THE EFFECTS OF MATERNAL IODINE SUPPLEMENTATION ON MATERNAL AND INFANT IODINE STATUS AND THYROID FUNCTION AND ON INFANT VISUAL INFORMATION PROCESSING

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Abstract: Iodine deficiency is one of the major causes of preventable brain damage in childhood. However, iodine supplementation during early pregnancy and lactation could prevent the ill effects of iodine deficiency. In this study we have assessed the effect of iodine supplementation to lactating mothers on visual information processing (VIP) of their 6-month-old infants in a community-based, randomized, supplementation trial. Mother infant dyads (n = 106) were recruited within the first week after delivery to participate in this study. Study participants were randomly assigned either to receive 225 µg of iodine as potassium iodide capsule (capsule group) daily for 26 weeks or iodized salt (I-salt group) (450 g) weekly for 26 weeks. Lactating women (n = 53) who had 26 weeks old infants were recruited to serve as controls. Mother-infant dyads in the two supplemented groups were not significantly different in any of the biomarkers and anthropometry measurements at baseline. At the end of 26 weeks, maternal thryroxine (T_4) decreased in the I-salt group ($p < 0.001$) but remained the same in the capsule group ($p = 0.45$), thyroid stimulating hormone (TSH) and triiodothyronine (T_3) significantly decreased in both groups but there was no change in thyroglobulin (Tg). Compared to the two treatment groups, TSH was significantly lower in the control group. Maternal goiter rate significantly decreased following iodine supplementation. Infants median (IQR) T_4 at 26 weeks was 10.8 (8.7, 13.6) µg/dL, 13.9 (10.6, 17.6) µg/dL and 19.6 (16.4, 25.9) µg/dL in the capsule, I-salt and control groups respectively. And the difference between the three groups was significant ($p < 0.001$). Compared to baseline, maternal and infant urinary iodine concentration (UIC) increased but breast milk iodine concentration (BMIC) decreased at the end of the 26 weeks. The percentage of infants who showed novelty preference to new stimuli above 0.55 was 26%, 51% and 47% in the capsule, I-salt and control groups respectively ($p = 0.024$), but other VIP tests did not show any effects of supplementation. In conclusion, iodine supplementation to iodine sufficient or mildly iodine deficient mothers did not show significant effects on infant VIP, T_4, TSH or maternal T_4, Tg, UIC and BMIC.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Background

Iodine is a chemical element found in trace amounts in the human body and is primarily obtained through the diet [1]. Iodine is essential for the synthesis of thyroid hormones, and thyroid hormones regulate the metabolic processes of most cells and play important roles in human growth and development [2].

Iodine deficiency is one of the most common nutritional problems in the world, and it is a major public health problem in many parts of the world [3]. Due to its multiple effects on human health, iodine deficiency is described as iodine deficiency disorders (IDDs) [4]. Iodine deficiency disorders can cause serious consequences on brain and physical development called endemic cretinism [5]. In neonates iodine deficiency and hence insufficient supply of thyroid hormones to the developing brain could cause neonatal goiter, neonatal hypothyroidism, endemic mental retardation, and increased susceptibility of the thyroid gland to nuclear radiation [6,7].

Maternal thyroid dysfunction could be a cause for developmental impairment in the offspring. Severe iodine deficiency during pregnancy causes maternal and fetal hypothyroxinemia [8]. Mild-to-moderate iodine deficiency could result in poor learning ability as well as increased risk of low birth weight, infant morbidity and mortality [9]. Infants under 2 years of age are one of the most vulnerable groups to be affected by iodine deficiency along with pregnant and lactating mothers [10]. Globally, it is estimated

that 241 million school age children, most of them in developing countries, suffer insufficient intake of iodine [11]. Moreover, millions of infants born every year are exposed to risk of cognitive damage from iodine deficiency [3]. However, the degree and types of damage produced by mild or moderate maternal iodine deficiency in cognitive and/or neurologic function in the offspring is not clear [12].

In Ethiopia, IDD has been a serious public health problem in all age groups for many years. Although data on the situation of IDD in infants are lacking, in school age children total goiter rate nationally was nearly 40% in a nationwide survey conducted in 2005 which indicates the country was severely affected by iodine deficiency [13]. In our study region (Southern Nations, Nationalities and Peoples Region (SNNPR)), prevalence of goiter in school age children was 56.2%, which was the highest rate of any region in the country [13]. Recently Ethiopia has been identified as one of the few African countries where iodine deficiency is still a moderate problem. Despite tremendous progress towards eliminating IDD around the world, IDD is reappearing even in developed countries [14].

IDD can be prevented and controlled by providing iodine and iodine can be provided in different ways. However, the United Nations Children's Fund and the World Health Organization jointly recommended salt iodization where iodized salt is accessible. Iodized salt is a safe, cost effective and sustainable strategy to ensure sufficient intake of iodine by all individuals and to improve maternal and infant health [15,16]. Globally, over 70% of households use iodized salt [10]. In places where iodized salt is not accessible, for pregnant and lactating women a daily dose of 250 μg iodine supplement or a single annual dose of 400 mg iodine as an iodized oil supplement is recommended. For children

below 2 years of age, a daily dose of 90 µg iodine or a single annual dose of 200 mg iodine as iodized oil supplement is recommended [3]. However, for infants 0 to 6 months of age, because significant amount of iodine is secreted into breast milk, iodine should be available through breast milk, provided that the lactating mother is receiving sufficient iodine and her child is exclusively breastfed [17].

According to the Ethiopia Public Health Institute, the national salt iodization program was launched in 2012 [18]. After we collected our data for this study in 2013, it was reported that the national iodized salt coverage (above 0 ppm) is 95.2% with 42.7% of the households greater than 15 ppm and 23.2% within the national standard (20 to 40 ppm) [18]. However, in our previously conducted study in 2009, in villages in Sidama zone, southern Ethiopia, there was not a single household that reported ever consuming iodized salt or any kind of iodine supplement [19]. Moreover, awareness of iodine deficiency was very low, and those who were aware that iodine was important associated its deficiency with goiter, but not with an effect on a child's ability to learn.

The visual information processing (VIP) paradigm has been used to assess infants' and children's information processing speed, recognition memory and attention in relation to various factors. For instance, infants whose mothers had high blood docosahexaenoic acid (DHA) concentration showed fast information processing speed over the first year as well as less distractibility in the second year [20]. A study conducted in Ethiopian infants showed that growth was associated with development of VIP [21].

VIP paradigm is a two-phase procedure (habituation to a single stimulus followed by a paired-comparison) that measures look duration, novelty preference, and shift rate to assess processing speed and memory. An infant's visual recognition memory

is the beginning of cognitive effort which can predict broad cognitive abilities in later childhood [21,22]. Habituation and recognition memory have been considered better predictors of later IQ compared to the standardized tests of developmental level [23].

Objectives of the study

The study had two major objectives

Objective one: i) to conduct an experimental study on the effect of iodine supplementation to lactating mothers on visual information processing (VIP) of their 6-month-old infants, ii) to assess efficacy of iodized salt to increase iodine concentrations in breast milk and in infants' urine and to improve mothers and infants thyroid function compared to daily intake of an iodine capsule for six months by lactating mothers,

Objective two: i) to assess the effect of the recently launched salt iodization program on urinary iodine concentration and rate of goiter in women age 15 to 49 years of age and school-age children and ii) to assess iodine content of household salt and drinking water.

Hypotheses

1. Infants 6 months of age from mothers supplemented with iodized salt or iodine capsule will perform better in visual information processing measurements than infants of the same age from non-supplemented mothers.
2. Infants 6 months of age from mothers supplemented with iodized salt or iodine capsule will have higher urinary iodine concentrations than infants from non-supplemented mothers.
3. Lactating mothers supplemented with iodized salt or iodine capsule will have higher iodine concentrations in their breast milk and urine, higher concentrations of thyroid

hormones, and lower concentrations of thyroid stimulating hormone and thyroglobulin in their plasma than non-supplemented lactating mothers.

4. Iodine content of salt at the household will be significantly lower than the requirement.

Definition of terms

- ***Visual information processing:*** The beginning of cognitive effort that emerges in early infancy and is related to processing speed and attention. It can also predict broad cognitive abilities in later childhood [22].
- ***Cognition:*** Refers to processes such as attention, language, perception, action, association, memory, concept formation, problem solving, and mental imagery [24].
- ***Exclusive breast feeding:*** Infants fed only breast milk and no other liquids or solids with the exception of drops or syrups consisting of vitamins, mineral supplements or medicines [25].
- ***Universal salt iodization:*** Universal salt iodization is the iodization of all salt in the country for human and livestock consumption in order to eliminate IDD [6].
- ***Endemic cretinism:*** Endemic cretinism is characterized by mental retardation, severe and irreversible alterations in brain development, and multiple neurological signs including deaf mutism, squint, spastic diplegia, motor rigidity, shuffling gait, and signs of severe thyroid insufficiency with dwarfism, myxoedma, and sexual immaturity [5,7].

Significance of the study

The results from this study will increase understanding of the effects of iodine on infant cognition (using visual information processing (VIP) as a measure of cognition). Although there is rich body of evidence that maternal IDD ultimately affects infant's cognition, the influence of infant IDD on VIP has not been studied. To the best of my knowledge in Ethiopia only one study has reported breast milk iodine concentration with limited information and no study has reported about infants' iodine status.

One of the best strategies to alleviate iodine deficiency in the community is implementing USI. However, because iodine content of salt may decrease due to handling, storage and utilization, determining the amount of iodine reaching the household was essential. Hence this study assessed household salt iodine level. We provided nutrition education to the community regarding the benefits, utilization and storage of iodized salt.

Limitations

- Because the ethics rule at Oklahoma State University, USA and Hawassa University, Ethiopia, do not allow depriving subjects from iodine supplement for study purpose, we did not have a control group that began at baseline and continued throughout the study. Instead we recruited mothers who had 6 ± 0.25 month old infants to serve as controls.
- The amount of salt that individual women consumed was not quantified.
- The setup of our VIP test was not ideal (lighting problem, small screen size) because of that we failed to test the reliability of the novelty preference.

Organization of the dissertation

Following the introduction, the dissertation reviewed literature identifying the recent prevalence of iodine deficiency in Ethiopia. The importance of iodine to human health, particularly its effect on brain function and methods used to evaluate infant cognition to date are reviewed. Availability, accessibility and utilization of iodized salt in Ethiopia also are reviewed. This is followed by the research methodology to explain the study design, the selection of research participants, and the collection and analysis of data. Finally, three separate manuscripts that include the results, discussions, and conclusions of the study are presented.

CHAPTER II

REVIEW OF LITERATURE

What is iodine?

Iodine (atomic weight 126.9) is a chemical element found in trace amounts in the human body and is primarily obtained through the diet [1]. Iodine was first discovered in 1811 by accident when Courtois was manufacturing gun powder from sea weed. Because it has a violet color Gay-Lussac named it iodine, from the Greek for "violet" and identified it as a new element. In 1895, iodine was found in the thyroid gland by Baumann [26].

Iodine is required for the synthesis of thyroid hormones and thyroid hormones act by regulating the metabolic pattern of most cells of the organism. Iodine plays a crucial part in the process of early growth and development of most organs, especially of the brain [2]. Iodine, and hence thyroid hormones, are essential for human life [9]. A constant supply of thyroid hormone is necessary for proper development of the brain and for body growth as well as to maintain basal metabolism and functional activity of most organs [27].

Sources of iodine

Iodine is widely distributed in the environment as iodide. Iodine found in the soil can be washed away by leaching, flooding and erosion, which leaves the soil and drinking water depleted of iodine. Plants grown in this soil will be low in iodine content and hence humans and animals that rely on these plants will most likely become iodine deficient [28]. Comparing plants grown in iodine deficient versus iodine sufficient soils,

plants grown in iodine-deficient soils might have iodine concentrations as low as 10 µg/kg, whereas plants grown in iodine-sufficient soils can have approximately 1 mg/kg dry weight. Iodine-deficient soils are commonly found in mountainous areas especially where there is frequent flooding [9].

For people who reside in iodine-deficient areas, the best ways to alleviate iodine deficiency are using iodized salt and diversifying local food with foods from iodine-sufficient areas. Plants and animals that are of marine origin are rich in iodine, because they can concentrate iodine from sea water. Iodine containing compounds such as fertilizers, livestock feed, and compounds used in irrigation and milk processing can increase iodine content in food as well as dairy products [9]. As a study conducted in Denmark indicated, the main sources of iodine for the Danish people other than dietary supplements were milk and other beverages such as water, tea, coffee, juice, soft drinks, beer and wine [29]. Major dietary sources of iodine in the United States in addition to iodized salt were milk and bread [30]. Similarly, in Switzerland, the main sources of iodine are bread and dairy products [31]. In the early 1920s, Switzerland was the first country to fortify salt with iodine in order to control IDDs [32].

Abuye and Kelbessa [33] in their study in Ethiopian schoolchildren from different regions showed that although insufficient amounts of iodine were consumed, the main sources of iodine were drinking water and various types of cereals and legumes. The amount of iodine consumed from animal products was very small. Additionally in some places of the country, cassava consumption could be a cause for increased iodine deficiency due to the compound called linamarin which is known to antagonize the thyroid gland [34].

Absorption and metabolism

Iodine exists as several chemical forms when it is ingested. It is rapidly and almost completely absorbed in the stomach and duodenum in the form of iodide. For instance, the iodate used in iodizing salt will be reduced in the gut to iodide before it is absorbed [9]. The main organs that clear iodine from the circulation are kidney and thyroid with fairly constant clearance by the kidney. Clearance by thyroid varies with iodine intake. No more than 10% of iodine absorbed is taken up by thyroid in times of adequate iodine intake, whereas during chronic iodine deficiency the uptake increases up to 80% [35-37]. Out of the ingested iodine, more than 90% is excreted in the urine, with only a small amount in the feces [9].

During lactation, the mammary gland secretes iodine into the breast milk after concentrating it to provide for the newborn [38]. Small amounts of iodine would be taken up by salivary glands, gastric mucosa and choroid plexus. Under normal circumstances, iodine in the blood is turned over rapidly and has a half-life of approximately 10 hours. But this is reduced during iodine deficiency or hypothyroidism and if the thyroid is overactive [35-37].

Once iodide (I^-) is absorbed in the cell, iodide migrates to the apical membrane after it is transported into the thyrocyte by the sodium/iodide symporter (NIS) at the basal membrane. The enzyme thyroperoxidase (TPO) and hydrogen peroxide (H_2O_2) oxidize iodide and attach it to tyrosyl residues in thyroglobulin (Tg) to produce the hormone precursors mono-iodotyrosine (MIT) and di-iodotyrosine (DIT). Then in the follicular lumen, residues couple to form thyroxin (T_4) and tri-iodothyronine (T_3) within the Tg molecule. By endocytosis Tg enters the cell and is digested to release T_4 and T_3 into the

circulation, and iodine on MIT and DIT is recycled within the thyrocyte [32]. During fetal and early postnatal life however, T_3 is entirely dependent on its local production from T_4 through type II deiodinase [7]. This pathway is well demonstrated in the figure 2.1.

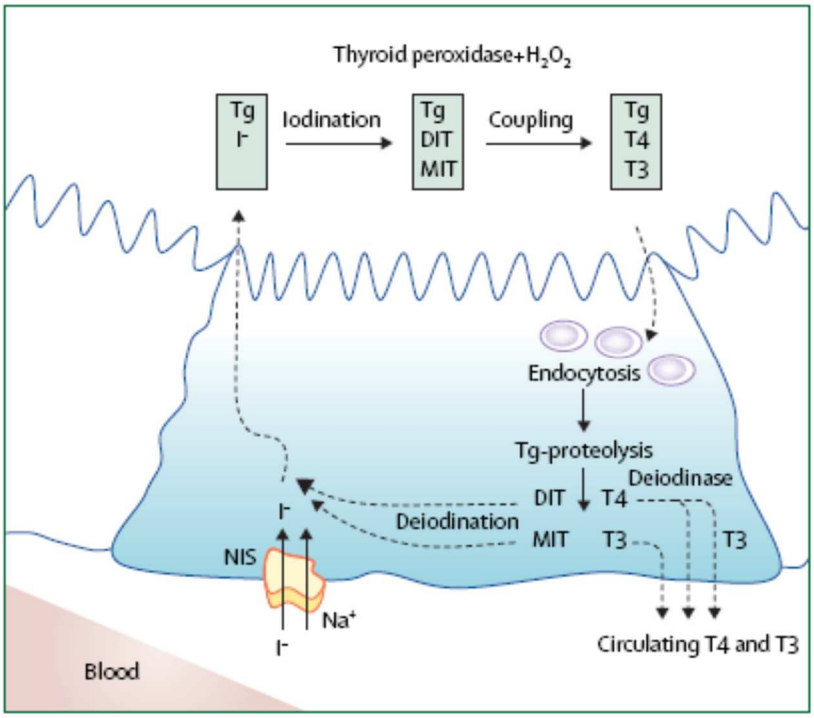

Fig 2.1. Iodine pathway in the thyroid cell. Adapted from Zimmermann et al 2008 [32].

Iodine deficiency

Iodine deficiency is one of the most common nutritional problems of the world. Globally, it is estimated that 2 billion people, most of them in developing countries, suffer inadequate intake of iodine [9]. The recent data estimated that 241 million (29.8%) school age children have insufficient iodine intake [11]. Tremendous progress towards eliminating iodine deficiency has been done. The number of iodine deficient countries decreased from 54 to 32 and the number of iodine sufficient countries increased from 67 to 105 in the last decade [11]. However, iodine deficiency is reappearing even in developed countries [14].

Due to its multiple effects on human health, iodine deficiency was called as iodine deficiency disorder (IDD) [4]. IDD refers to the ill effects of iodine deficiency that can be prevented through adequate iodine intake [39]. The detrimental effects of IDD in all age groups have been classified by Hetzel [4] and summarized by WHO [3], as shown in Table 2.1.

Table 2.1 The spectrum of iodine deficiency disorders

Physiological groups	Health consequences of iodine deficiency
All ages	Goiter, hypothyroidism and increased susceptibility to nuclear radiation
Fetus	Spontaneous abortion, stillbirth, congenital anomalies and perinatal mortality
Neonates	Endemic cretinism including mental deficiency with a mixture of mutism, spastic diplegia, squint, hypothyroidism, short stature and infant mortality
Children and adolescents	Impaired mental function, delayed physical development and iodine-induced hyperthyroidism
Adults	Impaired mental function and iodine-induced hyperthyroidism

The most critical period to be affected by IDD is from the second trimester of pregnancy to three years of age [40,41]. At these stages normal thyroid hormone concentrations are required for optimal development of the brain [3].

The WHO recommended daily intake of iodine is 90 µg/d for children less than 2 years of age and 250 µg/d for pregnant and lactating women. Iodine intakes more than 500 µg/d for lactating women and more than 180 µg/d for children do not have added health benefit [17].

Iodine deficiency occurs when iodine intake falls enough below the recommended intake that it fails to meet the needs of the individual and the thyroid gland will no longer be able to synthesize sufficient amounts of thyroid hormone. This leads to damage of the developing brain and all the aforementioned IDDs [3,4]. In children who are born and raised in areas with severe iodine deficiency, neurological cretinism could occur. Neurological cretinism which has the most marked central nervous system damage, leads to neurological abnormalities such as hearing and speech defects, mental deficiency and motor defects [42]. This damage is related to the mother's IDD and her inability to increase circulating thyroxine during pregnancy [43].

Iodine deficiency is the main cause of endemic goiter but it also can be caused by other goitrogenic substances that interfere with thyroid metabolism. Zimmermann and colleagues have compiled the following table on goitrogenic substances and micronutrient deficiencies and the possible mechanisms that affect thyroid metabolism [32].

