University of Venda

THE PREVALENCE OF ZINC DEFICIENCY AMONG CHILDREN AGED 3-5 YEARS IN THE VHEMBE DISTRICT OF LIMPOPO PROVINCE

The dissertation is submitted in fulfilment of the requirements of the degree of Master of Public Nutrition in the Department of Nutrition in the School of Health Sciences at the University of Venda.

By

SELEKANE ANANIAS MOTADI

BSCNUT (UNIVEN)

Student number: 11541509

Supervisor: Prof XG Mbhenyane

Co-supervisors: Mr NS Mabapa

Ms HV Mbhatsani

Year: 2014

DECLARATION

I, Selekane Ananias Motadi, hereby declare, that the dissertation submitted to the University of Venda for the degree of Master of Public Nutrition has not previously been submitted by me for a degree at this or any other University and that it is my own work. The sources that I have used or quoted have been indicated and acknowledged by means of complete references.

#178

Selekane Ananias Motadi

25/07/2014

Date

DEDICATION

Dedicated to my late father Selaelo Mathews Motadi and my mother Koena Josephina Motadi

My younger brother Evans Motadi

My younger sisters Koena, Molatelo, Kgabo, Moelelwa, Sebatiane

My son Phethego

ACKNOWLEDGEMENTS

I would like to thank:

- Prof XG Mbhenyane for her commitment, support, enthusiasm and guidance from the beginning to the end of this project.
- Mr NS Mabapa for his encouragement, guidance, tenacity and agility from the beginning to the end of this project.
- Ms HV Mbhatsani for her guidance and support from the beginning to the end of this project.
- The HoD at provincial level and district manager of the Department of Education, Vhembe district for giving me permission to conduct the study.
- Parents/guardians of the children for allowing your children to participate in this study.
- The children for showing interest and participating in this study.
- My research assistants Mabasa E and nurses from Ampath Laboratory without whom I could not have done it.
- Dr RL Mamabolo for your tenacity and guidance in helping me with statistics.
- Ampath Laboratory for analysing my data.
- Ms Matlou Sibaya for editing my masters research document.

ABSTRACT

Background. Children under five years constitute the most vulnerable group and their nutritional status is a sensitive indicator of community health and nutrition. Diet of many South Africans consists of porridge as staple food which is usually consumed with vegetables, legumes and a small amount of animal derived food. These animal derived food sources are not consumed extensively due to their high cost, limited supply and religious or cultural practices.

Objective. To determine the prevalence of zinc and iron deficiency among pre-school children aged 3-5 years in Vhembe district of the Limpopo province, South Africa.

Methods. This study was carried out on 400 pre-school children in Vhembe district, Limpopo province, South Africa. Municipalities were purposively selected and subjects were chosen by simple random sampling methods. Anthropometric measurements were made following standard techniques. Serum zinc, iron, ferritin, Tsauration, transferrin and CRP levels were measured by atomic absorption spectrophotometer.

Results. Of the 400 children, 349 were included in the final analysis of results. The prevalence of wasting, stunting and underweight was 1.4%, 18.6% and 0.3% respectively while 20.9% of the children were overweight and 9.7% were obese. The prevalence of zinc deficiency was 42.6% and anaemia was 28%, both were significantly higher in females as compared to males. When using both serum ferritin and Tsaturation levels as markers of iron deficiency 7(2%) children were found to have IDA. Combined iron and zinc deficiencies using ferritin as a marker of iron deficiency was found in 8(2.3%) of the children while when using Tsaturation as a marker of iron 42(12%) of the children had combined iron and zinc deficiencies.

Conclusion. Zinc deficiency and anaemia are common in pre-school children of Vhembe district, Limpopo province. Iron and zinc deficiency in children is associated with poor growth development, alteration in neurological function, immunological response and behaviour changes.

TABLE OF CONTENTS

CHAPTER TWO

CHAPTER FIVE

CHAPTER SIX

LIST OF TABLES

APPENDICES

ACRONYMS

ACC:	Administrative Committee on Coordination.
ADHD:	Attention Deficit Hyperactivity Disorder.
BAZ:	BMI for age
CDC:	Center for Disease Control and Prevention.
CRP:	C-reactive protein
DNA:	Deoxyribonucleic Acid.
DHS:	Demographic and Health Survey.
FAO:	Food and Agricultural Organization.
FNB:	Food and Nutrition Board.
HAZ:	Height for age
HB:	Haemoglobin
IZA:	International Zinc Association.
IZiNCG:	International Zinc and Nutrition Consultative Group.
MDGs:	Millennium Development Goals
NFCS:	National Food Consumption Survey.
NFCS-FB:	National Food Consumption Survey-fortification Baseline.
NHANES:	National Health and Nutrition Examination survey.
RDAs:	Recommended Dietary Allowances.
RNA:	Ribonucleic Acid.
TSATURATION:	Transferrin saturation
PEM:	Protein-Energy Malnutrition.
SADC:	Southern African Development Community.
SAVACG:	South African Vitamin A Consultative Group.
SCN:	Sub-Committee on Nutrition.

SPSS:	Statistical Package of Social Science.
UNICEF:	United Nations Children's Fund.
WAZ:	Weight for age.
WHZ:	Weight for age.
WHO:	World Health Organization.
WFP:	World Food Programme.

DEFINITION OF TERMS

The following terms are consistently used in this proposal and the definitions are operational

Fasting is defined as no food or beverage consumption for at least 8 hours.

Malnutrition is an incorrect or unbalanced intake of nutrients.

Nutritional status is a condition of health of individuals as influenced by utilization of the nutrients.

Stunting is defined as more than 2 standard deviations below the median value of the WHO International Growth Reference for length- or height-for-age.

Wasting is defined as more than 2 standard deviations below the median value of the WHO International Growth Reference weight-for-height median.

Underweight is defined as more than 2 standard deviations below the median value of the WHO International Growth Reference weight-for-age median.

Zinc is defined as an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement.

Zinc deficiency is caused by inadequate zinc in the diet or by liver disease or cystic fibrosis or other disease.

Recommended dietary allowance (RDA) is the amount of nutrients and calorie intake per day considered necessary for maintenance of good health, calculated for males and females of various ages and recommended by the Food and Nutrition Board of the National Research Council (Institute of Medicine/FNB, 2001)

CHAPTER ONE

INTRODUCTION

1.1 Background and Motivation

Despite the legislated fortification of staple foods in South Africa since 2003, there is a general shortage of zinc in a large part of the population's diets because 75% of zinc is destroyed in the milling process of wheat flour (Labadarios *et al.,* 2005). Furthermore, zinc deficiency still appears to be an important public health problem in many low–income countries (Dhansay, 2007). The global prevalence of zinc deficiency was estimated to be 31% (4%-73%) with a high prevalence of 37%-62% found in southern and central African regions (Caulfield and Black, 2004).

Zinc deficiency is an important cause of morbidity due to increased predisposition to infectious diseases and growth faltering among children (Dhingra *et al.,* 2009). Increased demand of zinc depends on rapid growth and decreased intake of zinc due to inadequate feeding practices predisposed to children, especially those living in communities of low socioeconomic status at an elevated risk of zinc deficiency (Black, 2003).

In recent decades, there has been heightened interest in the role of zinc in human nutrition research, as it plays an indispensable role as a key component of a host of enzymes crucial for optimal metabolism and body functioning [International Zinc Nutrition Consultative Group (IZiNCG, 2007)]. In addition, zinc is an anti-inflammatory and antioxidant agent and also functions in cell-mediated immune responses (Prasad, 2007). In childhood, it is required for optimal growth and development, as well as brain functioning (Gibson, 2006).

Data derived from Food and Agricultural Organisation National Food Balance Sheets (2004) suggests that, about half of the world's population is at risk of inadequate zinc intake (FAO, 2004). Maize meal porridge, rice and bread are the staple foods in the South African diet and are usually consumed with vegetables and a small amount of animal-derived food (Labadarios *et al.,* 2005). Low intake of animal source food leads to low intake of important micronutrients, such as zinc, iron, vitamin A, and calcium (Neumann, 2003).

Iron deficiency causes anaemia leading to compromised physical work capacity and poor growth and development, alteration in neurological function and immunological response (Hambridge, 2000; Hotz and Brown, 2004). Such deficiencies are caused by lack of financial means to acquire and consume red meat, chicken, fish in the amounts required (Iqbal *et al.,* 2006). High incidence rates of infectious diseases in combination with poor nutritional quality of traditional diets contribute to insufficient nutrient intake and are considered the main causes of malnutrition (Pinstrup-Andersen *et al.,* 1993; Waterlow, 1994).

The co-existence of iron and zinc deficiency is a major public health problem (Sreedhar *et al.,* 2004). Iron and zinc are essential micronutrients for human health. Deficiencies in these two nutrients remain a global problem, especially among women and children in developing countries (Adelekan, 2003). However, it seems dietary zinc reduces iron biochemical indices including ferritin, haemoglobin (Hb) and Transferrin Saturation (Paknahad *et al.,* 2007). Meat is the most important common dietary source of bioavailable iron and zinc. Phytates, oxalates, tannins and fibre on the other hand inhibit iron and zinc absorption leading to decreased bioavailability (Shan *et al*., 2003).

Iron and zinc are known to interact at the site of absorption as high intakes of iron can inhibit the absorption of zinc because of competition for similar transport pathways (Sreedhar *et al.,* 2004). Similarly, it has been shown that a high ratio of zinc to iron can inhibit iron absorption (Whittaker, 1998). Moreover, these two minerals have opposing effects on oxidant-antioxidant balance in either iron- or zinc-deficient intestines (Sreedhar and Nair, 2005). Although large nutritional national studies (Labadarios *et al.,* 1999; Labadarios *et al.,* 2005; Samuel *et al.,* 2010) have been conducted among South African children, little is known concerning the prevalence of zinc deficiency and its clinical sign in pre-school children in rural South Africa.

1.2 Problem Statement.

It was estimated that zinc deficiency affects about one-third of the world's population, with estimations ranging from 4% to 73% across sub regions in Sub Saharan Africa (WFP/CDC, 2005). This condition was responsible for 4% of deaths and disability-adjusted life years among children under five years old worldwide (Black *et al.,* 2008). Furthermore, zinc deficiency was responsible for approximately 16% of infections of the lower respiratory tract, 18.5% of malaria cases and 10% of diarrheal diseases (IZiNCG, 2004). In 2002, about 1.4% of deaths worldwide were attributed to zinc deficiency (WFP/CDC, 2005). According to Labadarios (2005) in South Africa, the level of zinc deficiency in children aged 1-9 years ranged between 27% and 58.5%, while the level of zinc deficiency in Limpopo and Mpumalanga provinces were found to be 27.3%. These findings are despite the legislated fortification of flour and maize meals since 2003 (Dhansay, 2007). According to Hotz and Brown (2004), the prevalence of serum zinc deficiency greater than 20% call for national nutrition interventions.

Iron deficiency (ID) and iron deficiency anemia (IDA) are two of the most common nutritional deficiencies among children (Scrimshaw, 1991). World Health Organisation (WHO) estimates that iron deficiency anemia affects quarter of the world population and this is more in pre-school children particularly in Africa (WHO, 2008). Malnutrition is a major underlying cause of deaths in 64% of children under the age of five in South Africa. One in five children is stunted and many are deficient in minerals vital to good health and optimal development (UNICEF, 2009). This study was the first to be conducted in Vhembe district of Limpopo province in order to establish the extent of zinc deficiency in pre-school children aged 3-5 years.

1.3 Aim of the Study

The purpose of the study was to determine the prevalence of zinc deficiency among pre-school children in Vhembe District of the Limpopo province in South Africa.

1.4 Objectives

1.4.1. To determine serum zinc concentrations among pre-school children using flame atomic absorption spectrophotometer.

1.4.2. To determine the presence of clinical signs of zinc deficiency among pre-school children using clinical assessment and liquid zinc.

1.4.3. To determine the anthropometric status (weight and height) of the pre-school children.

1.4.4 To determine the iron status of pre-school children using haemoglobin, serum ferritin, transferrin receptor and C- reactive protein (CRP).

1.4.5 To compare zinc status with anthropometric measurements and iron status.

Prevalence or nutrient status is normally determined by using more than two of the four assessment techniques which are anthropometric, biochemical, clinical and dietary measurements (Lee and Nieman, 2003). In this study three methods were used namely: anthropometric, biochemical and clinical measurements.

1.5 Expected outcomes of the study

The following were expected outcomes of the study:

- Iron and zinc status of pre-school children in Vhembe district.
- Association between zinc status and wasting, stunting and underweight.
- Association between iron status and wasting, stunting and underweight.

1.6 Significance

It is envisaged that findings of this study will help the Department of Health Authorities in understanding zinc and iron status of communities in Vhembe District and in South Africa. As such, intervention measures would be developed to control and prevent zinc and iron deficiencies. Additionally, such data would provide baseline information for future studies concerning zinc and iron status among children in the region, and possibly an intervention strategy should the prevalence be found to be of public health significance.

1.7 Structure of the dissertation

Chapter 1 introduces zinc and iron situation in South Africa. It also presents the aim, objectives, problem statement and the significance of the study conducted.

Chapter 2 deals with a discussion of the reviewed literature on zinc, relationship between anthropometric indices and zinc deficiency, clinical signs of zinc deficiency, determination of zinc status in children, nutritional status of children and iron status.

Chapter 3 describes the methods used to answer the research question.

Chapter 4 reports the findings of the study which include demographic data, anthropometric measurements, iron status, zinc status, combined iron and zinc status and finally correlations between biochemical and anthropometric are presented. Results are presented using tables, percentages, median and Interquartile range.

Chapter 5 discusses the findings of the study. Observed findings are compared to known literature.

Chapter 6 deals with the conclusion and recommendations.

Appendices and references used are listed at the end of dissertation in the order of use.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

Zinc deficiency is largely related to inadequate intake or absorption of zinc from the diet, although excessive losses of zinc during diarrhea may also contribute to this. The distinction between intake and absorption is important: high levels of inhibitors (such as fiber and phytates) in the diet may result in low absorption of zinc, even though intake of zinc may be acceptable. For this reason, zinc requirements for dietary intake are adjusted upward for populations in which animal products, the best sources of zinc, are limited, and in which plant sources of zinc are high in phytates (Cole *et al.,* 2010).

Severe zinc deficiency was defined in the early 1900s as a condition characterized by short stature, hypogonadism, impaired immune function, skin disorders, cognitive dysfunction, and anorexia (Prasad, 1991). Using food availability data, it is estimated that zinc deficiency affects about one-third of the world's population, with estimates ranging from 4% to 73% across sub regions of Africa. Although severe zinc deficiency is rare, mild-to-moderate zinc deficiency is quite common throughout the world (Sandstead, 1991).

Global estimates of zinc deficiency in children are lacking. However, based on an estimated prevalence of inadequate intakes obtained from various national food supplies (including absorbable zinc content), the global prevalence of zinc deficiency is estimated to be 31% (range is 4%–73%) with a high prevalence (i.e. 37%–62%) found in southern and central African regions (Caulfield and Black 2004). It was indicated that zinc deficiency at national level ranged between 27% and 58.5% in South Africa (Labadarios, 2005). In essence, this section contains a comprehensive discussion of what is known about the selected topic and espouses the conceptualisation of the study by means of a well suited theoretical framework. The information included in this section is presented under the following subtopics: Nutritional Status of Children under Five Years of Age; Definition of Zinc; Sources of Zinc; Recommended Dietary Intake (RDI); Function of Zinc and Serum Zinc Levels; Clinical Signs of Zinc Deficiency and the iron status of children.

2.2 Definition of zinc

Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement (IZiNCG, 2004). According to Hambridge (2000), zinc is a trace mineral essential for all forms of life because of its fundamental role in gene expression, cell development and replication.

2.3 Sources of zinc

Human colostrum (the first secretion from the mammary glands after childbirth) is a good source of zinc (Silverstre *et al.,* 2000, 2001; Turan *et al.,* 2001). The zinc content of most municipal drinking water is negligible. Common food sources of zinc are as follows according to Joshi (2002):

Rich sources: Beef, liver, oyster, spices, wheat bran.

Good sources: Cheddar cheese, crab, lamb, peanut butter, peanuts, popcorn, pork and poultry.

Fair sources: Beans, calms, eggs, fish, sausages and luncheon meats, turnip greens, wheat cereals, whole grain products (wheat, rye, oats, rice, barley).

Negligible sources: Beverages, fats and oils, fruits and vegetables, milk, sugar, white bread.

Supplemental sources: Wheat germ, yeast (torula), carbonates or zinc sulphates are commonly used where zinc supplementation is necessary (Joshi, 2002).

Zinc is also found in many cold lozenges and some over-the-counter drugs sold as cold remedies. Oysters contain more zinc per serving than other foods, but red meat and poultry provide the majority of zinc. Other good food sources include lean meats and poultry, beans, nuts and certain types of seafood (such as crab and lobster), whole grains, fortified foods including fortified breakfast cereals, and dairy products (Institute of Medicine/FNB, 2001).

The bioavailability of zinc in different foods varies widely from 5% to 50%. Meat, seafood (in particular Oysters) and liver are good sources of bioavailable zinc (Gibney *et al.,* 2002). It has been estimated that approximately 70% of dietary zinc is provided by animal products. In meat products the zinc content to some extent follows the colour of the meat, so that the highest content, approximately 50 mg/kg, is found in lean red meat, at least twice that in chicken

(Gibney *et al.,* 2002). However, in many parts of the world, most of the zinc is provided by cereals. In cereals, most of the zinc is found in the outer fiber rich part of the kernel, thus the degree of refinement determines the total zinc content (Gibney *et al.,* 2002).

Recommended Daily Intake (RDI) is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97% -98% of healthy individuals. The RDI is used to determine the daily value of foods (**http://healthyeatingclub.com/info/books-phds/foodfacts/htm/datasj.html**, Accessed 22/07/2011). Table 2 illustrates zinc per food serving.

Table 1.1: Zinc per serving

Source of zinc	**Amount**	**Zinc (mg)**
Meat and meat product	Beef stew (250g)	3mg
	Chicken boiled (100g)	2mg
Vegetables	Cabbage, raw (50g)	3mg
	Tomato, raw (120g)	2mg
Milk and milk products	Cow's milk, skim (30g)	1 mg
	Human's milk (100g)	3 mg
Egg and cheese dishes	Egg boiled (55g)	2 mg
	Egg fried (60g)	2 mg
Fruits	Apple, skin (100g)	1mg
	Banana (100g)	2 mg

Source: (http://healthyeatingclub.com/info/books-hds/foodfacts/htm/datasj.html, Accessed 22/07/2011).

Recommended dietary allowance (RDA) is the amount of nutrient and calorie intake per day considered necessary for maintenance of good health, calculated for males and females of various ages and recommended by the Food and Nutrition Board of the National Research Council (USDA, 2006). Phytates and fiber which are present in whole-grain breads, cereals, legumes and other foods-bind zinc and inhibit its absorption (Wise, 1995; Sandstorm, 1997). Thus, the bioavailability of zinc from grains and plant foods is lower than that from animal foods, although many grain-and plant-based foods are still good sources of zinc (Institute of Medicine/FNB, 2001). Table 1 illustrates the recommended dietary allowances (RDAs) for zinc.

Table 1.2: Recommended Dietary Allowance (RDAs) for Zinc

Age	Male	Female
Birth to 6 months	2mg	2mg
7 months to 3 years	3mg	3mg
4 to 8 years	5mg	5mg
9 to 13 years	8mg	8mg
14 to 18 years	11mg	9mg

Adapted from Institute of Medicine/FNB, 2001

2.4 Function of Zinc

Zinc is an essential mineral involved in normal growth and development (Spevackova *et al.,* 1996). Zinc deficiency is associated with poor growth, depressed immune function, hair loss, increased susceptibility to and severity of infections and neurobehavioral abnormalities by increasing number of opiate receptors in the hypothalamus in parallel with decrease of beta-endorphin and an increase to metenkephelin (Black, 1998; Bhutta *et al.,* 2000; Brown *et al.,* 2002; Fraker and King, 2004).

2.4.1 Normal growth and development

Normal growth is the result of a number of factors, such as growth hormones, genetic, nutrition, general health, intrauterine growth, emotional care/deprivation, and psychological factors, as well as the continuous and complex interaction between these factors during the whole period of growth (Eveleth and Tanner, 1990). Human development involves progressive changes in size (Rolfes *et al.,* 2006), as well as accompanying changes in skill and behaviour (Rovee-Collier and Hayne, 2000). Each living organism and each body system has its own unique schedule of growth and development, varying in rate, patterns and duration (Rolfes *et al.,* 2006).

The role of zinc in growth retardation is believed to be via zinc-finger proteins which attach to DNA at critical points and alter the expression of specific enzymes and hormones or other metabolic entities. Under these conditions, the synthesis of these zinc finger proteins become the most sensitive processes affected by zinc deficiency because the regulation, in these cases, is to control the synthesis of the respective enzyme proteins (Oberleas and Harland, 2010). Since thymidine triphosphate is an essential component of DNA replication, the lack of thymidine kinase slows DNA replication and thus explains the depression of growth rate (cell division) in zinc-deficient subjects (Oberleas and Harland, 2010).

2.4.2 Structural component

Zinc plays an important role in the structure of proteins and cell membranes. A finger-like structure, known as a zinc finger motif, stabilises the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzymes copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role (FNB, 2001; King *et al.,* 2006). The structure and function of cell membrane are also affected by zinc, as loss of zinc from biological membranes increases their susceptibility to oxidative damage due to inability to protect cell membranes from viruses, toxins and impairs their function (O'Dell, 2000).

2.4.3 Brain Function

Zinc is found in the vesicles of the mossy fiber system in the brain's hippocampus. These fibers play a role in enhancing memory and thinking skills (Standstead, 1986; Preiffer and Braverman, 1997). It acts as a neuromodulator at excitatory synapses and has a considerable role in the stress response and in the functionality of zinc-dependent enzymes (aromatase, diphosphoinositide kinase and choline acetyltransferase) contributing to maintaining brain compensatory capacity. There is much evidence that age-related changes are frequently associated with a decline in brain functions and impaired cognitive performances, brain shrinks in size, and brain cells (neurons) in certain areas are lost or become faulty (Mocchegiani *et al.,* 2005).

2.4.4 Cellular growth and differentiation

The significance of zinc in the body's response to infection is well known. Zinc is a component of thymic hormone which controls and facilitates the maturation of lymphocytes, and it also plays a role in cell division and DNA replication, thereby aiding in the production of immune system cells (Agget, 1985; Wu and Wu, 1987). The zinc in these enzymes is tightly bound and forms a variety of structures that are functionally important to the enzyme (Chesters, 1991). Numerous enzymes associated with DNA and RNA synthesis are also zinc metalloenzymes, including RNA polymerase (Wu *et al.,* 1992).

Connective tissue and fetal thymidine kinase may be depressed. Zinc functioning in enzyme systems, is largely involved in nucleic acid metabolism, protein synthesis, and carbohydrate metabolism. In rapidly growing tissues, Zinc deficiency greatly reduces synthesis of DNA, RNA, and protein, and hence, impairs cellular division, growth, and repair (Cha and Rojhani, 1997).

2.5 Causes of Zinc deficiency

Zinc deficiency is typically the result of inadequate dietary intake of zinc, disease states that promote zinc losses, or physiological states that require increased zinc. Populations that consume primarily plant based diets that are low in bioavailable zinc often have zinc deficiencies (Sandstead, 1991; Solomons, 2001). Diseases or conditions that involve intestinal malabsorption promote zinc losses. Fecal losses of zinc caused by diarrhea are one contributing factor (Castillo-Duran, 1988), often common in developing countries. Changes in intestinal tract absorbability and permeability due, in part, to viral, protozoal, and bacteria pathogens may also encourage fecal losses of zinc (Manary *et al.,* 2000). Physiological states that require increased zinc include periods of growth in infants and children (Gibson, 2006). Too much supplementary iron can interfere with zinc absorption because they compete for similar transport pathways making one to be more prevalent than the other (Sreedhar, 2004).

2.6 Determination of zinc status of children

Zinc plasma levels help to detect the zinc deficiency which can contribute to mental health problems. Zinc is necessary for immune and brain development. Serum or plasma zinc is the best available biomarker of the risk of zinc deficiency in populations (IZiNCG, 2007). The rationale for this selection is that serum or plasma zinc reflects dietary zinc intake; it responds consistently to zinc supplementation; and reference data are available for most age and sex groups.

Such indicators (biochemical and dietary) can be useful for identifying populations and specific subgroup that are at elevated risk for zinc deficiency and may thus be used to identify groups to whom interventions should be targeted (WHO, 2007; UNICEF, 2007 IAEA, 2007 and IZiNCG, 2007). Biochemical indicators may indicate the severity and extensiveness of zinc deficiency, although additional information will be needed to establish the specific cause of the deficiency. To date, serum zinc is the only biochemical indicator of zinc status known to meet these criteria.

Reference values for serum zinc concentration are based on results obtained from a large sample of presumably well-nourished Americans who participated in the NHANES II survey and were free from infection on the day of blood collection and not on any medications that may have affected their results (Hotz *et al.,* 2003). For population assessment, the recommended indicator to use is the percentage of the population with serum zinc concentrations below the age/sex/time of day-specific lower cut-off (Hotz *et al.,* 2003; IZiNCG, 2004; Hess *et al.,* 2007). This is considered to be elevated and of public health concern when the prevalence of low serum zinc's concentrations is greater than 20% (Hess *et al.,* 2007).

The recommended cut-off for children under 10 years of age is 65 µg/dL (9.9µmol/L) for samples collected in the morning hours in nonfasted state (IZiNCG, 2007). In the study conducted in the western region of Saudi Arabia, the range of serum zinc of all studied ages including infants and adults was reported to be 0.5-13.9 µmol/L, which is lower than the international standard of 7.65-22.95 µmol/L (Kumosani *et al.,* 1997). In Kenya, low serum zinc levels were used as an indicator of poor zinc status. The low plasma zinc level was considered to be < 10.7µmol/l (IZiNCG, 2004), while serum zinc levels between 12-18 µmol/l were considered to be within the normal range (Thompson, 1991; Brown *et al.,* 1998; Wood, 2000).

