, a thorough understanding of the causes, consequences, prevention and control of IDD is required. Therefore, increasing the knowledge and awareness of producers regarding the prevention and control of IDD via the correct iodization of salt may further strengthen their commitment towards the production of salt iodized according to the legal requirement. It is a standard recommendation that all countries that have implemented a national iodization programme should also have a functional monitoring system in place. Regular monitoring of the iodine concentration at the production site, and at the retail and

household levels, should be standard practice. Monitoring systems should include both process (e.g. the iodine concentration of salt, coverage of adequately iodized salt, etc.) and outcome indicators (e.g. urinary iodine, goiter rate) of IDD (Witten *et al.*, 2001).

One of the key issues that require attention is the vulnerability of low socio-economic groups to under- or non-iodized salt. A particular focus needs be developed to ensure a sustainable supply of adequately iodized salt to the poorer sector of the population. It is of great importance that the salt produced for this segment of the market is adequately iodized, particularly in view of the general susceptibility of low socio-economic groups to iodine deficiency. Consumption of non-iodized agricultural salt in households occurred predominantly among people in the low socio-economic groups in the three northern provinces, presumably because it is a cheap source of salt to those who have access to it. Unfortunately, mandatory iodization does not apply to agricultural salt used for animal nutrition and other agricultural purposes in South Africa. Therefore, a practical way to counteract the consequence of using non-iodized agricultural salt in food preparation, which deprives vulnerable people from consuming iodine-fortified salt, would be to iodize agricultural salt. This would also benefit animal production in iodine-deficient areas (Jooste *et al.*, 2001).

The success achieved in the national iodization programme supports and strengthens the continuation of the fight against IDD in South Africa. The challenge in the new decade for producers and health officials was to eliminate factors precluding coverage of 90% adequately iodized salt in the country, and to sustain the successes achieved until now (Witten *et al.*, 2001).

2.9.2. Iodine in processed foods

Household salt is not the only vehicle for iodine intervention (Jooste and Zimmermann, 2008). Worldwide, the use of iodized salt in the production of processed foods is considered as the second most important route of supplying iodine to consumers. Iodine is added to salt for the bread baking industry, animal foods, processed meat, fish sauce, and drinking or irrigation water. Iodine in milk is derived from iodophors i.e. iodine containing detergents used in dairy industry (Zimmerman and Anderson, 2012, Jooste and Zimmermann, 2008).

It was not mandatory to use iodized salt for the production of processed foods in South Africa, until 2007 and most producers used non iodized salt in their products to prevent unwanted effects of iodine on the properties of their products, for health reasons and financial consideration (Jooste and Zimmermann, 2008). The South African salt regulation was revised in 2007 to legislate the use of iodized salt in processed foods. This specification in the regulation is rather vague and probably not applied widely in food industries.

If the food industries were to use non iodized salt and only household salts were to be iodized in industrialised countries, where average daily salt consumption in adults is approximately 10g/day but only 1 to 2 g/day is from household salt, then total iodine intake from iodized salt would only be about 40µg/day, which is far below the daily requirements for all age groups. The use of iodized salt by the food industry can contribute to iodine sufficiency, in Denmark and the Netherlands for example, most salt used by the baking industry is iodized and is the major contributor to iodine sufficiency (Zimmerman and Anderson, 2012).

2.9.3. Drinking water iodization

In 1993, Fisch *et al.* proposed a new approach for combating iodine deficiency. This pilot study, based on the iodization of water, was conducted in a test village in Mali. The accompanying editorial by Hetzel welcomed this initiative and wished for trials on a larger scale. These villagers were severely affected by iodine deficiency, as demonstrated by a median urinary iodine concentration of 21µg/L in a representative sample of the population (n = 319).

After 12 months, a follow up evaluation was conducted, which included measurement of urinary iodine concentration and determination of the prevalence of goiter. The study found the median urinary iodine concentration to have increased significantly ($P<0.001$) to 174µg/L (n=278); only 0.4% still had a very low urinary iodine concentration. Concomitantly, the overall prevalence of goiter estimated in 2645 unselected inhabitants in the prefecture dropped from 60.9% to 44.4% while the prevalence of visible goiter declined from 10.7% to 2.5%. Because of the educational programme accompanying this trial, most of the participants were enthusiastic (Foo *et al.*, 1998).

Bourdoux (1995) demonstrated, in a large number of inhabitants in the Central African Republic, that the iodization of water is quite feasible. The present technique can be applied

immediately wherever populations are affected by iodine deficiency, providing that the water supplies come from wells or other controlled and contained sources. Moreover, the system can be adjusted to the number of persons and/or the severity of iodine deficiency. This makes it attractive in countries where only a limited number of regions are affected by iodine deficiency because it enables health practitioners to avoid the unnecessary and unjustified treatment of the whole population.

Since 1995, water iodization has been the principal intervention for control of iodine deficiency in longhouse communities in the interior of Sarawak, Malaysia. A common concern about water iodization is its high cost. For instance, in rural Sarawak, where populations live in longhouses, iodized water can be delivered at very low cost via a special drinking water pipeline separate from that used for washing and bathing purposes. The same approach could be used for the iodization of water in schools. In remote societies that consume little food from outside, use salt only in cooking, and share a common water source, iodization of the community water supply may be the only strategy that will effectively rid these societies of the scourge of iodine deficiency. Once implemented, water iodization is almost invariably associated with community-wide iodine repletion (Foo *et al.*, 1998).

2.9.4. Iodine supplementation in pregnancy

In some countries and areas with insufficient access to iodized salt for vulnerable groups of population, additional temporary strategies need to be considered to ensure optimal iodine nutrition for these groups while strengthening the salt iodization programme to reach universal coverage. The most vulnerable groups, pregnant and lactating women should be considered for supplementation with iodine using iodized oil capsules until salt iodization programme is scaled up (WHO/UNICEF/ICCIDD, 2007).

Urgent action is needed because there is evidence of significant and increasing iodine deficiency in pregnant and lactating women in the world. Similar recommendations have recently been made for European women (Zimmermann and Delange, 2004). The only exception to this recommendation for iodine supplementation is women with pre-existing thyroid disease who should be individually managed to ensure normal thyroid function during pregnancy. Urinary iodine excretion is an accurate indicator of dietary iodine intake as more than 90 per cent of ingested iodine is excreted in the urine (Berbel *et al.*, 2007).

The WHO working party has strongly recommended iodine supplementation for all pregnant and lactating women where USI is not established and where median urinary iodine excretion levels in pregnancy are less than 150µg/L. There are no concerns about the safety of iodine supplements at these dosages but the WHO committee does not recommend an intake above 500µg/day as there are no demonstrable benefits to mother and child above 250µg/day. There is little data on safety at intakes of more than 1000µg/day. It has been concluded that pregnant and lactating women should be taking iodine supplements in doses of between 100 and 200µg/day (Eastman, 2005).

WHO/UNICEF/ICCIDD recommends iodine supplementation in pregnancy and infancy in countries or regions where less than 90% of households are using iodized salt and the median UIC in school age children is less than 100ug/L (Zimmerman, 2009). An iodine supplementation of 250µg/d for pregnant and lactating women, 150µg/d for women of reproductive age or an annual dose of 400µg of iodized oil is recommended. Children less than 2 years should be given 90µg/d or 200µg per year. These are generic recommendations and intended to allow for the needs of countries with severe as well as less severe iodine deficiency (WHO/UNICEF/ICCIDD, 2007).

CHAPTER THREE

Methodology

3.1. Study design

The study design was a cross-sectional study in five municipalities of Mopani district in Limpopo province. A Cross-sectional study implies the collection of data on exposure and outcome at one point in time (Polit and Hungler, 1991). Cross-sectional studies are appropriate for describing the status of a phenomenon at a fixed point in time. In this study the phenomenon is the iodine status of pregnant women, iodine status of children, iodine concentration of table salt as well as iodine concentration of drinking water. The cross sectional data were also used in analytical data analysis to explore the relationship of independent variables with outcome responses variables. A quantitative research methodology was used. According to Polit and Hungler (1995) a quantitative study tends to be a highly structured investigation that yields numerical information amenable to statistical analysis.

3.2. Study population

3.2.1. Target population

The study was conducted in the Mopani district, one of the five districts in Limpopo province of South Africa. The target population was pregnant women in Mopani district. Mopani district consists of five municipalities namely Greater Giyani, Ba-Phalaborwa, Greater Tzaneen, Maruleng and Greater Letaba. The district borders Zimbabwe to the north, Mozambique to the east, Ehlanzeni district to the south, Sekhukhune district to the south west, Capricorn district to the south west and Vhembe district to the north west. The district has an estimated population of 964 195 and the language spoken by the majority of people is Xitsonga (Census, 2001). The estimated female population was 525 744 (54.5%), while the male population was 438 451 (45.5%).

3.2.2. Sampling

Mopani district was clustered into five units (i.e. five municipalities). According to Polit and Hungler (1991), clustering sampling proceeds through a series of different sampling units,

one begins with the largest, most inclusive unit, moving on to less inclusive unit, and then down to the most basic unit or element of population. This approach is often referred to as multi stage sampling. Simple random sampling was used to select four clinics from each municipality. The researcher requested a list of all clinics found in Mopani district from the district office. Clinics were grouped according to their municipalities. All clinics from each municipality were assigned numbers in small papers. These small papers were folded and put inside the container and the researcher picked one paper at a time from the container until four papers were picked for each municipality. Clinics which were represented by the papers were used as sampling frame for the study. A total of 20 clinics from the five municipalities were selected for the study. However, the desired population size of 500 could not be reached and an addition of 21 clinics was selected in order to address the short fall. The total number of clinics was 41 with 21 clinics from Greater Giyani, 7 from Greater Tzaneen, 6 from Maruleng, 4 from Greater Letaba and 3 from Ba-Phalaborwa. The additional clinics were mainly determined by accessibility of municipalities and the availability of funds; hence some municipalities had more clinics than others.

Convenient sampling was used to select pregnant women. Convenience sampling entails the use of most available persons for use as subjects in a study (Polit and Hungler, 1991). A total of 565 pregnant women were recruited for the study. A total of 116 children aged 6-12 years from the same households as that of the pregnant women were also sampled. Figure 3.1 illustrates the sampling process employed and number of samples collected in the study.