Table 2.2 Goitrogens and their mechanism

	Mechanism
Foods	
-Cassava, lima beans, linseed, sorghum, sweet potato	Contain cyanogenic glucosides; they are metabolized to thiocyanates that compete with iodine for thyroidal uptake
-Cruciferous vegetables such as cabbage, cauliflower, kale, broccoli, turnips, rapeseed,	Contain glucosinolates; metabolites compete with iodine for thyroidal uptake
-Soy, millet	Flavonoids impair thyroid peroxidase activity
Industrial pollutants	
-Perchlorate	Competitive inhibitor of the sodium/iodine symporter, decreasing iodine transport into the thyroid
-Others(e.g. disulphides from coal processes)	Competitive inhibitors of the sodium/iodine symporter, decreasing iodine transport into the thyroid
-Smoking	An important goitrogen; smoking during breastfeeding is associated with reduced iodine concentrations in breastmilk; high serum concentration of thiocyanate due to smoking might compete with iodine for active transport into the secretory epithelium of the lactating breast

Nutrients	
-Selenium deficiency	Accumulated peroxides might damage the thyroid, and deiodinase deficiency impairs thyroid hormone synthesis
-Iron deficiency	Reduces heme-dependent thyroperoxidase activity in the thyroid and might blunt the efficacy of iodine prophylaxis
-Vitamin A deficiency	Increases TSH stimulation and goiter through decreased vitamin A-mediated suppression of the pituitary TSHβ gene

Iodine deficiency in Ethiopia

In Ethiopia, iodine deficiency has affected a large percentage of the population for decades. The Ethiopian Health and Nutrition Research Institute (EHNRI) and UNICEF have reported that goiter prevalence in Ethiopia increased from 26% in 1981 to 40% in 2005. The rate in children was estimated to be 63% in some areas [44]. In 2005, Abuye and Berhane surveyed goiter status in Ethiopia and found that goiter rate among 15 to 49 years old women was 35.8%, which included 24.3% palpable and 11.5% visible goiter. They estimated that about 6 million women in this age category were affected by goiter. In the southern region of the country, out of 1702 women examined for goiter, 43.2% had palpable and 17.7% had visible goiter, which is a total goiter rate of 60.9%. These data show that Ethiopia, particularly the southern region, is severely affected by iodine deficiency [45]. Recently Ethiopia has been identified as one of the few African countries

with moderate iodine deficiency [14]. Although there are no available data on prevalence of cretinism in Ethiopia, it is estimated that in severely iodine deficient areas, cretinism may affect 5 to 15% of the population [46].

Other studies conducted in Sidama zone, near the town of Hawassa, showed the severity of iodine deficiency in the area. A study done in Wondo Genet indicated that out of 100 women of child bearing age participating in the study, 85% had either palpable or visible goiter and 99% had urinary iodine concentration (UIC) below 20 µg/L which is an indicator of severe iodine deficiency [47]. A cross-sectional study in three kebeles around Tula showed that of the 172 pregnant women who participated in a study conducted in 2010, more than 90% reported they have never heard about iodized salt and 90% never consumed iodized salt. Of the 158 pregnant women who were tested for urinary iodine concentration (UIC), 157 (99.4%) had UIC below 100 µg/L which indicates iodine deficiency [48]. A cross-sectional study in three other kebeles (Finchawa, Alamura and Tullo) showed that of the 204 women of child bearing age who participated in the study, 16% had visible or palpable goiter and 97% had UIC below 100 µg/L; the median UIC was 37.2 µg/L indicating the presence of moderate iodine deficiency [19]. Moreover, a study conducted in 7-9 years old school children in Hawassa town indicated that of the 116 participants who were tested for UIC, 18.9%, 63.2% and 16% had severe, moderate and mild iodine deficiency respectively [49].

Iodine deficiency and brain development

Iodine is a trace element essential for the synthesis of thyroid hormones, T_4 and T_3 that play a very important role in the process of early growth and development, especially the brain. Early human brain development occurs during the fetal and postnatal

period [50,51]. Thyroid hormones, through binding of T_3 to nuclear receptors, exert action to regulate the expression of specific sites in different brain regions following a precise developmental process [41]. The hormones also play a vital role in the timing, rate and quantity of brain cell proliferation by having an effect on RNA polymerase II in assembling messenger RNA, and influencing tRNA sulphurtransferase, which causes the release of polypeptide chains from ribosomes [52]. This shows the role of iodine and thyroid hormones in the growth and differentiation of the central nervous system [53].

Iodine is directly involved in cerebral functioning and intelligence. Cerebral development occurs mainly during the fetal period but it continues to develop up until the age of 3 years; frontal lobe development may even continue into the third decade. Deficiency of iodine or thyroid hormone during this critical period would result in slowing metabolic activity of all the cells and also would alter the development of the brain which later leads to irreversible mental retardation [54]. According to a review, the neurological disorders seen as a result of iodine deficiency are mediated by thyroid hormone deficiency [55].

Iodine deficiency is a preventable cerebral disease [54]. People living in areas affected by severe iodine deficiency were estimated to have an IQ of as much as 13.5 points below those of comparable communities in areas where there was no iodine deficiency [56]. In some regions of India, endemic cretinism in some children even caused an IQ deficit of 70 points [57]. In a recently published meta-analytical review it was noted that populations, specifically children who live in areas where iodine deficiency is chronic showed a mean reduction in IQ of 12 – 13.5 points [15]. In another systematic review and meta-analysis, it was reported that iodine sufficient children age 5 years and

younger had IQs that were 6.9 to 10.2 points higher compared to iodine deficient children [58]. The overall effect of this mental deficiency would have an immediate effect on children's learning capacity, the quality of life in communities, and economic productivity [3].

In randomized controlled trials, providing iodine supplements to women of childbearing age had significant effects on mental development. In Papua, New Guinea, an injection of intramuscular iodine before conception eliminated endemic cretinism [5]. In three other studies, children born to mothers supplemented prior to conception or in pregnancy showed better developmental levels compared to children born to non-supplemented mothers [59-61]. Furthermore, children of mothers who had higher thyroxin concentrations at 12 weeks of pregnancy had better psychomotor development at 10 months of age than children of mothers who had lower thyroxin levels [62]. In a cohort study in Spain, children from iodine sufficient areas showed decreased risk of attention deficit and hyperactivity disorders compared to children from iodine deficient areas [63].

Although there is not much evidence on the effect of mild iodine deficiency on children's intellectual development, a study in Tuscany showed mildly iodine-deficient (64 μg/d) 6 to 10 years old children had a delayed reaction time compared with iodine sufficient (142 μg/d) children [41,64]. A study in China revealed that mild prenatal iodine deficiency could affect infant's information processing ability and later cognitive function. In this study infants with elevated cord blood TSH showed poor performance in information processing tasks at seven months of age and poor cognitive development index at 13 months of age compared to infants with normal TSH levels [65].

In moderately iodine deficient areas, no signs and symptoms of endemic cretinism were observed but there were psychoneuromotor and intellectual development abnormalities in children. These abnormalities were demonstrated by different tests and the results showed that those moderately iodine deficient had low visual-motor performance, motor skills and neuromotor abilities, as well as low development quotients and IQ [40,66,67]. Severe iodine deficiency as mentioned earlier could cause serious consequences in physical and brain development which is named endemic cretinism [41,68].

Iodine needs in pregnancy, lactation and infancy

Iodine requirement of women is increased during pregnancy and lactation, because maternal iodine is essential for the brain development of her offspring during fetal and early postnatal life. During pregnancy, the requirement for T_4 increases to maintain normal metabolism in the mother, the fetus shares T_4 and iodide with the mother and there is an increased loss of iodide through the kidney [69]. Hence, compared to non-pregnant women, pregnant women have increased recommended dietary intake of iodine. The recommended dietary intake for non-pregnant women is 150 µg/d but according to the 2001 WHO report, the recommended intake for pregnant and lactating women was 200 µg/d [39]. A later WHO/UNICEF/ICCIDD (2007) report kept the recommended intake for non-pregnant women the same, but the recommendation for pregnant and lactating women in severely iodine deficient areas was increased to a 250µg daily dose of iodine supplement or 400 mg single annual dose of iodized oil supplement and children age 0 – 6 months should receive iodine through breast milk provided that the child is exclusively breastfed [3]. According to the Institute of Medicine (IOM) of the US Academy of

Sciences, the recommended daily intake for pregnant and lactating women is 220 µg/d [70], but others have suggested 175 to 230 µg/d [71,72].

Recommended intakes for infants are based on mean iodine intake of healthy full-term infants fed breast-milk, because functional criteria that reflect iodine intake in infants are not available. Hence, based on the IOM, adequate intake for iodine for infants age 0-6 months was 110 µg/d. This recommendation was based on two important points. First, the median breast milk iodine concentration in the early 1980's in the United States was 146 µg/L. Second, based on the estimated mean daily breast milk excretion, the mean amount of iodine obtained by an infant from human milk is approximately 115 µg/d [70]. However, WHO recommends 90 µg/d iodine intake for infants [3].

Breast milk iodine concentrations (BMIC) can be increased by high maternal iodine intake. In a study in 16 healthy lactating Boston area women BMIC was significantly increased after ingestion of 600 µg oral KI [73]. However, infants do not utilize iodine beyond their requirement. That means any extra iodine is excreted in the urine. Therefore, in iodine sufficient countries, the iodine requirement during lactation should not be based on the measured amount excreted in breast milk but based on infant balance studies [9]. Iodine retention of 7.3 µg/kg/d was reported in balance studies of full-term infants fed 20 µg/kg/d [74]. The estimated daily retention of iodine in a six-month-old-infant in positive balance is 50 µg if the reference body weight at six months of age is seven kg [9,70].

In a randomized, double blind, placebo-controlled supplementation trial in New Zealand, lactating mothers who had been supplemented with 75 µg/d or 150 µg/d of iodine for six months had significantly higher iodine concentrations in their breast milk

and urine than non-supplemented women. However, the amount of supplementation given was not sufficient to raise the mothers' and infants' iodine status to an optimal level based on the thyroid hormone measurement [75]. In studies conducted in lactating mothers from Iran and Morocco, BMIC was correlated with UIC ($r = 0.44$, $p < 0.0001$) and ($\beta = 0.675 – 0.739$, $p < 0.0001$) respectively [76,77]. In several other studies, as reviewed by Zimmermann iodine supplementation of different doses improved maternal UIC, and also thyroid hormones [78].

As indicated by Abuye and Kelbessa the median iodine content of breast milk in Ethiopian women was 5-16 µg/L which shows severe iodine deficiency [33]. Other countries categorized under severe iodine deficiency were Morocco with 27 µg/L and Congo with 15 µg/L [69,79,80]. On the contrary, high median iodine concentrations of breast milk were found in Korea and Japan, which were 892 and 661 µg/L respectively [69]. In another study in 50 Korean women considerable elevation of BMIC 198 to 8484 µg/L was reported [81]. Significant variation of BMIC ranging from 5.4 to 2170 µg/L and from 9 to 1267 µg/L were also reported [79,82]. A study in Boston area lactating women reported a median BMIC of 155 µg/L [83]. In a double blind, randomized, placebo-controlled study, 241 lactating women who received 400 mg of iodine as an iodized oil capsule had significant increases in median BMIC compared to baseline and to the placebo group [77]. However, although the sample size was small (only 13 lactating women) and short duration (24 hrs), iodized salt maintained better BMIC than single dose, 150 µg iodine, supplement daily for three days [84].

The epidemiological criteria for assessing iodine nutritional status for pregnant women of median urinary iodine concentration classified < 150 µg/L, 150 -249 µg/L, 250

- 499 µg/L and ≥ 500 µg/L as insufficient, adequate, above requirement and excessive iodine intakes respectively. For lactating women and children < 2 years of age, median urinary iodine concentration of 100 µg/l was recommended as adequate iodine intake, but no other categories were defined [3].

Prevention and control of IDD

Iodine deficiency is a major global public health problem, with consequences that are more severe for pregnant, lactating women and infants. Iodine deficiency is one of the worst causes of preventable brain damage in childhood. Due to this fact, it is classified as a primary problem to be eliminated worldwide [6].

The best strategy to control IDD is increasing iodine intake through supplementation or food fortification. Iodine supplementation can be given using iodized oil, which is now recommended in severely endemic areas with no access to iodized salt. But iodized salt, compared to iodized oil, is cheaper, has wider coverage, and does not require direct contact with each person [6]. Food fortification has been done in different ways previously. Bread, oil, milk and water have been fortified with iodine to increase iodine intake [85-87]. However, universal salt iodization was found to be the best strategy to meet the goal [6].

Universal salt iodization (USI) is the iodization of all salt for human and livestock consumption in order to eliminate IDD. USI is considered the best solution to iodine deficiency for the following reasons. i) salt is consumed by everybody, ii) it can be consumed throughout the year, iii) it can be produced by only a few producers which makes it easy to control, iv) salt iodization is easy to implement, is cheap ($ 0.4 to 0.5 cents/kg) and does not affect the color, odor or taste, and v) quality of iodized salt can be

easily monitored at different levels such as production, retail and household levels [6]. However, attention should be given to loss of iodine from salt because iodine in salt could be lost easily from improper storage, handling and cooking processes. In a study in Wukro town in Northern Ethiopia, it was confirmed that a great amount (57%) of the salt iodine content that was available at the production level had been lost when it reached the household. Moreover, it was estimated that 20% of the iodine will be lost during the cooking process [3,88].

The recommendation is to iodize salt at 20 to 40 mg iodine per kg of salt (assuming a person consumes 10 g of salt per day) [89]. The two forms of iodine used for fortification are potassium iodide and potassium iodate. The later is mostly recommended in hot and humid climates because it is more stable [89]. USI has to be done to this level not only to improve urinary iodine but also thyroid function [90]. A review in 30 developing countries showed that UIC has been positively and significantly correlated with household iodized salt availability [91].

In a prospective study in Denmark four years after a mandatory salt iodization program (13 ppm iodine) a lower median thyroid volume was observed in women aged 18 to 65 years. A larger relative decline of thyroid volume was observed in the younger compared to older females from a site where iodine deficiency was moderate [92].

A 12 year salt iodization program was evaluated in Tanzania (from early 1990's to 2004). School children aged 6 to 18 years were assessed for goiter rate (n = 140,358) and UIC (n = 4523). Total goiter rate significantly decreased from 61% in the 1980s before USI was implemented to 12.3% in 2004 and the median UIC was found to be 204

µg/L. However, some areas of the country were found to have low iodized salt coverage and others had excess coverage [93].

Worldwide, according to the WHO 2004 report, it was estimated that the iodine intakes of 36.5% (285 million) of school age children were insufficient. In Africa, it was estimated that 50 million children had low iodine intake and 42.6% of the general population had insufficient iodine intake (UI < 100 µg/L). Among the WHO regions in the world, iodine intake was insufficient in 54 countries and hence USI needs to be strengthened or implemented [6]. In 2007, it was reported that over 30 countries had achieved USI, which means more than 90% of households in those countries consumed iodized salt. In 2001 in Africa, the percentage of households having access to iodized salt was 63% [3].

By 2010 in Ethiopia, despite the serious iodine deficiency troubling the country, no iodization program had been implemented except the 1988 salt iodization program in Port Assab which is now separated from the country due to the dispute with Eritrea. This shows that minimal attention has been given to the importance of iodized salt. The average salt consumption in the country was estimated to be 8.4 ± 5.9 g per person per day. Only 4.2% of households reported consumption of iodized salt during the national survey in 2005, and in SNNPR, only 2.2% used iodized salt [13]. Moreover, among the 10,998 women respondents who participated in one study, 93% said they did not know the importance of iodized salt and 91% said they did not know the cause of goiter. In SNNPR, the percentages were 98.5 % and 95.7 % respectively. Legislation was initiated to implement USI, which includes prohibition of production, importation and distribution

of non-iodized salt in the country and this legislation was signed in 2011 and USI has been implemented since 2012 [18,45].

In 2011 it was reported that out of 94% of households who had salt tested for the presence of iodine, only 15% of households in Ethiopia and 12.2% of households in SNNPR were using iodized salt. Among these, 23% were urban households and 13% were rural households [94]. Criteria set by WHO for monitoring progress towards sustainable elimination of IDD as a public health problem are presented in the following table [39].

Table 2. 3. Criteria for monitoring progress towards sustainable elimination of IDD

Indicators	Goals
Salt iodization coverage	
Proportion of households consuming adequately iodized salt	> 90%
Urinary iodine	
√ Proportion of population with UI level below 100 µg/L	< 50%
√ Proportion of population with UI level below 50 µg/L	< 20%
Programmatic indicators	At least 8 of the 10
√National body responsible to the government for IDD elimination. It should be multidisciplinary, involving the different fields of nutrition, medicine, education, the salt industry, the media, and consumers, with the chairman appointed by the Minister of Health	
√ Evidence of political commitment to USI and elimination of IDD	

√ Appointment of a responsible executive officer for the IDD elimination program	
√ Legislation or regulation of USI	
√ Commitment to regular progress in IDD elimination, with access to laboratories able to provide accurate data on salt and UI	
√ A program of public education and social mobilization on the importance of IDD and the consumption of iodized salt	
√ Regular data on iodized salt at the factory, retail and household levels	
√ Regular laboratory data on urinary iodine in school age children, with appropriate sampling for higher-risk areas	
√ Co-operation from the salt industry in maintenance of quality control	
√ A database for recording results or regular monitoring procedures particularly for iodine, urinary iodine and , if available, neonatal thyroid stimulating hormone (TSH), with mandatory public reporting	

Side effects of iodine supplement and contraindications

Some populations, such as in some parts of Japan, who depend on sea foods for their daily food may consume as much as are 50,000 to 80,000 µg or 50 to 80 mg of iodine per day [95], although the safe upper limit for iodine supplement has been set at 1000 µg/d [70]. When taken according to the recommendations, iodine is known to be safe. However, some individuals might be hypersensitive to supplements even at the recommended dose. Hence, individuals who have pre-existing autoimmune thyroid

pathologies should be excluded from participating in studies that include iodine supplement [96].

Iodine and other nutrient interactions

Multiple micronutrients including selenium, iron and vitamin A are involved in iodine and thyroid metabolism. In areas of endemic goiter it is important to consider micronutrients which directly or indirectly affect the problem [97].

As reviewed by Zimmermann [98], in another study the effects of combined vitamin A and iodine deficiency on the thyroid axis of rats was studied. Accordingly, it was observed that compared to the control groups (rats that were vitamin A and iodine sufficient) the vitamin A and iodine deficient (ID) rats had increased serum TSH, TSHβ mRNA, and thyroid weight and had decreased FT_4 and TT_4. This study also confirmed that concurrent vitamin A deficiency (VAD) and ID were more likely to cause primary hypothyroidism compared to ID alone and had more effect on the pituitary thyroid axis compared to VAD alone [99]. Abnormality of the pituitary-thyroid axis caused by ID and VAD was reversed by supplementation with an iodine rich diet. Thyroid size and hyperstimulation were also reduced by vitamin A supplementation, and this was assumed the result of effect of vitamin A on pituitary TSHβ gene expression [100].

In an animal study comparing iron sufficient rats with deficient rats, decreased plasma triiodthronine (T_3) and thyroxin (T_4) concentrations were found in iron deficient anemic (IDA) rats [101]. Reduced TSH response to lower circulating T_3 and T_4 concentrations was observed in rats with severe IDA. On the contrary, iron-sufficient animals showed significantly higher T_3 turnover and elimination than iron- deficient animals. Moreover, anemic rats had decreased hepatic selenium-dependent 5' deiodinase

activity which is involved in the conversion and activation of the inactive thyroid hormone (T_4) to its active form (T_3) [102]. The impairment of 5'deiodinase activity was greater in severe IDA compared to mild IDA rats [103].

In human studies, adults who had severe goiter had increased retinol binding protein, serum retinol and transthyretin (TTR) concentrations. Furthermore, in children who were affected by severe IDD, TSH stimulation and thyroid size were increased and risk of hypothyroidism was decreased [98]. Significant decreases in serum retinol and retinol binding protein (RBP) were observed in Ethiopian children with visible goiter compared to children without goiter [104]. In an efficacy study, 6 to 14 years old Moroccan children were given 25 μg/g of iodized salt (IS) and were divided into two groups. The first group (IS group) received an oral placebo (sunflower oil) at 0 and 5 months, while the second group (IS + VA group) received an oral VA supplement (200,000 IU as retinyl palmitate) at 0 and 5 months. At 10 months, mean serum retinol, RBP, and RBP/TTR ratio increased significantly, median TSH and Tg levels decreased significantly and mean goiter volume and rate decreased significantly in the IS + VA group compared to the IS group. A significant reduction was also observed in the number of children with VAD in the IS + VA group [105].