Despite the legislated fortification of flour and maize since 2003 the level of zinc deficiency among children aged 1-9 years in South Africa ranged between 27.3% and 58.5% with the biochemical indicator of <65 µg/dL (Labadarios *et al.,* 2005). The province with the highest prevalence was Western Cape with 58.5% followed by North West with 41.1%, while Mpumalanga and Limpopo provinces recorded 27.3% of zinc deficiency (Dhansay *et al.,* 2007; Jooste *et al.,* 2007; Labadarios and Louw, 2007). Furthermore, Labadarios *et al.* (2008) indicated that nationally 45.3% of children aged 1-9 years still had an inadequate zinc status. According to IZiNCG (2004), a prevalence of serum zinc deficiencies greater than 20% called for national nutritional intervention.

2.7 Zinc deficiency in Africa/ South Africa

Dietary inadequacy is vital for the aetiology of zinc deficiency. Other aspects include disease states that induce excessive losses or impaired utilisation of zinc and physiological states that increase zinc requirements (Gibson, 2006). Achieving dietary adequacy of zinc may be difficult in developing countries, particularly in resource poor communities, as zinc rich foods, such as meat and fish are usually priced beyond the reach of many households. There is heavy reliance on cheaper plant based diets which are not only poor in zinc content but may also be hindering the bioavailability of zinc (Gibson, 1994).

Inadequate zinc intakes have also been identified among segments of the South African population, such as infants (weaning practices) (Oelofse *et al.,* 2002), children (poor feeding practices) (Faber *et al.,* 2001) and the elderly (physiological changes) (Charlton *et al.,* 2001). The 1999 National Food Consumption Survey (NFCS) was the first survey in South Africa that provided estimates on food consumption and the contribution various foods make to the nutrient intake of children aged 1 and 9 years using a nationally representative sample (Labadarios *et al.,* 2005).

Nationally, 45.3% of children of this age group (1-9) had inadequate zinc intake and were considered to be at risk of zinc deficiency by the authors while prevalence of zinc deficiency among under five children is 20% in Nigeria (UNICEF, 2011). Porridge and rice are the staple foods in the South African diet and are usually consumed with vegetables and a small amount of animal-derived food (Labadarios *et al.,* 2005). Low intake of animal source food leads to low intake of important micronutrients, such as zinc, iron, vitamin A and calcium (Neumann, 2003).

In the follow-up of the NFCS, the impact of food fortification on the nutritional status of South African children has been determined and has indicated that suboptimal zinc status remains a public health problem (Labadarios *et al.,* 2008; Steyn *et al.,* 2008). The province with the highest prevalence of zinc deficiency in SA was Western Cape with 58.5% followed by North West with 41.1%, while Mpumalanga and Limpopo provinces indicated 27.3% of zinc deficiency (Dhansay *et al.,* 2007; Labadarios and Louw, 2007). Perhaps, the differences in serum levels in different areas can be attributed to soil zinc concentrations (Dehghani *et al.,* 2011) and varying traditional diets (Downs *et al.,* 2009).

2.8 Clinical signs for zinc deficiency

Much of what is known about severe zinc deficiency was derived from the studies of individuals born with acrodermatitis enterpathica, a genetic disorder resulting from the impaired uptake and transport of zinc (Kings *et al.,* 2000). The symptoms of severe zinc deficiency include the following:

2.8.1 Growth retardation and development

Significant delays in linear growth and weight gain, known as growth retardation or failure to thrive, are common features of mild zinc deficiency in children. In the 1970s and 1980s, several randomised, placebo-controlled studies of zinc supplementation in young children with significant growth delays were conducted in Denver, Colorado (USA) and it was observed that modest zinc supplementation (5.7mg/day) resulted in increased growth rates compared to placebo (Walravens *et al.,* 1989; Black, 1998; MacDonald, 2000).

A number of studies in developing countries observed similar results with modest zinc supplementation (Black, 1998; Brown *et al.,* 2002). A data from zinc intervention trials confirmed the widespread occurrence of growth-limiting zinc deficiency in young children, especially in developing countries (Hambidge and Krebs, 2000). Although the exact mechanism for the growth-limiting effects of zinc deficiency are not known, research indicates that zinc availability affects cell-signalling systems that coordinate the response to the growth-regulating hormone, insulin like growth factor- (IGF-1) causing zinc absorption suppressed, reducing IGF-

1, which normally supports tissue growth (Fairweather-Tait, 1998; Brown *et al.,* 2002; MacDonald, 2000).

2.8.2 Delayed neurological and behavioural development in young children

Low maternal zinc nutritional status has been associated with diminished attention in new born infants and poorer motor function at six months of age. Zinc supplementation has been associated with improved motor development in very low-birth weight infants and more vigorous activity in infants and toddlers (Caulfield *et al.,* 1998). Additionally, zinc supplementation was associated with better neuropsychological functioning (e.g. attention) in Chinese first grade students, but this was observed only when zinc was provided with other micronutrients (Standstead *et al.,* 1998). Two other studies failed to find an association between zinc supplementation and measures of attention in children diagnosed with growth retardation. Some studies suggest that zinc deficiency may depress cognitive development in young children (Black, 1998).

2.8.3 Hair Loss

Deficiency in zinc can contribute to hair shedding and this result from seasonal changes in linear growth velocity, such that rapid periods of growth lead to a gradual depletion of tissue zinc pools because without zinc and other related minerals, hair shafts get weakened, causing hair breakage and very slow hair re-growth (Rushton, 2002). Zinc benefits for hair include promotion of cell reproduction, tissue growth and repair of broken tissues. It also maintains the oil-secreting glands that are attached to hair follicles, thus decreasing their chances of falling off (Yenigun *et al.,* 2004). Zinc deficiency does not only produce problems with hair loss, but also with variations in the scalp, the scalp may turn out to be too dry or flaky and may at times be irritated because of the deficient nutrients. Turning grey of hair can be stopped, and is the one area where zinc has shown to be effective (Alien, 2007). Even when hair is already grey or becoming thinner and balding, zinc can reverse the effects when taken in proper amounts (Capelle, 2007).

2.8.4 Dandruff

Dandruff is a mild inflammation of the scalp initiated by lethal or nonlethal cellular injury. Cellular injury may be caused by oxygen or nutrient deprivation (zinc), microorganisms causing flaking (McOsker and Hannon, 1967). These flakes are often extremely visible on the hair and often fall on the shoulder showing a high visibility of the problem. There is sometimes itching or soreness and the scalp can feel rather tight. Also highly effective in treating dandruff is zinc. A lot of anti-dandruff shampoos contain zinc pyrithione which seems to be very good at clearing the problem. Very often dandruff or a flaky scalp can be more socially unacceptable than body odour and bad breath. Some people feel that a lack of cleanliness causes dandruff, but this is far from the truth (http://www.stophairlossnow.com/Dandrff.htm). Zinc pyrithionine is an active ingredient in dandruff shampoos and is found to be very effective (Jenkins, 2006).

2.8.5 Delay in wound healing

Zinc helps maintain the integrity of the skin and mucosal membrane (Wintergerst *et al.,* 2007). Patients with chronic leg ulcers have abnormal zinc metabolism and low serum zinc levels (Lansdown *et al.,* 2007) and clinicians frequently treat skin ulcers with zinc supplements (Anderson, 1995). The authors of a systematic review concluded that zinc sulfate might be effective for treating leg ulcers in individuals who have low serum zinc levels (Wilkinson and Hawke, 2000). However, research has not shown that the general use of zinc sulfate in patients with chronic leg ulcers, arterial or venous ulcers is effective (Hawke and Wilkinson, 1998).

2.8.6 Rough skin

The skin is the largest organ in the body and provides a barrier between the body's internal and external environment. Its functions include protection and body temperature regulation. Zinc helps in the stabilisation of collagen and protein, which is necessary for healthy skin. It also helps control the amount of oil in the skin. With age, collagen starts to break down and the skin begins sagging. Zinc supplements are essential for a healthy glowing skin. Zinc sulphate and zinc gluconate help in preventing and reducing acne by normalising functioning of the oil-producing glands (sebaceous) in the skin (Chen *et al.,* 1985). Zinc supplements reduce skin inflammations, hormonal ill effects on the skin and heal blemishes (Jenkins, 2006).

Zinc has a soothing effect on skin and gives immediate relief. Zinc oxide acts as an astringent and drying agent. Zinc sulphate lotions prove effective in skin burns, irritations and surgical incisions. Zinc is an excellent sunscreen as applying lotions containing zinc helps protect skin from harmful ultraviolet rays of the sun. It has also been found beneficial to cure chapped lips. According to reports, zinc has been found to be effective in curing psoriasis but it is not proved safe yet (Jenkins, 2006).

2.8.7 Mild anemia

Iron-deficiency anaemia is a serious world-wide public health problem. Iron fortification programmes have been credited with improving the iron status of millions of women, infants and children. Fortification of foods with iron does not significantly affect zinc absorption, however, large amounts of supplemental iron (greater than 25mg) might decrease zinc absorption (Whittaker, 1998; Institute of medicine and FNB, 2001). Taking iron supplements between meals helps decrease its effect on zinc absorption (Whittaker, 1998). High zinc intake can inhibit copper absorption, sometimes producing copper deficiency and associated anaemia (Broun *et al.*, 1990; Willis *et al.,* 2005).

2.8.8 Attention deficit hyperactivity disorder (ADHD)

Attention deficit hyperactivity disorder (ADHD) is a developmental disorder characterised by inattention, hyperactivity, and impulsivity (Noorbala and Akhondzadeh, 2006). It is the most commonly diagnosed behavioural disorder of childhood, affecting between 3% - 5% of school-aged children (Kidd, 2000). Although many people sometimes have difficulties in sitting still, paying attention, or controlling impulsive behaviour, people with ADHD find that these symptoms greatly interfere with their everyday life. Generally, these symptoms appear before the age of 7 years and can lead to problems in school and in social settings. One- to two-thirds of all children with ADHD continue to have symptoms when they grow up. A diagnosis can be controversial, since there are no lab tests for ADHD and no objective way to measure a child's behaviour (Lamont, 1997).

Some experts also disagree about the best way to treat ADHD, but taking action early can improve a child's educational and social development. Zinc (35 mg per day); regulates the activity of brain chemicals, fatty acids, and melatonin, all of which are related to behaviour. A study of children with attention deficit hyperactivity disorder found that in those given the extra zinc their behaviour improved faster. Furthermore, several studies have found that zinc may help improve behaviour due to regulation of brain chemicals activity, melatonin, but only slightly (Sinn, 2008). Higher doses of zinc can be dangerous; zinc regulates the activity of brain chemicals, melatonin, all of which are related to behaviour. High doses of zinc may aggravate regulation of the activity of brain chemicals and some researchers have suggested that zinc may play a role in regulating the production of dopamine in the brain (Sinn, 2008).

2.8.9 White spot on the finger nails

Leukonychia punctata refers to the condition, where white spots develop on the nails. The other types are leukonychia totalis, leukonychia partialis and leukonychia striata. In leukonychia totalis, the entire nail becomes white, while eukonychia partialis refers to whitening of a part of the nail. On the other hand, leukonychia striata is the condition, where white or discoloured bands appear on the nail (Fatourechi *et al.,* 2002). Out of all these types, leukonychia punctata i.e. white spots on nails is the most common form which can be more commonly observed in young people and women. White spots that appear on several fingernails and keep appearing freshly and it indicates the deficiency of the trace mineral zinc (Admas, 1990).

2.8.10 Diarrhoea

Acute Diarrhoea is associated with high rates of mortality among children in developing countries (WHO/UNICEF, 2004). Zinc deficiency causes alterations in immune response that probably contribute to increased susceptibility to infections, such as those that cause diarrhoea, especially in children (Wintergerst *et al.,* 2007). Studies have shown that poor, malnourished children experienced shorter courses of infectious diarrhoea after taking zinc supplements. The children in these studies received 4-40mg of zinc a day in the form of zinc acetate, zinc gluconate or zinc sulfate (Black, 1998). Zinc supplement studies have found a significant reduction in the duration and severity of episodes of acute diarrhoea in children, especially those with low tissue zinc levels (Sazalwa *et al.,* 1995).

In addition, results from a zinc supplementation in developing countries suggested that zinc helps reduce the duration and severity of diarrhoea in zinc deficient or otherwise malnourished children (Bhutta *et al.,* 2000). Similar findings were reported in a meta-analysis review of zinc supplementation for preventing and treating diarrhoea (Fischer-Walker and Black, 2007; Lukacik *et al.,* 2008). Studies have shown that zinc supplementation reduces the prevalence of common childhood infections (Briefel *et al.,* 2000; Brown *et al.,* 2002; Bhandari *et al.,* 2002).

2.8.11 Night Blindness

A significant symptom of Vitamin A deficiency in developing countries can be due to deficiency of zinc, which is a coenzyme for conversion of retinol to retinaldehyde by retinol dehydrogenase (Mann and Truswell, 2007). In Vitamin A metabolism, zinc is particularly important in the process of retinol-binding protein synthesis, consequently in the hepatic mobilisation of retinol (Ferraz *et al.,* 2004). Zinc deficiency may contribute to Vitamin A deficiency even in the presence of adequate hepatic reserves of the vitamin A (Ahn and Koo, 1995; Christian and West, 1995). As such it is possible that the same mechanisms might be involved in humans.

2.9 Nutritional status of children under five years of age.

Malnutrition prevails everywhere in both developed and developing countries (Abdalla *et al.,* 2008). Besides protein-energy malnutrition, children also suffer from micronutrient deficiency. Pre-school children's age is an important stage of life where nutrition plays an important role and has long lasting effects in later life (Khan Khattak and Ali, 2010). Although good nutrition is universally accepted as a basic human right, it is estimated that globally, more than 800 million people suffer from malnutrition and that in developing countries, more than 20% of the populations are hungry (Khan Khattak and Ali, 2010).

Grant (1993) estimated that in 1990, 193 million pre-school children were underweight in Southern Africa. According to the literature, widespread outspoken hunger may not be a major problem, but the health and nutritional status of millions of Southern Africans are far from optimal. A recent comparison in the Republic of South Africa (RSA) of a few health indicators (infant mortality rate, life expectancy at birth and incidence of tuberculosis) and the money spent on health services compared with those of other countries at similar levels of economic development indicated non optimal use of resources (McIntyre *et al.,* 2001b).

South African national statistics currently use data from the three studies, Labadarios *et al*. (1995 and 2000) and the National Food Consumption Survey- Fortification Baseline (2005) for reporting the nutritional status of children. These studies indicated that nutritional status can be assessed by dietary, anthropometric, biochemical and clinical methods (Faber and Wenhold, 2007). A number of key indicators are useful in determining the level of malnutrition in children. The following are used extensively in research undertaken in South Africa, height-for-age (stunting), weight-for-height (wasting), and weight-for-age (underweight), and in addition micronutrient status including zinc assessment.

2.9.1 Underweight

Underweight is defined as more than 2 standard deviations below the WHO international growth reference for weight-for-age median. A low weight-for-age indicates that an individual is underweight. According to WHO (1995), when 20% - 40% of the population is underweight, it is considered to be a moderate public health problem. However, if more than 40% are underweight, the population is severely underweight (WHO, 1995).

The WHO classifications of the prevalence of underweight are :< 10%, low prevalence; 10-19%, medium; 20%-29%, high and > 30% very high (WHO, 1995). Worldwide, underweight prevalence was projected to decline from 26.5% in 1990 to 17.6% in 2015 (De Onis *et al.,* 2004). South Central Asia had the highest prevalence in the world (43.6%). In South-East Asia, it was 28.9% and in Asia overall, 29.0 %. Most (72%) of the underweight children in the world live in Asia, especially in South Central Asia. The fall in prevalence of underweight in Asia from 1995 to 2000 was 3%-4%, about the same as the decline on a global basis. The prevalence of low weight-for-age also increases with age (Allen and Gillespie, 2001).

The National Food Consumption Survey (Labadorios *et al.,* 2000), undertaken in 1999 in RSA, reported that one in ten children aged one to nine years was underweight. Furthermore, younger children (between 1-3 years) were most affected. The nutritional status of younger children (between 1-6 years) had neither improved nor deteriorated since the SAVACG national data of 1995. The 1999 survey placed emphasis on the high-risk segments of the population and captured a greater percentage of household of lower socioeconomic status than the SAVACG survey (Labadarios, 2000; Labadarios *et al.*, 2005).

2.9.2 Wasting

Wasting is defined as more than 2 standard deviations below the WHO international growth reference weight-for-height median. A healthy child gains approximately 2-3kg of body weight each year from the age of one until adolescence (Kruger *et al.,* 2007). A low weight-for-height reflects wasting, which occurs because of acute nutritional stress and severe food shortage or illness. According to WHO, a prevalence of 5%-10% in a population is regarded as a moderate prevalence, and more than 10% is considered a severe problem (de Onis and Blossner, 2003). Causes of wasting are inadequate food intake, poor feeding practices, disease and infections, or mostly, a combination of these factors are the known causes of wasting (Cogill, 2003).

The expected prevalence of wasting in developing countries is 2%-3%. When wasting rises to about 5%, mortality rates increase substantially (WHO, 1995b). In Asia, the overall prevalence is 10.4%, and is highest in South Central Asia (15.4%), 10.4% in South-East Asia, and lower in East Asia (3.4%). The global prevalence is 9.4% for developing countries. The prevalence in South Central Asia (15.4%) and West Africa (15.6%) are the highest in the world (Allen and Gillespie, 2001).

In 2005, nearly one out of every 20 children (5%) aged 1-9 years was wasted, while 1% of children in this age group was severely wasted. Equal proportions (5%) of 1-3 years and 4-6 years old were wasted in South Africa. The Northern Cape (19%) had the highest proportions of children who were wasted, followed by the Western Cape (12%), Limpopo with 7.5% in 1999 and in 2005 it was 4.4%. The prevalence of wasting in Western and Northern Cape was higher than the national prevalence (5%) while the prevalence of wasting in Limpopo was found to be lower than the national prevalence (Labadarios, 2007). The prevalence of children affected by wasting seems to have decreased in rural areas since 1999. However, it is of concern that the prevalence of wasting and severe wasting for children in urban areas increased from 3.7% in 1999 to 4.5% in 2005 (Berry *et al.,* 2010).

Another study reported that about one in ten of the under five-year old children in Limpopo were severely underweight. A similar situation was found in the North West. The results on wasting from Limpopo (15.0%) and North West (14.4%) reinforced the emerging pattern that in these provinces, the nutritional status of children under five was poorest (Nolunkcwe, 2007). These results are much higher than the observation by Labadarios (2007).

2.9.3 Stunting

Stunting is present when a child's height-for-age is less than -2 standard deviations from the median. A child, whose height-for-age score is less than -3 standard deviations, is severely stunted (WHO, 1995). Stunting usually becomes more prevalent after 3 months of age. The rate of decline in Z-scores starts to slow down at around 18 months and is very gradual after 24 months. Height Z-scores usually stop declining after around 3 years of age (Lindsey and Stuart, 2001). A healthy child grows by 5 – 7 cm each year from the age of one until adolescence (Kruger *et al.,* 2007).

Section 28 (1) (c) in the South African Constitution Bill of Rights gives every child the right to basic nutrition (South African Constitution, 1996). If children are stunted, they have been deprived of their right to basic nutrition over a period of time. The levels of stunting in the country are a good indication of the extent to which children are receiving the basic minimum nutrition needed for physical growth and development. Stunting remains the most-common nutritional disorder affecting children at a national level, with 18% of children aged 1 – 9 years affected in 2005 (Kruger *et al.,* 2007).

Data on stunting rate were derived from the most recent Demographic and Health Survey (DHS) and WHO surveys (WHOSIS, 2006; WHO, 2006; DAHS, 2006) and UNICEF population data of 2007. The results indicate that approximately 30% of the children under 5 years of age are stunted worldwide. The WHO considers a prevalence of stunting greater than 20% of the population to indicate a public health concern (IZiNCG, 2004). It has been estimated that 42% of children less than 5 years old who are living in low-income countries are stunted, a situation which has changed little over recent decades (de Onis *et al.,* 1993).

About 70% of the world's stunted children under five years of age live in Asia (Allen and Gillespie, 2001). Furthermore, Carlson and Wardlaw (1990) indicated that a third of pre-school children (34%) worldwide have stunted physical growth. The recognition of childhood stunting as an indirect indicator of a population's risk of zinc deficiency (Hotz and Brown, 2004), supports the proposition that inadequate zinc intake is a widespread public health problem. Furthermore, Nolunkcwe (2007) confirmed that stunting or chronic malnutrition is the most prevalent form of malnutrition amongst children less than five years in South Africa, as is the case in other developing countries. A comparison between South Africa and other Southern African Development Community member countries indicated that fewer (25%) under-fives in South Africa suffer from chronic malnutrition compared to countries such as Mozambique (41%), Angola (45%) and Lesotho (46%) (Labadarios, 2007). The civil wars in Mozambique and Angola could have contributed to this situation. On the other hand the poorer economy of Lesotho compared to that of South Africa could have been a contributing factor to the poor nutrition levels (Nolunkcwe, 2007).

Nationally (1999), 6% of children showed signs of severe stunting in South Africa. Children living in tribal areas were most affected with a rate of 6%. The high rates of severe stunting of 7% or more are of concern in the Northern Cape, Limpopo and Free State provinces. Nationally, nearly a quarter of children in the 1 – 3-year age group (23%) were affected by stunting, and 6% of this group was severely stunted. Stunting prevalence rates seem to have decreased since 1999, with the greatest improvement in rural areas. The prevalence rate remained the same in urban areas (Labadarios, 2007).

Stunting remains the most common nutritional disorder affecting children at a national level; with 18% of children aged 1-9 years affected in 2005 compared to 1999 where 23% of children were affected. Children living in formal rural areas were worse off; with one out of four children living in tribal areas stunted. The provinces with highest prevalence rates in 2005 than the national (18%) were Free State (28%), Northern Cape (28%) and Limpopo (24%) (Labadarios, 2007). According to the World Health Organization's criteria, these rates indicate a moderate prevalence of stunting (WHO, 1995). The rate of stunting in Limpopo province worsened by 1% from 1999 to 2005 (Labadarios, 2007).

A high prevalence of stunting was found in the Eastern Cape (32.8%) and Limpopo (27.1%) provinces (Nolunkcwe, 2007). These are the two provinces with the lowest mean income of R1 479 and R1 234 per month, respectively according to Census 2001. Western Cape (17.7%) and Gauteng (17.9%) had the lowest levels with the remaining provinces falling between the extremes. Once again, the pattern largely reflects the poor economic development in the other provinces (Nolunkcwe, 2007). The findings of Labadarios (2005) and Nolunkcwe (2007) on stunting, with regard to Limpopo varied by 3.1%.

2.10 Relationship between underweight and, stunting, wasting and micronutrient status (zinc and iron status).

Iron and zinc deficiencies may coexist in children that consume diets with insufficient amounts of animal-source foods (Walker *et al.,* 2005) and cause growth faltering (Brown *et al.,* 2002), delayed development (Idjradinata and Pollitt, 1993) and increased morbidity due to infectious disease (Bhutta *et al.,* 1999) that affect the health, development and well-being. The mediate causes of malnutrition (Under nutrition and micronutrient deficiencies) include poor quality of food, insufficient food intake, and severe or repeated infectious diseases (de Onis and Blossner, 2003).

Stunting is recognised as an indirect indicator of zinc status in some populations because zinc nutrition has been shown to have a significant impact on growth of young children. A meta-analysis of 25 studies of the effect of zinc supplementation on growth in children less than 13 years of age showed that zinc supplementation has a high statistically significant effect on linear growth and weight gain (Brown *et al.,* 1998; Hotz and Brown, 2004).

As a preliminary step in estimating the global prevalence of zinc deficiency, the risk can be estimated for each country based on existing values for the national prevalence of stunting in pre-school children. Data on stunting rates were derived from the recent DHS and WHO surveys

(WHOSIS 2006; WHO, 2006; DHS, 2006). A number of intervention trials have been carried out to assess the impact of zinc supplementation, particularly in low-income populations who are likely to suffer from zinc deficiency. These studies have shown that zinc supplementation increases growth among stunted children (Briefel *et al.,* 2000; Brown *et al.,* 2002; Bhandari *et al.,* 2002).

International Zinc Nutrition Consultative Group estimates that over 25% of the total population in India is at risk of inadequate zinc intake (Hotz and Brown, 2004). A study by Mishra (2005) using the National Family Health Survey (NFHS-2) found that in almost all the states of India, tribal households had a higher incidence of childhood stunting (52,3%) than non-tribal households (42, 8%). Several studies have also reported deficient intake of calories and protein among tribal populations relative to the Indian RDAs, which may be an explanation for the high rates of stunting among this group (Yadav and Singh, 1999; Agte *et al.,* 2005; Mittal and Srivastava, 2006).

In a meta-analysis, the results of more than thirty community-based intervention trials completed in different parts of the world were examined to determine the overall magnitude of growth responses to zinc supplementation. Notably, the responses to zinc supplementation were significantly greater in those studies that enrolled subjects with pre-existing nutritional stunting (Brown *et al.,* 2002). These results indicated that children with low height-for-age are likely to be zinc deficient, and they further suggest that the national prevalence of stunting among children less than 5 years of age can be used as indirect indicators of a population's risk of zinc deficiency (WHO, 1995).

Iron deficiency is particularly prevalent in infants and young children. These age groups of children have higher risk for developing iron deficiency because of the high demand for iron during the period of rapid growth (Gibson, 2005). Studies (Lozoff *et al.,* 1991; Ekiz *et al.,* 2003; Akman *et al.,* 2004) have shown that iron deficiency anaemia has an impact on psychomotor development, cognitive functions and growth as well as on increased risk or respiratory infections. Diet of many people in rural communities of South Africa consists of plant source with a very minimal amount of animal source which are rich sources of iron and zinc.

Consumption of this diet puts them at risk of health outcomes associated with lower intake of nutrients including iron (Labadarios *et al.,* 2005). Iron deficiency remains the most prevalent micronutrient deficiency worldwide (Gibson, 2005). The cause of nutritional anaemia is that the amount of iron absorbed is insufficient to meet body's requirements. This insufficiency may be due to inadequate iron intake from food and low bioavailability (Demaeyer *et al.,* 1989).