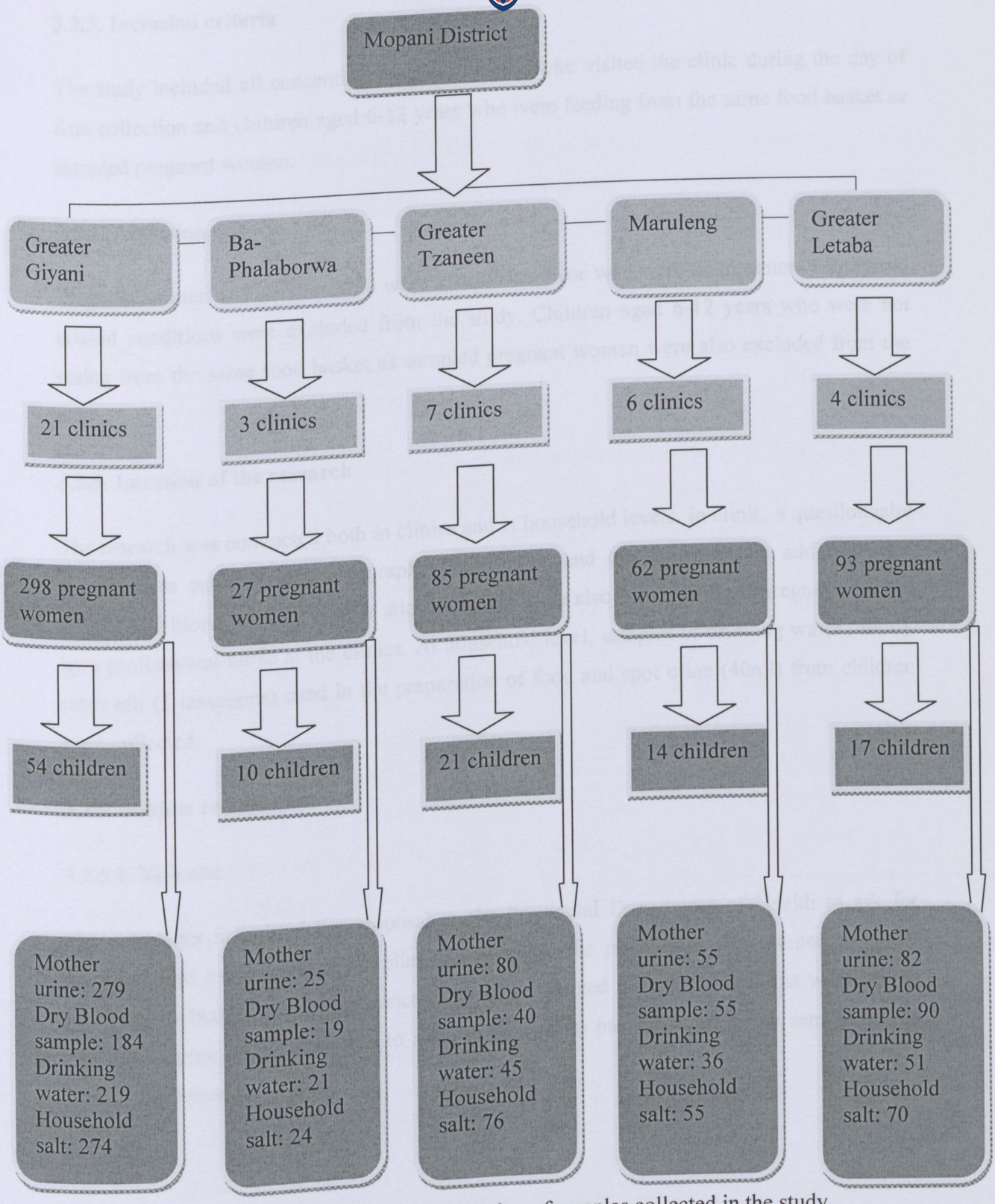

Figure 3.1 sampling process employed and number of samples collected in the study

3.2.3. Inclusion criteria

The study included all consenting pregnant women who visited the clinic during the day of data collection and children aged 6-12 years who were feeding from the same food basket as sampled pregnant women.

3.2.4. Exclusion criteria

Pregnant women and children who were ill and also those who were on treatment for thyroid related conditions were excluded from the study. Children aged 6-12 years who were not eating from the same food basket as sampled pregnant women were also excluded from the study.

3.2.5. Location of the research

The research was conducted both in clinics and at household levels. In clinic, a questionnaire was used to collect socio-demographic information and dietary intake. In addition urine (40ml) and blood samples (finger stick samples) were also collected from pregnant women by a professional nurse at the clinics. At household level, samples of drinking water (40ml), table salt (3 teaspoons) used in the preparation of food and spot urine (40ml) from children were collected.

3.2.6. Subject recruitment

3.2.6.1. Visit one

The researcher submitted the proposal to the Provincial Department of Health to ask for permission to use clinics as sampling points. Once the permission was granted, a list of clinics was obtained from the district office. The selected clinics from the list were visited and the purpose of the visit was to seek permission to include them in the sample and to explain the research.

3.2.6.2. Visit two

During the second visit the researcher conveniently recruited pregnant women and distributed consent forms. The research procedure and objectives were explained to the women before they participated in the study. Data was collected once women gave verbal assent and consented in writing. Verbal assent plus written consent forms were obtained from parents or guardians of participating children and pregnant women under the age of 18 years. Ethical considerations are addressed in Section 3.7 of this chapter.

3.3. Laboratory investigations

3.3.1. Urinary and water iodine content

Urine samples were collected from children and pregnant women using 40ml specimen containers with screw caps. The samples were kept in a cooler box with freezer blocks. The urine samples were aliquoted in 2.2-3ml safe lock Eppendorf micro tubules and packed in storage bags. Plastic Pasteur pipettes (3ml) were used for aliquoting, one pipette per sample. The samples were kept frozen at -20°C until analysis. The storage period was from February to July 2012. Urine samples were analyzed for iodine content using the Sandell-Kolthoff reaction (See appendix D for full analysis procedure). Analysis was done at the iodine laboratory of the Nutrition Intervention Research Unit of the Medical Research Council in Cape Town. The laboratory participated successfully in an international quality control programme, ensuring the Quality of Urinary Iodine Procedures (EQUIP) run by the Centers for Disease Control and Prevention (CDC) in Atlanta, USA and was internationally recognised and well equipped for iodine analyses. More information on quality assurance is given in Section 3.5.4 of this chapter. The analysis involved the spectrophotometric (microplate) measurement of iodine. Before the spectrophotometric measurement can be performed, urine samples must be treated to destroy organic matter and other substances in the urine that could interfere with the rate of reaction. The same procedure of urine collection and analysis was carried out for water samples. A total of 565 urine samples from pregnant women were collected and only 521 samples were analysed. The remaining 44 samples spilled during shipment to MRC. A total of 116 urine samples from children were collected and only 84 samples were analysed. Furthermore, a total of 565 water samples were collected and only 378 samples were analysed. The remaining 187 water samples spilled during shipment to MRC.

3.3.2. Iodine concentration of table salt

Salt samples were collected using small plastic bags with zip locks. The bags were filled with three teaspoons of salt (fine or coarse) used for preparing food at household level. The salt samples were collected by the researcher after the interviews at clinics. The salt samples were analysed by means of the iodometric titration method at the iodine laboratory at the MRC Nutritional Intervention Research Unit in Cape Town (See the full analysis procedure in appendix E). In the titration method, the iodine content of salt is determined by liberating iodine from a solution of iodized salt through the addition of sulphuric acid. Excess potassium iodide is added to keep the free iodine in a dissolved state. The free iodine is then titrated with sodium thiosulphate using starch as an external indicator. A total of 499 salt samples were collected and analysed.

3.3.3. TSH blood concentration

Spot finger stick blood samples were collected from pregnant women by a professional nurse using filter paper grade IDBS-226 obtained from Zurich, Switzerland (See appendix F for blood collection procedure). Dried blood spots on filter paper were analysed for whole blood thyrotropin (TSH) and serum T4 with an immunoassay. The analyses were done at the Swiss Federal Institute of Technology laboratory in Zurich. American Thyroid Association cut off reference values were used. A total of 404 blood samples were collected and only 388 samples were analysed. The remaining 16 blood samples were inadequate for analyses.

3.4. Survey questionnaire

A questionnaire was developed by the researcher following the objectives of the study. Literature was also reviewed to check how other researchers conducted the same research on iodine status of pregnant women and children aged 6 – 12 years in other parts of the world. This questionnaire was developed to collect data on demographic information, dietary iodine intake, patterns of eating goitrogenic foods as well as knowledge on iodine nutrition. The questionnaire was submitted to the Ethics Committee of the University of Venda for approval before data collection. It was also presented to the Department of Nutrition, University of Venda, for inputs before data collection. The questionnaire was pre-tested to check the clarity of questions in the instrument. The pre-test exercise was carried out on five staff members in the Department of Nutrition. The questionnaire was developed in English and translated into the local languages Xitsonga which is spoken in Mopani district. The translation was done by a specialist from the Department of Linguistics who was fluent in both English and Xitsonga.

3.5. Data collection procedures and quality assurance

3.5.1. Fieldwork arrangements

At the clinic, a working room was provided for the interview and also for drawing blood. This was done to ensure privacy for the study participants. The room was arranged such that two stations were assembled. At the first station, the professional nurse drew blood. At the second station, the interview was done by the research assistant and the researcher using the questionnaire. One pregnant woman was allowed into the working room per time. A session with one pregnant women lasted for about 30 minutes. Toilets were available for pregnant women to use for providing urine samples.

The researcher drove pregnant women to their households immediately after the last woman was interviewed. At the households, women were given the small plastic bags with zip locks to add three teaspoons of salt used for preparation of foods in the households. They were also given 40ml specimen containers with screw caps to collect drinking water. Both the samples were given to the researcher and the water samples were stored in the cooler box with freezer blocks until they were stored at -20°C in the nutrition laboratory.