Studies suggested that reduced activity of thyroid peroxidase (TPO), the iron-dependent enzyme in IDA, could be the reason for impaired thyroid hormone metabolism. In a study with iron sufficient and deficient diets, rats that received an iron deficient diet had decreased circulating T_3, T_4 and Hb concentrations, and TPO activity was also decreased compared with the control animals [106]. In humans, anemic women showed reduced T_3 and T_4 concentrations compared with healthy women but not

significant differences in TSH concentration [107]. In an intervention study in Côte d'Ivoire, goitrous children 6 to 12 years old were assigned into two groups. Children in group 1 were goitrous but without anemia and group 2 consisted of goitrous children with anemia. All children in both groups received iodized poppy seed oil containing 200 mg of iodine as a capsule. Thyroid volume and goiter rate were significantly reduced in group 1 at 15 and 30 weeks after intervention compared to group 2. Compared with baseline value, group 1 children had significantly reduced median TSH values at 15, 30 and 50 weeks. When both groups were combined, median TSH was significantly reduced and T_4 value was significantly increased in group 1 at 15 and 30 weeks [108,109].

Iron supplementation (60 mg Fe/d, 4 d/wk for 4 months) to iodized salt supplemented goitrous children in Côte d'Ivoire significantly decreased mean thyroid size twice as much as in the placebo group. A goiter rate of 43% or 62% was observed in the iron supplemented and the placebo group respectively ($p < 0.02$) [110]. In an area of borderline iodine deficiency, poor maternal iron status was found to predict lower and higher TSH concentrations in pregnant women [111]. In a randomized, double blind, controlled trial, children 6 to 15 years old were supplemented either with iodized salt (25 µg iodine/g salt) or with salt fortified with iodine (25 µg iodine/g salt) and iron (1g Fe/g salt). For several indicators children in the dual-fortified group showed better improvement compared to the control group. For instance, mean thyroid volume, prevalence of goiter and hypothyroidism were significantly decreased and serum thyroxine was significantly increased [112].

Selenium (Se) is one of the trace minerals involved in the synthesis and metabolism of thyroid hormone. The role of Se in organification, radioiodide uptake and

thyroid hormone synthesis has been observed in animal models. An inadequate Se supply decreased glutathione peroxidase (GPx) activity in the thyroid. Moreover, Se deficiency in animal models decreased renal and hepatic type I 5'-deiodinase and brain type II 5' deiodinase that play important roles in thyroid hormone metabolism [97]. In iodine-supplemented goitrous children, the positive effect of iodine on thyroid volume and serum TSH was inhibited by Se deficiency [108]. Selenium supplement (100μg sodium selenite daily for three months) also improved the T_3/T_4 ratio [113].

Cognition

Cognition refers to processes such as attention, language, perception, action, association, memory, concept formation, problem solving, and mental imagery [24]. Cognitive function refers to the processing of information taken in from the environment and reflected in the form of behavior [114].

Cognition includes visual and somatosensory perception, thinking, memory and learning and is considered to be an outcome of millions of metabolic processes where its main task is registering, encoding, selecting, maintaining, transforming, storing and retrieving information [115]. Cognition, as well as its elements that include reasoning, attention, memory, and psychomotor coordination, is complex. Memory for instance, a part of cognition, includes visual, long term, short term, verbal, spatial, declarative, strategic, and semantic aspects and requires several tools for assessment [116]. As another example, attention is widely considered to be a single unit involving several separable systems that are mediated by their own pathways [117].

Cognitive function, as defined by Wainwright and Colombo (2006), is the neural process that is necessary to support the flexible use of information to carry out adaptive

and goal-directed behavior. The ability to execute adaptive behavioral responses depends on the capacity to focus and maintain attention and these processes also depend on the holding capacity of information in working memory [118]. Other information processing functions that are known to be more complex are also involved. One example could be the ability to use conceptual information and organize it in a meaningful way for appropriate responses in different contexts and to hold back other competing behavioral responses [118].

Brain and cognitive function

The decision-making processes and information processing of the mammalian brain are mediated by neural circuits comprising a series of reciprocal cortical-subcortical loops. The cortex is the place from which these circuits originate and then project to various sub-cortical structures including basal ganglia and nucleus accumbens. Circuits then return to the region of the cortex, via the thalamus [119]. These structures and pathways are influenced by the inputs from many other regions of the brain and the presence of such functional interactions between these structures is essential to both behavioral and physiologic regulation [120,121]. The hippocampus, an important source of input to the nucleus accumbens, in addition to the prefrontal cortex, is necessary for the formation of long-term memory [120,122]. The hippocampus, by representing the relations between distinct stimuli, also plays a role in higher-level decision-making processes [122]. The hippocampus is also involved in novelty preference [123].

The prefrontal cortex, during the decision making process, generates possible behavioral alternatives in response to the specific nature and emotional categorization of sensory stimuli which can later be transferred to the nucleus accumbens [124]. Neural

transmission involving the hippocampus, prefrontal cortex, and nucleus accumbens is vital in enabling behavioral flexibility [125].

The amygdala, another source of input to the nucleus accumbens is involved in processing the affective emotion of sensory stimuli and passing on this information to the prefrontal cortex [120,126]. In addition, the amygdala also plays a vital role in stress responses during occurrence of perceived dangers [126]. According to McGaugh, the stress response includes the release of glucocorticoid hormones [127]. Appropriate concentrations of glucocorticoids are necessary for mnemonic functions to be accomplished effectively and the hippocampus together with the prefrontal cortex, are involved in the feedback regulation of these glucocorticoid concentrations [128].

Infant's visual recognition memory

Infant's visual recognition memory is the beginning of cognitive function which can predict broad cognitive abilities in later childhood [129]. Follow up data by Fagan and McGrath have shown the relation between infant visual recognition memory and later cognition [130]. Children tested for visual recognition memory at the age of 4 and 7 months were tested again for their performance on the Peabody Picture Vocabulary Tests and/or other vocabulary scales at the age of 4 and 7 years and a positive correlation was observed between the two tests. Predictive relations between infancy and 3-year IQ were found using the Fagan Test of Infant Intelligence [131,132].

The defining feature of infant visual recognition memory is the responsiveness to novelty, which is central to biological adaptation and to other cognitive development theories and intelligence. Infant visual recognition memory explains that previous knowledge can be inhibited by new events through the process of accommodation, and

the ability to deal with the new knowledge or events plays a major role in the human experimental theory of intelligence [133,134]. There exist individual differences in visual recognition memory and infant recognition memory show significant correlation with several cognitive abilities in later childhood [134]. Rose and colleagues (2004) also documented that visual recognition memory is affected by several factors that affect adult memory which include forgetting, speed of processing and attention [22].

In a study on chimpanzees by Fantz (1964) the chimps showed preference for novelty. He presented a photo for one minute, 10 times in succession, and each time he presented the photo paired with a new one. He observed that infant chimpanzees over two months of age showed an increasing attention to the novel picture and decreasing attention to the familiar pattern. The finding gave a strong base to the visual paired-comparison task, and hence to the study of visual recognition memory in infancy [135]. In recognition memory, the first measure is a 'novelty score' which is the percentage of looking during the test directed to the novel target [136,137]. The theory is that when infants come across a new stimulus, they try to match it with the stored information in their mind. If it is similar, their attention deviates. If it is not, attention remains engaged until enough information about the new stimulus is obtained and familiarized [138].

The principle of visual information processing is also explained by the theory of habituation and dishabituation. According to Sokolov (1963), when infants are presented continuously with the same stimulus their attention decreases, which is a result of the gradual construction of a memory trace of the visual stimulus. This phenomenon is called visual habituation. Short looking times show fast habituation and relatively longer

looking times show slow habituation[138]. This paradigm uses mean infant look duration as the dependent measure [139].

On the other hand, following habituation, attention diverts to a new stimulus encountered and this attention diversion is referred to as dishabituation. Hence, comparing the looking time towards the habituation stimulus at the end of the habituation phase with looking time towards the novel stimulus will indicate the strength of dishabituation. Both visual habituation and dishabituation would indicate basic information-processing skills. In a meta-analytical review, it was reported that it is possible to predict later childhood IQ from infant's habituation-dishabituation performance [140]. However, although habituation was related to later cognitive and language outcomes this relationship was recently criticized because it is unlikely that habituation can be considered as a primary component of intelligence but rather a starting effort of learning and cognition upon which higher level functions can be built later in childhood [139]. Habituation was also described as cognitive encoding and dishabituation was referred to as discrimination and memory [141]. Kavesk (2004) pointed out that dishabituation is sensitive in predicting later intelligence in at risk groups whereas in non-risk groups habituation is better [140].

Among the factors that influence immediate recognition, familiarization time (the time needed to encode what is seen) is most important. Familiarization time is affected by age; that is, older infants need a shorter time to become familiar with the stimulus compared with younger infants [22]. A study conducted by Rose (1983) measured familiarization time differences in infants age 6 and 12 months old. The test was to recognize three dimensional geometric figures at 10, 15, 20 and 30 seconds

familiarization. The 12 month old infants showed significant novelty scores at all familiarization times whereas the 6 month olds showed only at 20 and 30 seconds familiarization times. Hence, the ability to recognize at 10 and 15 seconds indicates those older infants recognized the stimuli faster than the young ones [142].

Other studies have demonstrated that infants can recognize delayed stimuli. In a study by Fagan (1973), infants aged 4 to 5 months were able to recognize abstract patterns after 2 days delay [143]. In another experiment, they were able to recognize a photograph of a man or a woman after 2 weeks delay. However, the familiarization time and pattern determined the recognition. For instance, infants recognized more when the practice or familiarization periods were distributed over relatively longer times than shorter times [22].

When familiarization times were relatively short, retention increased over the first year of life. Infants aged 5 to 6 months were able to recognize faces after 2 minutes delay following 10 seconds familiarization but infants aged 4 months could not [144]. Consistent with this, Rose (1981) found that 9 month old infants recognized patterns and faces immediately after 5 and 20 seconds and also after 2 to 3 months delay but 6 month olds only recognized the patterns and faces when displayed immediately [145]. Speed of processing is a very important variable because faster processing speed is related to better cognitive performance [20].

In general, as mentioned earlier, infants' visual recognition memory shows a true cognitive ability that is confirmed in later age in predicting broad cognitive abilities such as IQ and language. These predictive relations were similar for preterm and full-term infants and the results were consistent after controlling several confounding factors such

as maternal education, socio-economic status, medical risk and others. This indicates that the basis of cognition later in childhood can be found in infancy [22].

Factors affecting visual recognition memory

Factors that affect childhood cognition were found to affect infant visual recognition memory as well. Factors include prematurity, perinatal circumstances such as long labor, genetic abnormalities such as Down's Syndrome, prenatal exposure to drugs, nutritional deficiency, breast feeding, maternal education and others [65,146-151]. Preterm infants (average birth weight of 1.9 kg) and full-term infants at 4 months have been compared on four problems. The mean novelty score in the preterms was significantly lower than for full terms and also full terms showed recognition memory unlike preterms [152]. The memory deficit in preterms also showed in 12 month old infants [153]. Similarly, in another study, preterm and full term infants were tested with faces and abstract designs and full terms showed significantly better novelty recognition compared with preterms [154]. Differences within full-terms were also shown due to perinatal complications. Caron and colleagues (1983) found poor recognition memory in full-term infants who experienced different birth complications such as abnormally long labor, being small for gestational age, and low APGAR scores [147]. Moreover, preterm infants showed poor recall memory at one year of age that could persist into early childhood [153].

In a cross-sectional study, 100 healthy full term infants were divided into five different age groups including 14 weeks, 20 weeks, 26 weeks, 39 weeks, and 52 weeks. A series of eight different stimuli were shown to these infants to examine the process of look duration as a function of age and type of stimulus. Consistent with other studies the look duration decreased from 14 to 26 weeks for all stimuli types but after 26 weeks the

course of look duration deviated according to stimulus type. For instance, 6-month-old-infants showed more looking and increased heart rate to more complex stimuli than to simpler ones [155]. In a meta-analytical review, it was noted that in the first month of life, look duration increases followed by decreases at the age of 6 to 9 months [141].

A study in India on 5 to 12 month old infants showed nutritional status assessed by anthropometric measurements made a difference in visual recognition memory. Underweight infants showed poorer visual recognition memory and also failed to show age-related improvements observed in normal infants even after controlling for previous illness, birth weight, and parental education [150]. Kennedy and colleagues reported that development of visual information processing (VIP) in 6 to 8 month old infants was associated with growth. Moreover, they demonstrated that VIP can indicate infant cognitive development and it was also sensitive to nutritional factors [21].

Nutrition and cognition

Nutrition is one of the major factors affecting cognition [116]. Due to the fact that about 70% of the major development of the brain takes place during the prenatal period and most of the remaining 30% is during the first three years postpartum, optimal nutrition is most fundamental during pregnancy and the first 3 years of life [156].

Diet affects neurochemistry in two ways. First, neurotransmitters are synthesized by enzymes which require co-factors such as minerals and vitamins obtained from food. Second, neuronal functions are influenced by the composition of myelin sheath and cell membranes which are also affected by diet [157].

Cognition is influenced by various environmental factors at different stages of life; however, the effect of nutrition is thought to be crucial throughout life. The role of

nutrition on cognition is getting attention because it is now realized that the development of the brain occurs at more stages of the lifespan than previously understood [158]. Nutrition is one of the major environmental variables that can be manipulated relatively easily with regard to brain function. Not only does nutrition affect brain development but it also plays a significant role in maintaining the function of the brain [114].

A study conducted in Guatemala showed that slower reaction time on neuropsychological tests such as perception, attention, memory and reasoning was observed in vitamin B_{12} deficient school-aged children than those who were not deficient [159]. Several studies suggest that micronutrient deficiencies such as iron, zinc, vitamin A, and iodine bring about impairments in growth, immune competence and cognitive function among children [160,161]. Such adverse health consequences can lead to a reduction in both reproductive and intellectual potential in adulthood [162]. All domains of cognitive functioning were impaired by micronutrient deficiencies and were sensitive to change due to micronutrient status among children [163,164,41].

DHA and visual habituation

In a randomized, double blind, controlled study two groups of pregnant women were supplemented during the last trimester of the pregnancy with either eggs that contained 135 mg docosahexaenoic acid (DHA) per egg or ordinary eggs that contained 35 mg DHA per egg. The objective of the study was to evaluate the effect of maternal DHA on development of attention in their infants and toddlers. Colombo and colleagues found that infants of mothers consuming high DHA showed increased developmental attention and shorter look duration on the habituation stimuli that were pictures of children's faces. Thus they had decreased look duration indicating DHA affects attention

positively. Similarly, toddlers of mothers with high levels of DHA showed better developmental profiles on single-object measures and more optimal performance on distractibility assessments unlike the others [20]. Shorter looking time on stimuli during infancy has been associated with greater cognitive functioning later in childhood [140].

Summary

In conclusion, visual information processing as a measure of cognitive functioning is influenced by several nutritional and non-nutritional factors. Among the nutritional factors, iodine is one of the few nutrients that is well established to affect the brain, but its effect on visual information processing of infants is unknown. Several trials have shown cognitive disability as a result of iodine deficiency can be reversed by iodine supplementation. Its effect should be highly beneficial when implemented at an early stage. In a population where iodine deficiency is prevalent, iodized salt supplementation is found to be one of the best stable and safe interventions to alleviate iodine deficiency disorders.

CHAPTER III

METHODS

Data source and the study population

Participants for this study were selected from women and their infants living in rural areas of Sidama zone, southern Ethiopia. The study population depends on subsistence farming for their livelihood. Their major staple food is enset *(Enset ventricosum)* followed by unrefined maize [165]. The area is known for multiple micronutrient deficiencies and iodine deficiency is one of the major prevalent problems.

In selecting the site three important factors were considered. First, from our previously conducted research, we knew that the area is severely affected with iodine deficiency. Second, none of the study population had ever consumed iodized salt at the time of last study and hence, the effect of iodized salt would easily be seen. Third, due to the fact that we have done repeated research in the area and because Hawassa University has a strong link with the community, conducting intervention research is useful in order to plan for sustainable projects for the benefit of the community.

Objective one

Subject selection methodology

A total sample size of 159 subjects was calculated using an alpha of 0.05, 80% power and an effect size of 0.25. Of these 159 women and their infants, 53 women were to be selected to serve as controls and 106 were to be assigned to serve as treatment groups (53 in iodine capsule and 53 in iodized salt groups). Prior to data collection ethical clearance was obtained from Oklahoma State University, USA, the Ministry of Science

and Technology and the Food, Medicine, and Health Care Administration and Control Authority of Ethiopia. Following receipt of ethical clearance, study participants were selected with the help of health extension workers in the study area.

For selecting participants two important criteria were established. First, the women must have been lactating and must have infants of specific ages: a) less than or equal to one week old infants for the treatment groups, or b) 6 ± 0.25 month old infants for the control group. Second, mothers had to volunteer to participate in the study. Based on these criteria, participants were given a detailed explanation about the objectives of the research and consent was taken from the women for themselves and their infants before data collection was started.

Exclusion criteria

The exclusion criteria in this study were if mothers had any hyperthyroidism symptoms manifested by nervousness, anxiety, heart palpitations, rapid pulse, fatigability, tremor, muscle weakness, weight loss with increased appetite, heat intolerance, frequent bowel movements, or increased perspiration [166]. If infants had fever, cough or severe (>3/day) diarrhea, they would not be enrolled in the study. If mothers showed any allergic reaction such as acne, weakness or foul breath after supplementation started they would be excluded from the study as well. However, no one showed or reported any of the symptoms.

The research team (the PI and data collectors) first met with leaders and community health workers from the Kifle-Ketema (A small administrative unit which contains 12 kebeles) and explained the objectives of the research and procedures for data collection at baseline. With the help of community health workers, all mothers within one

week after delivery in each of the eight selected kebeles and their infants were invited to participate and study enrollment was continued until the required sample size was attained. These mothers were given iodized salt or iodine capsule based on the random assignment and served as the treatment groups. Subsequently, all women with 6 ± 0.25 month-old infants were recruited to serve as control. Once the required data were collected the mothers in the control group were provided with iodized salt for six months.

Research design

A community-based, randomized, controlled trial was used in this study. The study employed an experimental design in order to test hypotheses to establish cause-effect. The experimental groups were the groups manipulated by the researcher by giving different treatments; in this case, those lactating women and their infants who received iodized salt or iodine capsule supplement for 6 months beginning at the birth of the infant. The control group was composed of lactating women and their 6-month-old infants who were tested for VIP and provided all required data at baseline. The study was longitudinal in nature due to the fact that iodine status of lactating women and infants in the treatment groups were measured twice.

Eight kebeles were selected. At baseline, control groups were established by recruiting mothers of 53 six month old infants from all of the eight kebeles. Mother-child dyads were registered and VIP was tested in infants. The mothers then were given iodized salt supplements for themselves and their families for six months. For the experimental groups, 106 women and their infants from all of the eight kebeles were recruited for the study within one week of delivery. These mother-child dyads were randomly assigned to iodine capsule (n = 53) or iodized salt (n = 53) groups. The iodine

capsule group received 225 μg of iodine daily as a capsule of potassium iodide (Capsules were prepared for this study purpose by Pure Encapsulations, Inc. Boston, MA) daily for 6 months. Supplementation started within one week of delivery. Capsules were delivered to the mothers every day by the health extension workers. The experimenter supervised the mothers taking the capsules once a week. The iodized salt group was provided with iodized salt as 30 to 40 μg/kg of KIO_3 (1 packet (450 g) of salt per week per household) for 26 weeks) for themselves and their family's use starting within one week of delivery until 6 months. After 6 months, VIP was tested on the 106 infants whose mothers received supplemental iodine as iodine capsule or as iodized salt. These results were compared with the control group. Please see research design (Figure 1).