2.10.1 Haemoglobin

Iron is an essential component of the hemoglobin molecule, the oxygen-carrying pigment for red blood cells. Measurement of the concentration of hemoglobin in whole blood is probably the most widely used test for iron deficiency anaemia (Gibson, 2005). Iron deficiency anaemia develops only during the third stage of iron deficiency, when iron stores are exhausted and the supply of hemoglobin is therefore an insensitive measure of iron deficiency (Gibson, 2005). Hemoglobin differs according to gender and males have lower haemoglobin concentration than females (Domellof et *al.,* 2002a). The children are considered anaemic when the hemoglobin level is < 11g/dl (WHO, 2001).

2.10.2 Serum Ferritin

Ferritin is the most important intracellular iron storage protein found in reticuloendothelias system. Function of ferritin is not an intermediate protein during hemoglobin synthesis but also as a storage protein for iron released during hemoglobin denaturation. Serum ferritin is the only measure or iron status that can reflect a deficient, excess, or normal iron status (Gibson, 2005). Iron deficiency leads to lower iron stores: serum ferritin values fall progressively as the stores decline, before the characteristics changes in serum iron. Low concentration of serum ferritin is characteristic only of iron deficiency (Dellman *et al.,* 1996). Serum ferritin value of < 12 μg/dL was considered deficient (WHO, 2001).

2.10.3 Transferrin Saturation

The Tsaturation is the serum iron divided by the total iron-binding capacity (TIBC) which corresponds to circulating iron. The iron capacity reflects transferrin, the protein to which virtually all iron in the blood is bound. The ratio of serum iron to transferrin saturation index or percent (called total iron binding capacity) is the most specific indicator of iron deficiency, when is amply low. The transferrin saturation index of <15% indicates iron deficiency, while levels from 5% to 10% make the diagnosis of iron deficiency possible but not authoritative (Wish, 2006).

2.10.4 C-reactive protein

C- reactive protein (CRP), is a test which measures the concentration in blood serum of a special type of protein produced in the liver that is present during episodes of acute inflammation or infection. In the body, CRP plays an important role of interacting with the complement system, an immunologic defence mechanism. Concentration of C-reactive protein of less than 5mg/l was regarded as iron deficient (WHO, 2001).

Iron deficiency is the most prevalent micronutrient deficiency with 3 billion people affected worldwide (SCN, 2004). Iron deficiency exists in all countries, but its magnitude is higher in South East Asia (57%) and Africa (46%) (WHO, 2001). The prevalence of iron deficiency in urban and rural areas of Nigeria showed that more children from rural areas (61%) were more iron deficient than those from urban (45.6%) areas (Onyemaobi *et al.,* 2011). In a reviewed survey of Mexico it was found that children under 3 years of age living in Mexico city (42% for anaemia and 57% for iron deficiency) and in children of low socioeconomic status aged 18 to 36 months, living in rural areas of the centre plateau of Mexico (70% for anaemia and 48% for iron deficiency (Allen *et al.,* 2000).

2.11 Summary

Micronutrient malnutrition continues to be a burden in many developing countries in Africa. Malnutrition is manifested in most developing countries by early stunting and a high prevalence of micronutrients deficiencies. Dietary inadequacy is a key factor in the aetiology of zinc deficiency. Other aspects include disease states that induce excessive losses or impaired utilisation of zinc and physiological states that increase zinc requirements (Gibson, 2006). The findings of the first national survey of 1994 indicated that serum zinc levels in children aged 1-9 years old clearly indicate that zinc deficiency is a problem in this age group (NFCS-FB, 2005). The higher prevalence of zinc deficiency in the younger age group 1-3 years may reflect the poor dietary choices and feeding practices. Fortification may not entirely address the zinc deficiency problem because most of these staple foods are consumed with vegetables which contain high amounts of phytates, oxalates, and tannins which inhibit absorption of zinc and iron.

Zinc and iron deficiencies coexist in malnourished child population of developing nations because they share the same dietary sources. Poverty, poor food choices, lack of availability or decreased accessibility to certain foods, coupled with lack of knowledge about the importance of food group diversity for the health and growth of young children may limit the inclusion of micronutrient rich food in the diets of children. Low serum zinc and iron concentration are normally associated with low dietary intake of food rich in these micronutrients (Adelekan, 2003). Micronutrients rich foods are often expensive and the high cost of these micronutrients rich food make it difficult for poor people to meet their theoretical needs of iron and zinc because they cannot afford them (Ruel, 2003). Insufficient intake of these two micronutrients from their rich sources may lead to growth faltering, delayed development and contribute to mortality and morbidity from diarrhea, pneumonia and stunting in children in developing countries (Bhutta *et al.,* 1999; Brown *et al.,* 2002; Black, 2003). There are still unresolved questions, for instance of the role of zinc supplementation in public health policy to improve mortality, morbidity, growth and development in young children. The overall nutritional status of children as assessed by underweight, wasting and stunting directly influence zinc status as well as other micronutrients.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

Cross-sectional survey was used whereby the researcher aimed at describing the zinc and iron status of pre-school children in the Vhembe District. Cross sectional study is research methods that involve observation of all of a population or a representative subset at one specific point in time (Leedy and Ormrod, 2002). This type of research involved either identifying the characteristics of an observed phenomenon or exploring possible correlations among two or more phenomena (Leedy and Ormrod, 2002).

The research type was quantitative, because the information was collected by quantifiable measures e.g. biochemical variables, weight, height. According to Schneider *et al* (2003) this type of data can be used to assess the prevalence of acute or chronic conditions in a population.

3.2 Populations and Area

The target population was children in the Vhembe district and the accessible population was children aged 3-5 years in Makhado and Mutale municipalities. This target group was selected because children under five years constitute the most vulnerable group of any community. Their nutritional status is a sensitive indicator of community health and nutrition (Ibeanu *et al.,* 2012). Furthermore, pre-school children are at an important stage of life where nutrition plays an important role in growth and development and has long lasting effects in later life (Khan Khattak and Ali, 2010).

The study was conducted in Vhembe district which is one of five districts of Limpopo province. The district is divided into four municipalities namely Thulamela, Makhado, Musina and Mutale. According to 2011 census Vhembe district has a total population 1,294,722. 67.2% of this population speaks Tshivenda, 24.8% speaks Xitsonga, 1.3% speaks Afrikaans and 1.6% speaks Sepedi. For the purpose of this study Mutale and Makhado municipalities were selected. Mutale had an estimated population of 91870 and 148 villages whereas Makhado had an estimated population of 516,031 and 290 villages (Census, 2011).

3.3 Sampling

Sampling is a process of selecting a representative portion of the designated population (Leedy and Ormrod, 2002). The purpose of sampling is to increase the efficiency of a research study (Schneider *et al.,* 2003). According to Barlet *et al.* (2001), a sample is a physical representation of the target population. It should have the property that can identify every single element and include it in the sample.

Mutale and Makhado municipalities were purposively selected. Simple random sampling was used to select preschools and subjects. Simple random sampling is a sampling procedure which provides equal opportunity of selection for each element in a population (Katzenellenbogen *et al.,* 2003; Schneider *et al.,* 2003; Castillo, 2009). A total number of participants in the current study are a representative of all children aged 3-5 years in the chosen municipalities.

A list of pre-schools was obtained from the Department of Education in Vhembe district and each pre-school was assigned a number, these numbers were placed in a bowl and mixed thoroughly. The researcher picked one number at a time from the bowl blind-folded. Each number represented a pre-school. The same procedure was used to select subjects for the study. The following formula was used to calculate the total sample size used in the study: $n = N/ (1 + (N X e^2))$ where

n= sample size

N= total number of children in the pre-schools; and

e = the accepted level of error

$n = N/ (1 + (N X e^2))$

$n = 7873/ (1+ (7873 x 0.05)$

$n = 7873/20.6825$

$n = 380$

The formula yielded a total of 380 subjects and an addition of 10% was added for attrition.

Data was collected from 380 subjects in 16 pre-schools of Makhado and Mutale municipalities. The final sample size was 349 due to the missing values of some variables when data was analysed.

3.4 Inclusion and exclusion criteria

Children who were 3-5 years old (parents consented) and present on the day of data collection were included in the study. Children whose parents/guardians did not give consent were excluded from the study. The researcher did not have any reason to exclude children from the study except those whose parents did not give consent. Children who were included in the study were free from infection and not taking any medication.

3.5 Subject recruitment

First Visit

A list of pre-schools was obtained from the district office and pre-schools were randomly selected. The selected pre-schools were visited and the research aims and objectives were explained to the headmaster. In addition, permission to include pre-schools in the sample was requested.

Second Visit

The researcher gave children consent forms to take to their parents to sign if they agree to have their children included in the study. Children were instructed to return the consent forms to the headmaster the following day.

Third Visit

The researcher gave more explanation to parents who needed clarity before they could give consent for children to participate in the study. Data was also collected at this stage.

3.6 Measurements

The following measurements were carried out: anthropometric measurements, biochemical measurements and clinical signs.

3.6.1 Anthropometric measurements

In this study, anthropometric measurements included weight and height. These anthropometric measurements were used to assess body size. The weight and height were taken twice and the average was calculated. This was done to reduce error and to increase accuracy. Anthropometric measurements (weight and height) where analysed using WHO Antro and Antroplus version 3.2.2 (WHO, 2011).

3.6.1.1 Weight

The children were weighed without shoes and wearing light clothes. The subjects stood still in the middle of the platform of the scale without touching anything and with the body weight equally distributed on both feet. The weights were taken by registered nutritionist using Seca solar scale (model 0213). The Seca solar scale was also calibrated continuously against the electronic scale. The weight was recorded to the nearest 0.1kg (Lee and Nieman, 2003). A solar scale was used because some pre-schools did not have electricity.

3.6.1.2 Height

Height was taken using a height meter. The subject was barefooted and heels close together, arms to side, legs straight and knees together, looking straight ahead and wearing minimal clothes. The subjects were standing with shoulders relaxed and looking straight ahead. Heels, buttocks, scapular (shoulder blade) and back of the head were against the vertical surface in a Frankfurt position (Lee and Nieman, 2003). The height was recorded in centimetres. The height was recorded to the nearest 0.1cm (Lee and Nieman, 2003).

3.7 Clinical signs

A professional nurse checked the clinical signs. Zinc deficiency is associated with lack of gustin which leads to low salivary zinc concentration (Pluhator *et al.*, 1996), and can potentially lead to impaired taste acuity and loss of appetite (Bales *et al.*, 1986; Uchida *et al.*, 2000). Liquid zinc was used to determine whether the subject was zinc deficient and oral zinc test was done using liquid zinc to help determine whether children with impaired taste were zinc deficient or not (**see appendix C**). Children below 5 years are not considered to be able to express differences in taste perception by use of the preferential methods. A reliable estimation of difference particularly in children less than five years was achieved using the child's own spontaneous verbal judgment (Sjovall *et al.*, 1984; Uchida *et al.*, 2000).

The procedure to check clinical signs was to place 10ml (about two teaspoons) of the liquid in the mouth, hold the liquid zinc in the mouth for about ten to fifteen seconds and observe facial expressions and taste. A professional nurse asked the children what liquid zinc tasted like and recorded the results. Clinical signs like hair loss and white spot on the finger nails which could be identified by a naked eye were also checked.

3.8 Blood collection

3.8.1 Blood samples

A professional nurse collected the 380 blood samples from 16 pre-schools in Makhado and Mutale municipalities. The subject's skin was cleaned with alcohol-soaked gauze pads at the site of the antecubital vein and restrict occlusion of subject's arm with tourniquet for < 1 minute. A trace element-free siliconised 21-gauge minicath intravenous catheter was inserted into an antecubital vein, and blood was drawn into two trace element-free evacuated tubes that utilised siliconised rather than rubber. Rubber tip plunger was not used since rubber is a zinc contaminant. Once the blood was drawn it was kept for 5 minutes and centrifuged to separate serum from the blood. Serum was separated from the cells within 5 minutes after blood collection. Following the centrifugation serum was transferred into the new tube for storage in a cooler box with crushed ice until analysis at Ampath laboratories using standardised methods.

125I- Radioimmunoassay was used for the quantitative determination of zinc in serum (**see appendix D**). Access Ferritin assay is a two–site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with goat anti-ferritin-alkaline conjugate, and paramagnetic particles coated with goat anti-mouse: mouse anti-ferritin complexes (**see appendix E**). C-RP and Transferrin reagents, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems CAL 5 Plus, are intended for quantitative determination of C - reactive protein and transferrin concentration in human serum or plasma. CRP and Transferrin reagents are used to measure C-reactive protein and Transferrin concentration by a turbidimetric method (**see appendix F and G**).

3.9 Data collection procedure

3.9.1 Physical arrangements

A separate class room was requested to be used to ensure privacy. Two stations were arranged, the first station was for taking anthropometric measurements and demographic information such as age and gender. Weight and height were taken with minimal clothes without shoes on and with subjects relaxed and head in a Frankfort position.

In the second station a professional nurse was drawing blood. One child was brought to the room at a time because the researcher did not want those who were scared of needles to scare the rest of the study subjects. One needle was used per child. The researcher was present throughout the data collection

3.10 Tools and specific procedures

Data was collected using a record sheet. Literature on the same field of study was reviewed to gather information on how other researcher conducted their studies using the same procedure as in the current study. The following variables were measured: age, gender, anthropometric measurements, biochemical measurement and clinical signs (**see Appendix C**). The record sheet was developed and pilot tested to check for anything that could impede the instrument's ability to collect data in economical and systematic fashion. Additional information such as age, gender, time of day and time since last meal were included in the record sheet.

3.11 Field workers

Two professional nurses and a nutritionist were recruited as fieldworkers. Field workers were trained by the researcher and methods of data collection were standardised. Professional nurses were responsible for drawing blood as it is a requirement by the Health Professional Council of South Africa. A nutritionist was also recruited to take anthropometric measurements. The appointment of fieldworkers was based on their expertises which were required for the study and for good general practice.

3.12 Quality control

External and internal validity

The validity of a measurement instrument is the extent to which the instrument measures what it is supposed to measure. Furthermore, Reliability is the consistency with which a measuring instrument yields results with repeated measurements, preferably more than 10 repeats. Both validity and reliability, then, reflect the degree to which we may have errors in our measurements (Polit and Hungler, 2009).

For quality control Zinc, CRP and Transferrin were handled as though capable of transmitting infectious diseases because they are of human origin. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to Human Immunodeficiency Virus (HIV) and hepatitis C virus infection (HCV) and nonreactive for Hepatitis B surface antigen (HbsAg). At least two levels of control material were analysed daily. In addition, these controls were with each new calibration, with each reagent cartridge, and after specific maintenance or troubleshooting procedure as detailed in the appropriate system manual. The system must have a lot-specific parameter card and a valid calibration adjustment in memory before controls or patient sample can be run (**see appendix D**).

The Access Ferritin assay is a two-site immunoenzymatic (sandwich) assay. A sample is added to a reaction vessel with goat anti-ferritin-alkaline phosphate conjugate, and paramagnetic particles coated with goat anti-mouse: mouse anti-ferritin complexes. Serum or plasma (heparin) ferritin binds to the immobilized monoclonal anti-ferritin on the solid phase, while the goat anti-ferritin enzyme conjugate reacts with different antigenic sites on the ferritin molecules. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of ferritin in the sample. The amount of analytic in the sample is determined from a sorted, multi-point calibration curve.

3.13 Institutional approval

The research proposal was submitted to the Higher Degrees and Ethics committee for approval before data collection and ethical clearance certificate was issued (SHS/12/NUT/02/0212). A letter together with ethical clearance certificate and the research proposal were submitted to the Departments of Health and Education provincial office requesting permission to conduct research in pre-schools (**see appendix B**). Once permission was granted by the Departments of Health and Education a list of pre-schools was obtained from the Department of Education, Vhembe district. Permission to include pre-schools into the sample was sought with the headmasters.

3.14 Ethical consideration

No subjects were enrolled into the study until the protocol, subjects' information sheet and recruitment material was approved in writing by the Higher Degree and Ethics Committee of the University of Venda. The study was performed in accordance with principles of the Declaration of Helsinki (2008), Good Clinical Practice and the laws of South Africa. No subject entered the study without signed informed consent by parents after a full and adequate oral and written explanation of the study, including possible risks, has been provided by the investigator **(see appendix A)**. Subjects had the right to withdraw from the study at any time. If in the process of the research a subject was diagnosed with a particular nutrition related health risk or disease, the subject was informed; if informed, the subject was referred to appropriate health professional to address the diagnosed risk or disease. Data generated from the study was stored in a computer database, and in a manner that maintained subjects' confidentiality. For data verification and quality control purposes regulatory authorities and/ or members of the Higher Degree and Ethics Committee of the University of Venda might be allowed access to patient data under conditions of strict confidentiality. This was stated in the patient information sheet. The anonymity of subjects was ensured by using codes instead of names and in any publication of the data.

3.15 Data analysis

3.15.1 Nutritional status

Weight for age, height-for-age and weight-for-height were analysed using the Z-scores. The Z-scores system expressed the anthropometric values as a number of standard or Z-scores below or above the reference mean or median value (http://www.who.into/nutgrowthdb/into, Accessed 22/03/2013). Z-scores which were gender and age independent thus permitting the evaluation of children's growth status was determined by weight for age (WAZ), height for age (HAZ) and weight for height (WHZ). The Z-scores classification indicated in Table 3.1 was used for interpretation.

Table 3.1: Z-scores classification (http://www.who.into/nutgrowthdb/intro)

Z-scores	WAZ	HAZ	WHZ
<-3	Very low WAZ	Very low HAZ	Very low WHZ
Between -2 and -3	Low WAZ	Low HAZ	Low WHZ
Between -2 and +2	Normal WAZ	Normal HAZ	Normal WHZ
Between +2 and +3	High WAZ	High HAZ	High WHZ
Above +3	Very high WAZ	Very high HAZ	Very high WHZ

WAZ: Weight for age (underweight); HAZ: Height for age (stunting); WHZ: Weight for height (wasting)

For the purpose of establishing whether the observation on nutritional status are of public health importance, the following classification for assessing severity of Malnutrition by prevalence range was used (http://www.who.into/nutgrowthdb/into , Accessed 22/03/2013)

Table 3.2: Severity of malnutrition by prevalence ranges

Indicator	Severity of malnutrition by prevalence ranges (%)			
	Low	Medium	High	Very high
Stunting	<20	20-29	30-39	≥ 40
Underweight	< 10	10-19	20-29	≥30
Wasting	<5	5-9	10-14	≥ 15

3.15.2 Clinical signs

The following classification was used to categorise the clinical signs of zinc deficiency using liquid zinc:

3.3 Classification of zinc deficiency using liquid zinc (Crosby, 2009)

Zinc Status	Description
Good level (>9.9mmol)	If the zinc tastes bad, with a strong, unpleasant metallic taste and perhaps makes your tongue feel fuzzy you have a good level of zinc in your body.
Right amount (9.9mmol)	If there is a strong, but not unpleasant taste may be a little bit metallic, and the taste intensifies as time goes by, you have a right amount of zinc in your body.
Low level (<9.9mmol)	If at first there is little taste, but after ten seconds or so it tastes a little sweet or gives your tongue "fuzzy" sensation you are low in zinc.
Severe (<7mmol)	If it tastes like water or is "tasteless" you are extremely deficient in zinc.

3.15.3 Blood analysis

Comparisons were made to the normal values of International Zinc Consultative Group for children aged 1-9 (IZiNCG, 2007). The following blood constituents were analysed: serum iron, serum ferritin, transferrin saturation, C-reactive protein.

Table 3.4: An indication of lower cut-offs for serum zinc concentration (µmol/dL) by age group, gender, time of day and time since last meal (IZiNCG, 2007).

Time of day And Fasting status	<10 years	>or=10 years	
	Males and Females	**Non-pregnant females**	**Males**
Morning, fasting	Not available	70µg/dL	74µg/dL
Morning, Non-fasting	65µg/dL	66µg/dL	70µg/dL
Afternoon Non-fasting	57µg/dL	59µg/dL	61µg/dL

For conversion to µmol/L, divide by 6.54

3.16 Statistic methods

WHO Anthro was used to analyse anthropometric measurements. Weight for age, height for age and weight for height were analysed using the Z-scores. The Statistical Package for Social Sciences (SPSS) version 21 was used to analyse descriptive statistics (median and Interquartile range), Frequency, correlations and logistic regressions.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

This chapter presents the study results obtained from a sample of 349 subjects from Makhado and Mutale municipalities. The results presented herein include demographic data, anthropometric measurements, iron status, zinc status, combined iron and zinc interactions and lastly correlations between biochemical and anthropometric variables. The results are presented in the form of tables, percentages, standard deviations, means, medians, interquartile ranges and p-values.

4.1.1 Demographic Data

The study was carried out on 380 pre-school children aged 3-5 years and the number of children included in the analysis was 349. The sample reduction was due to missing values for some of the variables. About 186 (53.3%) of the study subjects were girls while 163 (46.7%) were boys. The minimum age for boys was 2.96 years and maximum 5.89 years while the minimum age for girls was 2.95 years and maximum was 5.89. Table 4.1 summarises distribution of subject by age and gender.

Table 4.1: Age and gender distribution

Gender	Sample size	Mean age (years)	Std deviation	Minimum age	Maximum age
Boys	163	4.5	0.73	2.96	5.89
Girls	186	4.5	0.65	2.95	5.89

4.2 Zinc status

Bad taste of liquid zinc was considered to be indicative of good serum levels of zinc while those who indicated that they had little taste of sweetness were considered to have low serum zinc levels. Children who indicated that liquid zinc was tasteless were considered to be extremely deficient in zinc (Crosby, 2009). Four percent (4.1%) of the children indicated that liquid zinc tasted bad while 30% indicated that there was little taste than sweet. About 65.9% indicated that liquid zinc was tasteless. In boys 1.7% indicated that liquid zinc tasted bad as compared to 2.4%

of girls. In girls, 17.2 % indicated that they had little taste as compared to 12.8% of boys. However, 32.1% of boys indicated that liquid zinc was tasteless as compared to 33.8% of girls. In addition 0.5% of the children showed symptoms of zinc deficiency. With respect to symptoms of zinc deficiency the prevalence was the same in both gender groups (0.5%) (Table 4.2).

Table 4.2: Zinc Taste Test

Description of taste	Boys (n=163) n %	Girls (n=186) n %	Total (n=349) n %
Taste Bad	6 (1.7%)	8 (2.4%)	14 (4.1%)
Little taste than sweet	45(12.8%)	60 (17.2%)	105 (30%)
Taste less	112 (32.1%)	118 (33.8%)	230 (65.9%)

Table 4.3 illustrates the prevalence of zinc deficiency using serum zinc. The prevalence of zinc deficiency was defined as serum zinc levels below 9.9 µmol/dl (IZiNCG, 2007). Of all the children, 149 (42.6%) were zinc deficient. The prevalence of zinc deficiency was virtually the same in boys and girls.

Table 4.3: Prevalence of zinc deficiency using serum zinc

Zinc classification	Boys (n=163) n (%)	Girls (n=186) n (%)	Total (n=349) n (%)
Deficient (<9.9 µg/dl)	65 (18.6%)	84 (24%)	149 (42.6%)
Normal (>9.9 µg/dl)	98 (28%)	102 (29.3%)	200 (57.3%)

4.2.1 Association between serum zinc and liquid zinc

Of all girls 8 (2.4%) who indicated that liquid zinc tasted bad 5 (1.4%) were zinc deficient when assessed by serum zinc levels while from 60 (17.2%) of girls who indicated that they had little taste than sweet 28 (8.1%) were zinc deficient as determined by serum zinc levels. From 118 (33.8%) girls who indicated that liquid zinc was tasteless 51 (14.6%) were zinc deficient when assessed by serum zinc levels. Of all boys 6 (1.7%) who indicated that liquid zinc taste bad 3 (0.8%) were zinc deficient when assessed by serum zinc levels while from 45 (12.8%) who indicated that they had little taste than sweet 25 (7.2%) were zinc deficient when assessed by serum zinc levels. Furthermore, 112 (32.1%) boys who indicated that liquid zinc was tasteless 37 (10.6%) were zinc deficient when assessed by serum zinc levels.

4.3 Anthropometric Measurements

Table 4.4 shows the median and Interquartile ranges of anthropometric measurements in the total population as well as by gender. There were no gender differences in anthropometric measurements.

Table 4.4 Anthropometric and biochemical variables of children by gender (median and Interquartile range)

Variables	Total (Median and Interquartile range)	Boys (Median and Interquartile range)	Girls (Median and Interquartile range)	P-Values
Weight (kg)	16.00(2.50)	17.00(2.75)	16.50(2.75)	0.599
Height (cm)	99.95 (7.45)	101.50 (7.90)	102.35(9.70)	0.954
WHZ	0.56(6.48)	0.10 (1.60)	0.19 (1.45)	0.321
HAZ	-1.02 (1.33)	-1.11 (1.34)	-0.92 (1.43)	0.078
WAZ	-0.23 (4.83)	-0.28 (1.09)	-0.22 (1.18)	0.466
BAZ	0.58 (1.40)	0.52 (1.34)	0.36 (1.42)	0.610

WAZ: Weight for age (underweight); HAZ: Height for age (stunting); WHZ: Weight for height (wasting); BAZ: BMI for age (Overweight).

The prevalence of wasting, stunting and underweight was 1.4%, 18.6% and 0.3% respectively while 20.9% of the children were overweight and 9.7% were obese (Table 4.5). Stunting was more prevalent in boys (10%) as compared to girls (8.6%). Furthermore, 3.1% of boys were wasted while no females were found to be wasted. About 0.6% of boys in the study were underweight while no girls were found to be underweight. Overweight and obesity levels were high in girls (11.2 and 5% respectively) as compared boys (9.7% and 4.7% respectively). Table 4.5 summarizes nutritional status of pre-school children.