3.5.2. Fieldworkers

A professional nurse collected finger stick blood samples of pregnant women. Training was provided on blood spot collection finger stick procedure (See appendix F for full blood collection procedure). A research assistant was a registered Nutritionist, doing Masters in Public Nutrition. The research assistant was trained on sample handling procedures as well as administering of a questionnaire. The research assistant was appointed based on his research experience and knowledge on the field of nutrition.

3.5.3. Quality assurance

Validity of a measurement instrument is the extent to which the instrument measures what it is intended to measure while reliability is the consistency with which a measuring instrument yield a certain result when the entity being measured has not changed. Both validity and reliability then reflect the degree to which we may have errors in our measurements (Schneider, 2004).

To ensure external validity of the urinary iodine analyses, the MRC laboratory participates in an international quality control programme run by the Centre for Disease Control and Prevention in Atlanta, USA. Urinary iodine measurements at the MRC laboratory agree exceptionally well with inductively coupled plasma mass spectrometry analyses in the USA at low, medium and high concentration. The inductively coupled plasma mass spectrometry measurements are internationally accepted as the gold standard in the analyses of iodine in urine samples.

Internal reliability of urinary iodine analysis is measured in terms of the variation observed with repeated analysis of the same sample, expressed as the coefficient of variation. For validity, commercial standard solutions were used for the construction of a calibration curve and at least two samples from a large pool of urine were also included in each batch of urine samples analysed to check for possible laboratory drift over the various batches analysed.

3.5.4. Statistical analysis

Data was analysed using Statistical Package for Social Sciences (SPSS) version 21. Descriptive statistics of frequencies, means, medians, standard deviation and standard error for all variables were calculated as appropriate.

Pearson correlation test was done to determine the statistical relationship between different variables. The strength of correlation was determined by the Pearson correlation coefficient (r). The strong correlation was $r>0.70$ or $r<-0.70$, moderate correlation was $r>0.30$ to 0.70 or $r<-0.30$ to -0.70 and weak correlation was $r=0.00$ to 0.30 or $r=0.00$ to -0.30. The P values were used to determine the statistical significance between variables. The correlation was significant at $P<0.05$ and $P<0.01$ level.

3.6. Institutional ethical approval

3.6.1. University of Venda

The study proposal was presented to the Higher Degrees Ethical Committee of the School of Health Sciences, for approval before data collection. The ethical clearance certificate was issued and the project registration number is SHS/10/NUT/003 (see appendix G).

3.6.2. Limpopo Department of Health

A permission letter was written to the Limpopo Provincial Health Department for the researcher to have access to the clinics in Mopani district (see appendix H). The Limpopo Provincial Health Department granted a permission letter (see appendix I) to be presented to the Mopani District Health Department. The district granted the permission for the researcher to have access to the clinics (see appendix J). Arrangements were made with sampled clinics for dates of data collection.

3.7. Ethical considerations

To minimize risk of infections through blood transmission when collecting blood samples, one needle was used per participant. There were no direct benefits to individual participants or community where the study was conducted.

During participants' recruitment, pregnant women who agreed to participate were given consent forms to sign. For those participants who were under the age of 18 years, parents/parents-in-laws, husbands or guardians were requested to sign on their behalf. A consent form included the aim of the study, all data and samples that were collected. It further indicated that participating in the study was by participant's individual choice and they could withdraw at any stage of data collection without being penalised (see appendix A).

The data generated from the study was stored in a computer database in a manner that maintains participant's confidentiality. For data verification and quality control purposes, regulatory authority and/ or UNIVEN Ethics committee members may be allowed to access participant data under the condition of strict confidentiality.

CHAPTER FOUR

Results

4.1. Introduction

This chapter provides the results of iodine status of pregnant women and children aged 6 to 12 years eating from the same food basket in the Mopani district of Limpopo province in South Africa. The frequencies of various groups and variables in the study are presented. The study comprised of 565 pregnant women and 116 children from five municipalities of Mopani district.

A total of 521 urine samples from pregnant women and 84 from child urine samples were analysed, representing 92.2% and 89.4% of samples that were successfully analysed respectively. The other urine samples spilled out during transport before analysis. A total of 388 dried blood spot samples from pregnant women were analysed, representing 68.7% of samples that were analysed. The other blood samples were inadequate for analysis. A total of 499 table salt samples were analysed, representing 88.2% of samples that were analysed. Other 11.8% did not bring salt sample because some households did not have salt available and some did not return salt samples. A total of 378 drinking water samples were analysed, representing 66.9% of samples that were successfully analysed. The balance of the water samples spilled out before analysis.

This chapter presents results on demographic data, dietary iodine consumption, UIC and TSH level of pregnant women, UIC level of children, iodine concentration of table salt and drinking water.

Table 4.1 Actual samples of various groups and variables measured in the study

Study groups and variable measured	Number: n					
	Municipality 1	Municipality 2	Municipality 3	Municipality 4	Municipality 5	MOPANI DISTRICT
Pregnant women (demographic and dietary information)	298	27	85	62	93	**565**
Urine samples of pregnant women	279	25	80	55	82	**521**
Pregnant women blood sample	184	19	40	55	90	**388**
Children aged 6 to 12 years	54	10	21	14	17	**116**
Urine samples of children	44	10	11	8	11	**84**
Household Salt samples	274	24	76	55	70	**499**
Drinking water samples	219	21	45	36	57	**378**

4.2. Demographic data

4.2.1. Age distribution of pregnant women

The age of the study participants ranged from less than 18 years to 45 years. The results showed that 81.7% of study participants were young women between the ages of 18 to 35 years while only 7.1% of participants were less than 18 years of age. The results also revealed that another 11.2% of participants were within the age range of 36 to 45 years. Age distribution of pregnant women is summarised in Table 4.2.

Table 4.2 Age distribution of pregnant women in Mopani District

Age	n	%
<18	40	7.1
18-24	229	40.5
25-35	233	41.2
36-45	63	11.2
Total	**565**	**100**

4.2.2. Economic status of study participants

The majority of the study participants (90.6%) were unemployed, while only 9.2% of participants were employed. Economic status of study participants is summarised in Table 4.3.

The income of the study participants ranged from those who did not get any income (i.e. none) to those who get more than R6000 per month. Most participants (40%) had an income ranging between R1001 to R3500 per month; only 4.8% of the participants did not have any income while 7.1% of the study participants had an income of more than R6000 per month.

Table 4.3 Economic status of study participants

	N	%
Employment		
Employed	52	9.2
Not employed	512	90.6
Did not respond	1	0.2
Household income per month		
None	27	4.8
Less than 500 rands	49	8.7
500 to 1000 rands	149	26.4
1001 to 3500 rands	226	40.0
3501 to 6000 rands	74	13.1
More than 6000 rands	40	7.1
TOTAL	**565**	**100**

4.2.3. Educational Level of study participants

With regard to educational level, 54.3% of the study participants had grades 11 and 12 whereas 26.5% had grades 8 to 10. About 9% of study participants had tertiary education while only 2.7% of study participants never went to school. Table 4.4 summarises educational level of the study participants.

Table 4.4 Educational Level of study participants

	N	%
Educational level (Grade)		
R - 7	33	5.8
8-10	150	26.5
11 - 12	307	54.3
College	38	6.7
University	17	3.0
Never went to school	15	2.7
Did not respond	5	0.9
Total	**565**	**100**

4.2.4. Marital status and the number of children of study participants

The majority of study participants (54.7%) were single, 44.8% were married whereas only 0.5% were widowed. When study participants were asked about the number of children they had, 43.2% indicated that they had only one child, 29.7% had two children, 14.5% had three children, 7.8% had four children, 4.6% had more than four children and 0.2% did not respond to this question.

4.3. Process factors of iodine nutrition

All factors operating in the supply chain of iodized salt, from the production stage to the consumption stage, which could influence the delivery of adequately iodized salt to the consumer or influence the iodine intake of a population in any way, are called the process factors.

4.3.1. Distribution and concentration of household salt in Mopani District

Table 4.5 below presents the result of household salt concentration in Mopani District. About 30 % of household salt had iodine concentration level less than 5 ppm, indicating non iodized salt. Around 18 % of households' salt had iodine concentration level between 5 to 14.9 ppm, indicating under iodized salt. Furthermore, more than 50% of household salt had iodine concentration level more than 15 ppm. The mean iodine concentration of household salt was 25 ppm indicating acceptable level of iodine concentration.

Table 4.5 Distribution and concentration of household salt in Mopani District (n=499)

Salt concentration (ppm*)	N	%
Non iodized (<5 ppm)	146	29.3
Under iodized (5 – 14.9 ppm)	91	18.2
Acceptable (15 – 64.9 ppm)	233	46.7
More than required (≥65 – 78.9 ppm)	15	3.0
Excessive (≥ 80 ppm)	14	2.8
Mean (ppm)	**25**	

*ppm = mg of iodine per kg of salt

4.3.2. Consumption pattern of salt by study participants

The results indicate that 98.9% of study participants consumed salt. More than 50% of the participants added salt before cooking while 43.5% add salt during cooking. About 40% of the study participants use fine salt, 47.1% use coarse salt while 12% use both fine and coarse salts. Table 4.6 summarises the consumption of salt by study participants.

Table 4.6 Consumption pattern of salt by study participants (n=565)

Characteristics	n	%
Salt consumption		
Eat salt	559	98.9
Do not eat salt	6	1.1
Addition of salt when cooking		
Before cooking	301	53.3
During cooking	246	43.5
After cooking	12	2.1
Did not respond	5	0.9
Type of salt used when cooking		
Fine salt	233	41.2
Coarse salt	266	47.1
Both	68	12.0
Did not respond	7	1.2

4.3.3 Distribution and concentration of iodine in drinking water in Mopani District

Table 4.7 presents the results of iodine concentration in drinking water in Mopani District. About 18% of household drinking water had iodine concentration level less than 5µg/L. Most of household drinking water (41.3%) had iodine concentration level greater than 60µg/L while 10.6% of household drinking water had iodine concentration between 20 to 39.9µg/L. The median iodine concentration of drinking water in Mopani District was 46.2µg/L.