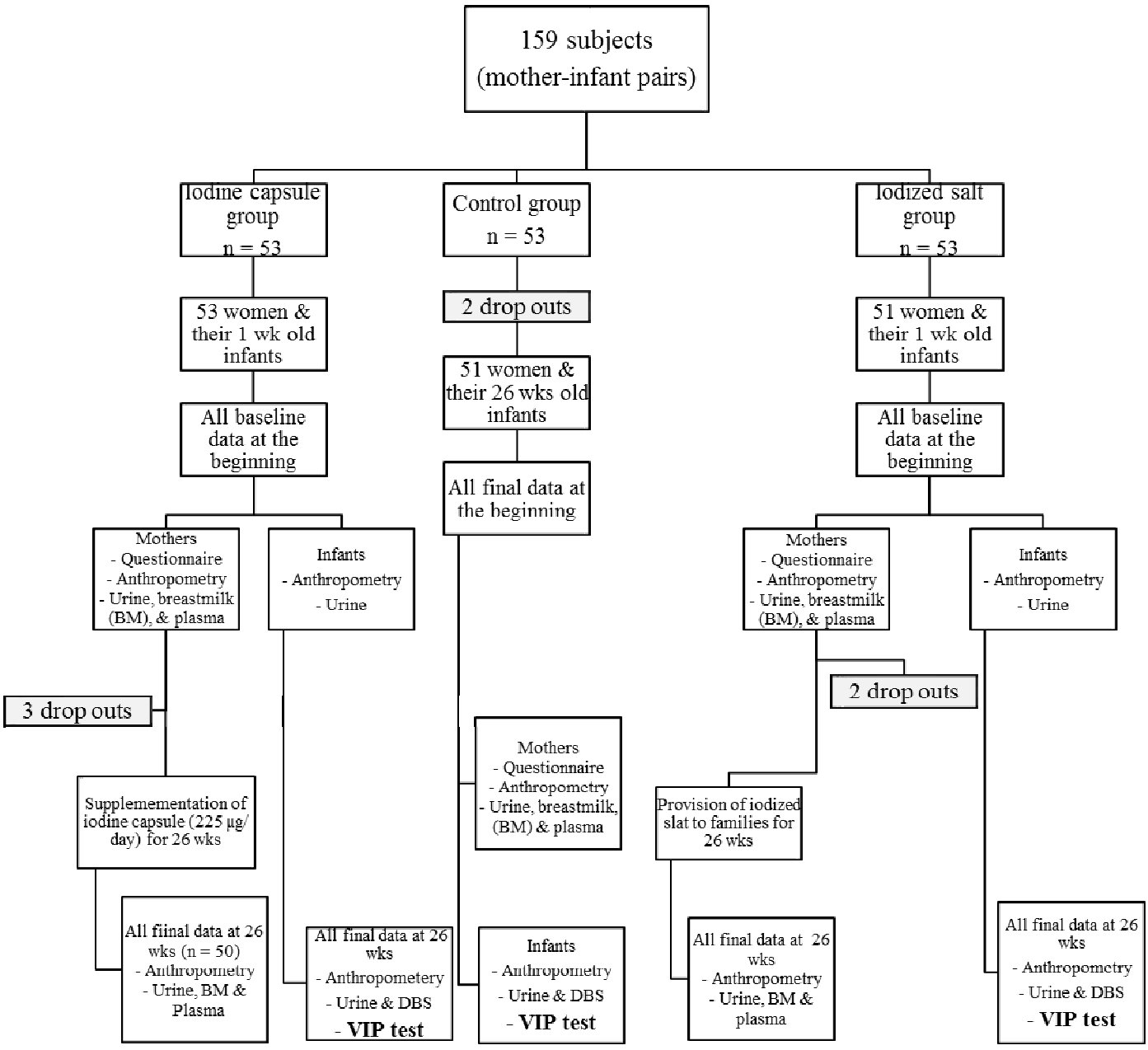

Fig 3.1. Research design

Data collection

Training was given to data collectors on the questionnaire, anthropometric measurements, VIP measurement and urine sample collection technique prior to data collection. The questionnaire and anthropometric measurements were pretested on 10 women and 10 infants who were not included in the study and the VIP test was also pretested in the same infants.

Data collection took place in eight kebeles, and data collectors including the PI went to the kebeles three times a week to collect the required data as infants reached the appropriate ages. For the control group, mothers with their six months old children were invited to come to the community health post. For the treatment groups, data collectors and the PI went home to home to collect the baseline data from mothers and their infants. When the infants reached six months of age, mothers and their infants were invited to come to the community health post for end point data and VIP test for the infants. Data were collected according to the following procedures. See also Table 3.1.

- **Questionnaire**

Due to the nature of the study it was important to assess several variables that were thought to affect the problem of interest. Hence, several instruments were used to measure the intended variables.

The questionnaire included three major sections. The first section asked about the socioeconomic status and demographic characteristics of women using a standardized questionnaire adopted from the Ethiopian Demographic and Health Survey Agency [167]. The second section included household food insecurity [168,169] and hunger [170]. And, the third section included questions about knowledge of the importance of iodine, barriers to

iodized salt procurement, and breastfeeding, complementary feeding and related practices. Data collectors read questions to mothers.

- **Anthropometric measurement**
    - **Materials**

As a measure of women's nutritional status, weight and height were measured to calculate body mass index (BMI = Wt (kg)/Ht^2 (m). Mid upper arm circumference (MUAC) was also measured. As indicators of the infant's nutritional status, head circumference, MUAC, weight and length were measured. Infant's weight-for-age, length-for-age, weight-for-length, MUAC and head circumference were converted to Z-scores using WHO Anthro software (Version 2.0.4).

- **Procedure**

Each woman's weight was measured on a solar digital scale (Uniscale, UNICEF, NY) and recorded to the nearest 100 grams. Women wore light clothing and removed shoes and heavy outer wear (e.g. sweaters) before obtaining weight. Height was measured to the nearest 0.1 cm using a single calibrated instrument (Adult Board, Shorr Productions, Olney, MD). Participants stood bare footed on a flat surface with weight distributed evenly on both feet, heels together, and the head positioned so that the line of vision was perpendicular to the body. Arms were hanging freely by the sides, and head back. Buttocks and heels were in contact with the vertical board. Mid upper arm circumference was measured using a plastic measuring tape. Anthropometric measurement instructions were adopted from Gibson [171]. All measurements were taken in duplicate and averages were taken for analyses.

Infant weight was measured using a Seca 345 infant scale and recorded to the nearest 2 grams. Infants wore light t-shirts of known weight and laid down on the scale. Their actual weight was calculated by subtracting the t-shirt weight from the total weight. The recumbent length was measured using a length-board (Shorr Productions, Olney, MD) and recorded to the nearest 0.1 cm. The head circumference and mid upper arm circumference were measured using a non-stretchable plastic tape and recorded to the nearest 0.1 cm. All measurements were taken in duplicate and averages were taken for analyses.

Visual information processing (VIP) administration

- **Materials**

For visual information processing measures, eight pictures of young adult Ethiopian faces without emotional expression (four males and four females) were used. Two laptop computers were used. One computer was used to control the presentation of stimuli. A second computer that has a built-in camera was used to follow and record the infant's looking behavior. Although the infant's looking behavior was coded live, the infant's looking process was recorded on this second computer for later reliability testing.

- **Procedure**

A quiet room was prepared for the data collection process. The infant sat on mother's lap in front of the camera, eyes forward, and half a meter away from the screen. A bed sheet was placed between the VIP tester and the infant and hence the researcher was out of sight of the infant. The tester adjusted the visibility and focus on the infant's face and eyes through a camera built in on the laptop. Once the focus was adjusted, the familiarization phase began. A randomly-selected picture was shown on the screen of the

laptop in front of the infant. The tester followed the infant's look on the laptop. If the infant looked at the picture, a square like shape (reflection) was visible in his/her pupil. At this time the researcher pressed down the left key of the mouse (computer key) and if the infant's eye turned away from the picture the researcher released the key. The computer recorded the time between stimulus onset and the look (response latency) and the duration of the look. If the infant looked away from the face for 1 second or longer, the face was replaced by a blank screen for 2 seconds. The face then reappeared. This continued until the criterion for habituation was met, namely two consecutive looks that were less than one half the mean duration of the two longest looks. Therefore, the minimum number of looks during the familiarization phase was four. After the habituation criterion was met, the comparison phase began without interruption of the procedure. In the comparison phase, the face that appeared during habituation (the "familiar" face) appeared again on one side of the screen and a novel face of the opposite gender appeared on the other side. The tester pressed down the left key of the mouse as the infant's eyes looked at the left picture and pressed down the right key of the mouse and released the other as the infant's eyes were looking at the right picture and released both fingers as the infant turned away from the pictures. While doing this, the second computer has recorded the infant's looking behavior for reliability testing, while the first computer automatically coded the infants' look duration at the pictures, shifts from one picture to the other, and length and number of turns away from the pictures. During the comparison phase the faces remained on the screen regardless of whether or not the infant was looking at one of them. Once the infant had accrued a total looking time at the faces of 5 seconds, the faces were replaced by a blank screen for 1 second, and then the faces

reappeared but on opposite sides of the screen. They remained until 5 additional seconds of looking accrued.

This VIP system has already been tested in rural villages near Wolayta by our research group. A generator was used to power the laptops and projector and the system worked well. The PI and the tester were trained on the testing of the VIP in the Department of Psychology at Oklahoma State University, USA.

Biomarkers in women

- **Materials**

Samples of urine and breast-milk were collected from mothers for assessment of iodine status. Samples of blood were also collected for assessment of thyroid hormones, thyroid stimulating hormone and thyroglobulin. All samples were collected in the morning.

- **Procedure**

  - **Urinary iodine**

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition because 90% of dietary iodine eventually appears in the urine. Urinary iodine concentration can be measured in spot urine specimens from representative samples [3]. Urine samples were collected in a cup from each participant and transferred to fill 2 mL tightly sealed vials in duplicate. Samples were frozen at -20 ^{0}C at Hawassa University until they were transported to OSU for analysis.

Urinary iodine was analyzed by inductively coupled plasma mass spectrometry (ICP-MS Elan 9000, Perkin Elmer, Norwalk, CT). All urine samples were diluted 10 fold (0.5 mL to 4.5 mL) with 2% ammonium hydroxide (NH_4OH) (Sigma-Aldrich, St. Lowis,

MO) in Millipore water. Standard solutions of iodine were prepared by dilution of certified standard solutions (Inorganic Ventures, Christiansburg, VA). Dilute working standards were prepared by dilution of an intermediate stock standard solution. The calibration standards were prepared in 2% NH_4OH solution at 0, 10, 20 and 100 µg/L. Tellurium (Perkin Elmer Life and Analytical Sciences, Shelton, CT) was utilized as an internal standard. Polypropylene plastic ware was used for standard and sample preparation (Sarstedt, Inc., Newton, NC).

- **Creatinine**

Creatinine reagent (Carolina Liquid Chemistries Corp. Brea, CA) for the quantitative determination of urine creatinine was analyzed using a BioLis 24i Clinical Chemistry Analyzer. Urinary iodine to creatinine ratio in a single urine sample is considered a reliable method to quantify urinary iodine in individuals and is beneficial in terms of time, cost and convenience compared to a 24-hr sample [172].

- **Breast milk**

Breast milk iodine concentration is relatively high compared to the UIC of the lactating mother even in areas of iodine deficiency [83]. Therefore, analysis of iodine concentration in breast milk is recommended in order to know the amount of iodine consumed by the infant because it is assumed that 95% of the iodine in breast milk is absorbed [9]. Hence, 10 ml of breast milk were collected from each mother in plastic vials for storage. Samples were frozen at -20 ^{0}C until analyzed.

Breast milk iodine concentration was analyzed by inductively coupled plasma mass spectrometry (ICP-MS Elan 9000, Perkin Elmer, Norwalk, CT). Iodine was extracted from breast milk using 25% Tetramethylammonium hydroxide (TMAH)

solution (Sigma-Aldrich, St. Louis, MO) and hydrogen peroxide (H_2O_2). TMAH solution (1.5 mL) and H_2O_2 (0.5 mL) were added to breast milk samples (3 mL) and were mixed well. The solution was incubated in an oven at 90 ^{0}C for 3 hours using DigiTUBE (CPS Science, Baie D'urfé, QC) and after cooling to reach room temperature, the solution was centrifuged at 3000 rpm for 15 minutes. All breast milk samples were diluted 25 fold (0.2 mL to 4.8 mL) with 5% TMAH (Sigma-Aldrich, St. Lowis, MO) in Millipore water. Standard solutions for iodine were prepared by dilution of certified standard solutions (Inorganic Ventures, Christiansburg, VA). Dilute working standards were prepared by dilution of an intermediate stock standard solution. The calibration standards were prepared in 5% TMAH solution at 0, 10, 25 and 50 µg/L. Tellurium (Perkin Elmer Life and Analytical Sciences, Shelton, CT) was utilized as an internal standard. Polypropylene plastic ware was used for standard and sample preparation (Sarstedt, Inc., Newton, NC). Non-fat milk powder (RM 1549, National Institute of Standards and Technology, Gaithersburg, MD) was used as an external control.

- **Plasma**

Fasting morning venipuncture blood samples were collected from mothers using a disposable 10 cc syringe coated with lithium heparin with a 21 gauge needle (Sarstedt, Inc., Newton, N.C.). Blood samples were collected using hygienic techniques by an experienced lab technician. The blood was centrifuged and plasma was separated. Plasma was kept frozen until analyzed for analysis of thyroglobulin (Tg), thyroid hormone concentrations (T_3 and T_4) and thyroid stimulating hormone (TSH).

Thyroglobulin, TSH and thyroid hormone concentrations were analyzed quantitatively based on a double antibody sandwich method (Alpco Diagnostics, Salem,

NH). Quality control samples (Bio-Rad Laboratories Inc., Irvine, CA) for Tg, TSH, T_3 and T_4 were utilized to verify method performance was within recommended ranges.

- **Goiter**

Total goiter of women was determined by health professional based on the following grades: grade 0, no palpable or visible goiter; grade 1, goiter palpable but not visible when neck is in the normal position; grade 2, goiter visible when neck is in the normal position [39].

Biomarkers in infants

- **Urinary iodine**

Urinary iodine concentration is a very good indicator to measure infant's iodine status [9]. Urine samples were collected from infants by placing cotton balls inside a plastic sheet available in the local market. The infants were checked every 10 minutes until urine samples were obtained. Urine samples were collected by squeezing urine out of the cotton balls. In order to avoid contamination, the health worker who helped with sample collection put on new trace mineral free gloves for each infant. Urine samples were transferred to fill tightly sealed vials and frozen for subsequent assessment of urinary iodine concentration. Samples were stored in a freezer at Hawassa University until transported to OSU for analyses. Infant's UIC and creatinine analysis were done by the same method as mother's UIC and creatinine.

- **Blood**

Blood drops from finger pricks, were collected on a Whatman 903™ specimen collection paper (GE Healthcare Bio-Sciences Crop. Westboroght, MA) [9,173]. Each specimen paper was placed in zip closure with indicating desiccant inside to prevent any

moisture build up and stored in freezer until analysis. Drops of blood were collected on a slide for hemoglobin analysis. Infants T_4 and TSH levels were determined using enzyme linked immunoabsorbent assay (Diagnostic Automation Inc., Calabasac, CA) by the sequential immunoenzymometric assay procedure.

- **Salt sample**

A 10 g salt sample was collected from each household for iodine content analysis. Salt iodine concentration (SIC) was analyzed with a portable digital electronic iodine checker (WYD, UNICEF). One gram of salt sample was weighed in 50 ml tube and dissolved with 10 ml of distilled water, 2 ml of KI-starch solution and 2 ml of H_3P0_4. Salt was thoroughly dissolved by shaking the tube. A small amount of the solution was filled in a cell and the cell was placed in the cell holder of the WYD. The concentration (mg/kg) of iodine was directly read out on the LCD of WYD iodine checker. A calibrated gray glass provided with WYD was used as a standard instead of using standard solution.

All salt samples were re-analyzed using inductively-coupled-plasma mass spectrometry (ICP-MS Elan 9000, Perkin Elmer, Norwalk, CT). A salt sample (1 gm) was diluted with 10 ml solution of 2% NH_3OH (Sigma-Aldrich, St. Lowis, MO) in Millipore water out of which 100 µL solution was taken and diluted in 5 ml solution of NH_3OH. Standard solutions of iodine were prepared by dilution of certified standard solutions (Inorganic Ventures, Christiansburg, VA). Dilute working standards were prepared by dilution of an intermediate stock standard solution. The calibration standards were prepared in 2% ammonium hydroxide solution at 0, 10, 20 and 100 µg/L. Tellurium (Perkin Elmer Life and Analytical Sciences, Shelton, CT) was utilized as an internal standard.

- **Iodine supplementation**

Iodine capsules that contained 225 µg iodine as potassium iodide (manufactured and formulated by Pure Encapsulations, Inc., Sudibury, MA) were purchased and stored at room temperature at Hawassa University until all were delivered to the study participants. Iodine capsules were delivered to the lactating women daily for 26 weeks by the health extension workers and health promoters who lived in the study kebeles.

Iodized salt was required by government mandate. Before iodized capsules were delivered to the women, their household salts were tested for iodine content using rapid test kits.

Table 3.1 Summary of data collection for objective 1 (control and treatment groups)

Subjects	Number of visits & data collection	
	At 0 to 1 week	At 26 weeks
Control groups		
- Mothers	NA	-Recruiting -Obtaining consent -Questionnaire (interview) -Measuring weight, height & MUAC -Collecting blood, urine & breast milk samples -Assessing goiter
- Infants	NA	-Measuring weight, length, MUAC & head circumference -Collecting urine & blood samples -Measuring VIP Distributing supplement of iodized salt or capsule as appropriate to the experimental design
Treatment groups		
- Mothers	-Recruiting -Obtaining consent	- Questionnaire (interview) -Measuring weight, height & MUAC

	-Measuring weight, height & MUAC -Collecting blood, urine & breast milk samples -Assessing goiter -Providing iodized oil or iodized salt as appropriate to the experimental design.	-Collecting blood, urine & breast milk samples
- Infants	-Measuring weight, length, MUAC & head circumference -Collecting urine samples	-Measuring weight, length, MUAC & head circumference -Collecting urine & blood samples -Measuring VIP

Objective two

Subject selection methodology

Out of the eight kebeles involved in objective 1, one kebele was randomly selected to participate in objective 2. A randomized sample of 40 families was drawn from the list maintained in the kebele office. All members of each selected household were invited to participate in the study. Parental consent was obtained for participation of any child under 18 years of age. Children 12 – 18 years of age provided assent for participation.

Study design

In one kebele, 40 randomly selected households were assessed for the impacts of availability of iodized salt and community iodine awareness on urinary iodine concentration (UIC). At baseline, urine was collected from members of 40 households (40 mothers and 76 children) who were available at home during data collection. Following that, education was given on consequences and causes of IDD, sources of dietary iodine particularly iodized salt, storage and utilization of iodized salt. All of the 40 households were provided with one packet of salt (450 g) per week for one month. At the end of the month and at two months urine samples were collected from each household member who provided urine at baseline.

Data collection

- **Socioeconomic status (SES) and salt utilization questionnaire**

Socioeconomic status was assessed by a combination of parental education and family wealth. Wealth criteria included possession of bicycles, lantern, or mobile phone and family ownership of animals and crops, land size, family size, type of home, roof and

windows, ownership of umbrella and shoes; children attending school or intention to send them; outside employment, water source and latrine. Questions about purchase, storage, and utilization of salt were included. Questionnaire was administered orally.

- **Urinary iodine**

Urine samples were collected from adults and children in cups. Urine samples were transferred to fill tightly sealed vials and frozen for subsequent assessment of urinary iodine concentration. See Table 3.2.

Table 3.2. Summary of data collection for objective 2.

Subjects	Number of visits and data collection		
	At baseline	At 1 month	At 2 month
-Parents & children over 18 years old	-Obtaining consent -Questionnaire (interview) -Collecting urine samples -Providing iodized salt to the household for 1 month	-Collecting urine samples -Salt will be tested for iodine content	-Collecting urine samples
-Children 12 to 18 years old	-Obtaining consent from parents -Obtaining assent from children -Collecting urine samples	-Collecting urine samples	-Collecting urine samples
Children under 12 years old	-Obtaining consent from parents -Collecting urine samples	-Collecting urine samples	-Collecting urine samples

Ethical consideration

In order to avoid any ethical issues of withholding iodine supplementation for the control group, six month old infants were identified and tested towards the end of the study to serve as controls. Right after testing, iodized salt was given to the mothers for 26 weeks for their household consumption.