Table 4.5: Anthropometric indices classification of nutritional status of the studied pre-school children

Anthropometric Index	Boys (n=163) n	%	Girls (n=186) n	%	Total (n=349) n	%
Wasting	5	1.4%	0	0	5	1.4%
Stunting	35	10%	30	8.6%	65	18.6%
Underweight	1	0.3%	0	0	1	0.3%
Overweight	34	9.7%	39	11.2%	73	20.9%
Obese	16	4.7%	18	5%	34	9.7%

4.4 Iron Status

Table 4.6 shows the median and interquartile ranges of biochemical measurements in the total study population as well as by gender. There were no differences in biochemical variables between the two gender groups.

Table 4.6: Median and Interquartile Range of biochemical measurements of boys and girls

Variables	Total (n=349)	Boys (n=163)	Girls (n=186)	P-Values
Hb(g/dl)	11.40(1.1)	11.40(1.1)	11.30(1.1)	0.662
IRON (umol/l)	13.20(8.3)	13.20(8.7)	13.90(8.3)	0.747
TRANSFERRIN (g/l)	2.60(0.5)	2.50(0.5)	2.50(0.4)	0.930
TSATURATION (%)	21.00(13.0)	20.00(14.0)	22.00(13.0)	0.670
FERRITTIN (ng/ml)	25.00(18)	28.00(17.0)	26.00(15.50)	0.257
CRP(mg/l)	0(2.00)	0(2.00)	0(2.00)	0.998
ZINC	10.15 (4.5)	11.40 (3.30)	10.30 (5.10)	10.30 (5.10)

HB- haemoglobin; T saturation: Transferrin saturation; CRP: C-reactive protein.

Anaemia was classified according to the WHO recommended cut-off value of less than (<) 11.0 g/dl for Hb (WHO, 1989). Hb concentration levels less than 7.0g/dl were considered as severe anaemia, 7.0 to 9.9 g/dl as moderate anaemia and Hb > 10.0 g/dl to < 11.0 g/dl as mild anaemia. Of all the children 14 (4%) had moderate anaemia while 84 (24%) had mild anaemia. About 2% of boys and girls had moderate anaemia. Boys 36 (10.3%) had mild anaemia as compared to 48 (13.7%) girls (Table 4.7).

Table 4.7: The prevalence of anaemia as determined by haemoglobin (Hb) Levels (11g/dl)

Hb levels	**Boys (n=163)**	**Girls (n=186)**	**Total (n=349)**
Severe (<7.0 g/dl)	0	0	0
Moderate (7.0-9.9 g/dl)	7(2%)	7(2%)	14(4%)
Mild (10.0 - 11g/dl)	36(10.3%)	48(13.7%)	84(24%)
Normal	120(34.5)	131(37.5%)	251(72%)

Table 4.8 illustrates the prevalence of micronutrient deficiencies as determined by the selected biochemical variables. Haemoglobin (Hb) levels below 11 g/dl were used as an indicator of anaemia (WHO, 1989). Of all the children 98 (28%) were anaemic. Ferritin levels below 12μg/dl were taken as indicative of iron deficiency and it was found that 7.1% of the children were iron deficient (WHO, 1989). Furthermore, 28.1% of the children were iron deficient when transferrin saturation (Tsaturation) below 15% was taken as an indicator of iron deficiency (Floyd, 2002; Wish, 2006). Using both serum ferritin and Tsaturation as markers of iron deficiency revealed that 15 (4.2%) of the children were iron deficient.

The prevalence of anaemia was high in girls (15.7%) as compared with 12.3% of boys. Of the girls 4% had low serum ferritin as compared to 3.2% of the boys. Furthermore, 15.2% of girls had low Tsaturation to 12.9% of boys. When iron deficiency anaemia (IDA) was determined by using Tsaturation as a marker of iron deficiency this indicated that 36 (10.3%) children had IDA, while when using serum ferritin levels it was found that 12 (3.4%) children had IDA. When using both serum ferritin and Tsaturation levels as markers of iron deficiency 7 (2%) children were found to have IDA.

Table 4.8: Prevalence of micronutrient deficiencies as determined by the selected biochemical variables

Variables	Boys (n=163)		Girls (n=186)		Total (n=349)	
	Low	**Normal**	**Low**	**Normal**	**Low**	**Normal**
Haemoglobin	43 (12.3%)	120 (34.4%)	55 (15.7%)	131(37.5%)	98 (28%)	251 (72%)
Ferritin levels	11 (3.2%)	152 (43.5%)	14 (4%)	172 (49.3%)	25 (7.2%)	324 (92.8%)
Tsaturation	45 (12.9%)	118 (33.8%)	53 (15.2%)	133 (38.1%)	98 (28.1%)	251 (71.9%)

4.5 Comparison between zinc and iron, anthropometry

4.5.1 The coexistence of iron and zinc

The coexistence of iron and zinc deficiency was determined using ferritin as a marker of iron deficiency was found in 8 (2.3%) of the children while when using Tsaturation as a marker of iron deficiency, it was found that 42 (12%) of the children had both iron and zinc deficiencies. When iron status was assessed using both serum ferritin and Tsaturation as markers of iron deficiency it was found that 6 (1.7%) of the children had combined iron and zinc deficiencies. Table 4.9 shows the median and Interquartile ranges of biochemical measurements in the stunted and non-stunted children. There were no differences in biochemical variables between the two groups.

Table 4.9: Median and Interquartile Range of biochemical measurements of stunted and non-stunted children

variables	Total (n=349)	Stunted (median and Interquartile) n = 65	Non Stunted (median and Interquartile) n = 284	P-Values
Hb (g/dl)	11.40(1.1)	11.50(1.00)	11.40(1.1)	0.615
IRON (umol/l)	13.20(8.3)	13.40(6.20)	13.90(9.3)	0.199
TRANSFERRIN (g/l)	2.60(0.5)	2.50(0.4)	2.60(0.4)	0.207
TSATURATION (%)	21.00(13.0)	20.00(11.5)	21.00(14.0)	0.272
FERRITIN (ng/ml)	25.00(18)	29.00(16.50)	26.00(17.50)	0.632

CRP (mg/l)	0(2.00)	0(3.00)	0(2.00)	0.137
ZINC (umol/l)	10.15(4.5)	11.40(3.30)	10.30(5.10)	0.071

HB- haemoglobin; T saturation: Transferrin saturation; CRP: C-reactive protein.

4.5.2 Correlations between biochemical markers and anthropometric measurements in the studied pre-school children.

Table 4.10 shows the correlation co-efficient between biochemical markers and anthropometric measurements in the studied pre-school children. In the total studied population serum C - reactive protein (CRP) was negatively correlated with transferrin (r= -0.110; P=0.040) and T saturation (r= -0.277; p<0.0001) but positively correlated with ferritin (r= 0.128; p= 0.017). Zinc was positively correlated with T saturation (r=0.107; p=0.045) but negatively with CRP (r= - 0.112; p=0.037). Furthermore, CRP was negatively correlated with weight (r= -0.156; p= 0.003). Height was positively correlated with ferritin (r= 0.112; p= 0.037) but negatively correlated with CRP (r= -0.154; p=0.004). WHZ was positively correlated with transferrin (r=0.169; p=0.002) but negatively correlated with zinc (r=-0.174; p=0.001). HAZ was negatively correlated with CRP (r=-0.145; p=0.007) and zinc (r=-0.122; p=0.022). WAZ was positively correlated with transferrin (r= 0.136; p=0.038) while BAZ showed a positive correlation with transferrin (r=0.24; p= 0.021).

Table 4.10: Correlation between biochemical markers and anthropometric measurements (n=349)

		Age (years)	WEIGHTKG	HEIGHTCM	WHZ	HAZ	WAZ	BAZ	CRP	ZINC
HB	r	0.046	0.054	0.075	0.013	0.086	0.028	-0.009	-0.129	0.003
	p	0.394	0.318	0.159	0.808	0.109	0.676	0.867	**0.016**	0.959
IRON	r	0.117	0.037	0.075	-0.071	0.015	-0.043	-0.051	-0.304	0.103
	p	0.028	0.491	0.164	0.185	0.774	0.514	0.341	**<0.0001**	0.055
TRANSFERRIN	r	-0.104	0.020	-0.027	**0.169**	0.045	**0.136**	**0.124**	**-0.110**	-0.093
	p	0.052	0.715	0.615	**0.002**	0.398	**0.038**	**0.021**	**0.040**	0.083
TSATURATION	r	0.158	0.064	0.107	-0.094	0.025	-0.047	-0.061	-0.277	0.107
	p	0.003	0.235	0.046	0.079	0.648	0.477	0.257	**<0.0001**	**0.045**
FERRITIN	r	0.169	0.057	**0.112**	-0.104	-0.018	-0.070	-0.056	**0.128**	0.079
	p	0.002	0.287	**0.037**	0.052	0.738	0.289	0.297	**0.017**	0.139
CRP	r	-0.081	**-0.156**	**-0.154**	-0.025	**-0.145**	-0.114	-0.017	-	**-0.112**
	p	0.132	**0.003**	**0.004**	0.638	**0.007**	0.084	0.753		**0.037**
ZINC	r	0.117	-0.077	-0.038	**-0.174**	**-0.122**	-0.116	-0.104	-0.112	-
	p	0.029	0.152	0.483	**0.001**	**0.022**	0.079	0.052	0.037	

WAZ: Weight for age; HAZ: Height for age; WHZ: Weight for age; BAZ: BMI for age; HB- haemoglobin; Tsaturation: Transferrin saturation; CRP: C-reactive protein.

4.5.3 Correlations between biochemical markers and anthropometric measurements in boys and girls.

In girls children CRP was negatively correlated with transferrin (r=-0.124; p=0.091) and Tsaturation (r= -0.237; p<0.0001). WHZ showed a negative correlation with serum ferritin (r= -0.161; p=0.028) and zinc (r= -0.194; p= 0.008). HAZ on the other hand was negatively correlated with CRP (r= -0.146; P =0.046), and BAZ was negatively correlated with serum zinc (r=-0.164; p=0.025) (Table 4.11).

Table 4.11: Correlations between biochemical markers and anthropometric measurements in girls (n=186)

		Age (years)	WEIGHTKG	HEIGHTCM	WHZ	HAZ	WAZ	BAZ	CRP	ZINC
HB	r	0.077	0.113	0.102	0.101	0.100	0.161	0.071	-0.087	-0.065
	p	0.293	0.124	0.167	0.169	0.174	0.068	0.338	0.239	0.376
IRON	r	0.142	0.019	0.094	-0.109	0.004	-0.124	-0.091	-0.254	0.017
	p	0.053	0.797	0.201	0.140	0.953	0.160	0.215	**<0.0001**	0.821
TRANSFERRIN	r	-0.079	0.028	-0.040	0.132	0.003	0.044	0.102	-0.124	0.005
	p	0.284	0.709	0.592	0.072	0.970	0.623	0.168	0.091	0.946
TSATURATION	r	0.178	0.044	0.124	-0.125	0.024	-0.112	-0.101	**-0.237**	0.002
	p	0.015	0.548	0.091	0.088	0.750	0.205	0.169	**<0.001**	0.975
FERRITIN	r	0.162	-0.023	0.107	**-0.161**	-0.005	-0.144	-0.128	0.135	0.035
	p	0.027	0.751	0.145	**0.028**	0.941	0.105	0.082	0.066	0.640
CRP	r	-0.079	-0.117	-0.138	0.101	**-0.146**	-0.055	0.076		-0.039
	p	0.286	0.112	0.061	0.170	**0.046**	0.534	0.304		0.593
ZINC	r	0.116	-0.125	-0.019	**-0.194**	-0.115	-0.115	**-0.164**	-0.039	-
	p	0.114	0.088	0.801	**0.008**	0.117	0.194	**0.025**	0.593	

WAZ: Weight for age; HAZ: Height for age; WHZ: Weight for age; BAZ: BMI for age; HB- haemoglobin; T saturation: Transferrin saturation; CRP: C-reactive protein.

Boys showed a negative correlation between serum CRP and Tsaturation (r= -0.325; p<0.0001). Zinc was positively correlated with Tsaturation (r= 0.225; p= 0.004) but negatively correlated with CRP (r= -0.188; p= 0.016). Transferrin was positively correlated with WHZ(r= 0.2; p= 0.011) and WAZ (r= 0.249; p=0.011). Ferritin was positively correlated with weight (r=0.167; p=0.033). CRP was negatively correlated with weight (r= -0.202; p= 0.010), height (r= -0.172; p =0.028), WHZ (r=-0.155; p=0.048) and WAZ (r= -0.198; p=0.045) (Table 4.12).

Table 4.12: Correlations between biochemical markers and anthropometric measurements in boys (n=165)

		Age (years)	WEIGHTKG	HEIGHTCM	WHZ	HAZ	WAZ	BAZ	CRP	ZINC
HB	r	0.009	-0.014	0.055	-0.076	0.075	-0.141	-0.097	-0.176	0.083
	p	0.911	0.856	0.483	0.334	0.344	0.155	0.218	0.025	0.292
IRON	r	0.095	0.059	0.052	-0.033	0.021	0.066	-0.007	-0.358	0.205
	p	0.228	0.455	0.507	0.678	0.793	0.507	0.931	**<0.0001**	0.009
TRANSFERRIN	r	-0.131	0.004	-0.002	**0.200**	0.107	**0.249**	0.132	-0.100	-0.213
	p	0.095	0.959	0.981	**0.011**	0.175	**0.011**	0.094	0.206	0.006
TSATURATION	r	0.142	0.099	0.084	-0.048	0.020	0.046	-0.001	**-0.325**	**0.225**
	p	0.070	0.208	0.288	0.547	0.804	0.645	0.989	**<0.0001**	**0.004**
FERRITIN	r	0.177	**0.167**	0.146	-0.040	0.003	0.044	0.009	0.123	0.122
	p	0.024	**0.033**	0.063	0.616	0.965	0.655	0.911	0.118	0.121
CRP	r	-0.077	**-0.202**	**-0.172**	**-0.155**	-0.141	**-0.198**	-0.111	-	**-0.188**
	p	0.328	**0.010**	**0.028**	**0.048**	0.072	**0.045**	0.157		**0.016**
ZINC	r	0.123	-0.014	-0.048	-0.143	-0.132	-0.089	-0.035	-0.188	-
	p	0.117	0.856	0.543	0.069	0.092	0.373	0.654	0.016	

WAZ: Weight for age; HAZ: Height for age; WHZ: Weight for age; BAZ: BMI for age; HB- haemoglobin; T saturation: Transferrin saturation; CRP: C-reactive protein.

4.5.4 Correlations between biochemical markers and anthropometric measurements in stunted and non-stunted preschool children.

In stunted children serum CRP was negatively correlated with Tsaturation (r= -0.292; p=0.018). Table 4.13 summarises correlations between biochemical markers and anthropometric measurements in stunted pre-school children

Table 4.13: Correlations between biochemical markers and anthropometric measurements in stunted pre-school children (n=65)

		Age (years)	WEIGHTKG	HEIGHTCM	WHZ	HAZ	WAZ	BAZ	CRP	ZINC
HB	r	0.171	-0.011	0.169	-0.006	0.088	-0.011	-0.080	-0.081	0.195
	p	0.174	0.930	0.179	0.962	0.485	0.942	0.528	0.519	0.119
IRON	r	0.249	0.106	0.079	-0.106	-0.098	0.076	0.056	**-0.342**	-0.042
	p	0.046	0.402	0.532	0.401	0.439	0.619	0.659	**0.005**	0.742
TRANSFERRIN	r	-0.161	-0.153	-0.068	0.164	0.018	0.103	-0.010	0.026	-0.144
	p	0.201	0.223	0.588	0.192	0.888	0.502	0.939	0.840	0.252
TSATURATION	r	0.249	0.177	0.083	-0.093	-0.076	0.150	0.115	**-0.292**	-0.052
	p	0.046	0.158	0.513	0.460	0.548	0.324	0.360	**0.018**	0.679
FERRITIN	r	0.140	0.184	-0.002	0.045	-0.138	0.088	0.168	0.192	0.020
	p	0.266	0.143	0.987	0.722	0.273	0.563	0.180	0.126	0.873
CRP	r	-0.125	-0.115	-0.137	0.036	-0.059	-0.145	-0.001	-	-0.154
	p	0.321	0.363	0.275	0.776	0.642	0.342	0.991		0.220
ZINC	r	0.287	0.200	0.081	-0.060	-0.178	0.079	0.147	-0.154	-
	p	0.021	0.110	0.522	0.634	0.156	0.607	0.243	0.220	

WAZ: Weight for age; HAZ: Height for age; WHZ: Weight for age; BAZ: BMI for age; HB- haemoglobin; T saturation: Transferrin saturation; CRP: C-reactive protein.

Table 4.14 summarise correlations between biochemical markers and anthropometric measurements in non-stunted children. Serum zinc was positively correlated with Tsaturation (r= 0.140; p= 0.018) while in the non-stunted children serum CRP was negatively correlated with transferrin (r= -0.133; p=0.025) and Tsaturation (r=-0.266; p<0.0001). Transferrin was positively correlated with WHZ (r=0.175; p=0.003), WAZ (r=0.156; P= 0.033) and BAZ (r=0.165; p=0.005). Serum Tsaturation was negatively correlated with WAZ (r=-0.161; p=0.027). Ferritin was positively correlated with weight (r=0.168; p=0.005) but negatively correlated with WHZ (r=-0.148; p=0.013). Furthermore CRP was negatively correlated with height (r=-0.132; p= 0.026) and HAZ (r= -0.137; p=0.021). Zinc was negatively correlated with WHZ (r= -0.216; p<0.0001), WAZ (r= -0.145; p=0.048) and BAZ (r= -0.190; p<0.0001). Logistic regressions were done and they revealed that no anthropometric and biochemical measurements predicted zinc deficiency in the children.

Table 4.14: Correlations between biochemical markers and anthropometric measurements in non-stunted pre-school children (n=284)

		Age (years)	WEIGHTKG	HEIGHTCM	WHZ	HAZ	WAZ	BAZ	CRP	ZINC
HB	r	0.015	0.090	0.099	0.021	0.152	0.065	-0.002	**-0.142**	-0.035
	p	0.798	0.132	0.096	0.725	0.010	0.379	0.974	**0.017**	0.551
IRON	r	0.090	0.009	0.033	-0.051	-0.039	-0.136	-0.052	**-0.288**	**0.130**
	p	0.132	0.886	0.579	0.390	0.512	0.063	0.380	**<0.0001**	**0.028**
TRANSFERRIN	r	-0.099	0.051	-0.085	**0.175**	-0.003	**0.156**	**0.165**	**-0.133**	-0.075
	p	0.097	0.395	0.155	**0.003**	0.957	**0.033**	**0.005**	**0.025**	0.208
TSATURATION	r	0.137	0.023	0.081	-0.083	-0.020	**-0.161**	-0.082	**-0.266**	**0.140**
	p	0.021	0.702	0.172	0.163	0.733	**0.027**	0.170	**<0.0001**	**0.018**
FERRITIN	r	0.177	0.033	**0.168**	**-0.148**	0.007	-0.118	-0.119	0.115	0.085
	p	0.003	0.576	**0.005**	**0.013**	0.903	0.107	0.045	0.054	0.152
CRP	r	-0.067	-0.170	**-0.132**	-0.058	**-0.137**	-0.087	-0.065	-	-0.109
	p	0.260	0.004	**0.026**	0.331	**0.021**	0.239	0.276		0.067
ZINC	r	0.091	-0.101	0.012	**-0.216**	-0.078	**-0.145**	**-0.190**	-0.109	-
	p	0.124	0.091	0.838	**<0.0001**	0.191	**0.048**	**<0.001**	0.067	

WAZ: Weight for age; HAZ: Height for age; WHZ: Weight for age; BAZ: BMI for age; HB- haemoglobin; Tsaturation: Transferrin saturation; CRP: C-reactive protein.

4.6 Summary of the results

4.6.1 Prevalence of zinc deficiency and clinical signs

The prevalence of zinc deficiency was 42.6% in the current study. The prevalence was significantly higher in girls (24%) than boys (18.6%). The prevalence of zinc deficiency using liquid zinc taste test was 30%, was high in girls (17.2%) and to a less extent in boys (12.8%). The prevalence is lower as compared to the prevalence determined by serum zinc. This means that liquid zinc taste test might not be a good indicator of serum zinc level because taste test depend on certain factors like oral hygiene, medication use and salivary gland disorder. In addition 0.5% of the children showed white spots on the finger nails (symptom) of zinc deficiency. With respect to symptoms of zinc deficiency the prevalence was the same in both gender groups (0.5%). The symptoms of zinc might not appear immediately in childhood but might have a greater consequence in later stage of life.

4.6.2 Anthropometric status

The prevalence of underweight, wasting, stunting was 0.3%, 1.4%, 18.6%, respectively. Malnutrition among pre-school children is a major public health problem with resultant morbidity, mortality, poor cognitive development and poor growth and development (Kwena *et al.*, 2003; UNICEF, 2008). Micronutrient deficiencies are the main manifestation of malnutrition besides protein energy malnutrition in children less than five years of age (UNICEF, 2008).

4.6.3 Iron status

The prevalence of anaemia was found to be 28% in the current study. Iron deficiency as indicated by low serum ferritin (<12μg/dl) and transferrin saturation (< 15%) were present in 7.2% and 28.1% of children respectively.

4.6.4 Comparison between zinc status and iron, anthropometric.

It was found that 2.3% of children in the study suffered from combined iron and zinc deficiency. Iron and zinc levels negatively correlated with HAZ, WHZ and BAZ in females. There were no correlations between zinc and anthropometric measurements in both stunted and non-stunted children in the current study. Although many studies have suggested stunting as the functional indicator of population zinc status (Brown *et al.*, 2002; Muller *et al.*, 2003; Fischer and Black, 2007) this seemed not to be the case in the current study.

CHAPTER FIVE

5.1 Demographic Data

Vhembe district is largely rural and the households are mostly headed by females. Males migrated to urban areas to find work. The area is faced with infrastructure backlogs of water, sanitation, electricity and poor roads which impact negatively on the health of these communities. The district relies on subsistence farming which is mostly dependent on rain. The aim of the study was to determine the prevalence of zinc and iron deficiencies among children aged 3-5 years in Vhembe district of the Limpopo province. Pre-school children are at an important stage of life where nutrition plays an important role in growth and development and has long lasting effects in later life (Khan Khattak and Ali, 2010). Children under five years constitute the most vulnerable group of any community; their nutritional status is a sensitive indicator of community health and nutrition (Ibeanu *et al.*, 2012).

There were no gender differences observed in both biochemical and anthropometric measurements in the current study. These findings are supported by previous studies which indicated that there were no significant gender differences in serum levels of iron and zinc concentrations (Arvanitidou *et al.*, 2007; Ohiokephai *et al.*, 2009; Dehghani *et al.*, 2011; Ibeanu *et al.*, 2012). In a study done by Wamani *et al.* (2007) on children aged 0-5 years it was indicated that there were no gender differences in anthropometric measurements. On the contrary Wamani *et al.* (2005) observed gender differences in anthropometric measurements and they attributed this to the normal pattern of dimorphism with males tending to be taller and heavier than females. Several other studies have reported that males are more vulnerable to zinc deficiency than females (Smit-Vanderkooy and Gibson, 1987; Gibson *et al.*, 1991; Cavan *et al.*, 1993; Parnell *et al.*, 2003), presumably because of higher requirements for zinc. This may be partly due to a higher growth rate and greater proportion of muscle per kilogram body weight as muscle contains a higher content of zinc than fat (Hotz and Brown, 2004).

5.2 Prevalence of zinc deficiency

5.2.1 Prevalence of zinc deficiency using serum zinc

The prevalence of zinc deficiency was 42.6% in the current study which signifies a public health problem. The prevalence is almost the same as the National food consumption survey done in

2005 where it was found that 45.3% of children aged 1-9 years had inadequate zinc intake and therefore considered to be at risk for zinc deficiency. However, the prevalence was higher when looking at provinces with Limpopo having 27.3% of zinc deficiency. The results warrant an immediate action since the prevalence is higher than 20% which is regarded as a public health concern by IZiNCG (2007). Perhaps this can be attributed to the fact that local villagers plough maize and take it to milling stations where there is no fortification. The milling process without fortification takes place despite the mandatory legislated fortification of flour and maize since 2003 (Dhansay *et al.,* 2007).

Most of the villagers consume the maize meal in the form of maize meal porridge together with vegetables and legumes that contain phytates, tannins and oxalates which may impede the absorption of zinc in the intestine (IZiNCG, 2004). The animal derived food sources are not extensively consumed due to high cost, limited supply and in some cases religious or cultural practices (IZiNCG, 2004). These inadequate zinc intake have also been identified among segments of SA infants due to improper weaning practices (Oelofse *et al.,* 2002) and children due to poor feeding practices (Faber *et al.,* 2001). Poor qualities of traditional diets accompanied by infectious diseases as well as insufficient food intake were considered the main causes of malnutrition in many populations (Pinstrup –Andersen *et al.* 1993; Waterlow, 1994). Many low income households in rural areas of South Africa are highly dependent for survival on government grants of which the old age and child grants are the most common and this makes it difficult for them to purchase food which could meet their daily requirements of nutrients because of the high cost of foods especially the protein rich food. The high cost of animal products makes these rich sources of absorbable zinc inaccessible to many of the world's poorer populations (IZiNCG, 2004).

Zinc deficiency is an important cause of morbidity due to infectious disease and growth faltering among children (Brown *et al.,* 2002). Increased demand of zinc due to rapid growth and decreased intake of zinc due to inadequate feeding practices predisposes pre-school children, especially those living in communities of low socioeconomic status to an elevated risk of zinc deficiency (Black, 2003). Zinc is very important for human growth and its deficiency is known to lead to growth disturbances, diarrhea, pneumonia, cognitive dysfunction, mortality, visual impairment and measles in children because zinc is important for activities of many important enzymes for DNA and RNA synthesis and cell proliferation (IZiNCG, 2007). C-reactive protein in these children is an indication of possible inflammation and infection in the current study. Infection and inflammation can cause low serum zinc levels and decrease in hepatic methalotionein where plasma protein bound by C-reactive protein. The presence of infections causes the immune system weak, so the ability to protect the body is reduced and makes pathogens including viruses to enter the body and causes diseases.