Table 4.7 Distribution and concentration of iodine in drinking water in Mopani District (n=378)

Iodine concentration in drinking water (µg/L)	n	%
< 5µg/L	68	18.0
5 – 19.9µg/L	68	18.0
20 – 39.9µg/L	40	10.6
40 - 60µg/L	46	12.2
>60µg/L	156	41.3
Median (µg/L)	**46.2**	

4.3.4. Consumption of foods containing goitrogens

Participants were asked questions to determine their consumption of commonly used foods containing goitrogens, such as cabbage, cassava and sweet potatoes. The questions asked were to determine whether the study participants eat foods containing goitrogens, the frequency of consumption as well as the preparation methods in which these foods were consumed.

4.3.4.1. Consumption of cabbage by study participants

Participants were asked questions to determine their consumption of foods containing goitrogens. Cabbage was consumed by 94.7% of the study participants. Only 3.2% of the study participants were consuming cabbage daily. About 30% of study participants were consuming cabbage occasionally. The majority of study participants (66.7%) indicated that they eat cabbage both cooked and uncooked. The consumption of cabbage is summarised in Table 4.8.

Table 4.8 Consumption of cabbage by study participants (n=565)

Characteristics	n	%
Cabbage consumption		
Eat cabbage	535	94.7
Do not eat cabbage	27	4.8
Did not response	3	0.5
Frequency of Cabbage consumption		
Daily	18	3.2
Once a week	75	13.3
Once a month	178	31.5
Twice a month	64	11.3
Once in six month	16	2.8
Occasionally	186	32.9
Did not respond	28	4.9
Preparation of cabbage		
Eat cooked	155	27.4
Eat uncooked	5	0.9
Both	377	66.7
Did not respond	28	4.9

*Occasionally means once after a period of six months

4.3.4.2. Consumption of cassava by study participants

Cassava was consumed by 23.5% of study participants while the majority of participants (76.3%) indicated that they did not consume cassava. About 10% of the participants indicated that they eat cassava occasionally while only 1.6% of them ate cassava once per week. About 20% of the study participants reported that they eat cassava cooked, while none of the participants indicated that they eat uncooked cassava. Only 1.6% of study participants eat cassava both cooked and uncooked respectively. The consumption of cassava is summarised in Table 4.9.

Table 4.9 Consumption of cassava by study participants (n=565)

Characteristics	n	%
Cassava consumption		
Eat cassava	133	23.5
Do not eat cassava	431	76.3
Did not respond	1	0.2
Frequency of cassava consumption		
Once a week	9	1.6
Once a month	36	6.4
Twice a month	6	1.1
Once in six month	22	3.9
Occasionally	58	10.3
Did not respond	434	76.8
Cassava preparation		
Eat cooked	124	21.9
Eat uncooked	0	0.0
Both	9	1.6
Did not respond	432	76.5

*Occasionally means once after a period of six months

4.3.4.3. Consumption of sweet potatoes by study participants

Sweet potatoes were consumed by 96.3% of the study participants. About 44% of the participants indicated that they consume sweet potatoes once in a month, while only 0.7% of study participants indicated that they consume sweet potatoes on a daily basis. The majority of study participants (80%) indicated that they consume sweet potatoes cooked, while 16.1% consume sweet potatoes both cooked and uncooked. The consumption of sweet potatoes is summarised in Table 4.10.

Table 4.10 Consumption of sweet potatoes by study participants (n=565)

Characteristics	n	%
Sweet potato consumption		
Eat sweet potato	544	96.3
Do not eat sweet potato	19	3.4
Did not respond	2	0.4
frequency of sweet potato consumption		
Daily	4	0.7
Once a week	25	4.4

Once a month	249	44.1
Twice in a month	44	7.8
Once in six month	27	4.8
Occasionally	195	34.5
Did not respond	21	3.7
Sweet potato preparation		
Eat cooked	452	80.0
Eat uncooked	2	0.4
Both	91	16.1
Did not respond	20	3.5

*Occasionally means once after a period of six months

4.4. Knowledge on dietary iodine

Participants were asked questions to determine their knowledge on iodine nutrition. The majority of study participants (88.3%) indicated that they did not know iodated salt while 90.6% indicated that they did not know what iodine was. Only 5.3% of the study participants correctly indicated that iodized salt is the main source of dietary iodine in South Africa while 0.2% indicated that fish/sea foods and vegetables are main sources of dietary iodine respectively. Knowledge on dietary iodine is summarised in Table 4.11.

Table 4.11 Knowledge on dietary iodine

Characteristics	n	%
Those who know iodated salt		
Know iodated salt	60	10.6
Do not know iodated salt	499	88.3
Did not respond	6	1.1
Main source of dietary iodine in SA		
Iodized salt	30	5.3
Fish/sea foods	1	0.2
Vegetables	2	0.4
Meat/meat products	1	0.2
Drinking water	2	0.4
Do not know	16	2.8
Did not know what iodine is	512	90.6

4.5. Impact factors of iodine nutrition

Factors that are used to measure the effectiveness (i.e. strength and/or the weaknesses) of the process factors are called impact factors. In this study, the maternal UIC, TSH and child UIC were used to measure effectiveness of iodized salt and dietary iodine intake of a population.

4.5.1. Iodine status of pregnant women

Iodine status of pregnant women was determined using UIC and TSH levels. The distribution of these indicators is presented as per pregnancy stage.

4.5.1.1. Distribution of pregnancy stages of study participants

Majority of study participants 295(52.2%) were in the third trimester of their pregnancies while 213(37.7%) were in the second trimesters. Only 57(10.1%) of study participants were in the first trimester of their pregnancies.

4.5.1.2. Distribution of maternal UIC of study participants

Table 4.12 presents the results of UIC level of pregnant women. An important proportion of pregnant women (44.9%) had UIC level less than 150µg/L. About 22.6% of the study participants had urinary iodine concentration level within 150 - 249µg/L while only 6.9% had urinary iodine concentration above 500µg/L. The maternal median UIC level of study participants was 164µg/L indicating maternal iodine sufficiency.

Table 4.12 Distribution of UIC of pregnant women

UIC (µg/L)	**n**	**%**
<150	234	44.9
150-249	118	22.6
250-499	133	25.5
≥500	36	6.9
Median UIC (µg/L)	164	

4.5.1.3. Maternal median UIC by pregnancy stages in Mopani district

The maternal median UIC levels in the first trimester was 145µg/L which is less than the recommended value of 150 to 249µg/L. Whereas maternal median UI in second trimester was 201µg/L which is within the recommended value of 150 to 249.9µg/L. Study participants in the third trimester had median UI less than 150µg/L. Table 4.13 summarises maternal median UIC of study participants.

Table 4.13 Maternal median UIC by pregnancy stages in Mopani District

Stages of pregnancy	N	%	Maternal UIC (µg/L)
First trimester	57	10.1	**145**
Second trimester	213	37.7	**201**
Third trimester	295	52.2	**132**

4.5.1.4. TSH level of pregnant women in Mopani district

TSH level of pregnant women were measured per trimester and almost all study participants had normal TSH levels. During the first trimester, TSH values ranged from 0.3 - 1.3 mIU/L however, there was TSH value of 2.1 mIU/L. The TSH cut off values used for this study were adopted from the American Thyroid Association (see table 2.3).

During the second trimester, the result of the study revealed that the TSH levels of the study participants were within the normal ranges of 0.2 – 2.8 mIU/L. A value of 5.6 mIU/L was observed during the third trimester which was the only value above the normal range of 0.3 – 3.0 mIU/L as stipulated by the American Thyroid Association.

4.5.2. Iodine status of children

Table 4.14 presents the results of UIC level of children aged 6 to 12 years in Mopani District. Most children (64.3%) had UIC level greater than 300µg/L. About 13% of children had UIC level ranging from 50 to 99µg/L while 14.3% of children had UIC level ranging from 100 to 199.9µg/L. The median UIC level of children was 386µg/L indicating excessive iodine status.

Table 4.14 Distribution of UIC of children (n=84)

UIC (µg/L)	n	%
<20	1	1.2
20-49.9	1	1.2
50 – 99.9	11	13.1
100-199.9	12	14.3
200-299.9	5	6.0
≥300	54	64.3
Median UIC(µg/L)	**386**	

4.6. Statistical correlation of maternal UIC and TSH, children UIC, salt iodine concentration and water iodine concentration

The correlations were done to determine the statistical relationship between different variables. The strength of correlation was determined by the Pearson correlation coefficient (r). The strong correlation was r>0.70 or r<-0.70, moderate correlation was r >0.30 to 0.70 or r-<0.30 to -0.70 and weak correlation was r=0.00 to 0.30 or r=0.00 to -0.30. The P values were used to determine the statistical significance between variables. The correlation was significant at P<0.5 and P<0.01 level.

The results indicated that there was a weak negative significant correlation between maternal UIC and TSH (r= -0.13, N=352, P<0.05). This means that an increase in maternal UIC was accompanied by decrease in TSH while decrease in maternal UIC was accompanied by increase in TSH. The median UIC in pregnant women was 164µg/L and median UIC in children was 386µg/L, these were significantly different (P<0.01). There was a positive correlation between median UIC in pregnant women and median UIC in children (r=0.30, N=76, P<0.01).

Iodine concentration in household salt was correlated with both maternal and children UIC. The results showed that there was a significant positive relationship between iodine concentration in household salt and maternal UIC (r=0.26, N=462, P<0.01), this means that when iodine concentration in household salt increased, maternal UIC also increased. However, there was no significant relationship between iodine concentration in household salt and UIC in children (r=0.17, N=83, P=0.12).

Iodine concentration in drinking water was significantly correlated with maternal UIC. The results indicated that there was a moderate positive significant relationship between iodine concentration in drinking water and maternal UIC (r=0.30, N=349, P<0.01), this means that when iodine concentration in drinking water increased, maternal UIC also increased. There was no significant relationship between iodine concentration in drinking water and UIC in children (r=0.11, N=72, P=0.36).