CHAPTER FOUR

MANUSCRIPT ONE

Title: Maternal iodine intake and its effect on breast milk iodine, maternal urinary iodine, and infants urinary iodine concentrations.

Abstract

Iodine deficiency during pregnancy and lactation could expose the infant to severe iodine deficiency disorders. This study compared methods of iodine delivery on breast milk iodine, and on maternal and infants' urinary iodine concentrations. An experimental randomized supplementation trial in lactating women was conducted. Women were randomly assigned either to receive 225 µg iodine as potassium iodide capsule daily for six months or 450 g of appropriately iodized salt (30 – 40 µg I as KIO_3/g of salt) weekly for household consumption for six months. Breast milk iodine and maternal and infant urinary iodine were measured at baseline and at six months. Median (IQR) breast milk iodine concentration (BMIC) at baseline was 154 (43, 252) µg/L and at six months was 105 (36, 198) µg/L, maternal urinary iodine concentration (UIC) at baseline was 107 (71, 161) µg/L and at six months was 130 (80, 208) µg/L, and infant UIC at baseline was 218 (108, 356) µg/L and at six months was 222 (117, 369) µg/L. The women did not differ in BMIC and UIC, and infants did not differ in UIC and anthropometric indices in a time by treatment interaction. However, significant correlation between the three variables was obtained in both groups at both times. In conclusion our findings suggest that appropriately iodized salt (30 – 40 µg I/g) had similar effects as a daily supplement of 225 µg I to lactating women on BMIC, maternal UIC, and infants UIC.

Key words: Breast milk iodine concentration, maternal iodine intake, Ethiopia

Introduction

Iodine is required for the synthesis of thyroid hormones; thyroid hormones in turn regulate the metabolic patterns of most cells. Iodine plays a crucial part in the process of early growth and development of most organs, especially the brain [1]. Severe maternal iodine deficiency during early pregnancy could result in permanent brain damage to their offspring [2,3]. Mild-to-moderate iodine deficiency could also result in poor learning ability, and increased risk of low birth weight, and of infant morbidity and mortality [4]. Infants under two years of age are among the most vulnerable groups to be affected by iodine deficiency along with pregnant and lactating mothers [5]. Hence special attention has been given to these population groups regarding preventing and controlling iodine deficiency [6].

Risk of iodine deficiency in early infancy is high even in iodine sufficient areas because infants' intrathyroidal reserve turnover rate is rapid [7]. Therefore in iodine insufficient areas recommendations for iodine for women during pregnancy and lactation are increased to 250 µg/d or to a 400 mg single annual dose of iodized oil supplement [8]. Because a large amount of iodine is secreted into breast milk, exclusively breastfed infants 0 – 6 months of age should receive sufficient iodine through breast milk [6,9].

Recommended intakes for infants are based on mean iodine intake of healthy full-term infants fed breast-milk, because functional criteria that reflect iodine intake in infants are not available. Hence, WHO recommends daily intake of iodine for infants as 90 µg [10]. Similarly, the Institute of Medicine (IOM) in the USA set the adequate iodine intake for infants 0-6 months of age at 110 µg/d. This recommendation is based on two important points. First, the median BMIC in the United States was 146 µg/L. Second,

based on the estimated daily breast milk excretion, the mean amount of iodine obtained by an infant from 0.78 L of human milk is approximately 115 µg/d [11]. Iodine intake greater than the requirement will be excreted in the urine [12].

Breast milk iodine concentration (BMIC) can be increased by increasing maternal iodine intake [12]. In a study in 16 healthy lactating US women, BMIC was significantly increased after a one time ingestion of 600 µg potassium iodide (456 µg of iodine) as measured hourly for eight hours [13]. In a randomized, double blind, placebo-controlled study, supplementation to lactating women of either 75 µg/d or 150 µg/d of iodine as potassium iodate for 6 months significantly increased breast milk and maternal urinary iodine concentration (UIC). However the amount of supplementation given was not sufficient to improve the mothers' or infants' thyroid hormones [14]. In another double-blind, randomized, placebo-controlled trial, one dose of 400 mg of oral iodine as a soft-gel capsule of iodized poppy seed oil to lactating mothers significantly increased UIC of their three-month old infants compared to a single direct dose of 100 mg iodine given orally as an iodized oil supplement to the infants [15].

As a strategy to alleviate iodine deficiency, increasing iodine intake through supplementation or food fortification has been recommended. Salt iodization programs have been found to be most useful because they are cost effective and feasible and salt is consumed by almost everyone [10]. In places where salt iodization is not feasible and/or unavailable, iodine supplements should be given to at risk groups including pregnant and lactating women or infants [8]. Our study aimed to compare efficacy of appropriately iodized salt in raising BMIC and maternal and infants UIC compared to daily intake of iodine capsules for six months by lactating mothers.

Materials and methods

Data source and study population

The study was conducted in rural areas of Sidama zone, southern Ethiopia. All lactating women (n = 101) in the study village who delivered between January and February, 2013 were recruited within a week after delivery to participate in the study. Informed consent was obtained from the women for themselves and for their infants. This study was conducted in accordance with the ethical principles for the protection of human subjects. Ethical approval was obtained from Oklahoma State University (OSU), USA, Hawassa University, Ethiopia, and the Ministry of Science and Technology, Ethiopia. The study employed an experimental randomized supplementation research design.

Mother-infant dyads were randomly assigned to receive either 225 µg of iodine as potassium iodide in capsule form (Pure Encapsulations, Inc. Boston, MA) daily to the mother for six months or 450 g (30 to 40 µg of I as KIO_3/g of salt) of iodized salt (I-salt) weekly for household consumption for six months. Capsules were delivered to the mothers daily or salt for the household was delivered weekly by health extension workers. Prior to randomization and delivery of supplements, baseline data including anthropometry, urine samples, breast milk samples and socio-demographic information were collected from mothers. Infant urine samples and anthropometric measurements were also obtained. Following six months of supplementation, similar data were collected from mothers and their infants.

Anthropometry and questionnaire

Weight (kg) and height (cm) of mothers were measured to calculate body mass index (BMI = weight (kg)/height $(m)^2$). Weight was measured on a solar digital scale

(Uniscale, UNICEF, NY) and recorded to the nearest 100 g and height was measured to the nearest 0.1 cm using a single calibrated instrument (Adult Board, Schorr Productions, Olney, MD). Mid upper arm circumference (MUAC) was measured to the nearest 0.1 cm using a non-stretchable plastic measuring tape. Weight (Kg) and length (cm) of infants were measured to assess growth. Infants' weight was measured using a Seca 345 infant scale and recorded to the nearest 2 g. Infants wore light t-shirts of known weight and laid on the scale. Their actual weight was calculated by subtracting the t-shirt weight from the total weight. The recumbent length was measured by a length board (Schorr Productions, Olney, MD) and recorded to the nearest 0.1 cm. The head circumference and mid upper arm circumference were measured using a non-stretchable plastic tape and recorded to the nearest 0.1 cm. A questionnaire was administered to assess socio-economic and demographic characteristics of the mothers participating in the study.

Measurement of biomarkers

Breast milk iodine

Ten ml of breast milk sample was collected from each mother in plastic vials for storage. Samples were frozen until analyzed. Iodine was extracted from breast milk using 25% Tetramethylammonium hydroxide (TMAH) solution (Sigma-Aldrich, St. Louis, MO) and hydrogen peroxide (H_2O_2). TMAH solution (1.5 mL) and H_2O_2 (0.5 mL) were added to breast milk samples (3 mL) and were mixed well. This solution was incubated in an oven at 90 ^{0}C for 3 hrs using DigiTUBE (CPS Science, Baie D'urfé, QC) and after cooling to room temperature, the samples were centrifuged at 3000 rpm for 15 minutes. All breast milk samples were diluted 25 fold (0.2 mL to 4.8 mL) with 5% TMAH (Sigma-Aldrich, St. Lowis, MO) in Millipore water. Breast milk iodine

concentration was analyzed by inductively coupled plasma mass spectrophotometer (ICP-MS, Elan 9000, Perkin Elmer, Norwalk, CT). Tellurium (Perkin Elmer Life and Analytical Sciences, Shelton, CT) was utilized as an internal standard. Non-fat milk powder (RM 1549, National Institute of Standards and Technology, Gaithersburg, MD) was used as an external control.

Urinary iodine

Mothers collected their own urine in a cup out of which samples were taken to fill 2 mL tightly sealed vials in duplicate. Urine samples were collected from infants by placing cotton balls appropriately inside a plastic sheet available in the local market. The infants were checked every 10 minutes until urine samples were obtained. Urine samples were collected by squeezing urine out of the cotton balls. In order to avoid contamination, the health worker who helped with sample collection put on new trace mineral free gloves for each infant. Samples were frozen at -20 ^{0}C at Hawassa University and then transported to OSU for analysis. Urinary iodine was diluted in 2% ammonium hydroxide and analyzed by inductively coupled plasma mass spectrophotometer (ICP-MS, Elan 9000, Perkin Elmer, Norwalk, CT), with tellurium as internal standard.

Urinary creatinine

Creatinine reagents (Carolina Liquid Chemistries Corp., Brea, CA) were purchased. Urinary creatinine was analyzed using a BioLis 24i clinical chemistry analyzer.

Statistical analysis

The main outcome variables in this study were BMIC, maternal and infants' UIC and infants' anthropometric measurements. All data were checked for normality using Kolmogorov-Smirnov test and skewed data were log transformed before analysis.

Weight-for-age Z score (W/A Z score), weight-for-length Z score (W/L Z score) length-for-age Z score (L/A Z score) and head-circumference-for-age Z score (HC/A Z score) of infants were calculated using the WHO Anthro software (version 3.2.2., 2011; WHO, Geneva, Switzerland). The time and treatment effect was assessed by linear mixed effect model analysis for repeated measures.

Correlations between outcome variables were assessed using Pearson's correlation for normally distributed data or Spearman's rho for non-normally distributed data. The relation between multiple variables and infant's UIC were modeled by multiple linear regression. Variables for regression were chosen based on correlation. Data are reported as mean (SD), median (25^{th}, 75^{th} percentile), or percentage (frequency) as appropriate. Level of significance was set at $p < 0.05$. SPSS (version 20; IBM Corp. Armonk) and SAS (Version 9.3; SAS Institute Inc., Cary, NC) were used for data analysis.

Results

Characteristics of study participants at baseline

All of the women who participated in the study exclusively breastfed in the first week after delivery and continued breast feeding to six months. However only 58% reported exclusive breast feeding at the age of six months (data not shown). The mean

frequency of breast feeding in the 24 hours preceding the survey in both groups was 12.8 (5.2) (Table 1).

Mother-infant dyads in the iodine capsule and I-salt groups did not differ in any of the socio-demographic characteristics presented in Table 1. Therefore, Table 1 shows both groups (capsule and I-salt groups) combined. The median age of the mothers was 22 (20, 25) years. The mothers' BMI was 21.5 (2.3) kg/m^2 and MUAC was 23.3 (1.7) cm. The mean gravidity and parity were 3 (2) and the mean household size was 6 (2.2). Mothers attended 3.8 (3.5) school years on average.

The median age of the infants at baseline was 6 (4, 8) days. There were 50 male and 51 female infants enrolled in the study.

Breast milk and urinary iodine concentration of mothers and their infants

Table 2 shows baseline and six months values for BMIC of mothers and UIC of mothers and their infants. The women were not significantly different in BMIC or UIC and the infants were not significantly different in UIC at the two time points. Because our data showed a large within group variation, we categorized BMIC into different groups as indicated in Table 2. At baseline 25.7% of the women had BMIC <50 µg/L, 22.8% had 50 – 149 µg/L, 26.7% had 150 – 250 µg/L and 24.8% had > 250 µg/L. At the end of the study at six months 31.7% of the women had BMIC < 50 µg/L, 32.7% had 50 – 149 µg/L, 21.8% had 150 - 250 µg/L and 13.9 % had > 250 µg/L. BMIC was lower at six months but there was no significant time by treatment interaction ($p = 0.656$). Of the women 11% and 10% had UIC below 50 µg/L at baseline and at six months respectively.

According to the WHO classification for iodine deficiency in lactating women, 47.5% of the women were iodine deficient (UIC < 100 µg/L) at baseline and 38.6% were

deficient at six months. Median UIC appeared to be slightly higher at six months but there was no time by treatment interaction ($p = 0.668$). Based on the median UIC, the mothers in both groups showed similar changes; the capsule group remained in the iodine sufficient category and the I-salt group moved from the iodine deficiency category (median UIC = 95 μg/L) to the iodine sufficient category (median UIC = 110 μg/L). Also maternal UIC adjusted for creatinine was not significantly different between groups or over time ($p = 0.748$).

The proportion of infants with UIC < 100 μg/L at baseline was 20.8% and at six months was 15.8%. The median UIC of infants was not significantly different in time by treatment interaction ($p = 0.322$) (Table 2).

As shown in Figure 1, UIC of infants at baseline was higher than BMIC ($p < 0.001$) and than maternal UIC ($p < 0.001$). Similarly, at six months, UIC of infants was higher ($p<0.001$) than maternal UIC and BMIC. Maternal BMIC was not significantly different from UIC at either baseline or endpoint.

Infants' anthropometric indices were calculated at baseline and at six months. Infants did not differ in any of the indices between groups. As shown in Figure 2, infants L/A Z-score was significantly decreased at six months ($p < 0.0001$) compared to baseline. The means of the three indices in both groups were above the cut-off for nutritional deprivation. However, some infants showed nutritional deprivation and this is illustrated in Figure 3.

Relations between BMIC, maternal UIC and infants UIC at six months were examined. As indicated in Figure 4A and 4B, BMIC was significantly correlated with maternal UIC ($r = 0.39$, $p < 0.001$) and infants UIC ($r = 0.44$, $p < 0.001$). The correlation

between BMIC and maternal UIC increased when maternal UIC was adjusted for creatinine as shown in Figure 4C, ($r = 0.51$, $p < 0.001$). Maternal UIC was correlated with infants UIC as shown in Figure 4D, ($r = 0.31$, $p < 0.001$). The correlation coefficients at baseline were similar to those illustrated at six months.

In Table 3, multiple regression models with four best-fitting variables predicting infants UIC are presented. The model at baseline with all four predictors produced an adjusted $R^2 = 0.290$ and at six months the model adjusted R^2 was 0.286. At baseline maternal UIC/Creatinine, BMIC and infant sex had significant regression weights, indicating infants whose mothers had higher maternal UIC and BMIC were expected to have high UIC and females were expected to have lower UIC than males. At six months maternal UIC adjusted for creatinine, BMIC and infants age had significant regression weights, indicating infants whose mothers had higher UIC and higher BMIC were expected to have higher UIC. Moreover, at six months, a one day increase in infants age was associated with a 0.174 μg/L increase in UIC.

Discussion

This study showed BMIC, maternal UIC and infants UIC were not significantly different between mothers who received 225 μg of iodine daily as a capsule for six months in addition to variable amounts (0 to 42 ppm (mg/kg)) of iodine in their existing household salt and mothers who received 450 g per household of appropriately iodized salt delivered weekly for six months. It was estimated that the average salt intake in Ethiopia nationally was 8.4 g and in the study region was 17.2 g per person per day which is more than two fold the national intake [16]. One reason the salt intake in this region is high is because people in the rural areas consume salt in their coffee several times a day in addition to other food items. From our follow up observation however, the amount of salt we provided (450 g) was enough for one week.

The dose of 225 μg of iodine provided was at the lower end of the recommended range for lactating women (225 to 350 μg daily) [17]. Measured iodine concentration of the salt was 30 to 40 mg KIO_3/kg of salt, consistent with the recommendation of the World Health Organization [18].

Infants' anthropometry was not different between groups for all indices. In all of the infants combined, length-for-age Z score was significantly decreased ($p < 0.0001$) at six months which indicates infants did not grow appropriately. The reasons for decline in growth could be various including multiple micronutrient deficiencies, infectious diseases and others.

Maternal iodine intake is critical during lactation in order to provide the breastfeeding infant's iodine requirement. Iodine is concentrated through the sodium iodide symporter mechanism, which promotes increased expression of iodine in milk

during lactation [19]. Thus, iodine is present in breast milk at higher concentrations than in plasma [20].

Iodine intake recommendations for infants up to six months of age are based on estimates of the amount provided by breast milk of well-nourished women. The average milk excretion is estimated to be 0.78 liter per day during the first 6 months of infancy. In iodine sufficient women (estimated to have a mean iodine consumption of 290 µg/d), this amount of breast milk would provide a mean of 114 µg of iodine [11]. In our study the median BMIC (154 (43, 252) µg/L) at baseline was within the normal range of 150 to 180 µg/L in iodine sufficient countries [21,22]. If the average daily milk volume was 0.78 L/day as estimated by IOM, the infants would receive a mean of 120 µg iodine per day.

Despite iodine supplementation of 225 µg daily or well iodized salt weekly for six months, median BMIC decreased from 154 (43, 252) µg/L at baseline to 105 (36, 198) µg/L at six months. Consistent with our study, Mulrine et al (2010) found that BMIC declined over the first 24 weeks in non-supplemented lactating women and supplementation with 75 µg or 150 µg of iodine daily did not increase BMIC to a level that could raise infants median UIC. In the women who received 75 µg of iodine, BMIC ranged between 35 to 57 µg/L and infants UIC was 50 (22, 60) µg/L. In the group who received 150 µg, BMIC ranged between 43 to 70 µg/L and infants UIC was 66 (36, 87) µg/L [14]. In another study, BMIC in non-supplemented mothers declined from 43 µg/L at baseline to 26 µg/L over a nine months period while milk from the supplemented mothers declined from 41 µg/L at baseline to 39 µg/L at nine months [15].

It should be noted that our baseline data was collected within one week (with more than 32% within four days) after delivery, when colostrum was still available.

Iodine concentration is high in colostrum and decreases over time until it reaches a point where it becomes steady in mature milk [21]. Because the decrease in iodine concentration in breast milk over time has not been measured in detail, it is difficult to quantify the effect our supplementation had on maintaining BMIC.

Our data showed a wide range in BMIC at baseline as well as at six months. At baseline the BMIC ranged from 8 µg/L to 965 µg/L with 49% below 150 µg/L. At six months the range was from 4 µg/L to 958 µg/L with 64% below 150 µg/L. In a study in 50 Korean women, whose diets frequently included sea-weed soup, considerable elevation and range of BMIC from 198 to 8484 µg/L was reported [23]. Significant variation of BMIC ranging from 5 to 2170 µg/L [22] and from 9 to 1267 µg/L [21] were also reported in two other reviews. This variation indicates that many infants may not get adequate amounts of iodine to meet their requirement. Moreover, of the women who had UIC > 100 µg/L, which is considered adequate for lactating women, 37% had BMIC < 150 µg/L.

Maternal median UIC was numerically high but not significantly different between the two types of iodine supplement. However, on the basis of the median UIC value of 100 µg/L for iodine sufficiency, the mothers in the I-salt group had increased their median UIC from 95 µg/L which indicated iodine deficiency to 110 µg/L which indicated iodine sufficiency. Based on the range (150 – 230 µg/L) of median maternal UIC as an indicator for optimal iodine nutrition during lactation [17], most of our study participants in both groups fell below the minimum value at baseline. Following six months of iodine supplementation however, most of the women in the capsule group met the minimum range whereas the women in the I-salt group remained below the range. For

the whole group at six months the median BMIC of those mothers with UIC below 150 µg/L was 83 µg/L and above 150 µg/L was 133 µg/L. This could indicate the dose given to these women might have been inadequate to raise iodine status.

The median maternal UIC of 107 µg/L at baseline indicated an improvement in the iodine status of the population because Ethiopia, including the study population, has been moderately to severely iodine deficient for decades (Median UIC ranged between 1 µg/L to 89 µg/L) [24-28]. Therefore, the change observed in this study can be attributed to the recently implemented salt iodization program in the country [29].