5.2.2 Prevalence of zinc deficiency using liquid Zinc Taste Test

The prevalence of zinc deficiency using liquid zinc taste test was 30%, was high in girls (17.2%) and to a lesser extent in boys (12.8%). In addition 0.5% of the children showed clinical signs (white spot on the fingernails) of zinc deficiency. With respect to symptoms of zinc deficiency the prevalence was the same in both gender groups (0.5%). The symptoms of zinc might not appear immediately in childhood but might have a greater consequence in later stage of life. The prevalence is lower as compared to the prevalence determined by serum zinc. The reason for the difference is that the study participants were children aged 5 years and below and children of that age range are considered to be unable to express differences in taste perception and a reliable estimation was achieved using the child's own spontaneous verbal judgement (Sjovallet *al.*, 1984; Uchida *et al.,* 2000). Zinc Taste Test (ZTT) can be useful when determining zinc status since impaired taste acuity has also been associated with suboptimal zinc status in children (Buzina *et al.,* 1980; Gibson *et al.,* 1989). Diminished taste acuity is an early symptom of zinc deficiency has been demonstrated in humans (Hambidge *et al.,* 1972; Henkin *et al.,* 1999; Tanaka, 2002; Goto *et al.,* 2000). Taste perception may be affected by several factors such as age and gender, smoking, medication and oral hygiene (Kostak *et al.,* 2008).

Individual responses to taste acuity testing have also been shown to vary in reaction to recency of food consumption (Glockner *et al.,* 1986; Zverev, 2004), age (Kettaneh *et al.,* 2005; Ikeda *et al.,* 2007) and gender (Brown and Toma, 1986; Zverev, 2004). However, the possibility of some being on medication could not be ruled out because medical history was not taken in the current study. Salivary zinc has been found to be associated with gustin, a metalloprotein that may be involved with providing nutrition to the taste buds (Youshida and Tomita, 2002). Zinc deficiency is associated with lack of gustin which leads to low salivary zinc concentrations (Pluhator *et al.,* 1996), and can potentially lead to impaired taste acuity and loss of appetite (Bales *et al.,* 1986; Uchida *et al.,* 2000), which may lead to decreased intake of zinc rich foods (Rolls, 1999; Shay and Mangian, 2000; Seiler *et al.,* 2002). Depletion of zinc can lead to decreased taste acuity but decreased taste acuity is not necessarily associated with depletion of body stores zinc (Catalanotto, 1978; Tanaka, 2002, Yoshida and Tomita, 2002). Liquid zinc taste test might not be a good indicator of serum zinc level because taste test depend on certain oral hygiene, factors, salivary gland disorders, medication use and age.

5.3 Nutritional status of the children

Malnutrition among pre-school children is a major public health problem with resultant morbidity, mortality, poor cognitive development and poor growth and development (Kwena *et al.,* 2003; UNICEF, 2008). Micronutrient deficiencies are the main manifestation of malnutrition besides protein energy malnutrition in children less than five years of age (UNICEF, 2008). Zinc status has been associated with disturbance in growth and diarrhoea, pneumonia, cognitive dysfunction, mortality, visual impairment and measles in children from developing countries because zinc is important for activities of many important enzymes for DNA and RNA synthesis and cell proliferation (IZiNCG, 2007). Brown *et al.* (2002) results confirm that zinc deficiency is an important cause of morbidity due to predisposition infectious disease and growth faltering among children. Increased demand of zinc due to rapid growth and decreased intake of zinc due to inadequate feeding practices predisposes preschool children, especially those living in communities of low socioeconomic status to an elevated risk of zinc deficiency (Black, 2003).

5.3.1 Underweight

The prevalence of underweight was 0.3% in the current study. Despite the national prevalence remaining statistically unchanged at 9.3%, the prevalence of underweight appeared to have increased in children living in the urban areas, and decreased in the rural areas, with the greatest improvement among children living in formal rural areas (4.9 – 3.8%) (Labadarios *et al.,* 1999; Labadarios *et al.,* 2005). Perhaps this low prevalence of underweight can be attributed to the Integrated Nutrition Programme (INP) that South Africa is using to address and prevent malnutrition in rural and urban areas with the vision of optimal nutrition for all through a comprehensive approach to address the underlying causes of malnutrition, through direct (e.g. nutrition education and promotion; micronutrient supplementation; food fortification; and disease-specific nutrition counselling and support) and indirect interventions (e.g. provision of healthcare services; improved access to food; parasite control and provision of clean and safe water), nutrition interventions which include service delivery as well as aspects of behaviour change (DoH, 2008). Low prevalence of underweight may be due to the fact that Vhembe district presented lowest poverty rates (19%) (de Cock *et al.,* 2013). Even though the prevalence of underweight was low in the current study the causes of underweight should be considered when studying the nutritional status of children under five years of age. Socio-economic disparities among rural communities, poor nutritional qualities of traditional diets as well as insufficient food intake are considered the main causes of malnutrition in many rural communities (Cogill, 2003). Household in rural area are mostly headed by female and relies on subsistence farming and this can influence the nutrition of household members directly by increasing income and production of nutrient-dense foods for family consumption which can result in low rate of underweight. They can also impact on nutrition and health indirectly by increasing income from the production and sale of high-value agricultural products (Arimond *et al.,* 2011). This, in turn, makes it possible for households to purchase more and better food and/or to acquire other goods and services that contribute to nutritional well-being and health (Hawkes and Ruel, 2006). Dietary intake and frequency of food consumption were not measured in this study as it would have given a better understanding of this low prevalence of underweight. Although good nutrition is universally accepted as a basic human right, many children still go to bed with an empty stomach which makes this burden of underweight difficult to solve (Roberts, 2004-2005).

5.3.2 Wasting

The prevalence of wasting in the current study was found to be 1.4%. It is lower than the study done by Zere and McIntyre (2003) where it was found that 13.1% of children under than five years of age in Limpopo were wasted. However, the prevalence was lower when compared with national prevalence (7%) of wasted children (Kimani-Murage, 2013). The current study was supported by study done in 2005 where it was found that 1 in 20 children (5%) aged 1-9 years was wasted (Labadarios *et al.,* 2005). The nutritional status of the current pre-school children might have been affected by the access that the community, household and individual had on food and utilization of the available food. Parents and caregivers make most food choices for meals consumed at home and these food choices are based on culture, beliefs, cost, time constraints and availability which may result in poor intake of vital nutrients (Kruger and Gericke, 2003; Black *et al.,* 2008). In addition, lack of knowledge on nutritional needs of children, inadequate food intake, poor feeding practices, low income, poor weaning and complementary feeding practices have shown to contribute to inadequate energy and protein intake leading to malnutrition (Mittal *et al.,* 2007; Cogill, 2003). Unfortunately, these were not measured in this study as it would have given a better insight into the problem faced by these children. The effect of wasting on children may not be apparent immediately but it has significant effects on levels of educational attainment, acute growth faltering, reduced physical capacity and poor resistance to infections and diseases in children less than five years of age (WHO, 1995; Ibeanu *et al.,* 2012). Wasting and childhood morbidity have a synergistic relationship, in such a way that illness can suppress appetite precipitating under nutrition while nutritional deficiencies increase susceptibility of children to infectious diseases (Pelletier *et al.,* 1995). Girls born with low birth weight are more likely to become poorly nourished children and poorly grown women and are, therefore, likely to give birth to low birth weight infants resulting in the vicious cycle of malnutrition (UNICEF/WHO, 2004).

The prevalence of overweight children in the present study was 20.9%. Overweight was more prevalent than underweight in the current study. The results is the same as the study done Mendez and Popkin (2004) where it was indicated that overweight in rural areas of South Africa is higher than underweight. Perhaps this can be attributed to dietary changes that have been observed in rural South African children from low fat diet to a typical westernised high fat diet (WHO, 2003). South Africa has become a market liberalised country in which many people in rural areas access fast food due to market globalisation which leads to change in dietary patterns and reduced levels of exercise (FAO, 2004). It has been suggested that lifestyle habits like diet and exercise levels may even track from childhood into adulthood. However, under nutrition at important stages of fetal development can also induce permanent physiological changes that result in obesity (Ravelli *et al.,* 1976). Undernutrition and over nutrition coexist in the current study like in many rural communities of South Africa. Interestingly in many communities where there is high prevalence of undernutrition there is also high prevalence of overweight and obesity (Kazemi, 2009). Historically, a heavy child was associated with a healthy child but today this perception has drastically changed on the basis of evidence that obesity and overweight in childhood are associated with a wide range of serious health complications and increased risk of premature illness and death later in life (Dietz, 1998). Overweight and obesity in children increase the risk of debilitating health problems such as type 2 diabetes mellitus, hypertension and cardio vascular diseases and increases the risk of mortality from these conditions (Solomon and Manson 1997; Owens *et al.,* 1999; Davison and Birch, 2001). In malnourished children, deficiency in overweight and obesity have also been associated with long term health complications in the form of metabolic syndromes and cardiovascular diseases (Pi-Sunyer, 1991; Katzmarzyk *et al*., 2001).

5.3.3 Stunting

Prevalence of stunting was 18.6% in the current study. The prevalence is almost the same as study done by Kruger *et al.* (2007) where it was found that 18% of children aged 1-9 years nationally were stunted. Furthermore, NFCS-FB (2005) also indicated that one in five children was stunted. The prevalence of stunting in the present study is slightly lower than the cut-off of 20% that would indicate elevated zinc deficiency (de Benoist *et al.*, 2007). Perhaps stunting in this current study as health indicator for chronic malnutrition can be attributed to socio-economic circumstances (WHO, 1995 and WHO, 1996).

This lack of adequate food and in particular high quality protein (which are also known to be a good source of zinc) due to socio-economic reasons, can lead to malnutrition in children which could result in growth retardation (UNICEF, 2008). The household's economic position is seen as having an impact on the probability of a child being stunted and underweight and as such the World Health Organization recommends stunting as a measure of overall social deprivation (Braverman, 1998). Long term nutritional deprivation in children often results in delayed mental development (Grantham- McGregor, 1995; WHO, 1995), poor school performance (WHO, 1995) and reduced intellectual capacity result in stunting (WHO, 1995; Kruger *et al.,* 2007) and zinc deficiency (IZiNCG, 2007). This is likely to perpetuate inequities and inequalities in health and other dimensions of household welfare. Children from the poorest households bear the greatest burden of malnutrition due to inequalities (Zere and MacIntyre, 2003).

Stunting and zinc deficiency in childhood are related to concurrent and possibly later delayed mental and motor development (Grantham- McGregor, 1995; IZiNCG, 2007), health and productivity in later life (WHO, 1995). Zinc deficiency has been associated with reduced growth and impaired immunity, impairment of immunity may lead to increased prevalence of and incidence of infectious diseases among children in developing countries (Brown *et al.,* 1998; Bahl *et al.,* 1998 and Bhutta *et al.,* 1999). Adequate nutrition is vital for child development particularly in the period from birth to five years of age (UNICEF, 2008) and long term nutritional deprivation in children often result in delayed mental development (Grantham-McGregor, 1995; WHO, 1995), poor school performance (WHO, 1995) and reduced intellectual capacity result in stunting (WHO, 1995; Kruger *et al.,* 2007). The prevalence of stunting was virtually the same in boys (10%) as compared to 8.6% of girls. Perhaps this gender differences may be due to differences in recommended daily intakes on nutrients.

5.4 Iron deficiency anaemia

The prevalence of anaemia was found to be 28% in the current study. It was lower when compared to the study done in Venda where it was found that 75% of children were anaemic (Heckman *et al.,* 2010). This high prevalence of anaemia is due to the fact that children consume less food than adults and their diet often consists of foods with low iron content and in which the bioavailability of iron is poor. This low proportion of foods rich in iron is also from the menus offered by the pre-schools (Vieira *et al.,* 2007). Unfortunately, pre -school menus were not analysed they would have given a better understanding of their dietary intake. Iron rich foods are poorly consumed in rural communities of South Africa because of their high cost and in some cases cultural or religious practices (Labadarios *et al.,* 2005). Even though South Africa had legislated fortification of flour and maize to reduce micronutrients malnutrition, food introduced during childhood includes small amounts of food rich in iron with a high content of substances such as Phytates and fiber that interfere with iron absorption (Cole *et al.,* 2010).

Lack of employment and education among mothers, guardians, women of child bearing age in rural areas are often the effects of underdevelopment and are primary cause of poverty and this could have resulted in poor food choices that could have reduced inclusion of iron rich food in their children's diet. Educational level and socioeconomic status have not been measured in the current study as this might have given a better understanding of high prevalence of iron deficiency anaemia. Poverty, poor food choices, limited food availability coupled with lack of knowledge about the importance of food groups' diversity for the health and growth of young children may limit the inclusion of micronutrient rich foods in the diets of children (Cole *et al.*, 2010). Many people particularly those in lower income settings of rural villages of Africa have limited access to diets that meet the theoretical requirements of both zinc and iron (IZiNCG, 2004; Cole *et al.,* 2010). Insufficient consumption of iron leads to physical work capacity and growth retardation and alterations in neurological function being compromised (Hotz and Brown, 2004). Most studies have found associations between iron-deficiency anaemia and poor cognitive and motor development and behavioural problems (Walter, 2003). Increased risk of child mortality due to severe anaemia has been documented (Macgregor, 1963; Bothwell and Charlton, 1981; Scholl and Heigiger, 1994).

5.4.1 Iron deficiency

Iron deficiency as indicated by low serum ferritin (12µg/dl) and transferrin saturation (< 15%) were present in 7.2% and 28.1% of children respectively which were lower than what was reported by Kruger *et al.* (1996) who found that 15.6% and 11% of children were deficient respectively. A large number of children came from poor background with poor sanitation and environment and were more prone to infections (Kruger *et al.,* 1996). Causes of low ferritin in children include iron deficiency and diet lacking in iron while inflammation and infections increase ferritin which is known to be an inflammatory marker together with CRP (Baumgartner *et al.,* 2013). The prevalence found in the current study of iron deficiency anaemia suggested that the serum concentrations of ferritin closely reflect the pool of iron stores. Lower iron status of the current study population maybe due to dietary factors and parasites, the most important one in the dietary factors might have been the low intake of heme-proteins in diet and the phytates, oxalates, tannins and fiber content found in legumes and vegetables consumed by the population which might have impeded absorption of iron (Brunvan *et al.,* 1995).

Deworming may have avoided the competition for nutrients in these children. High prevalence of inflammation as reflected by resulting protein energy malnutrition in the population studied may have resulted in low serum iron, low serum transferrin saturation and increased serum ferritin (Dallman *et al.,* 1993; Abshire, 1996). This is influenced by blood depleting parasites, chronic infections and some physiologic and hematologic conditions. This blood depleting parasites suck the blood voraciously and damage the mucosa while also competing for iron with the child's body (Markell *et al.,* 2006). However, the blood loss in the stools is not apparent. This produce iron deficiency anaemia by sucking blood from the host's intestinal walls and gives rise to a combination of intestinal inflammation and progressive iron deficiency anaemia (Markell *et al.,* 2006). C-reactive protein in these children suggests possible infection and inflammation in the current study.

5.4.2 Combined iron and zinc deficiency

It was found that small proportion (2.3%) of children in the study suffered from both iron and zinc deficiency. This could be attributed to insufficient intake of food rich in micronutrients and poor bioavailability which are the major causes of zinc and iron deficiency in developing countries (UNICEF/UNU/WHO, 2001). However, this was a small proportion given that zinc deficiency was 42.6% and iron deficiency was 28.1%. This then means that both do not always coexist in children. Lack of dietary diversity is a problem among rural populations; their diets are predominantly based on staple foods with low amounts of animal sources (Guansheng *et al.,* 2006). Low serum zinc and iron concentration are normally associated with low dietary intake of food rich in these micronutrients (Adelekan, 2003). Micronutrients rich foods are often expensive and the high cost of these micronutrients rich food make it difficult for poor people to meet their theoretical needs of iron and zinc because they cannot afford them (Ruel, 2003).

Insufficient intake of these two micronutrients from their rich sources may lead to growth faltering, delayed development and contribute to mortality and morbidity from diarrhoea, pneumonia and stunting in children in developing countries (Bhutta *et al.,* 1999; Brown *et al.,* 2002; Black, 2003). Iron and zinc interact either at the site of absorption or post absorption because they compete for similar transport pathways making one to be more prevalent than the other (Sreedhar, 2004). Iron and zinc compete for absorption or interact at other site; therefore, the benefit of each may be less than if either was given alone (Whittaker, 1998; Kordas and Stoltzfus, 2004; Walker *et al.,* 2005). Sandstrom *et al* (1985) found that increasing the molar ratio of iron to zinc from 1:1 to 2.5:1 did not affect absorption of zinc. An iron and zinc ratio of 25:1 decreased zinc absorption but addition of the zinc ligand histidine decreased the inhibitory effect of this dose of iron and zinc absorption increased from 34% to 47%. Furthermore, Sandstrom *et al* (1985) also observed no inhibitory effect on zinc absorption when iron and zinc were given with a meal. Baqui *et al* (2005) found that addition of zinc to weekly iron supplementation improved children' zinc status but had no differential effect on iron status compared to iron alone. Iron did not affect zinc absorption since iron deficiency was high in the current study.

5.5 Association among biochemical and anthropometric variables

C-reactive protein correlated negatively with transferrin and Tsaturation, and positively with ferritin. Ferritin and C- reactive protein are known to be inflammatory markers and the positive correlation between the two suggests possible inflammation and infections in the current study. Unfortunately, deworming was not done prior data collection as this would have ensured that children are free from infections. Inflammation in these children may be attributed to high levels of poverty resulting from exposure to stressful conditions such as early development and persistence of different social norms of poor diet, high body mass index (Broyles *et al.,* 2012). Causes of low ferritin in children include diet lacking iron and iron deficiency. Inflammation and protein energy malnutrition are closely related to each other (Dulger *et al.,* 2002). In malnourished children there are altered mechanisms during early infectious process, which induce an acute phase response with increased synthesis of inflammatory mediators such as cytokines and acute-phase proteins including C-reactive protein (Schneider and Furst, 2000), due to deterioration of the immune response which seriously compromises lymphoid tissues and cellular immunity (Savino *et al.,* 2007).

HAZ correlated negatively with C-reactive protein and Zinc. This means that HAZ increased as C-reactive protein and zinc decreased. HAZ indices were used to assess the degree of stunting. C-reactive protein has the benefit of providing a potentially objective marker of inflammation and may reveal low grade infection in the current study. Children in developing countries fail to achieve their growth potential due to malnutrition and infections (Ulijaszek, 1994; Verhoef *et al.*, 2002). When infections burden is high and nutrition is poor the high price is paid in the form of stunting and growth retardation (Tarini *et al.,* 1999). Poor nutrition especially of iron and zinc deficiency in children is associated with retardation of growth (Salgueiro *et al.,* 2002; Hotz and Brown, 2004), and poor cognitive development (Pollit, 1997). In support of the above reports HAZ was found to be negatively correlated with C-reactive protein but the negative association with zinc is unexplainable as decreased zinc levels are to affect growth negatively (Bao *et al.,* 2010).

In the current study children with wasting had the depletion of transferrin may be the direct result of a reduced rate of synthesis of proteins mediated either through the changes in iron status or by dietary zinc and iron deficiency (Morlese *et al.,* 1996). It has been reported that there is decreased availability of transferrin in children with wasting when plasma transferrin becomes more saturated with iron, thereby markedly reducing the iron binding capacity of malnourished children (Morlese *et al.,* 1997). These mean that when BAZ increased transferrin decreased. There is increasing evidence that inflammatory produced by excess adipose tissue in overweight individuals stimulate the secretion of hepcidin by the liver and has a crucial role in iron homeostasis. Its synthesis is up-regulated in chronic inflammation and iron excess (Zimmerman *et al.,* 2008; Aeberli *et al.,* 2009; Viatte and Vaulont, 2009).

Iron and zinc levels negatively correlated with HAZ, WHZ and BAZ in females. Even though there was high prevalence of both zinc and iron deficiencies, stunting and overweight the results suggest that zinc and iron deficiencies are not a major cause of stunting, overweight in the present study but it is also known that socioeconomic factors and genetics are the other underlying causes because of their indirect influence on the health and nutrition of the population (UNICEF, 2006). Less intake, poor absorption, poor bioavailability of iron and zinc in the diet, presence of iron and zinc inhibitors such as phytates, oxalate tannins and fiber in the children's diet and systematic effect of infection may be responsible for their low iron and zinc status (WHO, 1999). Zinc was negatively correlated with iron in males. The inverse relationship between zinc and iron in males supports the existing knowledge of iron and zinc interaction either at the site of absorption or post absorption because they compete for similar transport pathways making one to be more prevalent than the other (Whittaker, 1998; Sreedhar, 2004; Olivares *et al.,* 2007).

Furthermore, Sandstrom *et al* (1985) also observed no inhibitory effect on zinc absorption when iron and zinc were given with a meal. Zinc and iron deficiencies coexist in malnourished child population of developing nations (Walker *et al.,* 2005). High prevalence of both shows other causes for both deficiencies. Diet of many South African consists of staple food and this are consumed with vegetables and legumes which are poor zinc and iron source. Iron and zinc deficiencies coexist because the diet consumed with insufficient amount of animal source foods (Walker *et al.,* 2005). Poverty, poor food choices, lack of availability or decreased accessibility to certain foods, coupled with lack of knowledge about the importance of food group diversity for the health and growth of young children may limit the inclusion of micronutrient rich food in the diets of children (Cole *et al.,* 2010).

Malnutrition is manifested in most developing countries by early stunting and a high prevalence of micronutrients deficiencies. If these micronutrient deficits persist, the likelihood of catch-up growth is diminished. Zinc deficiency plays a role in aetiology of stunting because it contributes to low birth weight, growth failure and impaired immunity (Pelletier *et al.,* 1993; Grantham-McGregor, 1995; Sazawal *et al.,* 1998). Overweight and obesity may be associated with poor quality of diets if overweight and obese individuals are lower in iron since the absorption of iron may be reduced because of increased circulating hepcidin in overweight and obesity which may reduce iron absorption because iron is trapped inside the macrophages (where iron is stored) (Hunter *et al.,* 2002). Increased malnutrition often has an association with diseases and mainly infectious diseases that can also affect nutritional status in several ways such as reducing nutrient intake of children due to inflammatory response, reduced absorption of food caused by damaged intestines and increased nutrient requirements due to increased metabolism (Hughes, 2004).

There were no significant correlations between zinc and anthropometric measurements in both stunted and non-stunted children in the current study. Although many studies have suggested stunting as the functional indicator of population zinc status (Brown *et al.,* 2002; Muller *et al.,* 2003; Fischer and Black, 2007) this seemed not to be the case in the current study. Even though there was high prevalence of both zinc deficiency and stunting, the results suggest that zinc deficiency is not a major cause of stunting in the present study but it is also known that socioeconomic factors and genetics are the other underlying causes because of their indirect influence on the health and nutrition of the population (UNICEF, 2006). This relationship should be considered when studying zinc deficiency and stunting. It is known that stunting result from insufficient feeding, inadequate care and nutrient depletion caused by repeated bouts of illness, depressed appetite and poverty (UNICEF, 2000).

Serum Tsaturation correlated negatively with WAZ while ferritin correlated negatively with WHZ, C-reactive protein correlated negatively with height. The high prevalence of stunting in this study strongly supports the existing knowledge of the contribution of iron deficiency anaemia to growth retardation. However, transferrin was positively correlated with WHZ, WAZ and BAZ suggesting a relationship between body composition and iron status a finding which has been reported in adults (Baumgartner *et al.,* 2013). Iron requirements in growing overweight children are higher than in their normal weight counterparts. Thus, at a similar level of iron intake the iron status of overweight deteriorate to a greater extent than in the normal weight children during periods of growth (Baumgartner *et al.,* 2013). Overweight children have higher C-reactive protein interleukin (IL)-6, leptin and hepcidin concentrations that predict poor iron status (Aeberli *et al.,* 2009). Despite this, studies have indicated that iron deficiency does not appear to contribute to growth faltering (Ramakrishna *et al.,* 2004; Torbjorn *et al.,* 2004). Inadequate intake and low content of bioavailable iron are known to have a direct impact on nutritional status of young children leading to anaemia in underweight, wasted and stunted children (Labadarios *et al.,* 2005).

Zinc correlated positively with WHZ, WAZ and BAZ. Many studies have assessed growth velocity associated with modest dietary supplements and have confirmed the effect of zinc supplementation in increasing height and weight (Brown, 1998; Brown *et al.,* 2002; Radja and Charles-Homles, 2002; Maret and Sandstead, 2006). Daily intake of zinc is required especially during childhood because the body has no specialised zinc storage system (Rink and Gabriel, 2000). Varying traditional diets, differences in dietary intake of zinc in some areas along with different soil zinc levels may be other reasons to explain this negative association (Downs *et al.,* 2009; Dehghani *et al.,* 2011). The main source of zinc for these children were foods of plant origin which has been shown to have poor zinc bioavailability due to the presence of inhibitors such as phytates, oxalic acid and polyphones (NFCS-FB, 2005).

CHAPTER SIX

6.1 Conclusions

Zinc deficiency is a major public health concern among aged 3-5years in Vhembe District. The purpose of the study was to determine the prevalence of zinc deficiency among pre-school children in Vhembe District of Limpopo province in South Africa. The objectives of the study were to determine serum zinc concentration among pres-school children using flame atomic absorption spectrophotometer, to determine the presence of clinical signs of zinc deficiency among pre-school children using clinical assessment and liquid zinc, to determine the anthropometric measurements of pre-school children, to determine the iron status of pre-school using haemoglobin, serum ferritin, transferrin receptor and C-reactive protein (CRP), lastly to compare zinc status with anthropometry and iron status.

Data was collected using record sheets from 349 children aged 3-5 years. Weight and height for all subjects were measured by the researcher and the results were entered to WHO Anthro, (2007) and Antroplus software to calculate z-score of weight-for-age, height-for-age and weight-for-height. A total of 349 blood samples were collected.

- The study demonstrated that high percentage of zinc deficiency was found among children (42.6%), these results are despite mandatory legislated fortification of flour and maize meal to reduce micronutrient malnutrition in the country.