CHAPTER FIVE

Discussion of results

5.1. Demographic and socio-economic data

The majority of study participants were from poor socio economic background. The socio economic level was indicated by high unemployment rate among study participants. About 90% of study participants were unemployed which is almost four times higher than the national unemployment rate of 25%. High unemployment rate among study participants may be caused by a low number of people with post matric education; only 9.7% of study participants had post matric educational level. More than 30% of study participants lived with a monthly income of less than R1200.00 which indicated that they lived below the poverty line; this is according to Statistic SA (2012), when using an average of three members per household.

5.2. Process factors of iodine nutrition

5.2.1. Iodine in household salt

The introduction of mandatory salt iodization in South Africa impacted favourably on the iodine content of household salt (Jooste *et al.*, 2001). The national survey on a nationally and provincially representative basis was carried out in South Africa in 1998 to investigate the iodine content of household salt by means of the titration method. The data showed that 95.4% of households in South Africa used salt regularly and 2.9% occasionally (Jooste and Zimmerman, 2008). Similar results were found in the current study, 98.9% of households used salt regularly providing confirmation that salt is an effective fortification vehicle penetrating virtually all households in the country as well as in the study area. This means that if all types of salt consumed by the population can be adequately iodized, almost all households in the country would get sufficient iodine in their diet.

Although almost all households indicated that they used salt regularly in this current study, 53.3% added salt before cooking while 43.5% added salt during cooking, and only 2.1% indicated that they add salt after cooking their foods. This shows that if all salt used by these households is adequately iodized, consumption of iodine may be adequate because only about 20% of the iodine will be lost during cooking (WHO/UNICEF/ICCIDD, 2007). Consumers could be advised to add iodized salt toward the end of cooking.

The situation in South Africa was that people at the lower end of socio-economic spectrum were more likely to suffer the consequences of using under iodized salt because more of them used agricultural salt and more of them used coarse salt that contained less iodine than fine salt (Jooste and Zimmermann, 2008). More households (47.1%) in this current study tended to use coarse salt while 41.2% used fine salt. This is an indication that even though fine salt contains a higher iodine concentration, coarse salt remains the type mostly consumed by households in the low socio-economic category. The price differential between non-iodized agricultural coarse salt and iodized fine salt aggravates shortcomings in national salt iodization. It is therefore important to revisit the guidelines of implementation of national salt iodization programme to include iodization of agricultural coarse salt since the majority of people, particularly from low socio-economic spectrum tend to use this type of salt. This would assist in increasing the coverage of households using adequately iodized salt. According to Mabapa (2005), fine salt had a significantly higher mean iodine concentration than coarse salt; the study also confirmed that consumption of non-iodized agricultural salt was higher than adequately iodized fine salt.

The purpose of determining iodine content of salt at household level was to give a reflection of the iodine concentration which study participants were consuming on a daily basis. The mean iodine concentration of household salt was 25ppm in Mopani district, indicating adequate level of iodine concentration. This is similar to South African national mean iodine concentration of 27ppm as reported by Jooste *et al.* (2001). However, this mean iodine concentration differs from the study done by Mabapa (2005), the mean iodine values were lower than 15ppm in both Vuwani and Mutale sub district of Limpopo province of South Africa and more than 80% of households were using salt containing less than 15ppm of iodine.

In this current study, 52.5% of households used adequately iodized salt containing more than 15ppm. This was considerably lower than the international goal of 90% of households using adequately iodized salt. This is an indication that despite the success of mandatory iodization programme at the national level in South Africa, there are some areas, particularly in the Limpopo province, were the implementation is poor. This is mainly caused by the use of cheap non-iodized agricultural salt bought in local spaza shops and small scale salt producers. A national survey of the iodine content of household salt in 1998 showed that 62.4% of households used adequately iodized salt containing 15ppm or more iodine, with considerable

variation among provinces. The Eastern Cape province with a coverage of 77% of households came closest to the international goal of 90% of households using adequately iodized salt, while less than half of households in the three northern provinces (Limpopo, Mpumalanga and North West) used adequately iodized salt (Jooste and Zimmermann, 2008).

According to the South African National Food Consumption survey-Fortification Baseline (NFCS-FB) in 2007, the national mean iodine concentration in household salt was 39.7ppm. The provincial mean concentration varied significantly from 20ppm in Limpopo province to 51.8ppm in the Western Cape province. The percentage of households in the country using salt containing more than 15ppm was 76.9%, and these percentages varied from 45.3% in Limpopo province to 87.7% in the Western Cape province (Jooste *et al*., 2007). The households with adequately iodized salt in the current study showed a slight increase as compared to the results of NFCS-FB (2007) where only 45.3% of household salt were adequately iodized in Limpopo and less than 20% in the study done by Mabapa (2005) in Vuwani and Mutale districts of Limpopo province. This slight increase could be an indication of positive progress in the implementation of national iodization programme in the study area. However, the household coverage of adequately iodized remain very low in all studies done in Limpopo province over the past 15 years including the current study. This is an indication of a weak salt iodization programme seriously in need of corrective action in Limpopo province.

5.2.2. Iodine in drinking water

Iodine in drinking water may serve as an indication of the amount of iodine occurring naturally in the environment. Except for Northern Cape and Limpopo provinces of South Africa, the iodine concentration in water may be seen as generally low and comparable with that found in other countries. Thus, the limited contribution of iodine in water to the total daily iodine intake would usually not be considered as adequate in meeting the requirements of children and women. In contrast, the iodine in water in the Limpopo province in the 2005 NFCS-FB survey may have contributed as much as 50% of iodine requirement of children and about 40% of adult women assuming a conservative water consumption of 1 liter per day (Jooste *et al*., 2007).

In the present study, the median iodine concentration in water was 46.2µg/L and most household drinking water (41%) had iodine concentrations greater than 60µg/L. This

confirms the findings that the iodine concentration of drinking water in Limpopo is higher than that in other provinces except for Northern Cape Province and may contribute significantly to the iodine requirement of children and women. Based on the median iodine concentration in drinking water and a conservative estimate of 1 litre of water consumed per day, it was calculated in this current study that 38.5% of the daily iodine requirement of children aged 6 to 12 years could be met by iodine in drinking water and 18.5% in the case of pregnant women. This data clearly shows that drinking water is a significant potential source of iodine intake in vulnerable groups in the population, although many studies on iodine nutrition tend to overlook this important potential source of dietary iodine.

5.2.3. Food containing goitrogens

Dietary substances that interfere with thyroid metabolism can aggravate the effect of ID and are termed goitrogens (Zimmermann, 2009). Goitrogens are naturally occurring substances found in various foods and they have an ability to cause goiter. In addition to promoting goiter formation, goitrogenic foods can act like antithyroid drugs, slowing down the thyroid and ultimately causing hypothyroidism. Goitrogens are able to disrupt normal thyroid function by inhibiting the body's ability to use iodine, block the process by which iodine is taken up into the thyroid, inhibiting the actual secretion of thyroid hormones and disrupt the peripheral conversion of T4 to T3. Goitrogenic foods such as cabbage contain glucosinolates; their metabolites compete with iodine for thyroidal uptake, whereas foods such as cassava and sweet potatoes contain cyanogenic glucosides; these may be metabolized to thiocyanates that also compete with iodine for thyroidal uptake (Zimmermann, 2009).

In this present study, commonly consumed goitrogenic foods such as cabbage, cassava and sweet potatoes were studied to determine their contribution in the utilisation of iodine by the body. Sweet potatoes were the most commonly consumed goitrogenic food followed by cabbage. Most of the study participants indicated that they consumed these foods on a monthly basis and some reported that they consumed them occasionally. This indicate that the consumption of these goitrogenic foods by the study participants were not at a level to inhibit the body's ability to use iodine. Furthermore, 80% of study participants stated that they consumed cooked sweet potatoes while 66.7% consumed both cooked and uncooked cabbage. The enzymes involved in the formation of goitrogenic material in plants can be partially destroyed by heat, allowing people to consume these foods, cooked. According to

Zimmermann (2009), it appears that most of the goitrogenic substances do not have a major clinical effects unless there is coexisting ID in that population.

It thus can be concluded that the consumption pattern of sweet potatoes, cabbage and cassava in the study area did not pose a threat to the iodine metabolism of the participants. This is because the frequency of consumption was low, the food was mostly cooked before consumption and there was no existing ID in children.

5.3. Knowledge of iodine nutrition

Given the public health importance of eliminating iodine deficiency and the health consequences of its deficiencies, it could theoretically be expected that consumers are informed and educated about iodine nutrition and IDDs (Jooste *et al*., 2005). Knowledge of iodine nutrition is a process indicator often neglected in salt iodization programmes because of the usual emphasis placed on iodine in salt and its impact on iodine and goiter status. Internationally, from the limited information available on knowledge of iodine nutrition, it appears that the knowledge level of iodine nutrition varies from very low level in a country such as India to a level where people are well informed, such as in Iran (Jooste and Zimmermann, 2008).

In the present study, the majority of study participants (88.3%) stated that they did not know what iodated salt was, while only 5.3% correctly indicated that iodized salt was the main source of dietary iodine in South Africa. The low level of knowledge about iodine nutrition in this current study suggests that the international message about brain damage resulting from iodine deficiency has not been conveyed successfully to the consumer level in the country. Neither is it that consumers will choose or demand iodized salt for the benefit of children and women. This general low level of knowledge on iodine nutrition may also have a negative impact on the progress of the national iodization programme.

Similar results were found in a South African national study assessing the knowledge of iodine nutrition of adults, only 15% of study participants correctly identified salt as the primary dietary source of iodine. Knowledge was even poorer in low socio-economic households where participants were less informed as compared to high socio-economic households (Jooste and Zimmermann, 2008). More specifically, the study participants in this current study, as elsewhere in the country, were generally unaware of iodized salt and the

dietary sources of iodine. The knowledge of iodine nutrition in areas such as those in this current study, where a large percentage of households used non-iodized agricultural salt from local spaza shops and small scale salt producers, could be very important. They would be in a position to ask for or demand iodized salt every time that they buy salt.