The proportion of women with median UIC below the cut-off value for iodine sufficiency decreased by 10% (from 42% to 32%) in the capsule group and by 8% (from 53% to 45%) in the I-salt group between baseline and the end of the study. A double-blind, randomized, placebo-controlled study reported a 14% increase in UIC at six months after giving the lactating mother a 400 mg iodized oil capsule [15]. The study in New Zealand showed that a daily supplement of either 75 µg or 150 µg of iodine daily for six months during lactation increased maternal UIC significantly but maternal UIC remained below 100 µg/L [14]. On the contrary, a study conducted in Thailand reported median UIC of lactating women (199 µg/L), who received a multivitamin/mineral tablet that contained 200 µg of iodine daily for the second and third trimesters of pregnancy was not significantly different from non-supplemented women (120 µg/L) at two months postpartum. This could be due to the increased salt iodization coverage observed in the country during the study period [30]. In the current study, although the UIC increase was not significant, the proportion of women with UIC above 150 µg/L was increased from

40% at baseline to 50% at six months in the capsule group and from 20% at baseline to 43% at six months in the I-salt group.

The recommended dietary intake (225 - 350 µg) of iodine daily for lactating mothers and 90 µg of iodine daily for neonates and infants was set on the basis that when these requirements are met the expected UIC output in the neonates and infants would be 180 - 225 µg/L [17]. In the current study UIC of infants was within the range of the expected outcome in both groups and at both times. Surprisingly the median UIC of infants obtained in this study was high (218 µg/L at baseline and 222 µg/L at six months) compared to iodine sufficient countries except the USA, which was 921 µg/L. In the rest of iodine sufficient countries infants median UIC was 162 µg/L in The Netherlands, 148 µg/L in Canada, and 112 µg/L in Sweden [12].

The median infants UIC at baseline was significantly higher than BMIC ($p < 0.001$) and maternal UIC ($p < 0.001$) and although not significant median BMIC was higher than maternal UIC. This pattern was similar to other studies that reported these variables [14,15]. The majority of iodine in lactating women is excreted in the breast milk; infants need small amounts to meet their daily requirement, and the remaining iodine will be excreted in their urine supporting this pattern [12,17].

Significant correlations were obtained between maternal UIC, BMIC and infants UIC (Figure 4). The correlation between maternal UIC and BMIC increased when maternal UIC was adjusted for creatinine. A study conducted in Denmark reported that urinary iodine predicted BMIC more precisely when it was adjusted for creatinine [31]. Another study reported that urinary iodine was not associated with median thyroid

stimulating hormone (TSH) and thyroxine (T_4) but was significantly associated when it was adjusted for creatinine [32].

In our regression model at baseline, maternal urinary iodine adjusted for creatinine and BMIC were positive predictors ($p = 0.002$ and $p = 0.006$ respectively) and infants sex was a negative predictor ($p = 0.046$) of infants UIC. At six months maternal urinary iodine adjusted for creatinine and BMIC were positive predictors ($p < 0.001$ and $p = 0.001$ respectively) and infants age was a negative predictor ($p = 0.014$) of infants UIC. This is consistent because as infants grow older the likelihood increases that they will not be exclusively breastfed.

This study compared the results from providing salt iodized at the level recommended by WHO with a daily supplement of 225 µg as an iodine capsule in addition to household salt on BMIC and maternal and infants UIC. Most studies have not compared these three major outcome variables. The method we used to collect infants urine samples was cost effective and feasible. Thus far there was only one study that reported breast milk iodine content (in a range of 5 to 16 µg/L) in Ethiopia, at a time that only 4.2% of households consumed iodized salt [28]. The study used the Moxon and Dixon method [33] which requires dry-ashed milk sample which we think could cause loss of iodine before analysis. Other strength of this study is that we recruited all of the women who delivered within the data collection time period.

We analyzed water iodine concentration in this study and all of the water samples from different sources were below the detection limit of 1 µg/L (Data not shown). As it is well known the only source of iodine in the study population is iodized salt. As infants begin to get a higher percent of energy from low iodine complementary foods, their total

iodine intake will go down. In Ethiopia in general and in the study region (SNNPR) in particular, the median duration of exclusive breastfeeding was 2.3 and 2.2 months respectively [34].

The limitation of this study is that we did not analyze iodine content of complementary food. This is important because starting complementary food early affects frequency of breastfeeding and this affects the amount of iodine which could go to the infant through breast milk [35]. However, the increase in infant UIC at six month indicates iodine intake increased.

Conclusions

In conclusion our findings suggest that appropriately iodized salt (30 – 40 μg I as KIO_3/g) had a similar effect on BMIC and maternal and infants UIC as a daily supplement of 225 μg I as potassium iodide to lactating women. As a way to combat iodine deficiency disorders, universal salt iodization has been recommended. However, it is important to ensure that the salt is homogenously iodized and contains the required amount. The salt used in this study was iodized for research purposes. The special order was required because the salt sold in the Ethiopian market at the time of the study varied notably in a range of 0 to 42 ppm (data not shown) which would result in variable iodine intake. Despite the variability however, the recently implemented salt iodization program in Ethiopia seems to show promising progress. Although data are limited on previous breast milk iodine concentrations in the country, the iodine concentration obtained in the study participants would put Ethiopia among the iodine sufficient countries [12]. However, our data does not represent the whole country and hence a nationwide study would be of great importance.

Salt is recommended as the best vehicle for delivery of iodine, but attention needs to be given to quality control for iodization and to losses through storage, cooking and handling processes [10]. How much iodine is lost through these processes is not clear, but well-designed research is required to evaluate losses and to identify strategies to avoid such losses.

Key messages

- Appropriately iodized salt (30 – 40 μg iodine as KIO_3/g of salt) showed similar effects as 225 μg of potassium iodide on BMIC, and maternal and infants' UIC.
- The median BMIC and infants' UIC obtained in this study were similar to those in iodine sufficient countries.
- Exclusive breast feeding while consuming salt iodized at levels recommended by WHO could provide an infant with sufficient amounts of iodine.
- Salt should be adequately and homogenously iodized in order to ensure appropriate intakes of iodine.
- The daily intake of 225 μg of potassium iodide may not be enough to raise iodine status.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

TG was involved in designing the study, data collection, laboratory and data analyses and writing the manuscript; BJS was involved in designing the study, laboratory and data analyses and writing the manuscript.

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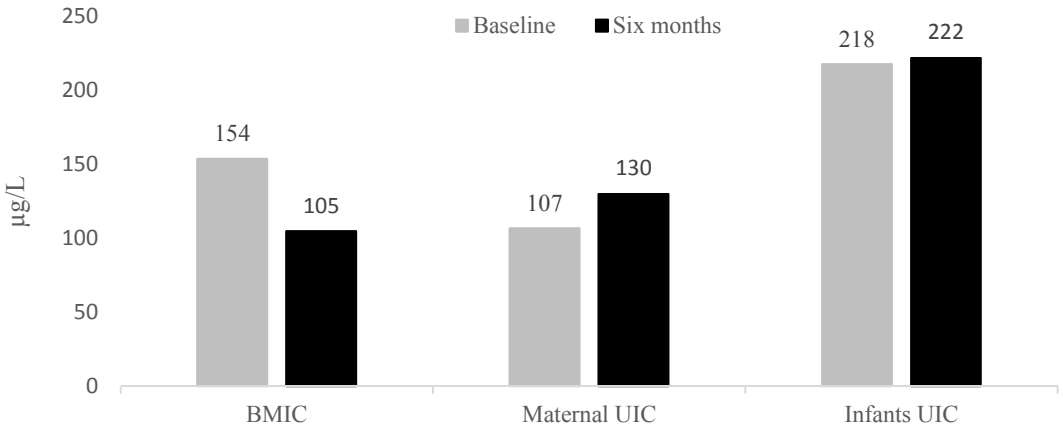

Fig. 1. Median BMIC, maternal UIC and infants UIC at baseline and at six months (n = 101)

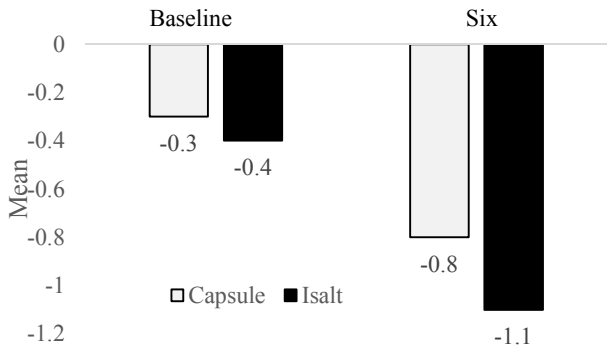

Fig. 2A. Length-for-Age Z-scores of infants at baseline & six months

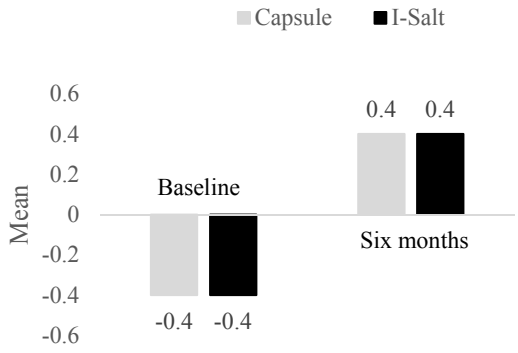

Fig. 2B. Weight-for-length Z-score of infants infants at baseline & six months

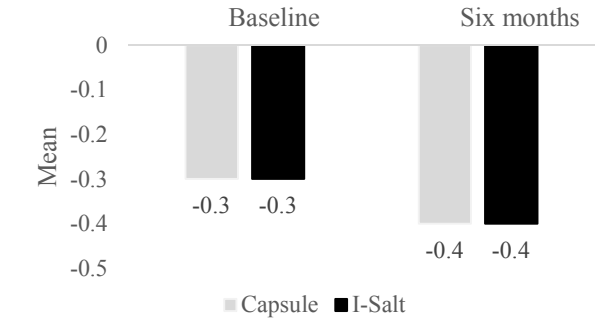

Fig. 2C . Weight-for-age Z-score of infants at baseline and six months

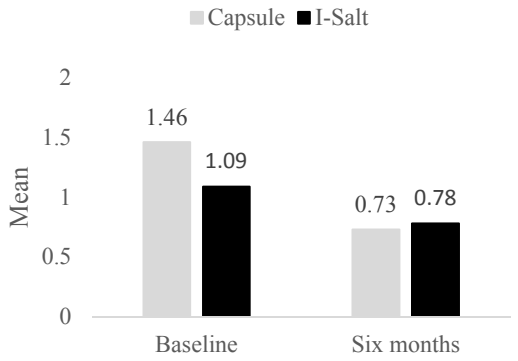

Fig. 2D. Head circumference-for-Age Z-score of infants at baseline & six months

Fig 2. Z scores of anthropometric indices of infants at baseline and six months

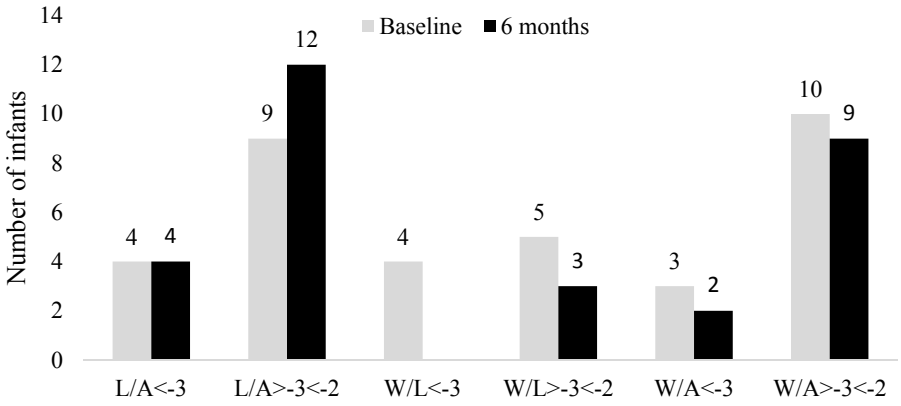

Fig. 3. Nutritional deprivation of infants at baseline & at six months by categories of L/A, W/L & W/A Z-Scores (n = 101)

Fig 4. Correlations between BMIC, maternal UIC and infants UIC

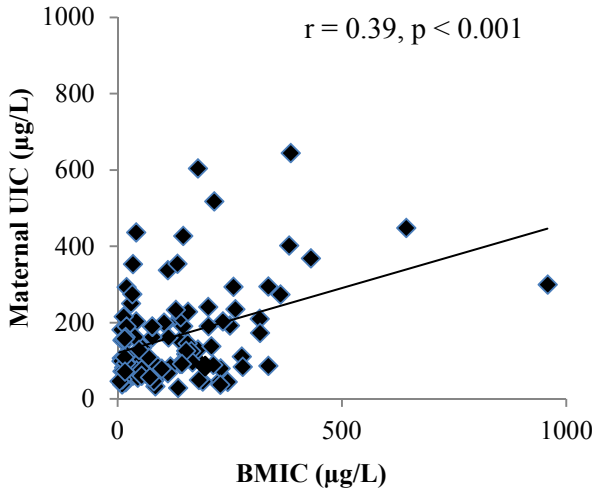

Fig 4A. BMIC & maternal UIC at 6 months (n = 101)

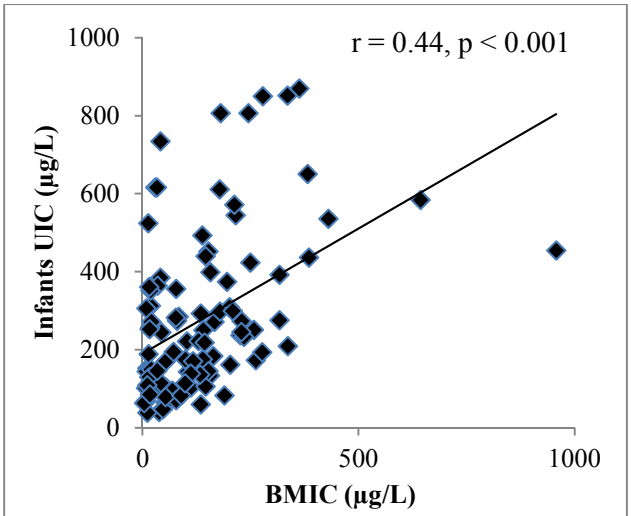

Fig 4B. BMIC & infants UIC at 6 months (n = 101)

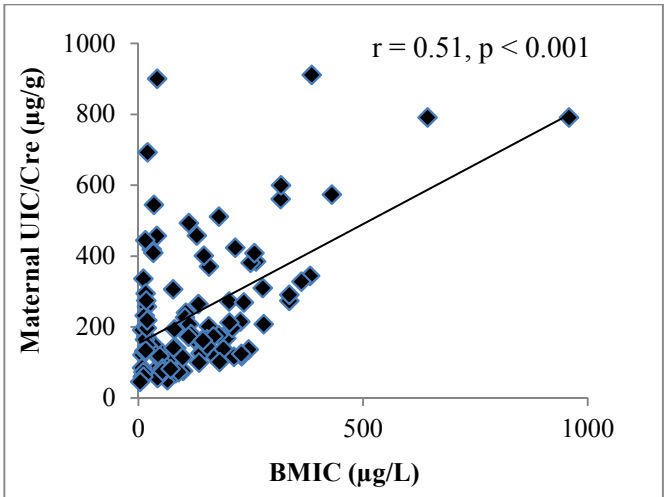

Fig 4C. BMIC & maternal UIC/Cre at 6 months (n = 101)

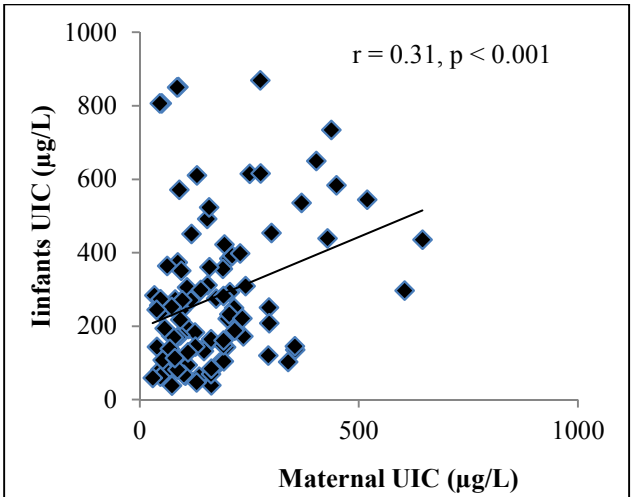

Fig 4D. Maternal UIC & Infants UIC at 6 months (n = 101)

Table 1. Socio-demographic characteristics of mother-infant dyads at baseline (n = 101)

Variables	Median, mean, or %
Mothers	
- Age (y)	22 (20, 25)
- MUAC (cm)	23.3 (1.7)
- BMI (kg/m^2)	21.5 (2.3)
- Gravidity	3 (2)
- Parity	3 (2)
- School years	3.8 (3.5)
- Household size	6 (2.3)
- Frequency of breast feeding last 24 hours	12.8 (5.2)
Infants	
- Age (days)	6 (4, 8)
- Sex	
- Male	49.5%
- Female	50.5%

Data are median (IQR), mean (SD), % (n/N).

MUAC: mid-upper-arm circumference, BMI: body mass index.

Table 2. BMIC and UIC of mothers and infants in subjects who received iodine as a capsule (n = 50) or iodized salt (n = 51)

	Capsule group		I-salt group	
Mothers	Baseline	6 months	Baseline	6 months
BMIC (µg/L)	149 (46, 266)	104 (39, 197)	157(29, 243)	111 (34, 202)
BMIC < 50 µg/L	26%	34%	25%	29%
BMIC 50 - 149 µg/L	24%	30%	22%	35%
BMIC 150 – 250 µg/L	22%	22%	31%	22%
BMIC > 250 µg/L	28%	14%	22%	14%
UIC (µg/L)	136 (76, 173)	150 (86, 220)	95 (64, 142)	110 (73, 191)
UIC < 100 µg/L	42%	32%	53%	45%
UIC ≥ 100 µg/L	58%	68%	47%	55%
I/Cre (µg/L)	214 (142, 292)	176 (126, 383)	203 (139, 268)	173 (117, 292)
Infants				
UIC µg/L	234 (121, 379)	254 (130, 400)	193 (107, 331)	195 (108, 352)
UIC < 100 µg/L	16%	8%	18%	23.5%
UIC ≥ 100 µg/L	84%	92%	82%	76.5%

Data are median (IQR) or percentage. Continuous data were analyzed using a mixed effects model. BMIC: breast milk iodine concentration, UIC: urinary iodine concentration, Cre: creatinine, I-salt group: iodized salt group.

Table 3. Multiple regression predicting UIC of infants at baseline and 6 months (n = 101)

Variables	Baseline		At 6 months	
	β	p	β	p
Maternal UIC/Creatinine	0.297	0.002	0.407	0.000
BMIC	0.258	0.006	0.251	0.001
Infant's age	-0.158	0.083	0.174	0.014
Infant's sex	-0.180	0.046	-0.081	0.123
Adjusted R-square	0.290		0.286	

The dependent variable (Infants UIC) and the independent variables including maternal UIC/Creatinine and BMIC were analyzed as log transformed data because they were not normally distributed.