- The prevalence of underweight, wasting and stunting was 0.3%, 1.4%, 18.6% respectively. Malnutrition in South Africa still exists among children in rural areas despite the Bill of Rights in the Constitution that indicates that every child has the right to basic nutrition (South African Constitution, 1996), Section 28 (1) (c).

- The prevalence of anaemia was 28% and of iron deficiency, as indicated by low serum ferritin (<12µg/dl), and transferrin saturation (<15%) were 7.1% and 4.2%. These indicate high prevalence of inflammation in children which have resulted in low serum iron, low serum transferrin saturation and increased serum ferritin.

- Only 2.3% of children suffered from combined iron and zinc deficiency in the current study. Iron and zinc coexist in the population because they share the same dietary sources.

- There were no correlations between zinc and anthropometric measurements in both stunted and non-stunted children in the current study. Although many studies have suggested stunting as the functional indicator of population zinc status (Brown *et al.*, 2002; Muller *et al.*, 2003; Fischer and Black, 2007), this seemed not to be the case in the current study. Even though there was high prevalence of both zinc deficiency and stunting, the results suggest that zinc deficiency is not a major cause of stunting but it is also known that socioeconomic factors and genetics are the other underlying causes because of their indirect influence on the health and nutrition of the population (UNICEF, 2006).

6.2 Recommendations

- Improving nutritional status should follow an integrated approach tackling both malnutrition and micronutrients deficiencies at the same time considering behavioural approach.

- Nutrition education programme targeted at mothers, guardians and women of child bearing age on the importance of iron and zinc and also foods introduced at complementary feeding and weaning should include food from animal origin as they are good sources of zinc and iron.

- Dietary diversification (Inclusion of animal products) of meals for pre-scholars and improved food processing/handling could be of help.

- Supplementation as a short term strategy to address this zinc intake.

- Regular surveys of zinc and iron status should be carried out to determine whether the programmes are being efficiently implemented.

- Further studies on zinc must look at increasing bioavailability by increasing the quantity of micronutrients and decreasing inhibiting factors such as phytates, oxalates, tannins and fiber in food through bio- fortification would have profound and direct influence on the nutritional status of the children of rural communities where animal products are of a high cost.

- Zinc prevalence studies should also determine dietary intakes of all nutrients.

References

ACC/SCN. 2000. Fourth Report on the world Nutrition Situation. Geneva: ACC/SCN in collaboration with the International Food Policy Research Institute.

Abdalla F. Mutharia J, Rimal N, Bilutha O, Talley L, Handzel T and Bamrah S. 2008. Malnutrition and micronutrients deficiencies among Bhutanese refugee children. MMWR Morb. Mortal. Wkly. Rep., 57:370-373.

Abshire TC.1996. The anemia of inflammation: a common cause of childhood anemia. Pediatric Clinics of North America. 43: 623–637.

Admas C. 1990. What causes white spot on the fingernails, The Nails in Disease, Samman and Fenton, 1986.

Adel ET, Francoise M, Salaheddin E, Najeeb AM, Ahmed B and Gerard L .2007. Nutritional status of under five children in libya-a national population based survey. Libyan J Med 65(4):425-432.

Adelekan DA. 2003. Multiple micronutrients deficiency in developing countries. Nutrition; 19:473-4.

Aeberli I, Hurrell RF and Zimmermann MB. 2009. Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable to normal weight children. Int J Obesity; 33: 1111–1117.

Aggett PJ. 1985. Physiology and metabolism of essential trace elements: an outline, Clin Endocrinol. Metabol; 14 (3): 513-43.

Agte VV, Chiplionkar SA and Tarwadi FE. 2005. Fcators influencing zinc status of apparently healthy infants. Journal of American College of nutrition, 24: 334-341.

Ahn J and Koo SI. 1995. Effects of zinc and essential fatty acid deficiencies on the lymphatic absorption of vitamin A and secretion of phospholipids. J Nutr Biochem.; 6; 595-603.

Akman M, Cebeci D, Okur V, Angin H, Abali O and Akman AC. 2004. The effects of iron deficiency on infants development test performance; 93:1391-1396.

Alien S. 2007. Zinc for hair loss/zinc deficiency (American chronicle). June 18.

Allen LH and Gillespie SR. 2001. What works/ A review of the efficacy and effectiveness of nutrition intervention. United Nations Administrative Committee on Coordination/Sub-Committeeon Nutrition, Geneva. Nutrition policy Paper No 19. Asian Development Bank, Manila.

Allen LH, Rosado JL, Casterline JE, Hernandez AB and Sosa S. 2000. Supplementation in Anemic Mexican preschoolers with multiple micronutrient deficiencies. Am J Clin Nutr; 71: 1485-1494.

Arcasoy A, Akar N, Ors U, Delilbasi L and Karayalcin S. 1990. Ultra structural changes in the mucosa of the small intestine in patients with geophagia (Prasad's syndrome). J Pediatr Gastroenterol Nutr; 11:279–82.

Arvanitidou V, Voskaki I, Tripsianis G, Athanasopoulou H, Tsalkidis A and Filippidis S. 2007. Serum copper and zinc concentrations in healthy children aged 3-14 years in Greece. Biol Trace Elem Res; 115:1-12.

Amaya D, Varrieta R, Gil N, Mmolano N, Medrano I and Castejon H. 1997. Zinc plasma, arch levels in a low socioeconomic status population of children in Maracaibo, Venwzuela Latinoan Nutrition; 47 (1): 23- 28.

Anderson, I. 1995. Zinc as an aid to healing. Nurs Times; 91:68, 70.

Bahl R, Bhandari N, Hambidge K and Bhan, M.K. 1998. Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting. American Journal Clinical Nutrition. 68: 414–417.

Barlet JA, Kortlik JW and Higgines C. 2001. Sampling Statistics; Determining Appropriate Sample Size for Survey Research. Journal ITLP; 19 (1): 43-50.

Berry L, Hall K and Hendricks M. 2010. Nutrition- Stunting in Children. Statistics on children in South Africa, www.childrencount.ci.org.za. 23/04/2010.

Bales CW, Steinman LC, Freelandgraves JH, Stone JM and Young RK. 1986. The effect of age on plasma zinc uptake and taste acuity. Am J Clin Nutr 44, 664–669.

Bao B, Prasad AS, Beck FWJ, Fitzgerald JT, Snell D, Bao GW, Singh T, and Cardozo LJ. 2010. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am J Clin Nutr; vol. 91 no. 6: 1634-1641.

Baqui AH, Fischer Walker CL and Zaman K. 2005. Weekly iron supplementation does not block increases in serum zinc due to weekly zinc supplementation in Bangladeshi infants. Journal of Nutrition, Vol. 135, no 1, pp. 2187-2191.

Bhutta ZA, Nizami SQ and Isani Z. 1999. Zinc supplementation in malnourished children with persistent diarrhea in Pakistan. Pediatrics; 103:1–9.

Bhutta ZA, Bird S and Black RE. 2000. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. Am J Clin Nutr.; 72(6): 1516-1522.

Bhandari N, Bahl R and Taneja S. 2002. Substantial reduction in severe diarrhea morbidity by daily zinc supplementation in young north Indian children. Pediatrics 109, e86.

Black MM. 1998. Zinc deficiency and child development. Am J Clin Nutr.; 68 (2 suppl): 464S-469S.

Black RE. 1998. Therapeutic and preventive effects of zinc on serious childhood infectious disease in developing countries. Am J Clin Nutr.; 68 (2 Suppl): 476S-479S.

Black RE. 2003. Zinc deficiency, infectious disease and mortality in the developing world. J Nutr; 133: 1485S-1489S.

Black R, Allen, L and Bhutta Z. 2008. Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet; published online January 17. D01;10. 10/S0140-6736907)61690-0.

Blanchard R and Cousins R. 1997. Upregulation of rat intestinal uroguanylin mRNA by dietary zinc restriction. Am J Physiol; 272 (5 Pt1):G972–8.

Bleichrodt N. and Born M. 1994. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stansbury JB, ed. The damaged brain of iodine deficiency. New York: Cognizant Communication Corporation, 195-200.

Bomela N. 2007. Child nutritional status and household patterns in South Africa. African Journal of food agriculture nutrition and development, Vol 7 No5, ISS 1684-5374.

Bothwell T and Charlton R. 1981. Iron deficiency in women. Washington DC, Nutrition Foundation.

Broyles ST, Staiano AE, Drazba KT, Gupta AK, Sothern M and Katzmarzy PT. 2012. Elevated C-Reactive Protein in Children from Risky Neighborhoods: Evidence for a Stress Pathway

Linking Neighborhoods and Inflammation in Children. PLoS one 7(9): e45419. doi:10.1371/journal.pone.0045419.

Broun ER, Greist A and Tricot G. 1990. Excessive zinc ingestion: a reversible cause of sideroblastic anemia and bone marrow depression. JAMA; 264:1441-1443.

Briefel RR, Troiano RP, Carroll MD and Bialostosky K. 2000. Zinc intake of US population: findings from the third National Health and Nutrition Survey 1988-1994. 130: 1367 S.

Brown KH; Wuehler SE and Peerson JM. 2001. 'The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency', Food and Nutrition Bulletin 22(2), 113–125.

Brown KN, Peerson JM and Allen LH. 1998. "Effect of zinc supplementation on children's growth: a meta-analysis of intervention trials". Bibliotheca Nutroitio-Et Dieta, 54: 76-83.

Brown J and Toma R. 1986. Taste changes during pregnancy. Am J Clin Nutr;43: 414–418.

Brown KH, Peeraon JM, Rivera J and Allen LH. 2002. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr; 75 (6): 1062-1071.

Brock JH, Halliday JW, MJ P and Powell LW. 1994. Iron metabolism health and disease, London, W.B Saunders Company Ltd.

Butrimovitz GP and Purdy WC. 1977. The determination of zinc in blood plasma by atomic absorption spectrometry. Anal Chim Acta, 94: 63-73.

Buzina R, Jusic M, Sapunar J and Milanovic N. 1980. Zinc nutrition and taste acuity in school children with impaired growth. J Clin Nutr:33: pp2262-2267.

Baumgartner J, Smuts CM, Aleberli I, Malan L, Tjalsma H and Zimmermann MB. 2013. Overweight impairs efficacy of iron supplementation in iron-deficient South African children: a randomized controlled intervention. International journal of Obesity; 37, 24-30.

Bourne LT, Lambert EV, Steyn K. 2001. Where does the black population of South Africa stand on the nutrition transition? Public Health Nutrition; 5: 157–62.

Brunvan L, Henrickson C, Larsson M and Sandberg AS. 1995. Iron deficiency among pregnant Pakistanis in Norway and content of phytic acid in their diet. Acta Obstet Gynecol Scand; 74: 520-525.

Byanes RD and Stipanuk MH. 2000. Biochemical and physiological aspects of human nutrition. Philadelphia, PA; WB Saunders company: 71; 1-40. The expenses associated with meats may limit access for children.

Capelle K. 2007. Zinc for hair loss. http://worldvillage.com/zinc-for-hair-loss (February 11th, 2007).

Caulfield L and Black RE. 2004. 'Zinc deficiency', in Ezzati M. A.D, Rodgers L A. and Murray C.J.L (eds.), Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors vol. 1, pp. 257–279, World Health Organization, Geneva.

Castillo JJ. 2009. Experimental Research> Simple Random Sampling.

Castillo-Duran C, Vial P and Uauy R 1988. "Trace mineral balance during acute diarrhea in infants". J. Pediatr. 113 (3): 452–7.

Catalanotto F. 1978. The trace metal zinc and taste. Am J Clin Nutr; 31:1098–1103.

Cameron N. 2003. Physical growth in a transitional economy: the aftermath of South African apartheid. Economics and human biology 1: 29-42.

Carlson BA and Wardlaw TM. 1990. "A global, regional and country assessment of child Malnutrition." UNICEF Staff Working Paper No. 7.New York.

Caufield LE, Zavaleta' N, Shanker AH and Merialdi M. 1998. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr.; 68 (2 Suppl): 499S-508S.

Cha MC and Rojhani A. 1997. Zinc deficiency inhibits the direct growth effect of growth hormone on the tibia of hypophysectomized rats. Biol Trace Elem Res; 59 (1-3):99-111.

Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M and Solomons NW. 1993. Growth and body composition of peri-urban Guatemalan children in relation to zinc status: a cross-sectional study. Am J Clin Nutr 57, 334–343.

Chester JK. 1991. Trace element-gene interactions with particular reference to zinc. Proc. Nutr.Soc; 50: 123-129.

Charlton KE, Bourne LT, Steyn K and Laubscher JA. 2001. 'Poor nutritional status in older black South Africans', Asian Pacific Journal of Clinical Nutrition 10(1), 31-38.

Christian P and West KP. 1995. Interactions between zinc and vitamin A. Am J Clin Nutr suppl: 4355-4415.

Chen XC, Yin TA, He JS, Ma QY, Han ZM and Li LX. 1985. Low levels of zinc in hair and blood, pica, anorexia and poor growth in Chinese preschool children, J Clin Nutr, 42. 694-700.

Clegg MS. Keen CL, Lonnerda BO, Hurley LS. 1981. Influence of ashing technique on the analysis of trace elements in animal tissue. I. Wet Ashing Biol Trace Element Res 3: 107-115.

Coleman JE. 1998. “Zinc enzymes” Current Opinion in Chemical Biology. 2: 222-234.

Cole CR, Grant FK, Swaby-Ellis ED, Smith JL, Jacques A, Northrop-Clewes CA, Caldwell K L, Pfeiffer CM, and Ziegler TR. 2010. Zinc and iron deficiency and their interrelations in low-income African American and Hispanic children in Atlanta. Am J Clin Nutr. Apr 2010; 91(4): 1027–1034.

Clarke N, Grantham-McGregor SM and Powell C. 1991 Nutrition and health predictors of school failure in Jamaican children. Ecol. Food Nutr. 26:1-11.

Crosby. 2009. Zinc Diagnostic Tests. http//:www.diagnoseme.com.

Cogill B. 2003. Anthropometric Indicators Measurements Guide. Food and Nutrition Technical Assistance. Revised Edition.

Crosby. 2009. Zinc Diagnostic Tests. http//:www.diagnoseme.com.

Dallman PR, Looker AC, Johnson CL and Carroll M. 1993. Influence of age on laboratory criteria for the diagnosis of iron deficiency anemia and iron deficiency in infants and children: Hallberg L, Asp N-G (eds) iron Nutrition in Health and Disease. John Libbey and Co, London, pp. 65-74.

Dannhauser A, Bester CJ, Joubert G, Badenhorst PN, Slabber M, Badenhorst AM, Du Toit E, Barnard HC, Botha P and Nogabe L. 2000. Nutritional status of preschool children in informal settlement areas near Bloemfontein, South Africa. Public Health Nutrition: 3(3), 303±312.

Davison KK and Birch LL. 2001.Weight status, parent reaction, and self-concept in five-year-old girls. Pediatrics; 107: 46–53.

Dietz WH. 1998. Health consequences of obesity in youth: childhood predictors of adult disease. Pediatrics; 101: 518-25.

De Benoist B, Darnton-Hill I, Davidsson L, Fontaine O and Hotz C. 2007. Food and Nutrition Bulletin. The United Nations University. Vol. 28, no. 3 (supplement).

De Cock N, Haese MD, Vink N , van Rooyen CJ, Staelens L , Schönfeldt HC and Haese LD. 2013. Food security in rural areas of Limpopo province, South Africa. The Science, Sociology and Economics of Food Production and Access to Food. 5:269–282.

De Onis, M and Habicht JP. 1996. Anthropometric reference data for international use: recommendations from a World Health Organization Expert Committee. American Journal of Clinical Nutrition 64: 650-658.

De Onis M, Monteiro C, Akre J and Glugston G. 1993. The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global Database on Child Growth. Health Organ 71: 703-712.

De Onis M and Blossner M. 2003. The world Health Organisation Global Database on Child Growth and Malnutrition: methodology and applications. Int J Epidemol; 32: 518-526.

Demaeyer EM, Dallman P, Gumey SM, Hallberg L, Sood SK and Srikantia SG. 1989. Prevention and controlling iron deficiency anemia through primary health care. Geneva: World Health Organization.

Department of Health. 2008. Integrated nutrition programme booklet.

Domellof M, Lonerdal B, Dewey KG, Cohen RJ, Rivera IL and Hornell O. 2002a. sex difference in iron status during infancy. Pediatrics 110: 545-552.

Dhansay MA, Marais CD and Labadarios D. 2007. Zinc status. In: Labadarios D. National food consumption survey: fortification baseline, Chapter 9D: South Africa 2005. Stellenbosch: Department of Health.

Dehghani SM, Katibeh P, Haghighat M, Moravej H and Asadi S.2011. prevalence of zinc deficiency in 3-18 years old children in Shiraz-Iran. Iran Red Crescent Med J; 13(1):4-8.

Dhingra U, Hiremath G, Menon, V.P, Dhingra P, Sarkar A and Sazawal S. 2009. Zinc Deficiency: Descriptive Epidemiology and Morbidity among Preschool Children in Peri-urban Population in Delhi, India, J Health popul Nutr; 27(5):632-639.

Disease Control Priorities in developing countries. 2006. Second edition.

Downs SM, Arnold A, Marshall D, McCarran MJ, Raine KD and Willows ND. 2009. Associations among the Food Environment, Diet Quality and Weight Status in Cree Children in Quebec. Public Health Nutrition; 12(9): 1504- 1511.

Dulger H, Arik M, Sekeroglu MR, Tarakcioglu M, Novan T and Cesur Y. 2002. Pro-inflammatory cytokines in Turkish children with protein-energy malnutrition. Mediators Inflamm; 11: 363-5.

Demographic and Health Survey. http://wwwmeasuredhs.com/accesssurveys/search/start.cfm.Accessed 11/06/06.

Eveleth PB and Tanner JM. 1990. Worldwide variation in human growth. Cambridge University Press, Family and Relationships - 397 pages.

Ekiz C, Agaoglu L, Karakas Z, Gurel N and Yalcin I. 2003. The effect of iron deficiency anaemia on the function of the immune system; 56:243-245.

Faber M, Jogessar VB and Benade AJS. 2001. "Nutritional status and dietary intakes of children aged 2-5 years and their caregivers in a rural south Africa community", international Journal of science and Nutrition 52 (5), 401-411.

Faber M and Wenhold F. 2007. Nutrition in contemporary South Africa. Water SA, 33: (3).

Fairweather-Tait SJ. 1998. Iron-zinc and calcium-iron interactions in relation to zinc and iron absorption, Proc Nutri Soc 54: 465.

Fatourechi V, Ahmed DD, Schwartz KM. 2002. Thyroid acropachy: report of 40 patients treated at a single institution in a 26-year period. J Clin Endocrinol Metab;87: 5435–5441.

Ferraz IS, Daneluzzi JC, Vannucchi H, Jordao AA Jr, Ricco RG, Del CIampo LA, Martinelli Jr, CE, D'Angio Engelberg AA, Custodio VIC, Bonilha Luis RCM. 2004. Detection of deficiency in Brazilian preschool children using the serum 30-day dose-response test. Eur J Clin Nutr; 58:1372-1377.

FAO (Food and Agriculture Organization of the United Nations). 2004. Globalization of food systems in developing countries: Impact on food security and nutrition. FAO Food and Nutrition Paper 83. Rome: FAO. (2006). The state of food insecurity in the world 2006.

Fesharakinia A, Zarban A and Sharifizadeh GR. 2009. prevalence of zinc deficiency in elementary school children of south Khorasan province (east iran). Irn Pediatr J; 19:249-54.

Fischer Walker CL and Black RE. 2007. Micronutrient and diarrheal disease. Clin Infect Dis; 45 Suppl 1: S73-77.

Food and Nutrition Board, Institute of Medicine Zinc. 2001. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, D.C: National Academy Press; 442-501. (National Academy press).

Fraker PJ and King LE. 2004. Reprogramming of the immune system during zinc deficiency. Annu Rev Nutr.; 24:277-98.

Floyd BA. 2002. Anemia. Bloomington, IN: Author House.

Frongillo EA Jr, de Onis M and Hanson KMP. 1997. Socioeconomic and demographic factors are associated with worldwide patterns of stunting and wasting of children. Journal of Nutrition; 127: 2302–9.

Grant JP. 1993. Halving child malnutrition by the year 2000. An ethical imperative. Address to the 15th International Congress on Nutrition, Adelaide, Australia. Unpublished.

Gibney MJ, Vorster HH and Kok FJ. 2002. Introduction to Human Nutrition (edited on behalf of the Nutrition Society), Blackwell science Ltd, pp 197-200.

Gibson RS, Smit-Vanderkooy PD, MacDonald AC, Goldman A, Ryan B and Berry M. 1989. A growth limiting mild zinc deficiency syndrome in some Southern Ontario boys with low growth percentiles. Am J Clin Nutr; 49: 1266-1273

Gibson RS. 1994. Zinc nutrition in developing countries. Nutrition Research Reviews 7: 151-173.

Gibson RS. 2005. Principles of Nutritional Assessment, second edition. Published by oxford university press.

Gibson RS. 2006. 'Zinc: The missing link in combating micronutrient malnutrition in developing countries', Proceedings of the Nutrition Society 65(1), 51-60.

Gibson RS. Manger MS, Krittaphol W, Pongcharoen T, Gowachirapant S and Bailey KB. 2007. 'Does zinc deficiency play a role in stunting among primary school children in NE Thailand? British Journal of Nutrition 97(1),167-175.

Goto T, Komai M, Bryant BP and Furukawa Y. 2000. Reduction in carbonic anhydrase activity in the tongue epithelium and submandibular gland in zinc-deficient rats. Int J Vitam Nutr Res;70: 110–118.

Goto T, Komai M, Suzuki H and Furukawa Y. 2001. Long-term zinc deficiency decreases taste sensitivity in rats. J Nutr;131:305–310.

Glöckner L, Fikentscher R and Ulrich F. 1986. Taste perception in fasting overweight patients. Z Gesamte Inn Med; 41:244–246.

Grantham-McGregor SM. 1995. A review of studies of the severe malnutrition on mental. J Nutr. Suppl. 2233S-2238S.

Guansheng M, Yanping L, Ying J, Songming D, Frans J K and Xiaoguang Y. 2006. Assessment of intake inadequacy and food sources of zinc of people in China. Public Health Nutrition:, page 1 of 7

Gunshin H, Mackenzie and Berger UV. 1997. Cloning and characterization of mammalian proton coupled metal-ion transporter. 388: 482-488.

Gunter EW, Turner WE, Neese JW and Bayse DD. 1985. Laboratory procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey (HANES II) 1976-1980. Revised edition. Atlanta, GA: US Department of Health and Human Services.

Hawke CI and Wilkinson EA. 1998. Does oral zinc aid the healing of chronic leg ulcers? A systematic literature review. Arch Dermatol; PubMed abstract; 134: 1556-1560.

Hambidge K, Hambidge C, Jacobs M and Baum J. 1972. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediatr Res; 6: 868–874.

Hambidge M and Krebs N. 2000. Zinc and growth. In: Roussel AM, ed. Trace elements in man and animals 10: Proceedings of the tenth international symposium on trace elements in man and animals. New York; Plenum Press; 977-980.

Hambridge M. 2000. Human zinc deficiency. J Nutr; 130: 1344-1349.

Hess SY, Peerson JM and Brown KH. 2007. Use of serum zinc concentration as an indicator of population zinc status. Food Nutr Bull; 28 (suppl): S403-429.

Henkin R, Martin B and Agarwal R. 1999. Decreased parotid saliva/carbonic anhydrase VI secretion: An enzyme disorder manifested by gustatory and olfactory dysfunction. Am J Med Sci; 318:380–391.

Henkin R, Schechter P and Firedewald W. 1976. A double blind study of the effects of zinc sulfate on taste and smell dysfunction. Am J Med Sci; 272: 285–299.

Heckman J, Amidou S, Bessong P, Ntsieni M, Hamandi H, Kohler M, Milam B, Scriver J and Dillingham R. 2010. Anaemia among clinically well under-fives attending a community health centre in Venda, Limpopo Province; S Afr Med J; 100(7): 445–448.

Hsu DJ, Daniel JC and Gerson SJ. 2004. Effect of zinc deficiency on keratin in buccal epithelium. Vol 36, issue 10, page 759-763.

Hotz C, Peerson, JM and Brown KH. 2003. Suggested lower cutoffs of serum zinc concentration for assessing population zinc status: A reanalysis of the second US National Health and Nutrition Examination Survey data (NHANES II: 1976-1980). Am J Clin Nutr; 78:756-764.

Hotz C and Brown KH. 2004. International Zinc Nutrition Consultative Group. Technical Document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull.; 24(1): S94-203.

Hotz C and Brown KH. 2004. Assessment of the risk of zinc deficiency in population and options for its control. Food Nutr Bull; 25: S91-204.

Hughes DA. 2004. Diet and Human Immune Function. New Jersey: Humana Press Inc.

Hunter HN, Fulton DB, Ganz T and Vogel HJ. 2002. "The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis". J Biol Chem. 277 (40): 37597–603.

HPCSA. 2007. Health Profession Council of South Africa. Guidelines for good practice in the Health Care profession: General Ethical Guidelines for Health Researches. 1st Reissue: Booklet 3.

Hawkes C and Ruel MT. (Eds.). 2006. Understanding the links between agriculture and health. Agriculture and nutrition linkages: Old lessons and new paradigms (Focus 13, Brief 4 of 16).

http://www.stophairlossnow.com/Dandrff.htm (March 27th, 2010)

http://www.unicef.org/nutrition/files/ENAcute Diarrhea reprint.pdf (March 3rd, 2010).

Crosby J. 2009. Uses of liquid zinc (internet): Available from: http://www.livestrong.com/article/30263- uses-liquid-zinc (July 27th, 2011).

Ibeanu V, Okeke E, Onyechi U and Ejiofor U. 2012. Assessment of anthropometric indices, iron and zinc status of preschoolers in a peri-urban community in south east Nigeria. International Journal of Basic and Applied Science Vol: 12 No: 05; 1213005-2727.

Iqbal A, Khalil IA, Ateeq N and Khan MS. 2006. Nutritional quality of important food legumes. Food chem.; 97:331-335.