5.4. Impact factors of iodine nutrition

5.4.1. Iodine status of pregnant women

The overall median UIC in pregnant women was 164µg/L in the current study, indicating maternal iodine sufficiency. This overall median UIC is sufficient for the proper functioning and development of brain of fetus during pregnancy. Iodine sufficiency in pregnant women can be associated with mean iodine concentration of household salt, which was 25ppm in the study area although only 52.5% of households had adequately iodized salt. Consumption of drinking water which contained the median iodine concentration of 46µg/L contributed 18.5% of dietary iodine intake which could also be associated with this iodine sufficiency in pregnant women. The statistical analysis in this current study showed a significant relationship ($P<0.01$) between maternal UIC with both iodine concentration in household salt and iodine concentration in drinking water. Similar results were reported by Ujowundu *et al.* (2010) and Wang *et al.* (2009), where median UICs were 163µg/L and 169µg/L respectively. Women in iodine sufficient regions typically begin pregnancy with adequate intrathyroid iodine stores and were able to meet the increased demands of pregnancy as long as they maintained optimal dietary iodine intake (Pearce, 2012).

However, the results in this current study showed that there were variations in median UICs when measured in trimesters of pregnancy. The median UIC in the first and third trimesters were below 150µg/L, indicating iodine insufficiency, while UIC in the second trimester was 201µg/L, indicating iodine sufficiency. This is an indication that the overall median UIC during pregnancy does not give an accurate reflection of iodine status of pregnant women.

Therefore, all studies conducted to determine iodine status of pregnant women should have a methodological consideration to measure the median UIC per trimester. These outcomes are consistent with the results from other studies which showed, even in areas with overall adequate iodine status, a significant proportion of pregnant women have UICs below the recommended level, particularly in the third trimester. Similar trends of variations were observed by Wang *et al.* (2009). The possible causes of this variation in median UIC in

different trimesters were not clear in this current study, neither Wang nor other researchers had explained these variations. It may, however, be speculated that the demand for iodine increased in the third trimester because of the increased demand by the growing fetus and its developing thyroid gland. The implication of this finding is that the need for supplemental iodine becomes more acute in the third trimester, particularly if the maternal diet provides an inadequate amount of iodine, as was found in the current study.

Several studies reported insufficient maternal median UIC in different countries. In this current study, pregnant women in the first and third trimester had insufficient iodine status. Consequences of iodine deficiency are most severe for pregnant women and their fetuses and, depending on the severity of the deficiency, may include goiter, cretinism, intellectual impairments, growth retardation, neonatal hypothyroidism, increased pregnancy loss and infant mortality (Pearce, 2012). Zimmermann (2011) further indicated that the consequences of ID during pregnancy depend upon the timing and severity of the hyperthyroidism. Because iodine deficiency during pregnancy can impair cognitive and motor development of the offspring, it is important that women enter pregnancy with adequate iodine status (Hussein *et al.*, 2013). In addition, in areas where the iodine intake is inadequate in the last trimester, such as in the study area, supplements containing iodine should be given to the pregnant mother.

TSH is also an indicator of iodine status of pregnant women. According to Pearce (2012), if adequate iodine is not available to produce thyroid hormones in pregnancy, TSH rises and consequently maternal and fetal goiter may develop. The pituitary gland secretes TSH in response to circulating level of T4. Serum TSH rises when serum T4 concentration are low and fall when they are high. Iodine deficiency lowers circulating T4 and raises the serum TSH, so iodine deficient populations generally have higher serum TSH concentration than do iodine sufficient group (WHO/UNICEF/ICCIDD, 2007).

In this present study, TSH values in all trimesters were within the normal level, except for one study participant in third trimester which was higher than the recommended value. This TSH indicates as well that the iodine status of pregnant women in this current study was sufficient to produce adequate thyroid hormones (T4) despite the marginal iodine deficiency. Therefore it may be concluded that the pregnant women in this study had sufficient iodine

reserves in the thyroid gland to maintain euthyroidism (production of a sufficient amount of T4 and T3, which resulted in an unchanged TSH concentration).

Under these circumstances thyroid hormone concentrations are poor indicators of iodine status, particularly for older children and adults. In iodine deficient population, serum T3 and TSH rise or remain unchanged and serum T4 usually falls. However, these changes are often within the normal range and the overlap with iodine sufficient population is large enough to make thyroid concentrations an insensitive measure of iodine nutrition (Zimmermann *et al.*, 2008, WHO/UNICEF/ICCIDD, 2007). In this current study, although the results showed variation in median UIC in different trimesters, TSH showed consistency. This confirms that TSH is not a good indicator of iodine status in pregnant women who have marginal iodine deficiency and some iodine reserve in the thyroid because TSH hardly changed and remained within the normal range.

5.4.2. Iodine status of children

The median UIC in school age children was traditionally used for assessment of iodine nutrition in populations. If the median UIC is adequate in school age children, it was usually assumed iodine intakes are also adequate in the remaining population, including pregnant women (Gowachirapant *et al.*, 2009). Urinary iodine is an excellent indicator of recent iodine intake because more than 92% of dietary iodine is absorbed and in a healthy, iodine-replete adult, more than 90% is excreted in the urine within 24 to 48 hours (Zimmermann and Anderson, 2012). For this reason, UIC values were used for population estimates of iodine status.

In this study, the median UI in children was 386µg/L indicating excessive iodine status while the majority of children (64.3%) had UIC level greater than 300µg/L. This shows that both the median UIC and the distribution indicate a clear excessive iodine status in children aged 6 to 12 years. The excessive median UIC in the current study is a reason for concern; this is because the long term effects of such high excessive median UIC have not been studied. Excessive iodine intake may lead to iodine induced hyperthyroidism; this occurs more commonly in older population with pre-existing nodular goiter that had been exposed to longstanding ID followed by a rapid increment in iodine intake (Jooste *et al.*, 2007).

The reasons for high UIC values in children in the present study are not clear. It could be the high level of iodine concentration in drinking water in the study area, but the results showed that there was no significant relationship (P=0.36) between median UIC in children and iodine concentration in drinking water. This means that the iodine concentration level in drinking water was not a justification for higher median UIC in children. The other reason could be the consumption of processed foods which contain a significant amount of iodine. All children in this study were benefiting from the National School Nutrition Programme (NSNP), which uses iodated salt for food preparation, which could have increased their consumption of iodine. However, all the reasons mentioned in this section are not sufficient to explain the excessive iodine status of children in this study. It is therefore assumed that these children could have consumed high amount of iodine from other sources which are not known.

The results of the current study differ significantly from the study conducted by Mabapa (2005) where school children in Vuwani and Mutale districts of Limpopo province had median UIC levels of 75µg/L and 94µg/L respectively, indicating mild iodine deficiency. However, the median urinary values of two schools in those districts were extremely high with one school having median UI of 304.9µg/L and 323µg/L in another school. Again, it was not clear what the reason was for the unexpectedly high median UICs in two of the schools.

The result in this current study showed a significant difference (P<0.01) between median UIC in children (386µg/L) and median UIC in pregnant women (164µg/L), even though they were feeding from the same food basket. This is an indication that the traditional theory which assumed that if median UIC of children aged 6 to 12 years is adequate, the iodine status of other population groups, including pregnant women is also adequate is not correct.

The most likely explanation for why children had a sharply higher median UIC than median UIC in pregnant women is that dietary iodine intake for both children and pregnant women was in the same range since they were feeding from the same food basket. If so, the median UIC in children aged 6 to 12 years with smaller daily urine volume would be higher than that of pregnant women with higher water intake and greater urine volume. However, this explanation is insufficient to justify this huge difference between median UIC of children and that of pregnant women.

The results from the study conducted by Gowachirapant *et al.* (2009) also demonstrated a significant difference between median UIC in school age children (200µg/L) and median UIC in pregnant women (108µg/L), although they were feeding from the same basket. Therefore, the iodine status of pregnant women as well as the iodine status of children should be measured in population estimates of iodine deficiency. It is clear from the current results that these two groups should both be included as study groups in population estimates of iodine status.

High intake of iodine should be avoided, especially in populations with a history of chronic ID, because a rapid and large increase in iodine intake may precipitate autoimmune thyroid disease and/or hyperthyroidism. Based on the national median UIC in 2011, an international analysis reported that eleven countries have iodine intakes above the 300µg/L threshold that WHO classifies as excessive. These data emphasise the importance of regular monitoring of iodine status to detect not only low but also excessive intake of iodine (Zimmermann and Andersson, 2012).

CHAPTER 6

Conclusion and recommendations

6.1. Conclusion

6.1.1. Iodine in household salt

- Although fine salt contains a higher iodine concentration, non-iodized agricultural coarse salt remains the type mostly consumed by households, particularly in low socio-economic category. This is caused by price difference between non-iodized agricultural salt and iodized fine salt.
- Despite the success of the national salt iodization programme in South Africa, only 52.5% of household salt was adequately iodized in the current study in the Limpopo province. This was considerably lower than the international goal of 90% of households using adequately iodized salt. The household coverage of adequately iodized salt remained low in all studies in the Limpopo province over the past 15 years including the current study, and this is an indication of a weak iodization programme in Limpopo Province.

6.1.2. Iodine in drinking water

- Drinking water, in the study area had a significant amount of iodine concentration. It can then be concluded that drinking water in the study area is a significant potential source of iodine intake in vulnerable groups.

6.1.3. Consumption of foods containing goitrogens

- The consumption pattern of sweet potatoes, cabbage and cassava in the study area did not pose a threat to the iodine metabolism of the participants. This is because the frequency of consumption was low, the food was mostly cooked before consumption and there was no existing ID in children.

6.1.4. Knowledge of iodine nutrition

- The study participants in this current study, as elsewhere in the country, were generally unaware of iodized salt and the dietary sources of iodine.

6.1.5. Iodine status of pregnant women

- Results of the overall median UIC in the current study show that pregnant women in the study area have an adequate iodine status. However, when the results were stratified according to trimester, ID existed in the first and third trimesters.
- The level of TSH values in all trimesters were within the normal ranges, regardless of variation in median UIC, which confirms that changes in TSH level is low and sometimes

remain within the normal range. It can be concluded that TSH is a poor indicator of iodine status in pregnant women.