- Infants sex was coded as 0 for Female and 1 for Male

CHAPTER FIVE

MANUSCRIPT TWO

Title: Maternal iodine supplementation during lactation and its effect on maternal and infant thyroid function and infants visual information processing

Abstract

Iodine deficiency is a major global public health problem, which has particularly severe consequences in pregnant women and young children. Iodine deficiency is one of the major causes of preventable brain damage in childhood. However, iodine supplementation during early pregnancy and lactation can prevent the ill effects of iodine deficiency. The objective of this study was to assess the effect of iodine supplementation on mothers and infants thyroid function and infants visual information processing (VIP). A community-based, randomized, supplementation trial was used. Mother infant dyads (n = 106) were recruited within the first week after delivery to participate in this study. Study participants were randomly assigned either to receive a potassium iodide capsule (225 µg iodine) daily for 26 weeks or appropriately iodized salt weekly for 26 weeks. Lactating women (n = 53) who had 26 week old infants were recruited to serve as a control group. Maternal thyroxine (T_4), triiodothyronine (T_3), thyroid stimulating hormone (TSH), thyroglobulin (Tg), urinary iodine concentration (UIC), breast milk iodine concentration (BMIC) and infant T_4, TSH, UIC and VIP were measured as outcome variables. At baseline, mother infant dyads in the two groups (capsule and iodized salt (I-salt) groups) were not significantly different in any of the biomarkers and anthropometry measurements. Maternal TSH and goiter rate significantly decreased following iodine supplementation. Infant T_4 was significantly different between groups ($p < 0.001$). The percentage of infants who showed novelty preference above 0.55 to new stimuli in the capsule, salt and control groups were 26%, 51% and 47% respectively. However, VIP tests did not show any significant differences between groups. In

conclusion, a dose of 225 μg of iodine supplement and initiating supplementation during lactation may not be enough to affect thyroid and brain function in the infant.
Key words: Iodine supplement, infant visual information processing, southern Ethiopia

Introduction

Iodine is essential for the synthesis of thyroid hormones, and thyroid hormones regulate the metabolic processes of most cells and play important roles in human growth and development [1]. Iodine deficiency is a major global public health problem, and it is one of the most severe causes of preventable brain damage in childhood [2].

While thyroid hormone is essential for development of several neurological functions the timing of its deficiency also determines the type of deficit [3]. Thyroid hormone deficiency during early pregnancy may affect visual processing, motor, and visuomotor skills whereas deficiency in the early neonatal period may affect visuospatial abilities. If the problem extends to the postnatal period and further in infancy it may affect sensory motor, language, fine motor, auditory processing, attention and memory skills. Late treatment may result in deficits in executive processing during childhood [3].

On the other hand, iodine supplementation during early pregnancy and lactation could prevent the deleterious effects of iodine deficiency [4]. During these developmental periods, the mother is the only source of iodine to the fetus and neonate [5]. The fetus gets T_4 from the mother in order to produce T_3 (active thyroid hormone) which indicates the importance of early intervention [4-6]. In areas of severe iodine deficiency, iodine supplementation to pregnant women produced better psychomotor development scores and higher IQ scores in the offspring compared to the offspring of non-supplemented pregnant women [6]. In Papua, New Guinea, an injection of intramuscular iodine before conception eliminated endemic cretinism [7].

Nonetheless, although previous studies confirmed the beneficial effects of iodine in improving cognition, recent research findings are showing inconsistent results. In a

mildly iodine deficient population in New Zealand and Albania, iodine supplemented children had better motor performance and information processing rates compared to placebo [8,9]. However, in another study in Spain, an iodine supplement of 200 or 300 µg daily to women in their first trimester did not show any significant change in either mental development or psychomotor development scales (PDS) in infants between the ages of 6 and 18 months [10]. Furthermore, a study from Spain reported that infants (1 year of age) of mothers who received iodine supplement > 150 µg/day scored lower on the PDS than infants of women who received < 100 µg/d of iodine supplement [11].

The inconsistent results could be for various reasons. When measuring psychomotor or mental development it is crucial to identify the appropriate measurement instrument that is sensitive enough to detect the targeted outcome. The Institute of Medicine has developed seven criteria in choosing appropriate measurement instruments. These include, age appropriateness, potential long-term consequences, sensitivity, brain-behavior links, cross-species generalizability, function specificity, and ease of administration [12].

One of the best known and most widely used tests of infant development is the Bayley Scales of Infant Development (BSID). However the BSID is generally good an overall measure of infant development but unable to predict latter IQ [13,14]. On the other hand visual information processing (visual recognition memory) is the beginning of cognitive function which can predict broad cognitive abilities in later childhood [15,16]. Moreover, this measure can be administered during the first year [14], has links to the central nervous system [17], can assess specific functions [14], and is relatively easy to administer [18].

In the current study we assessed infant cognitive function using a visual information processing (VIP) paradigm. The objective of this study was to assess the effect of iodine supplementation on mothers and infants thyroid function and on infants VIP.

Materials and methods

Subjects and study design

The study was a community-based, randomized, supplementation trial conducted in 2013. Participants for this study were selected from women and their infants living in rural areas of Sidama zone, southern Ethiopia. The study population depends on subsistence farming for their livelihood. Their major staple food is enset *(Enset ventricosum)* followed by unrefined maize [19]. The area was known for severe iodine deficiency prior to implementation of salt iodization in 2012 [20-22].

Subject selection methodology

A total sample size of 159 mother-infant dyads was calculated using an alpha of 0.05, 80% power and an effect size of 0.25. For selecting participants two important criteria were set. First, the women must be lactating and must have an infant of a specific age: a) less than or equal to one week old for the treatment group, or b) 6 ± 0 .25 months old for the control group. Second, they had to volunteer to participate in the study. Based on these criteria, all mother-infant dyads who met the criteria were invited and enrollment was continued until the required sample size was attained.

Exclusion criteria

The exclusion criteria for mothers in this study were any hyperthyroidism symptoms manifested by nervousness, anxiety, heart palpitations, rapid pulse, fatigability, tremor, muscle weakness, weight loss with increased appetite, heat

intolerance, frequent bowel movements, or increased perspiration [23]. If infants had fever, cough or severe (>3/day) diarrhea, the mother-infant dyad would not be enrolled in the study. If mothers showed any allergic reaction such as acne, weakness, or foul breath after supplementation was started they were excluded from the study as well. But none of the subjects showed any of the symptoms.

Because the ethics rules at Oklahoma State University, USA and Hawassa University, Ethiopia did not allow a control group with no iodine supplement for study purposes, we did not begin the control group with infants less than one week of age. Instead we recruited mothers and their 6 ± 0.25 month old infants to serve as a control group for our treatment groups at 6 months of age.

Ethical clearance

Ethical approval was obtained from Oklahoma State University, USA, Hawassa University, Ethiopia, Ministry of Science and Technology, and Food, Medicine, and Health Care Administration and Control Authority of Ethiopia. Following receipt of ethical clearance, study participants were enrolled with the help of health extension workers working in the study area. Study participants were given a detailed explanation of the objectives of the research and consent was taken from the women for themselves and their infants before data collection was started.

Randomization

Of the 159 women recruited, 53 women who had 6 ±0.25 month-old infants were designated as the control group and the remaining 106 mother-infant (<1 wk of age) dyads were randomly assigned either to the iodine capsule group or to the iodized salt (I-salt) group.

Procedures

The capsule group (n = 50) received 225 μg of iodine daily as a capsule of potassium iodide (Pure Encapsulations, Inc. Boston, MA) for 26 weeks. Iodine capsules were delivered daily to mothers by health extension workers. The I-salt group was provided with iodized salt with 30 to 40 μg/kg of KIO_3 (1 packet (450 g) of salt per week per household) for 26 weeks. (Guts Agro Industry PLC, Addis Ababa, Ethiopia). Supplementation was started within one week of delivery of the infant and continued for 26 weeks.

Prior to beginning supplementation, baseline data were collected from both mothers and infants. From mothers, urine, breast milk and blood samples and urine samples from infants were collected. Blood samples were collected from each woman using a disposable 10 cc syringe coated with lithium heparin with a 21 gauge needle (Sarstedt, Inc., Newton, N.C.). The blood was centrifuged and plasma was separated immediately. Plasma was kept frozen at – 20 ^{0}C and was used for analysis of thyroid hormone concentrations (T_3 and T_4), thyroid stimulating hormone (TSH) and thyroglobulin (Tg).

Mothers collected urine in a cup out of which urine samples were taken to fill tightly sealed vials in duplicate. Ten ml of breast milk samples were collected from each mother in a plastic vial for storage. Infant urine was collected by squeezing urine out of cotton balls in a disposable diaper; urine samples were transferred to tightly sealed vials. All samples were frozen for subsequent assessment of iodine concentration.

Goiter of each woman was determined by palpation based on the following grades: grade 0, no palpable or visible goiter; grade 1, palpable goiter but not visible

when neck is in the normal position; grade 2, visible goiter when neck is in the normal position [24]. Goiter grade was determined by a health professional.

Maternal anthropometry including weight and height to calculate body mass index (BMI = Wt_{kg} Ht_{m2}), and mid upper arm circumference (MUAC) were measured. Infant anthropometry including head circumference, MUAC, weight and length were measured. Infant's weight-for-age (WAZ score), length-for-age (LAZ score), weight-for-length (WLZ score), MUAC and head circumference were calculated using WHO Anthro software (Version 2.0.4).

Mothers were interviewed using an individual questionnaire adapted from the Ethiopian Demographic and Health Survey Agency. The questionnaire included socioeconomic status and demographic characteristics of women [25].

Following 26 weeks of supplementation, samples similar to baseline were collected from both mothers and infants of the two treatment groups (Capsule and I-salt). At 26 weeks similar samples were also collected from a control group. Moreover, after 26 weeks blood samples were collected from all infants in the three groups by finger prick on specimen paper for TSH and T_4 analysis and infants were tested using the VIP test.

Administration of visual information processing (VIP)

Materials

For the VIP, eight pictures of young adult Ethiopian faces without emotional expression (four male and four female) were used. Two laptop computers were used. One laptop computer was used to control the presentation of stimuli. A second computer that had a built-in camera was used to follow and record the infant's looking behavior.

Although the infant's looking behavior was coded live, the looking process was recorded on this second computer for later reliability testing.

Procedure

A quiet room was prepared for administration of the VIP test. The infant sat on the mother's lap in front of the camera, eyes forward, and half a meter away from the screen. A bed sheet was placed between the VIP tester and the infant and hence the researcher was out of sight of the infant. The tester (who was blind to the study) adjusted the visibility and focus of the infant's face and eyes on the laptop that had the camera. Once the focus was adjusted, the familiarization phase began. A randomly-selected picture was shown on the screen of the laptop in front of the infant. The tester followed the infant's look on the laptop. If the infant looked at the picture, a square like shape (reflection) was visible in his/her pupil. At this time the tester pressed down the left key of the mouse (computer key) and if the infant's eye turned away from the picture the tester released the key. The computer recorded the time between stimulus onset and the look (response latency) and the duration of the look. If the infant looked away from the face for one second or longer, the face was replaced by a blank screen for two seconds. The face then reappeared. This continued until the criterion for habituation had been met, namely two looks that were less than one half the mean duration of the two longest looks. Therefore, the minimum number of looks during the familiarization phase was four. After the habituation criterion had been met, the comparison phase began without interruption of the procedure. In the comparison phase, the face that appeared during habituation (the "familiar" face) appeared again on one side of the screen and a novel face of the opposite gender appeared on the other side of the screen. The tester pressed down the left key of

the mouse as the infant's eyes were looking at the left picture and pressed down the right key of the mouse and released the left as the infant's eyes looked at the right picture. The tester released both fingers as the infant turned away from the pictures. While doing this, the second computer recorded the infants' looking behavior for reliability testing, while the first computer automatically coded the infant's look duration at the pictures, shifts from one picture to the other, and length and number of turns away from the pictures. During the comparison phase, the faces remained on the screen regardless of whether or not the infant was looking at one of them. Once the infant accrued a total looking time at the faces of 5 seconds, the faces were replaced by a blank screen for 1 second, and then the faces reappeared but on opposite sides of the screen. They remained until an additional 5 seconds of looking had accrued.

Visual recognition memory is composed of various constructs. Novelty preference was used to measure recognition memory and speed of processing [26]. Look duration was a measure of processing speed and attention [27], average look duration measures the speed at which recognition was achieved [28] and total shift was used as a measure of how active the infant was in looking back and forth between the two faces in order to compare them. A reliability test was performed by a University student who was blind to the study. This involved the student independently coding the recorded videos of randomly selected 30 infants. Reliability was then calculated as the correlation between the original coding and the reliability coding.

Measurement of biomarkers

Urinary iodine concentration (UIC) and BMIC were analyzed by inductively coupled plasma mass spectrometer (ICP-MS Elan 9000, Perkin Elmer, Norwalk, CT).

Detailed analysis methods for UIC and BMIC are described elsewhere. Creatinine was analyzed using a BioLis 24i Clinical Analyzer (Carolina Liquid Chemistries Corp. Winston-Salem,NC). Total thyroxine (T_4), total triiodothyronine (T_3), thyroid stimulating hormone (TSH) and thyroglobulin (Tg) were quantitatively determined by ELISA assay (ALPCO Diagnostics, Salem, NH). Quality control for T_4 and T_3 was obtained from Bio-Rad Laboratories (Irvine, Ca). Infant's T_4 and TSH also were determined by ELISA assay (Diagnostic Automation, Calabasas, CA).

Statistical analysis

Data were analyzed using selected descriptive and analytical statistical measures. In the descriptive section percentages, frequency distributions, means (SD) and medians (IQR) were used to describe the socio-economic status, demographic characteristics, and iodine status of lactating mothers and their infants. All data were checked for normal distribution and skewed data were log-transformed before analysis. To test whether or not the means of the three groups were all equal we used Tukey's honestly significant test (Tukey's HSD) for normally distributed data and Kruskal-Wallis H test for non-normally distributed data. To compare the means between two unrelated groups (Capsule group vs salt group at baseline) we used independent-sample t tests for normally distributed data and Mann-Whitney U test for non-normally distributed data. For comparison of related samples (baseline vs 26 weeks), we used paired-samples t tests for normally distributed data and Wilcoxon Signed-Rank Test for non-normally distributed data. To test time by treatment effect we used mixed effect design.

Pearson's correlation coefficient or Spearman's rho were used to examine relations between variables, particularly between iodine status and VIP of infants.

Multiple regression analyses were used for predicting VIP tests. All variables were checked for collinearity using variance inflation rate (VIF) and the variables that met the criteria (VIF ≤ 4) were entered into the models. All of the biomarkers analyzed and infants anthropometric indices were entered into the models. Logistic regression was used to measure the relation between categorical dependent variables and one or more independent variables. Chi-square test of independence of categorical variable was used to test whether the effect of one variable depends on the value of another variable. Ordered Logistic Regression was used to test significant difference between groups in ordinal variable (goiter rate).

Results

The baseline characteristics of study participants are presented in Table 1. Neither mothers nor infants were significantly different between groups at baseline. The median maternal TSH, T_4, T_3 and Tg were all within the normal range. For both groups combined at baseline, the median BMIC was 154 (42, 252) µg/L, median maternal UIC was 107 (71, 161) µg/L and median infant UIC was 218 (108, 356) µg/L. Of the women, 23% had palpable goiter and 52% had visible goiter.

The median mothers' age was 22 (20, 25) years, mean body mass index (BMI) was 21.5 (2.3) and mid-upper-circumference (MUAC) was 23.3 (1.7) cm. The median infants' age was 5 (3, 7) days. There were 50 (49.9%) male and 51 (50.5%) female infants participating in the study. Infants did not differ in any of the anthropometric indices.

As shown in Table 2, the median maternal TSH and T_3 significantly decreased at 26 weeks in both groups. The median maternal T_4 decreased in both groups but the

decrease was significant only in the I-salt group ($p < 0.001$). The median maternal Tg did not show any significant change. Except T_4 ($p = 0.014$), none of the biomarkers have shown treatment by time interaction (data not shown).

The proportion of mothers with subclinical hypothyroidism significantly decreased at 26 weeks compared to baseline in both groups. The incidence of hypothyroxinemia in mothers significantly increased in the salt group ($p = 0.004$). The median UIC of mothers and their infants was not significantly different at 26 weeks compared to baseline. Breast milk iodine concentration decreased at 26 weeks in the capsule group but not in the I-salt group. The median UIC in infants did not show significant changes at 26 weeks.

As shown in Table 3, the median maternal TSH at 26 weeks indicated that maternal TSH in the capsule group was significantly higher than in the control group ($p = 0.05$). Maternal T_4 was significantly higher in the capsule group than in the I-salt group ($p = 0.002$) and T_3 was significantly higher in the I-salt group than in the control group ($p = 0.015$). However, the median maternal Tg, BMIC and UIC were not different among groups.

Prevalence of goiter in mothers was significantly higher in the control group followed by mothers in the salt and capsule groups ($p < 0.001$). As shown in Figure 2, percentage of goiter was significantly decreased following iodine supplementation ($p < 0.001$). Goiter rate decreased from 76% at baseline to 30% at 26 weeks in the capsule group and from 74% at baseline to 43% at 26 weeks in the salt group. From the mothers who were recruited at 26 weeks after delivery to serve as control, 92% had goiter. Maternal TSH at baseline was associated with goiter ($X^2 = 5.8$, $p = 0.016$) (data not

shown).

Infants' TSH and T_4 were within the normal range in all of the groups and infants' TSH levels were not significantly different between groups (Table 3). However unlike mothers, infants T_4 was significantly different in all three groups; T_4 values were lowest in the capsule group, highest in the control group and T_4 in salt group was in middle ($p < 0.0001$). Among the infants 2% in the capsule, 8% in the salt, 10% in the control groups had subclinical hypothyroidism. None of the infants in any group had overt hypothyroidism or hypothyroxinemia. Incidence of subclinical hyperthyroidism was high in all groups but there was no significant difference between the groups ($p = 0.098$).

Infants in the three groups did not differ in WAZ, WLZ, LAZ or in HCAZ scores. The mean of these anthropometric indices shows that infants in all groups were above the cutoff level set for nutritional insult ($\leq$ -2 Z score).

In the visual information processing tests infants were significantly different between groups in total novelty quotient but not in the other variables. A novelty quotient > 0.55 indicates the infants remember the familiar face and anything below 0.55 means they did not remember. Based on this classification, 26% of the infants in the capsule, 51% in the salt and 47% in the control groups remembered the familiar face.

Table 4 shows significant predictors of the VIP tests using stepwise multiple linear regression analysis. Accordingly, in the two groups (capsule and I-salt groups) infants one unit increase in UIC/Cre at 26 weeks increased total shift by 0.24 units, maternal T_4 at baseline increased total shift by 0.23 units and maternal T_3 at 26 weeks decreased total shift by 0.21 units. About 12% of the variation in total shift was explained by the model. Maternal T_3 at baseline was associated with a 0.26 unit increase in average

longest look and Tg at baseline was associated with a 0.24 unit decrease in average longest look and 10.4% of the variation was explained by the model. Similarly, maternal T_3 at baseline was associated with a 0.24 unit increase in longest look and Tg at baseline was associated with a 0.25 unit decrease in longest look and 10% of the variation was explained by the model. In the three groups at 26 weeks average look was predicted by maternal T_3 ($\beta = 0.215$, $p = 0.011$) and TSH ($\beta = 0.227$, $p = 0.008$) at 26 weeks (Data not shown).

In the two supplemented groups, sex was significantly associated with total novelty quotient ($X^2 = 4.54$, $p = 0.033$) and number of looks ($X^2 = 6.6$, $p = 0.011$) and males performed better than females. When the control group was also considered, sex was again associated with total novelty quotient ($X^2 = 4.08$, $p = 0.028$) and similarly males performed better than females (Data not shown).

A logistic regression analysis was used to predict total novelty quotient using infants' sex, WAZ score at baseline, LAZ score at 26 weeks, maternal T_3, TSH and T_4 at 26 weeks as predictors. These predictors were selected based on significant correlation. Hence comparing to infants of mothers with hypothyroidism ($T_3 < 0.8$ ng/mL), infants of mothers with hyperthyroidism ($T_3 > 1.9$ ng/mL) were 93% less likely to score novelty preference above 55% and females were 65% less likely to score novelty preference above 55% compared to male infants (Table 5). The reliability correlation for the longest look was 0.78. The correlation for looks during the novelty preference phase was very low and varied highly between the left ($r = -0.02$) and right ($r = 0.50$) stimulus positions as well as among the infants.

Discussion

We examined the effect of iodine supplementation on thyroid hormone function, thyroglobulin, goiter, UIC and BMIC of lactating women and thyroid hormones, UIC and visual information processing in infants at age 26 weeks. Mothers in the two supplemented groups (capsule and I-salt) were not significantly different in any of the biomarkers and other baseline characteristics. Likewise, infants were not different in any of the measurements.