Ikeda M, Ikui A and Kamiyama A. 2007. Causative factors of taste disorders in the elderly and therapeutic effects of zinc. J Laryngol Otol; e-pub ahead of print: 1–6.

Idjradinata P and Pollitt E. 1993. Reversal of developmental delays in iron deficient anaemic infants treated with iron. 341:1-4.

International Zinc Nutrition Consultative Group (IZiNCG). 2004. Assessment of the risk of zinc deficiency in populations and options for its control. Hotz C, Brown KH, eds. Food Nutr Bull; 25 (suppl 2): S91-204.

International Zinc Nutrition Consultative (IZiNCG). 2007. Quantifying the risk zinc deficiency, Technical Brief, No 1.

International Zinc Association (IZA). Zinc Protects: zinc for better health. Available at http://www.zincworld.org/zinc health. html accessed 27th January 2008.

Institute of Medicine, Food and Nutrition Board. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academy Press

International Center for Diarrhea Disease Research. Dhaka, Bangladesh. Zinc supplementation in the treatment of childhood diarrhea. Indian J Pediatr 1995; 62:181-193.

Jenkins T. 2006. Beneficial .Effects of Zinc, June 12th, (www.Healthinsuredirect.co.za).

Joshi SA. 2002. Nutrition and Dietetics, second edition (2nd), Tata McGraw-Hill publishing company limited, pp134-135.

Jooste PL, Labadarios D, Nel H and Strydom E. 2007. Iodine content of household salt, drinking water and iodine status of women and children. In: Labadarios D. National food consumption survey: fortification baseline, Chapter 8: South Africa 2005. Stellenbosch: Department of Health.

Katzenellenbogen JM, Joubert G and Abdool-Karim SS. 2003. Epidemiology: a manual for South Africa. Auckland, Bangkok, Cape Town: Oxford University Press.

Kazemi TF. 2009. Prevalence of Overweight and Obesity in 7 to 18 Year-Old Children in Birjand/ Iran. Iranian J Paediatrics, 19(2):135-140.

Kettaneh A, Paries J and Stiremann J. 2005. Clinical and biological features associated with taste loss in internal medicine patients: A cross-sectional study of 100 cases. Appetite; 44:163–169.

Khor GL and Tee ES. 1997. Nutritional assessment of rural villages and estates in peninsular Malaysia. II. Nutritional status of children aged 18y years and below. Malaysian J Nutr 3:21-47.

Khan Khattak, MMA and Ali S. 2010. Malnutrition and associated risk factors in pre-school children Pakistan. J Med Sci; 10:34-39.

Kidd P. 2000. Attention deficit/hyperactivity disorder (ADHD) in children: rationale for integrative management. Altern Med Rev; 5(5): 402-428.

Kimani-Murage EW. 2012. Exploring the paradox: double burden of malnutrition in rural South Africa. Glob Health Action. 6:19249.

Kimani-Murage EW. 2013. Exploring the paradox: double burden of malnutrition in rural South Africa. Global Health Action. Jan 24; 6: 19249.

King JC and Cousins RJ. 2006. Zinc. In: Shils ME. Shike M, Ross, A.C, Caballero B, Cousins, R.J, eds. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams and Wilkins; 271-285.

King JC. 1990. Does poor zinc nutriture retard skeletal growth and mineralization in adolescents? Am J Clin Nutr; 64: 375.

King JC, Shames DM and Woodhouse LR. 2000. Zinc homeostasis in humans. 130; 1360S-1366S.

Kostka E, Wittekindt C and Guntinas-Lichius O. 2008. Tongue coating, mouth odor, gustatory sense disorder—earlier and new treatment options by means of tongue scraper. Laryngorhinootologie 87: 546–550.

Kordas K and Stoltzfus RJ. 2004. New evidence of iron and zinc interplay at the enterocyte and neural tissues. J Nutr 134: 1295-1298.

Kruger M, Badenhorst CJ, Mansvelt EPG, Laubscher JA and Benade AJS. 1996. Effects of iron fortification in a school feeding scheme and anthelmintic therapy on the iron status and growth of six- to eight-year-old schoolchildren. Food Nutr. Bull. 17:11–21.

Kruger R and Gericke GJ. 2003. A qualitative exploration of rural feeding and weaning practices, knowledge and attitudes on nutrition. Public Health and Nutrition, 6(2):217-223, April 2003.

Kruger HS, Swart R, Labadarios D, Dannhauser A and Nel JH. 2007. Antropometric status. In: Labadarios (ed) 2007. The National Food Consumption Survey- Fortification Baseline: The knowledge, attitude, behaviour and procurement regarding fortified foods, a measure of hunger and the anthropometric and selected micronutrient status of children aged 1-9 years and women of child bearing age; South Africa, 2005. Pretoria; Directorate; Nutrition, Department of Health.

Kumosani TA, Abdul-Jabbar HS and Al-Tazi ZA. 1997. Serum zinc levels of healthy individuals living in the Western Province of Saudi Arabia. Ain Shams Med J; 48:797-802.

Kuhn LC. 1996. Control of cellular iron transport and storage at the molecular level. P 17-29.

Kwena AM, Terlouw DJ, de Vlas SJ, Phillips-Howard PA, Hawley WA, Friedman FJ, Vulule JM, Nahlen BL, Sauerwein RW and Ter Kuile FO. 2003. Prevalence and severity of malnutrition in pre-school children in a rural area of western Kenya. Am J Trop Med Hyg, vol 68 No 4 suppl 94-99.

Labadarios D, Van Middelkoop A, Coutsoudis A, Eggers RR, Hussey G, Ijsselmuiden C and Kotze JP. 1995. Children aged 6 to 71 months in South Africa, 1994: their anthropometric, vitamin A, iron and immunization coverage status. South African Vitamin A Consultative Group (SAVACG), Isando. 335 pp.

Labadarios D. 2000. Dietary reference intakes (DRLs): Antioxidant micronutrients. The South African J purnal of Clinical Nutrition Aug; 13(3): 111.

Labadarios D, Steyn NP, Maunder E, MacIntryre U, Gericke G, Swart R, Huskisson J, Dannhauser A, Vorster HH, Nesmvuni AE and Nel JH. 1999. The National Food Consumption Survey (NFCS): South Africa, Public Health Nutr. 2005 Aug; 8(5):533-543.

Labadarios D. Folate status. 2007. In: Labadarios D. National food consumption survey: fortification baseline, Chapter 9C: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D and Louw R. 2007. Iron Status. In: Labadarios D. National food consumption survey: fortification baseline, Chapter 9B: South Africa 2005. Stellenbosch: Department of Health.

Labadarios D, Swart R, Maunder EMW, Kruger HS, Gericke GJ and Kuzawayo PMM. 2005. 'Executive summary of the National Food Consumption Survey Fortification Baseline (NFCS-FB-I), South Africa, 2008' South African Journal of Clinical Nutrition 21(3, suppl. 2), 245–300.

Labadarios D, Swart R, Maunder EMW, Kruger HS, Gericke GJ and Kuzawayo PMM. 2008. Folate, iron, vitamin A and zinc status of children 12-108 months and women 16-35 years old in South Africa: the national food consumption survey-fortification Baseline. Abstract 70. 2008 Nutrition congress: Evidence based Nutrition Leading the Way in Innovation, South Africa p202.

Lamont J. 1997. Homoeopathic treatment of attention deficit hyperactivity disorder; a study. Br Homoeopath J; 86: 196-200.

Lansdown AB, Mirastschijski U, Stubbs N, Scanlon E and Agren MS. 2007. Zinc in wound healing: theoretical, experimental, and clinical aspects. Wound Repair Regen; PubMed abstract. 15; 2-16.

Leedy PD and Ormrod JE. 2002. Practical Research; Planning and Design (8th edition). Pearson Merill Hall.

Lee RD and Nieman DC. 2003. Nutritional Assessment; 3rd Edition, San Francisco: McGraw Hill. Inc. pp 164-165.

Levin HM, Pollitt E, Galloway R and McGuire J. 1993. Micronutrient Deficiency Disorder. In: Jamison DT, Mosley WH, Measham AR, Bobadilla, J.L (eds). Disease Control Priorities in Developing Countries. Oxford: Oxford University Press, pp 421-451.

Lozoff B, Menez E, Hagen S, Mollen E and Wolf AW. 2000. Poorer behavioural and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics: 105; E51.

Lozoff B, Jimene E and Wolf A. 1991. Long term development outcome of infants with iron deficiency. Engl J Med; 325: 687-694.

Lozoff B. 2000. Perinatal iron deficiency and the development brain. Pediatr Res: 48; 137-139.

Lukacik M, Thomas RL and Aranda JV. 2008. A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea. Pediatrics, PubMed abstract, 121: 326-336.

Lukowski AF, Koss M, Burden MJ, Jonides J, Nelson CA and Kaciroti N. 2010. Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long term deficits in executive function function and recognition memory. Nutr Neurosci; 13 (2): 54-70.

Lui J, Raine AD, Venables PH and Mednick S A. 2004. Malnutrition at Age 3 Years and Externalizing Behavior Problems at Ages 8, 11, and 17 Years. American Journal of Psychiatry. 161:2005–2013.

Laura EC, Richard SA and Black R. 2004. Nutrition as underlying cause of malaria morbidity and mortality in children less than five old. Am J Trop Med Hyg, vol. 71 no. 2 suppl 55-63.

Mann J and Truswell SA. 2007. Essential of human nutrition, 3rd (Third Edition) oxford New York, press inc, pp 139-141.

Manary MJ, Hotz C and Krebs NF. 2000. "Dietary Phytate reduction improves zinc absorption in Malawian children recovering from tuberculosis but not in well children". J. Nutr. 130 (12): 2959–64.

Mahan LK and Escott-Stump S. 2008. Krause's Food and Nutrition Therapy, 12 edition; p114-120.

McDonald RS. 2000. The role of zinc in growth and cell proliferation. J Nutr, 130 (5S Suppl): 1500S-1508S.

MacIntyre, U.E, Venter, C.S and Vorster, H.H. 2001b. 'A culture-sensitive quantitative food frequency questionnaire used in an African population: 2. Relative validation by 7-day weighed records and biomarkers', Public Health Nutrition 4(1), 63–71.

McOsker DE and Hannon DP. 1967. Ultra structural studies of dandruff involved scalp tissue. The Toilet Goods Association. 47:5-8.

Macgregor M. 1963. Maternal anemia as a factor in prematurity and perinatal mortality. Scottish Medical Journal, 8:134.

Mascotti DP, Rup D and Thach RE. 1995. Regulation of iron meatbolism: Translational effects medicated by iron, heme and cytokines. Ann. Rev. Nutr., 15: 239-261.

Marti A and Allred CG. 1999. Role of zinc in wound healing. Adv in wound care; 12: 137-138.

Maret W and Sandstead HH. 2006. Zinc requirements and the risk and benefits of zinc supplementation. J Trace Elem Med Biol; 20:3-18.

Markell EK, John DC and Petri WH. 2006. Markell and Voge's medical parasitology (9th ed.). St. Louis, Mo: Elsevier Saunders 0-7216-4793-6.

McGregor G and Ani C. 2001. A review of studies on the effect of iron deficiency on cognitive development in Children. J Nutr; 131: 649S-66S; discussion 666S-668S.

Mendez M and Popkin B. 2004. Globalization, urbanization and nutritional change in the developing world. In Globalization of food systems in developing countries: Impact on food security and nutrition. FAO Food and Nutrition Paper 83: pp. 55–80.

Mishra NR. 2005. Dynamics of caste-based deprivation in child under-nutrition in India. Report Presented at the National Seminar on Development of Scheduled Castes in India; An Appraisal of Implementation of Programmes at MPISSR, Ujjain, Madha Pradesh, January 17-19.

Mittal PC and Srivastava S. 2006. Diet, nutritional status and food related traditions of Oraon tribes of New Mal (West Bengal), India. The International Electronic Journal of Rural and Remote Health Research, Education, Practice and Policy, 6: 385.

Michaelsson G, Juhlin L and Vahlquist. 1977. Effects of oral zinc and vitamin A in acne. American Journal of Medical Association vol 113, No 1.

Mocchegiani E, Bertoni-Freddari C, Marcellini F and Malavolta M. 2005. Role of zinc in brain function, Apr; 75(6): 367-390 PubMed

Mocchegiani E and Muzzioli M. 2000. Therapeutic Application of Zinc in Human Immunodeficiency Virus against Opportunistic Infections. J Nutr, Vol 130, No 5: 1424S-1431S.

Morgan G and Harmon R. 1999. Sampling and External Validity. Journal of the American Academy of child and adolescent Psych; 28: 1051-1053.

Morlese JF, Forrester T, Badaloo A, Del Rosario M, Frazer M and Jahoor F. 1996. Albumin kinetics in protein-energy malnourished children. Am. J. Clin. Nutr. 64:952–959.

Morlese JF, Forrester T, Del Rosario M, Frazer M and Jahoor F. 1997. Transferrin kinetics is altered in children with severe protein-energy malnutrition. ; 127(8):1469-74.

Monteiro CA, Conde WL and Popkin, BM. 2002. Is obesity replacing or adding to undernutrition? Evidence from different social classes in Brazil. Public Health Nutrition; 5: 105–12.

Muller O. Garonne M. Reitmaier P. Baltussen A and Zweeden V. 2003. Effect of zinc supplementation on growth in West African children: a randomized double-blind placebo-controlled trial in rural Burkina Faso. International Journal of Epidemiology. 32: 1098-1102.

National Food Consumption Survey- Fortification Baseline. 2005. The knowledge, attitude, behaviour and procurement regarding fortified foods, a measure of hunger and the anthropometric and selected micronutrient status of children aged 1-9 years and women of child bearing age; South Africa, Pretoria; Directorate; Nutrition, Department of Health.

Neuman WL. 2003. Social Research Methods-Qualitative and Quantitative Approaches. 5th edition. Allyn and Bacon. Boston.

Noorbala AA and Akhondzadeh S. 2006. Attention-deficit/hyperactivity disorder; etiology of a pharmacotherapy. Arch Iran Med; 9(4); 374-380.

Nolunkcwe B. 2007. Child Nutritional Status and Household Patterns in South Africa, volume 7, no 5.

O'Dell BL. 2000. Role of zinc in plasma membrane function. J Nutr.; 130 95s suppl): 1432s-1436s.

Oelofse A, Van Raaij, JMA, Benadé, AJS, Dhansay M.A, Tolboom JJM and Hautvast JGAJ. 2002, 'Disadvantaged black and coloured infants in two urban communities in the Western Cape, South Africa differ in micronutrient status', Public Health Nutrition 5(2), 290-294.

Oberleas D and Harland BF. 2010. The True Story of Zinc Nutrition and Homeostasis, sight and life magazine; 2: 13-19.

Ohiokpehai O, David DM and Kamau J. 2009. Serum zinc levels of school children on corn-soy blend feeding trial in primary schools in Suba district Kenya.

Onyemaobi GA, Onimawo IA, Uwaegbute CA. 2011. Prevalence of Iron Deficiency Anaemia among under-five childre in Imo state, Nigeria. Australian Journal of Basic and Applied Sciences, 5(2): 127-130.

Olivares M, Pizarro F, Gaitan D and Ruz M. 2007. Acute inhibition of iron absorption by zinc. Nutrition Research 27: 279-282.

Owens S, Gutin B, Allison J, Riggs S, Ferguson M, Litaker M and Thompson W. 1999. Effect of physical training on total and visceral fat in obese children. Med. Sci. Sports Exerc. 31:143–148.

Paknahad Z, Mahdavi R, Mahboob S, Ghaemmaghami SJ, Omidvar N, Ebrahimi M, Ostadrahimi A and Afiat Milani SH. 2007. Iron and Zinc Nutritional and Biochemical Status and Their Relationship among Child Bearing Women in Marand Province. Pakistan Journal of Nutrition 6 (6): 672-675.

Parnell W, Scragg R, Wilson N, Schaaf D and Fitzgerald E. 2003. NZ Food, NZ Children; Key Results of the 2002 National Children's Nutrition Survey. Wellington: Ministry of Health.

Pelletier D, Frongillo EA and Habicht JP. 1993. Epidemiological evidence for potentiating effect of malnutrition on child mortality. Am J Public Health; 83:1130-1133.

Pelletier DL, Frongillo EA, Schroeder DG and Habicht JP. 1995. The effects of malnutrition on child mortality in developing countries. Bull World Health Organ 73:443-448.

Pelletier DL. 1994. The relationship between child anthropometry and mortality in developing countries: implications for policy, programs and future research. J Nutr 124 (suppl10): 2047S-2081S.

Pfeiffer CC and Braverman ER. 1997. "Zinc, the brain, and behaviour". Biological Psychology, 17: 513-530.

Pinstrup-Andersen P, Burger S, Habicht JP and Peterson K. 1993. Protein-energy malnutrition. In: Jamison DT, Mosley WH, Measham AR, Bobadilla JL (eds). Diseases Control Priorities in Developing Countries. Oxford: Oxford University Press, pp. 391-420.

Pi-Sunyer FX. 1991. Health implications of obesity. Am J Clin Nutr. 53(Suppl 1): S595– 603.

Pilch SM and Senti FR. 1984. Assessment of the zinc nutritional status of the US population based on data collected in the second National Health and Nutrition Examination Survey, 1976-1980. Bethesda, MD: Life Science Research Office, Federation of American Socities for Experimental Biology.

Pluhator MM, Thomson ABR and Fedorak RN. 1996. Clinical aspects of trace elements: zinc in human nutrition – assessment of zinc status. Canad J Gastroenterol 10, 37–42.

Pollit E. 1997. Iron deficiency and educational deficiency. Nutr Rev; 55: 133-141.

Polit DF and Hungler BP. 2009. Essential Nursing research: Principles and methods 6th edition. JB Linpincott Company.

Prasad AS, Halsted JA and Nadimi M. 1961. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med; 31:53246.

Prasad AS, Oberlelas D, Miller ER and Luecke RW. 1963. "Biochemical effects of zinc deficiency: changes in activities of zinc –dependent enzymes and ribonucleic acid and deoxyribonucleic acid content of tissues" Journal of laboratory and clinical medicine, 77: 144-152.

Prasad AS. 1995. Zinc: an overview Nutrition II (Suppl): 93-99.

Prasad AS. 2003. Zinc deficiency editorials, BMJ volume 326: 409-10.

Prasad AS. 2007. Zinc: mechanisms of host defence. J Nutr. 2007 May; 137(5):1345-9.

Ramakrishnan U, Aburto N, McCabe G, Martorell R. 2004. Multimicronutrient Interventions but Not Vitamin A or Iron Interventions Alone Improve Child Growth: Results from Three Meta-Analyses. Journal of Nutrition. PubMed;134(10):2592–2602.

Radja N and Charles-Holmes R. 2002. Acrodermatitis enteropathica lifelong follow-up and zinc monitoring. Clin Exp Dermatol; 27:62-63.

Ravelli GP, Stein ZA and Susser MW. 1976. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med; 295: 349–53.

Ripa S and Ripa R. 1995. "Zinc and immune function". Minerva Medica, 86 (7-8): 315-8.

Rink L and Gabriel P. 2000. Zinc and the immune system. Proc Nutr Soc; 59:541-552.

Roy SK, Beherens R, Haider R, Akramuzzaman SM, Mahalanabis D, Wahed MA and Tomkins AM. 1992. "Impact of zinc supplementation on intestinal permeability in Bangala-deshi children with acute diarrhea and persistent diarrhea syndrome" Journal of Pediatric Gastroenterology and Nutrition, 15 (3): 289-296.

Roberts B. 2004-2005.Empty stomachs, empty pockets: poverty and inequalities in post apartheid South Africa. State of the nation.

Rolfes SR, Pinna K, Whitney E and Wadsworth T. 2006. Understanding Normal and Clinical Nutrition. 7th Edition. Thomson Wadsworth. Austraila.

Rovee-Collier C and Hayne H. 2000. Memory in infancy and early childhood. In Tulving E and Craik FIM (Eds.), The Oxford handbook of memory (pp. 267-282). New York: Oxford University Press.

Rolls B. 1999. Do chemosensory changes influence food intake in the elderly? Physiol Behav 66, 193–197.

Rushton DH. 2002. Nutritional factors and hair loss. Clin Exp Dermatol. Jul; 27(5): 396-404.

Ruel MT. 2003. Operationalizing dietary diversity: a review of measurement issues and research priorities. J Nutr. 2003 Nov;133(11 Suppl 2):3911S-3926S.

Sadler PJ. 1976."Zinc in enzymes". Nature, 262: 258.

Samuel FO. Egal AA, Oldewagen-Theron WH, Napier CE and Venter CS, 2010. Prevalence of zinc deficiency among primary school children in poor peri-urban informal settlement in South Africa. 1Institute of Sustainable Livelihoods, Vaal University of Technology, South Africa.

Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA , Weill R, José RE and Boccio P. 2002. The role of zinc in growth and development. Volume 18, issue 16; p510-19.

Sayed NE, Gad A, Nofal L and Netti G .1999. Assessment of the prevalence and potential determinats of nutiritional anemia in Upper Egypt. Food Nutr Bull; 20:417-421.

Sazalwa S, Black RE, Bahn MK, Ghandari N, Sinh A, Jalla S. 1995. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med 1995; 333: 839-844.

Sandstead HH. 1991. American Journal of Diseases of Children; 145:853-9.

Sandstrom B, Davidsson L, Cederblad A and Lonnerdal B. 1985. Oral iron, dietary ligands and zinc absorption. J Nutr 1985; 115:411–4.

Sandstorm B. 1997. Bioavailability of Zinc. Eur J Clin Nutr; 51 (1 suppl): s17-19.

Sandstead HH, Penland JG and Alcock NW. 1998. Effects of repletion with zinc and other micronutrients on neuropsychological performance and growth of Chinese children. Am J Clin Nutr.; 68 (2 suppl): 470S-475S.

Sandstead HH. 1986. “Nutrition and brain function: trace elements”. Nutrition Review, 44: 37-41.

Sandstead HH. 1991. "Zinc deficiency. A public health problem?" Am. J. Dis. Child. 145 (8): 853–9.

South African Constitution .1996. Bill of Rights Section 28 (1) (c)

Savino W, Dardenne M, Velloso L and Silva-Barbosa S. 2007. The thymus is a common target in malnutrition and infection. Br J Nutr. 98 (Suppl 1): 11-6.

Sazawals S, Black RE, Jalla S, Mazumdar S, Sinha A and Bhan MR. 1998. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children; a double blind controlled trial. Pediatr; 102: 1-5.

Shatzman AR and Henkin RI. 1981. Gustin concentration changes relative to salivary zinc and taste in humans. Proc Natl Acad Sci USA 78: 3867– 3871.

Schneider Z, Elliot D, LoBiondo-Wood G and Haber J. 2003. Nursing research: Methods and Critical Appraisal and utilization 2nd edition.

Schneider V and Furst P. 2000. New concepts on nutritional management of severe malnutrition: The role of protein. Curr Opin Clin Nutr Metab Care; 3: 31-8.

Scrimshaw NS. 1991. Iron deficiency. Sci Amer; 265: 46-52.

SCN. 2004: 5th report of the world nutrition situation: nutrition for improved outcome. New York: standing committee on Nutrition, United Nation stytemss.

Scholl TO and Hediger ML. 1994.Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. American Journal of Clinical Nutrition, 59:492S–500S.

Sen CK, Khanna S and Venojarvi M. 2002. Copper-induced vascular endothelial growth factor expression and wound healing. Am J Phys –Heart Circ Phys ;282(5):H1821– 1827.

Seiler WO, Itin P and Stahelin HB. 2002. Zinc deficiency, a problem of old age frequently not recognised. Ernahrungs- Umschau 49, 260.

Sinn N. 2008. Nutritional and dietary influences on attention deficit hyperactivity disorder. Rev. Oct; 66 (10): 558-568.

Sharieff W, Bhutta Z, Schauer C, Tomlinson G and Zlotkin S. 2006. "Micronutrients (including zinc) reduce diarrhoea in children: the Pakistan Sprinkles Diarrhoea Study". Archives of Disease in Childhood, 91 (7): 573-579.

Shankar A and Prasad A. 1998. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr; 68:447S–463S.

Sjövall J, Fogh A, Huitfeldt B, Karlsson G and Nylen O. 1984.Methods for evaluating the taste of paediatric formulations in children: a comparison between the facial hedonic method and the patient's own spontaneous verbal judgement. Eur J Ped; 141: 243- 247.

Skinner JD, Carruth BR and Houck KS. 1997. Longitudinal study of nutrient and food intakes of infants aged 2 to24 months. J Am Diet Assoc; 97: 496-504.

Shan, Ji Li, Jihye Kim, Hyojee Joung and Hee Young Paik, 2003. The Effect of Dietary Phytate Content on Iron Absorption and Status in Young Korean Women: Int. J. Human Ecol., 1: 35-44.

Shay NF and Mangian HF. 2000. Neurobiology of zinc-influenced eating behaviour. J Nutr 130, Suppl. 5S, 1493S–1499S.

Spevackova V, Benes B, Smid J. 1996. Comparison of concentration of Cu and zinc in children population, central Eur J Public Health.; 4 (1): 102-105.

Sreedhar B, Subramaniyan R and Nair KM. 2004. A protective role for zinc on intestinal peroxidative damage during oral iron repletion. Biochem Biophys Res Commun; 318: 992-997.

Sreedhar B and Nair KM. 2005. Modulation of aconitase, metallothionein, and oxidative stress in zinc-deficient rat intestine during zinc and iron repletion. Free Radic Biol Med ; 39: 999-1008.

Sreedhar B and Krishnapillai MN.2007. Concurrent repletion of iron and zinc reduces intestinal oxidative damage in iron- and zinc-deficient rats. World J Gastroenterol 2007 November 21;13(43): 5707-5717.

Steyn NP, Wolmarans P, Nel JH and Bourne LT. 2008. National fortification of staple foods can make a significant contribution to micronutrient intake of South African adults. Public Health Nutrition; 11(3): 307-313.

Solomons. 2001. Dietary Sources of zinc and factors affecting its bioavailability. Food Nutr. Bull. 22: 138-154.