6.1.6. Iodine status of children aged 6 to 12 years

- Iodine status of children (median UIC=386µg/L) in this study was excessively high and it is a reason for concern. It was more than two times higher than the iodine status of pregnant women (median UIC=164µg/L). It is difficult to explain this significant difference of iodine status of these two groups since they were feeding from the same food basket. It can then be concluded that the median UIC of school aged children may not be an appropriate surrogate for monitoring iodine nutrition in pregnant women as was previously assumed.

6.2. Recommendations

- The coarse salt which is meant for human and animal consumption should be iodized and be regulated by the Iodized Salt Regulation.
- The implementation of the salt iodization programme in the Limpopo province should be revised to detect factors that hinder the progress. A provincial strategy should be drafted to overcome the many weaknesses of the salt iodization programme in this province and to strengthen the programme in order to achieve the goal of a household iodized salt coverage of 90%.
- The community needs to be educated on the use of iodized salt and its benefits for brain function and development.
- Drinking water in the Limpopo province should be considered as a significant source of dietary iodine.
- All studies conducted to determine iodine status of pregnant women should have a methodological consideration to measure median UIC per trimesters. This is because the overall median UIC tend to hide/cloud variations that occur in different trimesters.
- A regular monitoring of iodine status is necessary to detect not only low, but also excessive intake of iodine as demonstrated by the high median UIC of children in this study.
- No information is available on pregnant women iodine status in South Africa. Therefore, a wider survey should be undertaken to establish the iodine status and needs of this vulnerable group.

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APPENDICES

APPENDICES

APPENDIX A

CONSENT FORM

The aim of this study is to assess iodine status of pregnant women in Mopani district, Limpopo province, South Africa. Pregnant women are required to provide urine and blood samples for determination of iodine status. Water and salt samples will also be required to determine iodine content. Information on demographic and factors influencing iodine status will be collected by means of questionnaire. Urine of children aged 6 to 12 years is also required. The information provided in this study will be kept confidential i.e. names will not be recorded but codes will be used instead. If you agree to participant, please sign below.

I understand that:

1. The study deals with the prevalence of iodine status of pregnant women in Mopani district, Limpopo province, South Africa.
2. Any question that I may have regarding the research or related matters will be answered by the researcher.
3. The researcher will require urine, blood, water and salt samples for determination of iodine content. Questionnaire will be used for demographic information and factors influencing iodine status.
4. The research protocol, i.e. the aim, objectives and methods have been explained to me.
5. Participation in this study is by my choice, and I may withdraw my participation at any stage without any action taken against me.

I…….. agree to participant in the study

Signature…… Date……..

(Respondent)

Signature…… Date……..

(Researcher)

Cell No: 079 016 6133

E- mail: Eric.Mabasa@univen.ac.za

Enq: *Mr E Mabasa*
Cell: *079 016 6133*
E-mail: *Eric.Mabasa@univev.ac.za*

The Manager
Primary Health Care
Mopani District
Giyani

Dear Sir/Madam

Re: PERMISSION TO USE MOPANI DISTRICT'S CLINICS FOR RESEARCH PROJECT

I, Mabasa Eric of student number 11521103 and ID number 790202 5779 083, hereby request permission to use clinics in Mopani district as sample frames for pregnant women. I am currently studying Masters in Public Nutrition at the University of Venda. As part of my studies, I am required to do a research project. My research topic is: **ASSESSMENT OF IODINE STATUS OF PREGNANT WOMEN IN MOPANI DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA**

I have enclosed research proposal and ethical certificate from the institution for your perusal. Your assistance will be highly appreciated.

Please direct any queries to the above name.

I thank you in advance

Yours faithfully
Mabasa E (Researcher)

_____

APPENDIX C

QUESTIONNAIRE

ASSESSMENT OF IODINE STATUS OF PREGNANT WOMEN IN MOPANI DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

NAME OF INTERVIEWER:.....

DATE OF INTERVIEW:.....

CODE

SECTION A

DEMOGRAPHIC INFORMATION

1. Name of the respondent.....

2. Age

1.	< 18	
2.	18- 24	
3.	25- 35	
4.	36- 45	
5.	> 45	

3. How long is your pregnant?

1.	0 – 3 months	
2.	> 3 – 6 months	
3.	> 6 – 9 months	

4. Are you employed?

1.	Yes	
2.	No	

5. If yes, specify.....

6. Household income per month (in rands)

1.	None	
2.	< 500	
3.	500-1000	
4.	1001- 3500	
5.	3501- 6000	
6.	> 6000	

7. Formal education level (in STDs)

1.	0-5	
2.	6-8	
3.	9-10	
4.	College/ Technical education	
5.	University education	

6.	None	
7.	Other, specify	

8. Marital status

1.	Married	
2.	Single	
3.	Widowed	

9. How many children do you have including the one you are carrying?

1.	One	
2.	Two	
3.	Three	
4.	Four	
5.	Other, specify	

SECTION B

FACTORS INFLUENCING IODINE STATUS OF PREGNANT WOMEN

10. Do you eat salt?

1.	Yes	
2.	No	

11. If yes, when do you add salt when cooking?

1.	Before cooking	
2.	During cooking	
3.	After cooking	
4.	Other, specify	

12. Do you know about iodized salt?

1.	Yes	
2.	No	

13. Which salt do you use?

1.	Fine	
2.	Coarse	
3.	Both	
4.	No salt	

14. Does your pregnancy sometimes make you feel that you do not like salty food?

1.	Yes	
2.	No	

15. If yes, how do you respond?

1.	Avoid salt completely	
2.	Reduce salt	
3.	Other, specify	

16. Do you eat cabbage?

1.	Yes	
2.	No	

17. If yes, how often do you eat cabbage?

1.	Daily	
2.	Once in a week	
3.	Once in a month	
4.	Twice per month	

5.	Once in six month	
6.	Other, specify	

18. How do you eat your cabbage?

1.	Cooked	
2.	Uncooked	
3.	Both	
4.	Other, specify	

19. Do you eat mitsumbula/cassava?

1.	Yes	
2.	No	

20. If yes, how often do you eat it?

1.	Daily	
2.	Once in a week	
3.	Once in a month	
4.	Twice per month	
5.	Once in six month	
6.	Other, specify	

21. How do you eat your mitsumbula/cassava?

1.	Cooked	
2.	Uncooked	
3.	Both	
4.	Other, specify	

22. Do you eat sweet potato?

1.	Yes	
2.	No	

23. If yes, how often do you eat it?

1.	Daily	
2.	Once in a week	
3.	Once in a month	
4.	Twice per month	
5.	Once in six month	
6.	Other, specify	

24. How do you eat your sweet potato?

1.	Cooked	
2.	Uncooked	
3.	Both	
4.	Other, specify	

25. Which food is the main source of iodine in the people of South Africa?

1.	Iodized salt/ iodated salt	
2.	Fish/ sea foods	
3.	Vegetables	
4.	Meat or meat products	
5.	Dairy products e.g. milk, cheese	
6.	Drinking water	
7.	Other, specify	
8.	Do not know	
9.	Do not know what iodine is	

26. Where do you usually buy or obtain salt that is used for food in your house?

1.	Buy in a shop like Pick 'n Pay, Shoprite, Spar etc.	
2.	Agricultural coarse salt from farmer, employer etc.	
3.	Spaza shop	
4.	Informal sector: street vendor or hawker	
5.	Direct from salt producers	
6.	No salt in the household	
7.	Other, specify	

27. Do you read labeling on the salt package when you buy salt to make sure that salt is iodated?

1.	Yes	
2.	No	
3.	Cannot read	
4.	Do not know what iodine is	

28. Do you have any concern about iodine being added to salt?

1.	Yes	
2.	No	
3.	Unsure	
4.	Do not know what iodine is	

METHOD FOR MEASURING URINARY IODINE USING AMMONIUM PERSULFATE

NB: This same procedure was also used to determine iodine concentration in drinking water

Principle

Urine is digested with ammonium persulfate. Iodide is the catalyst in the reduction of ceric ammonium sulfate (yellow) to cerous form (colourless), and is detected by rate of colour disappearance (Sandell-Kolthoff reaction).

Equipment

Heating block (vented fume hood not necessary), colorimeter, thermometer, test tubes (13 x 100 mm), reagent flasks and bottles, pipettes, balance scales.

Reagents

1. Ammonium persulfate (analytical grade)
2. As2O3
3. NaCl
4. H2SO4
5. Ce(NH4)4(SO4)4
.2H2O
6. Deionized H2O
7. KIO3

Solutions

1.0 M Ammonium persulfate: Dissolve 114.1 g H2N2O8S2 in H2O; make up to 500 ml with H2O. Store away from light. Stable for at least one month. 5 N H2SO4: Slowly add 139 ml concentrated (36 N) H2SO4 to about 700 ml deionized water (careful - this generates heat!). When cool, adjust with deionized water to a final volume of 1 litre. Arsenious acid solution: In a 2000 ml Erlenmeyer flask, place 20 g As2O3 and 50 g NaCl, then slowly add 400 ml 5 N H2SO4. Add water to about 1 litre, heat gently to dissolve, cool to room temperature, dilute with water to 2 litres, filter, store in a dark bottle away from light at room temperature. The solution is stable for months.

Ceric ammonium sulfate solution: Dissolve 48 g ceric ammonium sulfate in 1 litre 3.5 N H2SO4. (The 3.5 N H2SO4 is made by slowly adding 97 ml concentrated (36 N) H2SO4 to about 800 ml deionized water (careful – this generates heat!), and when cool, adjusting with

deionized water to a final volume of 1 litre). Store in a dark bottle away from light at room temperature. The solution is stable for months.

mol/l): Dissolve□g iodine/ml (7.9 □Standard iodine solution, 1 0.168 mg KIO3 in deionized water to a final volume of 100 ml (1.68 mg KIO3 contains 1.0 mg iodine; KIO3 is preferred over KI because it is more stable, but KI has been used by some laboratories without apparent problems). It may be more convenient to make a more concentrated solution, e.g., 10 or 100 mg g/ml. Store in a dark bottle. The solution is□iodine/ml, then dilute to 1 g/l.□stable for months. Useful standards are 20, 50, 100, 150, 200, and 300

Procedure

1. Mix urine to suspend sediment.

l of each urine sample into a 13 x 100 mm test tube.□2. Pipette 250 Pipette each iodine standard into a test tube, and then add H2O as needed to l. Duplicate iodine standards and a set of internal□make a final volume of 250 urine standards should be included in each assay.