Solomon CG and Manson JE. 1997. Obesity and mortality: a review of the epidemiologic data. Am. J. Clin. Nutr. 66: 1044S–1050S.

Smit-Vanderkooy P and Gibson RS. 1987. Food consumption patterns of Canadian pre-school children in relation to zinc and growth status. Am J Clin Nutr 42, 165–169.

Silvestre MD, Lagarda MJ, Farre R, Martinez-Costa C, Brines J, Molina A and Clemente G. 2000. A study of factors that may influence the determination of copper, iron, and zinc in human milk during sampling and in sample individuals. Biol. Trace Elem. Res. 76, 217–227.

Silvestre D, Martinez-Costa C, Lagarda MJ, Brines J, Farre R, and Clemente G. 2001. Copper, iron, and zinc contents in human milk during the first three months of lactation: a longitudinal study. Biol. Trace Elem. Res. 80, 1–11.

Taheri F, Fesharakinia A, Saadatjoo AR. 2001.Study of Malnutrition in 6-12 year-old children in Birjand. J Birjand Univ Med Sci; 8(1): 22-27.

Tenaud I, Sainte-Marie I and Jumbou O. 1992. In vitro and complications in elective microsurgery. Plast Reconstr Surg; 89(3):490.

Tanaka M. 2002. Secretory function of the salivary gland in patients with taste disorders or xerostomia: Correlation with zinc deficiency. Acta Otolaryngol Suppl:134–141.

Tarini A, Bakari S and Delisle H. 1999. The overall nutritional quality of the diet is reflected in the growth of Nigerian children. Santé. 9: 23-31.

Tomkins A and Watson F. 2003. Malnutrition and infection: a review. United Nations Administrative committee on coordination/subcommittee on Nutrition 5 ACC/SCN state of the art series, Nutrition policy Discussion Paper No5.

Torbjorn L, Lonnerdal Bo, Hans stenlund, Indria LG, Djauhar I, Seswandhana R and Larke Ake P. 2004. A community based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. Am J Clin Nutr. 80: 729-736.

Thompson RPH. 1991. Assessment of zinc status. Pro Nutr Soc: 50; 19-28.

Troiano RP, Flegal KM and Kuczmarski RJ, Campbell SM and Johnson CL. 1995. Overweight prevalence and trends for children and adolescents. Arch. Pediatr. Adolesc. Med. 149:1085–1091, The Constitution of the Republic of South Africa, 1996, was approved by the Constitutional Court (CC) on 4 December 1996 and took effect on 4 February 1997.

Turan S, Saygi S, Kilic Z and Acar O. 2001. Determination of heavy metal contents in human colostrum samples by electrothermal atomic absorption spectrophotometry. J. Trop. Pediatr. 47, 81–85.

The Constitution of the Republic of South Africa, 1996, was approved by the Constitutional Court (CC) on 4 December 1996 and took effect on 4 February 1997.

Ulijaszek SK. 1994. Between-population variation in pre-adolescent growth. Eur J Clin. Nutr 48:S5-14.

Uchida T, Miyanaga Y, Tanaka H, Wada K, Kurosaki S, Ohki T, Yoshida M and Matsuyama K. 2000. Quantitative evaluation of the bitterness of commercial medicines using a taste sensor. Chem Pharm Bull; 48 (11): 1843-1845.

UNICEF. 2000. The progress of the nation no 2.

UNICEF/UNU/WHO. 2001. Iron Deficiency Anaemia: Assessment, Prevention, and Control. A guide for programme managers. WHO/NHD/01.3.

UNICEF. 2006. Progress report on children's nutrition No 4.

UNICEF/WHO. 2004. Low Birth weight: Country, regional and global estimates, UNICEF, New York, No 3.

United Nations Children's Fund (UNICEF). 2007. Vitamin A supplementation: Decade of progress. ISBN: 978-92-806-4150-9.

United Nations Children's Fund (UNICEF). 2008. Child survival where we stand. The state of the world children, New York. (Online) (Cited 2009, October 2). Available from URL: http://www.unicef.org/sowc08/docs/sow08.

United Nations Children's Fund (UNICEF). 2009. State of the World's Children

UNICEF. 2011. Overview of nutritional status of Nigerians. A seminar presented on high quality protein: the missing link in development at Ikeja, Lagos on the 8th march 2011.

USDA. 2006. Dietary Reference Intakes (DRI) and Recommended Dietary Allowances (RDA) resources USDA, NAL. food and Nutrition information center: 8; 1-9.

Verhoef H, West CE, Veenemans J, Beguin Y and Kok FJ. 2002. Stunting may determine the severity of malaria associated anemia in African Children Pediatrics. 110:1-5.

Viatte L and Vaulont S. 2009. Hepcidin, the iron watcher. Biochimie; 91: 1223–1228.

Vieria F, Ana Claudia, Diniz AS, Cabral CP, Oliveira RS, Lola MMF, Silva MMS and Kolsteren P. 2007. Nutritional assessment of iron status and anemia in children under 5 years old at public daycare centers. J Pediatr (Rio J); 83(4):370-376.

Walter T. 2003. effect of iron-deficiency anemia on cognitive skills and neuromaturation in infancy and childhood. Food Nutr Bull. 24 (4 suppl): S104-S110.

Walker CR, Kordas K, Stolzfus RJ and Black RE. 2005. Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. Am J Clin Nutr; 82: 5-12.

Waterlow JC. 1994. Causes and mechanisms of linear growth retardation (stunting). Eur J Clin Nutr; 48 (suppl.1): S1-S4.

Walravens PA, Hambidge KM and Koepfer DM. 1989. Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double-blind, controlled study. Pediatrics; PubMed: 83 (4): 532-538.

Wamani H, Åstrøm AN, Peterson S, Tumwine JK and Tylleskär T. 2005. Predictors of poor anthropometric status among children under 2 years of age in rural Uganda. Public Health Nutr. 9:320-326.

Wamani H, Astrøm AN, Peterson S, Tumwine JK and Tylleskar T. 2007. Boys are more stunted than girls in Sub-Saharan Africa: a meta-analysis of 16 demographic and health surveys. BMC Pediatrics.7:17.

WFP/CDC. 2005. A Manual; Measuring and Interpreting Malnutrition and Mortality, Rome

WFP/UNICEF. 2006. Global Plan of Action. Draft for Review. Ending child hunger and under-nutrition initiative UN/UNICEF.

Whittaker P. 1998. Iron and zinc interactions in humans. Am. J. Clin. Nutr; 68(suppl.):442S-446S.

Winter ES, Maggini S and Horing DH. 2007. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab; PubMed abstract; 51: 301-323.

Wilkinson EA and Hawke CI. 2000. Oral zinc for arterial and venous leg ulcers. PubMed abstract; Cochrane Database Syst Rev; (2): CD001273.

Willis MS, Monaghan SA, Miller ML, McKenna RW, Perkins WD, Levinson BS, Bhushan V and Kroft SH. 2005. Zinc-Induced Copper Deficiency. Am J Clin Pathol, 123:125-131.

Wise A. 1995. Phytate and zinc bioavailability. Int J Food Sci Nutr; PubMed abstract; 46: 53-63.

Wintergerst ES, Maggini S and Hornig DH. 2007. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab, 51:301-323.

Willows ND; Barbarich BN; Wang LC; Olstad DL and Clandinin MT. 2010. Dietary inadequacy is associated with anemia and suboptimal growth among preschool-aged children in Yunnan Province, China, Nutr Res; 31(2):88-96.

Wish JB. 2006. Assessing iron status beyond serum ferritin and transferrin saturation CJASN. Vol 1, No supplement 1, S4-S8.

Wood RJ. 2000. Assessment of marginal zinc status in humans. Journal of Nutrition 130: 1350S-4S.

Report of a WHO scientific group. Nutritional anaemias. Geneva, World Health Organization, 1968. (WHO Technical Report Series, No.405). Available at http://whqlibdoc.who.int/trs/WHO_TRS_405.pdf.

World Health Organization. 1992. World Declaration on Nutrition ± Plan of Action. Geneva: World Health Organization, Division of Food and Nutrition.

World Health Organization. 1995. Integrated management of the sick child. Bull World Health Organ; 73: 735-740.

World Health Organization. 1996. Guidelines for the control of iron deficiency anemia in countries of the Eastern Mediterranean, Middle East and North Africa. World Health Organization, Regional Office for the Eastern Medditerranean, Alexandria, Egypt; pp8-9.

World Health Organization. 1999. Management of severe malnutrition. A manual for physicians and other senior health workers. Geneva: 4.

World Health Organization. 2001. Iron deficiency anemia; assessment, prevention and control: a guide for programmer managers, Geneva.

World Bank. 2009. Report on nutrition in Sub-Saharan Africa.

WHO. 2002. The World Health Report 2002. "Reducing Risks, Promoting Healthy Life". World Health Organization, Geneva, Switzerland.

World Health Organization. The World Health Report 2002: Reducing risks, promoting healthy life. Geneva,World Health Organization, 2002.

WHO. 2001: Iron deficiency anemia. Assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization.

World Health Organization and United Nations Children Fund. 2004. Clinical management of acute diarrhea. WHO/UNICEF joint Statement, August.

World Health Organisation. 2006. WHO growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organisation.

WHO/UNICEF/IAEA/IZiNCG. 2007. Executive summary, Recommendations for indicators of population zinc status. Report of WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. Food Nutr Bull; 28:S399-S400.

WHO Statistical Information System (WHOSIS). 2006. Includes data from World Health Statistics 2006 and The World Health Report 2006 Edition; World Health Organisation 2006. http://www.who.int/en/. Accessed 11/02/06.

Wu FY and Wu CW. 1987. "Zinc in DNA replication and transcription". Annual Reviews Nutrition, 7: 251-272.

Wu FY, Huang WJ, Sinclair RB and Powers. 1992. The structure of the zinc sites of Escherichia coli DNA- dependent RNA polymerase. J. Biol. Chem; 267: S25560-25567: 59.

Yadav RJ and Singh P. 1999. Nutritional status and dietary intake in tribal children of Bihar. Indian Pediatrics, 36: 37-42.

Yenigun A, Ozkinay F, Cogulu O, Coker C, Cetiner N, Ozden G, Aksu O and Ozkinay C. 2004. Hair zinc level in Down syndrome. Down Syndrome Research and Practice; 9(2);53-57.

Yoshida S and Tomita H. 2002. A case of Cronkhite-Canada syndrome whose major complaint, taste disturbance, was improved by zinc therapy. Acta Otolaryngol Suppl;546:154–158.

Zaliah MS, Bond JT and Johnson NE. 2000. Nutritional status of primary school children from low income households in Kuala Lumpur. Malaysian J Nutr 6: 17-32.

Zere E and McIntyre D. 2003. Inequities in under-five child malnutrition in South Africa. International Journal for Equity in Health, 2:7.

Zimmermann MB, Zeder C, Muthayya S, Winichagoon P, Chaouki N and Aeberli I. 2008. Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. Int J Obesity; 32: 1098–1104.

Zverev Y. 2004. Effects of caloric deprivation and satiety on sensitivity of the gustatory system. BMC Neurosci; 5:5.

APPENDICES

APPENDICES

APPENDIX A

CONSENT FORM

The Prevalence of Zinc Deficiency among Children Aged 3-5 Years in the Vhembe District

Researcher's Name: Motadi Selekane Ananias

The aim of this study is to determine the prevalence of zinc deficiency among preschool children in the Vhembe District. The children are required to provide blood for determination of zinc serum/plasma levels and to assess the nutritional status using anthropometric measurements. If you agree to have your child participate, please sign the form below.

The following measurements will be taken: weight and height, blood sample.

Anthropometric measurements mentioned above will require children to wear minimal clothes and to be without shoes.

I,……………………………………, hereby agree to have my child participate in the study

I realize that:

- The study deals with the prevalence of zinc deficiency disorder in the Vhembe District of Limpopo Province.
- The researcher will answer any question that I may have regarding the research or related matters.
- The experimental protocol, i.e. the extent, aims and methods of the research have been explained to me.
- A participant in this research does it voluntarily and I can withdraw the child from participation at any stage.
- I understand that my child will be examined and required to provide a sample of blood. There may be discomfort during the drawing of blood.

Name of child………………………… Date of Birth……………………………..

Signature of parent/guardian………………… relationship (mother/father/grandparent)

Name……………

Signed at……………………… this……………………………day of………………2010.

CONSENT FORM

Mutodulusi: Motadi Selekane Ananias

Ndivho ya thoduluso ndi u lavhavhelesa tshivhalo tsha vhana vhane vha vha na thahelo ya zinc muvhilini wavho. Kha vhupo ha Vhembe. *Vhana vha do dzhiwa malofha uri hu kone u* sedzuluswa uri vha na zinc ingafhani malofhani. Vha do dovha vha kaliwa tshileme na vhulapfu. Arali vha tshitenda uri nwana wavho a vhe tshipida tsha thoduluso (research0 kha vha saine fomo iyi.

Zwithu zwi tevhelano zwi do kaliwa: tshileme, vhulapfu na zinc kha malofha.

Vhana vha do kaliwa vha sina zwienda nahone vho ambara zwiambaro zwituku.

Nne ………………………………………… Ndi khou tenda uri nwana wanga a dzhenelele kha thoduluso iyi.

Ndi khou pfesesa zwa uri:

- Thoduluso ikhou lavhelesa tshivhalo tsha vhana vhane vha vha na thahelelo ya zinc malofhani kha tshitirik tsha Vhembe Limpopo Province.
- Murisetshi (Mutodulusi) u do fhindula mabudziso dzanga dzine dza livhana na risetshe kana u yelana na risetshe.
- Ndivho na zwithu zwine zwa do itiwa kha iyi risetshe ndo talutshedziwa nga hazwo.
- U dzhenelela iyi risetshe a si khombe-khombe. Nahone u ngalitshasa nwana wau kha u dzhehelele tshifhinga tshinwe na tshinwe.
- Ndi a pfesesa uri nwana wanga u do toliwa a dovha a dzhiiwa malofa. Musi a tshi dzhiwa malofa zwinga di vhavha zwituku.

Dzina la nwana……………………………..…. Duva la Mabebo………………………………

Tsaino ya Mubebi…………………………….. Vhushaka ná nwana (Mme, Khotsi, Makhulu)

Dzina la mubebi…………………………………..

Hosainwa…………………… ngadzi…………………….. Duvha ………………… 2012

APPENDIX B

TO: DEPARTMENT OF HEALTH

PROVINCIAL OFFICE

POLOKWANE

0700

FROM: DIRECTOR OF RESEARCH AND INNOVATION

PROF C V NIKODEM

DATE: 30 May 2012

Dear Sir/Madam

RE: PERMISSION TO USE PRE-SCHOOLS AS A SAMPLING FRAME FOR RESEARCH

With regard to the abovementioned matter the department of Research and Innovation would like to request your office to grant Mr Motadi SA (11541509) permission to use pre-school children as subjects for his MSc project. The title of the project is "*The Prevalence of Zinc Deficiency among Children Aged 3-5 Years in Vhembe District, Limpopo Province*" and the project no is SHS/12/NUT/02/2012. The permission to conduct this project is already been granted by the Provincial Department of Education (**see attached letter**). The envisaged date for data collection is July 2012. Zinc deficiency is a public health problem affecting 33% of children under five years of age. Furthermore the prevalence of the deficiency is not known in Vhembe district, therefore there is a need to investigate the magnitude of the problem. The ethical clearance certificate and the proposal methods of the study are enclosed at your perusal. The Department is looking forward to your response.

For more information regarding the project please contact Mr SA Motadi at 015 962 8685/0797455449 or evans@webmail.co.za.

....

Supervisor (Mr. N.S Mabapa)

....

Director of Research and Innovation

APPENDIX C

Date of data collection……………………

RECORD SHEET

1. Preschool…………………………………………………………

2. Name of child……………………………………………………

3. (i.) Code………………………………………………………

   (ii.) Date of Birth……………… day……… Month………………year

   (iii.) Age…………………………………

4. Gender:

Male	Female

5. (a) Body weight…………………………………… kg

   (b) Body weight………………………………… kg

   Average weight……………………………………… kg

3. (a) Height……………………………… cm

   (b) Height……………………………… cm

   Average height……………………………………… cm

Blood sample taken:

Yes	No

3.1 Time of blood collection……………………………………

3.2 Time of the previous meal……………………………………

4. Types of symptoms/signs observed

1. Hair loss	
2. Rough skin	
3. White spot on the finger nails	
4. Loss of taste and smell	

8. Liquid zinc

1. Place 10ml (about two teaspoons) of the liquid in the mouth.
2. Hold the liquid zinc in the mouth for about ten to fifteen seconds.
3. How is the taste of the zinc?

    a. Taste bad

    b. Taste strong and metallic

    c. Little taste than sweet.

    d. Taste like water/tasteless.

b. Eye diminishing (Taste strong and Metallic)

d. Neutral (Tasteless)

Nose wrinkle (Taste bad)

c. Eye widening (Little taste than sweet)

APPENDIX D

MATLOU SIBAYA

P O Box 1106 CELL: 084 255 2164
Faunapark OFFICE: 015 287 0709
POLOKWANE EMAIL: matlou.moloto@gmail.com
0787

EDITING LETTER 21 November 2013

TO WHOMIT MAY CONCERN

This letter serves as confirmation that I Matlou Sibaya have edited the research project of Selekane Ananias Motadi, entitled **"The prevalence of Zinc deficiency among children aged 3-5 years in the Vhembe district of Limpopo province** "to be submitted for his BSc in Nutrition (BSC NUT) at the University of Venda. I therefore, declare that the document is to my knowledge devoid of language errors which may deprive Mr Motadi's work from being accepted for the qualification Bsc.

I am the English Communication Lecturer at a University and am registered with a professional editing body: the Professionals Editors' Group (PEG).

I am contactable at the above contact details should you have queries.

Regards

Matlou Sibaya (Miss)

PhD Candidate (TUT) MPhil Second Language Studies (Stellenbosch) BEd Language Methodology (University of Limpopo) BA Languages (University of Limpopo) HED (University of Limpopo) and [Member of the Professionals Editors' Group]

APPENDIX D

1. PRINCIPLE

INTENDED USE

C-RP reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems CAL 5 Plus, is intended for quantitative determination of c-reactive protein concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

C-RP measurements are useful in the clinical evaluation of stress, trauma, infection, inflammation, and surgery.1,2

METHODOLOGY

C-RP reagent is used to measure C-reactive protein concentration by a turbidimetric method. In the reaction, an anti-CRP antibody-coated particle binds to C-reactive protein in the patient sample resulting in the formation of insoluble aggregates causing turbidity.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio *used is one part sample to 50 parts reagent*. The System monitors the change in absorbance at 600 nanometers. This change in absorbance is proportional to the concentration of C-RP in the sample and is used by the System to calculate and express C-RP concentration based upon a single-point adjusted, predetermined calibration curve.

CHEMICAL REACTION SCHEME

SPECIMEN

TYPE OF SPECIMEN

Serum samples are recommended. Plasma samples (Lithium Heparin, and Sodium Heparin) can be used.

Serum or plasma samples should be collected in the manner routinely used for any clinical laboratory test.2 Freshly drawn serum or plasma from a fasting individual is preferred. Anticoagulants tested are listed in the PROCEDURAL NOTES section of this chemistry information sheet.

Chemistry Information Sheet A18485 AG C-RP

AUGUST 2011 Page 1 of 10

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.3
2. If serum samples are not assayed within 8 hours, samples should be stored at +2°C to +8°C. If samples are not assayed within 72 hours, samples should be stored frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.3
3. Plasma samples can be stored at +2°C to +8°C for up to 72 hours. Plasma samples should not be frozen.

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME
A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.
CRITERIA FOR UNACCEPTABLE SPECIMENS
Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens. Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION
Special instructions for patient preparation as designated by this laboratory:
SPECIMEN HANDLING
Special instructions for specimen handling as designated by this laboratory:
C-RP Chemistry Information Sheet A18485 AG
Page 2 of 10 AUGUST 2011
REAGENTS
CONTENTS
Each kit contains the following items:
Two C-RP Reagent Cartridges (2 x 200 tests)
One lot-specific Parameter Card
VOLUMES PER TEST
Sample Volume 6 µL
ORDAC Sample Volume 3 µL
Total Reagent Volume 300 µL
Cartridge Volumes
A 250 µL
B 50 µL
C –
REACTIVE INGREDIENTS
REAGENT CONSTITUENTS
CRP Antibody (particle bound goat and mouse anti-CRP antibody)
11 mL Reaction Buffer 61 mL Bovine Serum Albumin 0.1% (w/v)
Also non-reactive chemicals necessary for optimal system performance.

CAUTION
Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).
EUROPEAN HAZARD CLASSIFICATION
C-Reactive Protein Reagent (Compartment A)
N;R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in aquatic environment. S60 This material and/or its container must be disposed of as hazardous waste. S61 Avoid release to the environment. Refer to special instructions/Safety data sheets.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT
SYNCHRON® Systems CAL 5 Plus
At least two levels of control material Saline

Chemistry Information Sheet A18485 AG C-RP
AUGUST 2011 Page 3 of 10
REAGENT PREPARATION
1. Invert cartridge several times prior to loading onto the SYNCHRON LX System.
2. Check for bubbles or foam in compartments; break any bubbles.
ACCEPTABLE REAGENT PERFORMANCE
The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.
REAGENT STORAGE AND STABILITY
C-RP reagent when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE. Reagent storage location:
CALIBRATION
CALIBRATOR REQUIRED
SYNCHRON® Systems CAL 5 Plus
CALIBRATOR PREPARATION
No preparation is required.
CALIBRATOR STORAGE AND STABILITY
SYNCHRON® Systems CAL 5 Plus is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

CAUTION
Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.4 C-RP Chemistry Information Sheet A18485 AG Page 4 of 10 AUGUST 2011 Calibrator storage location:

CALIBRATION INFORMATION
1. The system must have a lot-specific parameter card and a valid calibration adjustment in memory before controls or patient samples can be run.
2. Under typical operating conditions the C-RP reagent cartridge must be calibrated every 30 days and also with certain parts replacements or maintenance procedures, as defined in the

SYNCHRON LX Maintenance Manual and Instrument Log, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LXOperations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, consult the Troubleshooting Manual, or the UniCel DxC 600/800 System

Instructions For Use (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME SAMPLE TYPE STORAGE

Chemistry Information Sheet A18485 AG C-RP

AUGUST 2011 Page 5 of 10

Table 1.0 Quality Control Material, Continued

CONTROL NAME SAMPLE TYPE STORAGE

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations. For detailed testing procedures, refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

CALCULATIONS

The SYNCHRON® System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

The C-RP reference interval values were established using the SYNCHRON LX® 20 PRO Systems CRPH assay, for a population of 551 apparently healthy, non-smoking, ≥ 18 years of age, male and female adults from a Southern California blood bank, and were verified with the

SYNCHRON Systems C-RP assay. Each laboratory should establish its own reference intervals based upon its patient population.

2. PRINCIPLE

INTENDED USE

TRFN reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems CAL 1, is intended for quantitative determination of transferrin concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

The measurement of transferrin aids in the diagnosis of malnutrition, acute inflammation, infection, assessment of renal function and red blood cell disorders, such as iron deficiency anemia.

METHODOLOGY

TRFN reagent is used to measure the TRFN concentration by a turbidimetric method.1,2 In the reaction, TRFN combines with specific antibody to form insoluble antigen-antibody complexes. The SYNCHRON® System(s) automatically dilutes sample and dispenses the appropriate sample and reagent volumes into a cuvette. The ratio used is one part diluted sample to 37.5 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is proportional to the concentration of transferrin in the sample and is used by the System to calculate and express the transferrin concentration based upon a single-point calibration.

CHEMICAL REACTION SCHEME

SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.3 Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood or urine are not recommended for use as a sample.

Chemistry Information Sheet A18562 AG TRFN

AUGUST 2010 Page 1 of 10

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.4

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed. 4 Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE PREPARATION

Sample preparation is not required. All samples are diluted automatically by the system using the DIL1 Cartridge.

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

TRFN Chemistry Information Sheet A18562 AG

Page 2 of 10 AUGUST 2010

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two TRFN Reagent Cartridges (2 x 150 tests)

One lot-specific Parameter Card

VOLUMES PER TEST

Sample Dilution Volumes

Sample Volume 15 µL

Diluent Volume 285 µL

Diluted Sample Volume (1:20 dilution) 6 µL

Total Reagent Volume 225 µL

Cartridge Volumes

A 200 µL

B 25 µL

C – –

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Reaction Buffer 43.0 mL

Antibody Monospecific for human Transferrin (Goat) 6.2 mL

Also non-reactive chemicals necessary for optimal system performance.

CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems CAL 1

DIL 1 Cartridge

At least two levels of control material

Chemistry Information Sheet A18562 AG TRFN
AUGUST 2010 Page 3 of 10

REAGENT PREPARATION

No preparation is required.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

TRFN reagent when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

DIL 1 stored unopened at room temperature is stable until the expiration date indicated on each cartridge. Once opened, DIL 1 is stable for 60 days on instrument or until the expiration date, if sooner. Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems CAL 1

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems CAL 1 is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C. DO NOT FREEZE.

CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.5 TRFN Chemistry Information Sheet A18562 AGPage 4 of 10 AUGUST 2010 Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have a lot-specific parameter card and a valid calibration adjustment in memory before controls or patient samples can be run.
2. Under typical operating conditions the TRFN reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the SYNCHRON LX Maintenance Manual and Instrument Log, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LXOperations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual for information on this feature.

3. For detailed calibration instructions, refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.
4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the SYNCHRON LX Diagnostics and Troubleshooting Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

TRACEABILITY
For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL
At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility. Table 1.0 Quality Control Material

CONTROL NAME SAMPLE TYPE STORAGE
Chemistry Information Sheet A18562 AG TRFN
AUGUST 2010 Page 5 of 10
TESTING PROCEDURE(S)
1. If necessary, load the reagent onto the system. A lot-specific parameter card must be loaded one time for each lot.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations. For detailed testing procedures, refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

CALCULATIONS
The SYNCHRON® System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

REPORTING RESULTS
Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.