3. Add 1 ml 1.0 M ammonium persulfate to each tube.

4. Heat all tubes for 60 minutes at 100oC.

5. Cool tubes to room temperature.

6. Add 2.5 ml arsenious acid solution. Mix by inversion or vortex. Let stand for 15 minutes.

l of ceric ammonium sulfate solution to each tube□7. Add 300 (quickly mixing) at 15-30 second intervals between successive tubes. A stopwatch should be used for this. With practice, a 15 second interval is convenient.

8. Allow to sit at room temperature. Exactly 30 minutes after addition of ceric ammonium sulfate to the first tube, read its absorbance at 420 nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

Calculation of results

Construct a standard curve on graph paper by plotting iodine concentration of each standard on the abscissa against its optical density at g/l (OD405) on the ordinate.□405

Notes

1. This is modified from the former method, substituting ammonium persulfate for chloric acid (more toxic) as digestant.

2. Since the digestion procedure has no specific end-point, it is essential to run blank and standards with each assay to allow for variations in heating time, etc.

3. The exact temperature, heating time, and cooling time may vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of the reading must be the same for all samples, standards, and blanks.

4. With the longer ceric ammonium sulfate incubation and with 15 second interval additions of CAS, up to 120 tubes can be read in a single assay.

5. The volumes and proportions of samples and reagents can be varied to achieve different concentrations or a different curve shape, if conditions warrant. If different tube sizes are used, corresponding sized holes in the heating block are also needed.

6. If necessary, this method could probably be applied without a heating block, using a water, oil, or sand bath, but this is not recommended. It is essential that all tubes be uniformly heated and that the temperature be constant within the range described above.

7. Test tubes can be reused if they are carefully washed to eliminate any iodine contamination.

8. Various steps of this procedure are suitable for automation. For example, the colorimetric readings can be done in microtiter plates with a scanner, and the standard curves plotted and read on a simple desk computer.

APPENDIX E

TITRIMETRIC METHOD FOR DETERMINING SALT IODATE CONTENT

a. Description of reaction

The iodine content of iodated salt sample is measured using an iodometric titration, as described by WHO/UNICEF/ICCIDD (2007). The reaction mechanism can be considered into two steps:

b. Reaction1: Liberation of free iodine from salt

- Addition of H_2SO_4 liberated free iodine from the iodate in the salt sample.
- Excess KI is added to help solubilise the free iodine, which is quite insoluble in pure water under normal conditions.

c. Reaction 2: Titration of free iodine with thiosulfate

- Free iodine is consumed by sodium thiosulfate in the titration step. The amount of thiosulfate used is proportional to the amount of free iodine liberated from salt.
- Starch is added as an external (indirect) indicator of this reaction, and reacts with free iodine to produce a blue colour. When added towards the end of titration (that is, when only a trace amount of free iodine is left) the loss of blue colour, or endpoint, which occurs with further filtration, indicates that all remaining free iodine has been consumed by thiosulfate.

d. Reaction Steps for Iodometric Titration of Iodate

1. $IO_3 + 5I + 6H \rightarrow 3I_2 + 3H_2O$
2. $2Na_2S_2O_3 + I_2 \rightarrow 2NaI + Na_2S_4O_6$

e. Reagent preparation

The preferred water for this method should be boiled distilled water, which requires provision of a distillation unit. As a simpler alternative, regular tap water treated with a mixed bed deionising resin can be used, thus avoiding the need for an expensive distillation unit.

- **0.005M Sodium thiosulfate ($Na_2S_2O_3$):** Dissolve 1.24g $Na_2S_2O_3 . H_2O$ in 500ml water. Store in a cool, dark place. This volume is sufficient for 100 – 200 samples, depending on the iodine content of samples. The solution is stable for at least 1 month, if stored properly.
- **2N Sulfuric acid (H_2SO_4):** Slowly add 6 ml concentrated H_2SO_4 to 90 ml water. Make up to 100 ml with water. This volume is sufficient for 100 samples. The solution is stable indefinitely. Always add acid to water, not water to acid, to avoid excess heat formation and splitting of acid. Stir solution while adding acid.

- **10% Potassium iodide (KI):** Dissolve 100 g of KI in 1000 ml of water. Store in a cool dark place. This volume is sufficient for 200 samples. Properly stored solution is stable for six months, provided no change occurs in the colour of the solution.
- **Starch indicator solution:** Dissolve reagent-grade sodium chlorine (NaCl) in 100 ml double distilled water. While stirring, add NaCl until no more dissolves. Heat the content of beaker until excess salt dissolves. While cooling, the NaCl crystals will form on the sides of the beaker. When it is completely cooled, decant the supernatant into a clean bottle. This solution is stable for six to twelve months. Dissolve 1 g chemical starch in 10 ml double distilled water. Continue to boil until it completely dissolves. Add the saturated NaCl solution to make 100 ml starch solution. This volume is sufficient for testing 20 to 45 samples. Prepare fresh starch solution every day, since starch solution cannot be stored.

Procedure

- Weigh 10 g of salt sample into a 250 ml Erlenmeyer flask with a stopper.
- Add approximately 30 ml water, swirl to dissolve salt sample.
- Add water to make volume up to 50 ml.
- Add 1 ml 2N H_2SO_4
- Add 5 ml 10% KI. The solution should turn yellow if iodine is present.
- Stopper the flask and put in the dark (drawer) for 10 minutes.
- Rinse and fill burette with 0.005M Na2S2O3, and adjust to zero.
- Remove flask from drawer, and add some Na2S2O3 from the titration burette until the solution turns pale yellow.
- Add approximately 2 ml of starch indicator solution (the solution should turn dark purple) and continue titration until the solution become pink, and finally colourless.
- Record the level of thiosulfate in the burette and convert to ppm using the conversion table.

BLOOD SPOT COLLECTION - FINGERSTICK PROCEDURE

- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Hold finger down over the first circle on the blood collection card, but do not touch the circle
- When a full hanging drop of blood is formed, gently touch the blood drop inside the first circle.
- Blood should be applied from only one side of the paper and appear as an even, uniform layer.
- The recommended collection technique is to absorb the blood directly from the collection site onto the paper while watching the circle to ensure that it completely fills.
- If needed, continue to apply blood drops onto open areas of the first circle until it is completely covered. **Do not layer blood drops on top of one another.** It is acceptable for blood to extend outside the circle.
- Fill the first circle completely before going onto the second circle.
- Fill the second circle completely, as above, before going on to the third circle, etc.
- Completely fill all circles on the blood collection card. Failure to fill all circles completely may result in a sample insufficient for testing.
- After filling all of the circles completely, apply an adhesive bandage to the finger.
- Label the blood collection card with the identification information required by the protocol or laboratory.
- Air-dry the specimen for at least 3 to 4 hours on a flat, nonabsorbent surface in a horizontal position.
- Do not allow the blood collection card to be exposed to direct sunlight or extreme temperature or humidity.

APPENDIX G

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:

Mr. E. Mabasa

PROJECT TITLE: Assessment of iodine status of pregnant women in Mopani District, Limpopo Province, South Africa.

PROJECT NO: SHS/10/NUT/003

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof. X.G Mbhenyane	University of Venda, Nutrition	Supervisor
Prof. L.O Amusa	University of Venda, Biokinetics, Recreation and Sport Science	Co-Supervisor
Mr. N.S Mabapa	University of Venda, Nutrition	Co-Supervisor

ISSUED BY:

UNIVERSITY OF VENDA, HEALTH, SAFETY AND RESEARCH ETHICS COMMITTEE

Date Considered: 23 November 2010

Decision by Ethical Clearance Committee Granted

Signature of Chairperson of the Committee: .....

Name of the Chairperson of the Committee: Prof. X.G Mbhenyane

UNIVERSITY OF VENDA
PRIVATE BAG X5050, THOHOYANDOU, 0950, LIMPOPO PROVINCE, SOUTH AFRICA
TELEPHONE (015) 962 8504/8484 /8313 FAX (015) 962 8439

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APPENDIX H

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH & SOCIAL DEVELOPMENT

Enquiries: Selamolela Donald
Ref: 4/2/2

07 February 2011
Mabasa E
University of Venda
Thohoyandou
0950

Dear Sir

Re: Permission to conduct the study titled: Assessment of iodine status of pregnant women in Mopani District, Limpopo Province, South Africa

1. The above matter refers.
2. The permission to conduct the above mentioned study is hereby granted.
3. Kindly be informed that:-
   - Further arrangement should be made with the targeted institutions.
   - In the course of your study there should not be any action that will disrupt the services
   - After completion of the study, a copy should be submitted to the Department to serves as a resource
   - The researcher should be prepared to assist in the interpretation and implementation of study recommendation where possible

Your cooperation will be highly appreciated

Head of Department
Health and Social Development
Limpopo Province

Private Bag x 9302 Polokwane.
18 College Str. Polokwane 0700. Tel.: (015) 293 6000 Fax: (015) 293 6211 Website: http/www.limpopo.gov.za

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APPENDIX I

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

Ref: S4/2/1
Enq: Mogale DI

Mabasa Eric
University of Venda
THOHOYANDOU
0950

RE: PERMISSION TO CONDUCT RESEARCH ON "ASSESSMENT OF IODINE STATUS OF PREGNANT WOMEN IN MOPANI DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA".

1. The above matter refers.
2. It is with great pleasure to inform you that permission to conduct research on the above-mentioned topic at Mopani District clinics is granted and also approved with effect from March 2011 to 30 August 2011.
3. We therefore, request you, to provide us with a copy of your thesis on completion as it will serve as a resource for our Department.
4. Please note that when conducting research you should not disrupt any services in the clinics.
5. We hope that you will find this in order.

DISTRICT EXECUTIVE MANAGER

DATE 23/03/11

Private Bag X628, GIYANI, 0826
Tel: (015) 811 6500 Fax: (015) 812 3162 Website: http://www.limpopo.gov.za

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