Maternal iodine supplement did not show any effect on infants' anthropometric measurements between groups. In the two treatment groups the mean change in LAZ was 0.61 (1.08), in WAZ was 0.01 (1.22), and in HCAZ was 0.2 (1.16). The two groups did not differ in any of these anthropometric indices. However, 22% of the infants showed growth decline at the end of six months (Data not shown).

Our study population was known to have severe iodine deficiency for decades. This was confirmed with repeated studies in different population groups at different times [20-22,29]. However recently, Ethiopia has been categorized as a moderate iodine deficient country [30,31]. This current study was conducted in 2013, following the implementation of salt iodization program in 2012. Perhaps as the result of this, our study population showed higher UIC and BMIC than previous studies. The median UIC within one week after delivery of all lactating women was 107 (71, 161) µg/L and their infants was 218 (108, 356) µg/L and the median BMIC was 154 (43, 252) µg/L. This indicates the study population was iodine sufficient [32].

Following 26 weeks of iodine supplementation, plasma TSH in lactating women significantly decreased by 54% in the capsule group and by 42% in the I-salt group compared to baseline. However, the improvement in TSH was not significantly different

between the mothers who received daily iodine capsule and weekly iodized salt for 26 weeks. A significant decrease in the proportion of women with subclinical hypothyroidism was observed in both groups. Consistent with this, Santiago and colleagues reported there was no significant difference in maternal TSH between pregnant women who were supplemented with iodized salt and those who were supplemented with either 200 µg iodide per day or 300 µg iodide per day [10]. Although we do not have baseline data for the control group, lactating women recruited at 26 weeks after delivery had unexpectedly lower TSH compared to women in the capsule group at 26 weeks. A study compared control group to women received 300 µg potassium iodide during pregnancy and lactation reported that plasma T_4 and T_3 were significantly higher and TSH was significantly lower in the control group than in the counterparts [33].

Infants' TSH at the age of 26 weeks was significantly lower and T_4 was significantly higher than their mothers ($p < 0.001$ in both). This could be due to the fact that the majority of breast feeding mothers iodine intake is excreted into the breast milk which provides enough iodine to the infant [5]. All of the infants in the current study had normal T_4 and TSH levels except for 10 infants who had TSH above the cutoff for their age. Nonetheless, a high proportion of infants were observed to have subclinical hyperthyroidism which indicates a low level of TSH and normal values of T_4 and T_3 [34].

The effect of iodine supplement in improving thyroid hormone markers does not seem consistent in this study. Maternal TSH and T_3 decreased significantly following iodine supplementation but the change in Tg wasn't significant. Maternal T_4 decreased in both groups with a significant decrease in the I-salt group which resulted in a significant increase in the proportion of women with hypothyroxinemia. However, in a double-blind,

randomized, controlled trial conducted in Morocco, a single capsule of 400 mg of iodine as iodized oil given to the breast-feeding mother or a single oral supplement of 100 mg of iodine given to her newborn baby did not produce any significant change in maternal and infant TSH or T_4 at 3 months, 6 months or 9 months after supplementation [35]. Such inconsistent thyroid hormone response to iodine supplement was also reported in other studies [33,36]. Pregnant women showed no change or increase in maternal TSH following iodine supplement of 50 μg daily, 200 μg daily or 300 μg daily for six months [10,37,38]. In two other randomized controlled trials, maternal free T_4 concentration decreased in both controls and treated groups and no difference was observed between groups [39,40]. However, a longitudinal study showed TSH was negatively correlated with urinary iodine excretion in a moderately iodine deficient population [41].

Thyroid hormone response to iodine supplement may depend on level of deficiency and age group. In a recently published review on biomarkers of iodine, it was suggested that T_4 and T_3 are sensitive markers in areas of severe iodine deficiency and TSH was considered as a sensitive marker in newborns [31]. Thyroglobulin has been recommended as a more sensitive indicator than TSH and T_4 in iodine deficient and excess areas in children, because thyroglobulin is synthesized only in the thyroid [31,42]. Another group commented that goiter rate, Tg and TSH are good markers to assess iodine status in a population but thyroid hormones are fairly imprecise to detect iodine deficiency [43]. However unlike children, no cutoff point for median Tg for adults has been set related to iodine status [42]. Serum Tg was correlated with severity of iodine deficiency assessed by urinary iodine [31]. In the current study Tg positively correlated with T_4 ($r = 0.203$, $p = 0.043$) and negatively correlated with TSH ($r = -0.261$, $p = 0.009$) but not with

UIC. Maternal T_3 negatively correlated with TSH ($r = -0.366$, $p = 0.000$) and positively correlated with T_4 ($r = 0.366$, $p = 0.000$). When thyroid markers were categorized to examine association with goiter rate, TSH was the only variable that showed significant association with goiter ($p = 0.016$) (Data not shown).

Maternal goiter significantly decreased in both capsule and I-salt groups at six months. As previously mentioned, maternal iodine supplementation for 26 weeks did not show any significant difference in T_4, T_3, TSH and Tg compared to the control group. However, goiter rate was significantly lower in the supplemented groups compared to the control group. In goitrous children age 6 to 12 years in Côte d'Ivoire, a 200 mg iodine supplement produced a significant and continuous decrease in thyroid gland volume over 30 weeks. However, similar to our results there was no significant change in serum T_4. But TSH decreased significantly in the Côte d'Ivoire study [44]. In contrast, in a severely iodine deficient area in Senegal 480 mg of iodine given as iodized oil to adults significantly increased T_4 and decreased rate of goiter, T_3 and TSH [45].

The effect of iodine supplement on maternal thyroid disorders and in children's neurocognitive development in severely iodine deficient areas is well established [46]. It is unclear however, whether iodine supplement to pregnant women in areas of mild-to-moderate iodine deficiency affects cognitive function of the offspring [6,47]. Children of pregnant women who had received iodine supplement from their first trimester compared with those who did not receive supplement showed no difference in mental development index measured by Bayley Scales of infant development at the age of two years [33]. In another study in mildly iodine deficient and iodine sufficient area in Spain, consumption

of 150 μg/day of iodine supplement by pregnant women did not improve infant neuropsychological development at one year of age [48].

In the present study, infants whose mothers received iodine supplement for 26 weeks starting within one week after delivery, were not different from the control group in most of the visual information processing measures. However, infants in the control and I-salt groups were significantly better in novelty quotient (NQ) than infants in the capsule group. In total (all three groups together) the mean NQ was 0.52 (0.19) which is higher than the mean NQ reported by Kennedy and colleagues [49].

The look duration or the length of the longest look during the familiarization phase (habituation phase) as a measure for attention and processing speed were not significantly different between groups. The mean of the longest look duration in both groups was 21.1 (18.0). Shorter fixation indicates better attention and faster processing speed and predicts later childhood IQ [15,27]. In the current study more than 70% of the infants in both groups were below the mean with 26% below 10. The average look duration, which indicates the speed at which recognition of the familiar stimulus is achieved [28], was not significantly different between groups. Although there is no established standard for number of looks a child would make during the habituation phase or trials to criterion, infants were not significantly different between groups. However, the longer it takes an infant to reach the habituation criterion, the longer it is taking the infant to learn the face. In this regard 41% of the infants had number of looks above the median and 39% had number of looks below the median of 6 (5, 7.7) which indicates the later learned the habituation face quickly [50].

The visual information processing (VIP) paradigm has been used to assess infants' and children's information processing speed, recognition memory and attention in relation to various factors. Infants whose mothers had high blood DHA concentration showed fast information processing speed over the first year as well as less distractibility in the second year [51]. A study conducted in Ethiopian infants showed that growth was associated with development of VIP [49]. On the other hand increased prenatal alcohol exposure was correlated with longer look, indicating a slower information processing [52]. Similarly, exposure to toxic substances during pregnancy resulted in poorer recognition memory during the first year of the child [53].

In the present study we have checked for any association between VIP and thyroid hormones, thyroglobulin, UIC and children's anthropometry but no association was confirmed. Nonetheless, infants of the mothers who had hypothyroidism remembered the familiar face better than infants of the mothers who had hyperthyroidism. Moreover, male infants remembered the familiar face and learned faster than female infants. Not much evidence is available regarding iodine intake on infants' cognition performance by sex. However, one study done in 1982 in school children from an area of endemic goiter reported that girls showed better cognition improvement as a result of iodine supplement than boys [54]. Another study from iodine sufficient and mildly iodine deficient area reported similar results [11].

In our multiple regression analyses infant UIC/Cre at 26 weeks and maternal T_4 at baseline were positive predictors and maternal T_3 at 26 weeks was a negative predictor of total shift. For average longest look and longest look, both variables that indicate speed of processing, maternal T_3 at baseline was a positive predictor and maternal Tg at baseline

was a negative predictor. None of the infants UIC, T_4, TSH or anthropometric measures predicted any of the VIP measures. We performed reliability checks for longest look and novelty preference. The correlation for longest look was 0.78. However, correlations for novelty preference were unacceptably low. This poor reliability was likely due to the fact that the stimuli were presented on a single laptop screen, which made it difficult to tell on the recorded video whether the child was looking at one of the pictures or away from the pictures. For the future this problem could be solved by using two laptops screens that can be placed farther apart.

In conclusion, although the study population had a long history of iodine deficiency, the present study showed a median UIC that indicated iodine sufficiency. Hence, consistent with other studies [48] maternal iodine supplementation may not show an effect in an iodine sufficient population. Moreover, all of the biomarkers measured in this study may not respond to iodine supplement in the time of iodine sufficiency. Due to this reason, the VIP test did not show any significant change between groups. Except for TSH none of the biomarkers showed improvement with supplementation in this study. However, the rate of goiter decreased significantly in both supplemented groups and the difference with the control group was remarkably high. The only marker that showed association with goiter was TSH. The association observed between VIP tests and infants' sex may also require further investigation.

Findings from this study showed that perhaps, the comments given on the sensitivity of different thyroid function markers in different population groups might need further investigation. Moreover, this study confirmed that iodized salt showed similar effect as iodine capsule supplement if it is adequately iodized and carefully

monitored. Results from the present study should be evaluated with caution because data were collected immediately after salt iodization program was launched in Ethiopia. Therefore, it is too early to comment on the iodine status of the population. Large scale and repeated studies are warranted to evaluate the current iodine status of the Ethiopian population.

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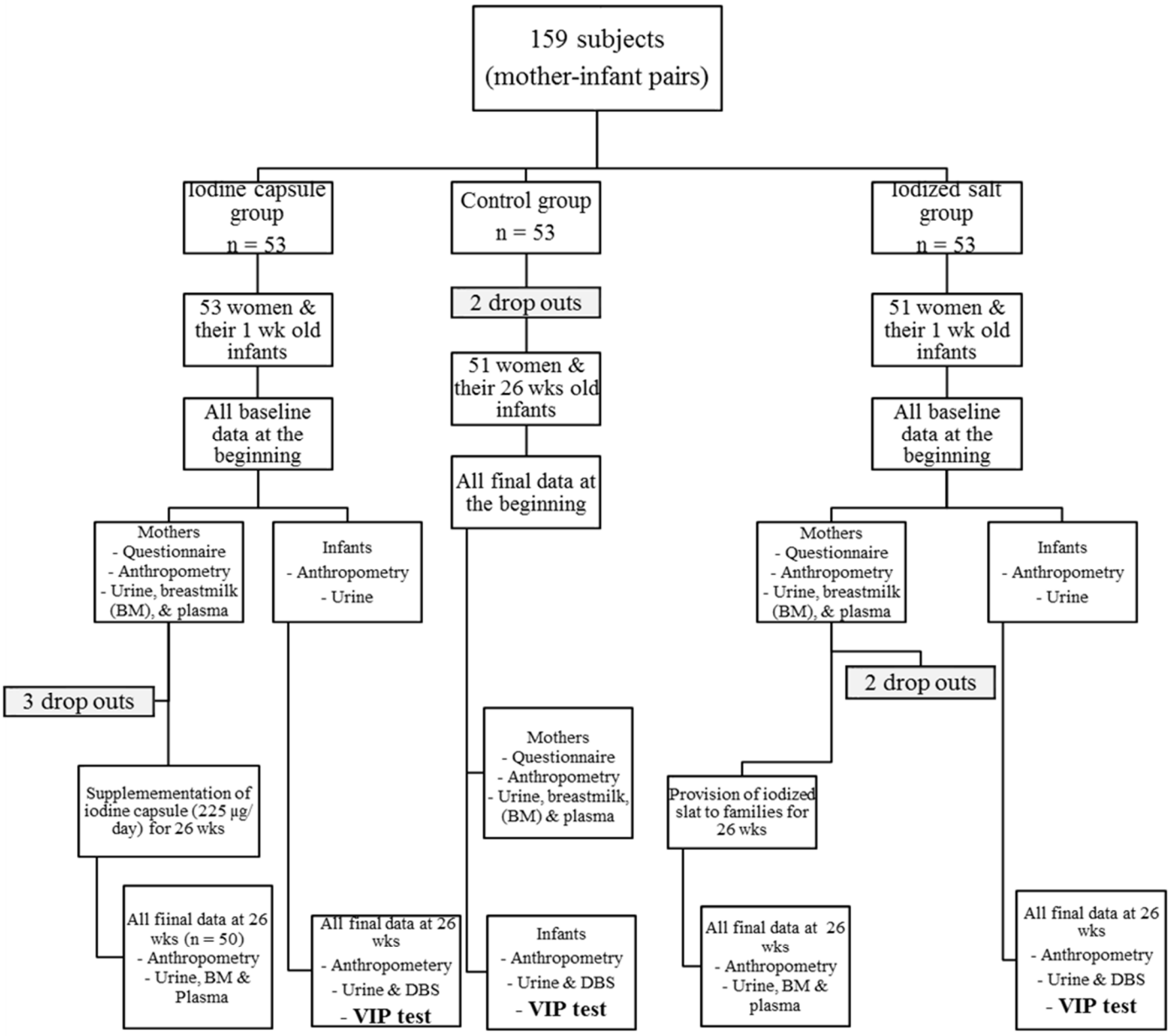


Fig 1. Research design

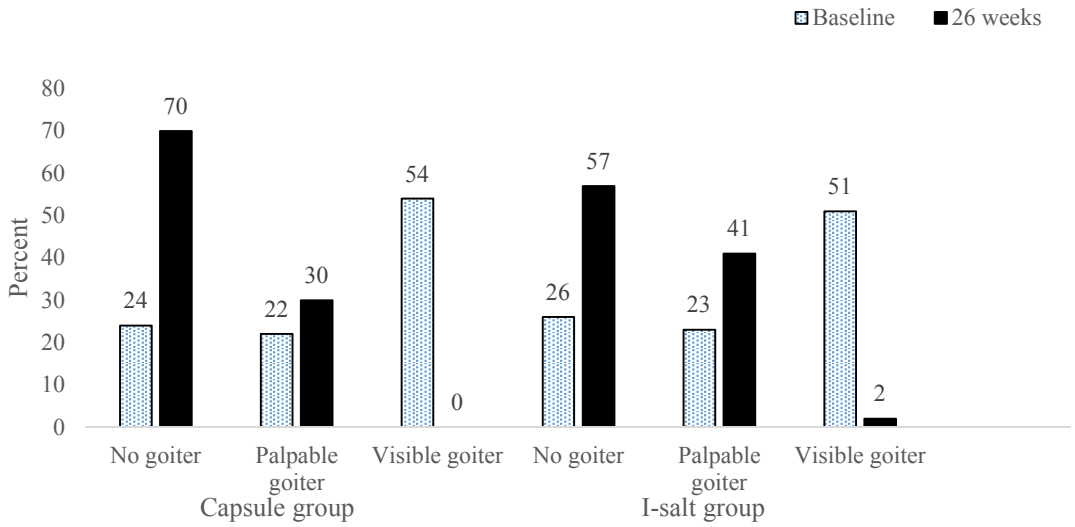
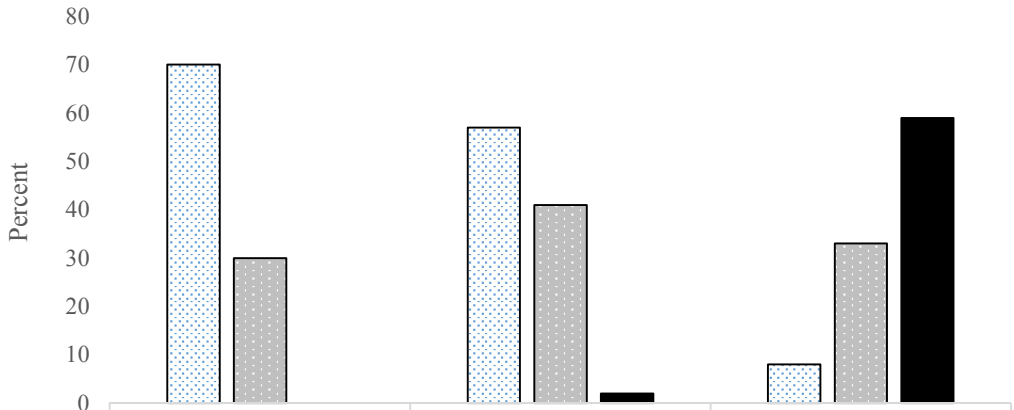


Fig. 2. Prevalence of goiter in women at baseline and 26 weeks in the capsule vs salt groups (n = 101)



	Capsule group	Isalt group	Control group
No goiter (%)	70	57	8
Palpable goiter (%)	30	41	33
Visible goiter (%)	0	2	59

Fig.3. Prevalence of goiter of women at 26 weeks in all of the three groups (n = 152)

Table 1. Socio-demographic and clinical characteristics of mother-infant dyads at baseline (n = 101)

	Capsule group (n = 50)	I-salt group (n = 51)	p - value
Mothers	Mean (SD), median (IQR) or f (%)	Mean (SD), median (IQR) or f (%)	
- Age (years)	23 (20, 27)	21 (20, 25)	0.14
- MUAC (cm)	23.2 (1.8)	23.3 (1.7)	0.62
- BMI (kg/m2)	21.2 (2.5)	21.8 (2.0)	0.15
- Gravidity	2 (1.75, 4)	2 (1, 5)	0.41
- Parity	2 (1, 4)	2 (1, 4)	0.38
- School years	2 (2, 3)	2 (1, 3)	0.42
- T_3 (ng/mL)	1.3 (1.0, 1.7)	1.4 (1.2, 1.7)	0.052
- T_4 (µg/dL)	7.0 (5.9, 8.1)	6.8 (5.4, 8.0)	0.57
- Tg (ng/mL)	4.2 (1.4, 10)	4.5 (2.3, 11.9)	0.25
- TSH (µIU/mL)	2.6 (1.1, 4.4)	1.9 (0.8, 3.2)	0.11
- Goiter			
- Grade 0	12 (24%)	13 (26%)	0.96
- Grade 1	11 (22%)	12 (23%)	
- Grade 2	27 (54%)	26 (51%)	
- UIC (µg/L)	136.3 (76, 173)	95 (64, 142)	0.091
- BMIC (µg/L)	149 (46, 266)	157 (29, 243)	0.97
- I/Cre (µg/g)	213.6 (142, 292)	203.3 (139, 268)	0.99

	Capsule group (n = 50)	I-salt group (n = 51)	p - value
Infants	Mean (SD), median (IQR) or f (%)	Mean (SD), median (IQR) or f (%)	
- Age (days)	5 (3, 7)	6 (5, 8)	0.05
- UIC (µg/L)	234 (121, 379)	193 (107, 331)	0.39
- I/Cre (µg/g)	2097 (1001, 3957)	2025 (1397, 3811)	0.94

Data are median, mean and percentage. Continuous data were analyzed using T-Test and Mann-Whitney U test, categorical variable was analyzed using Chi-square test and ordinal variable was analyzed using Ordered Logistic Regression. Normality was checked using Kolmogorov-Smirnov. UIC = urinary iodine concentration, BMIC = breast milk iodine concentration, I/Cre = iodine to creatinine ratio, T_3 = triiodothyronine, T_4 = Throxine, Tg = thyroglobulin, TSH = thyroid stimulating hormone, MUAC = mid-upper-circumference, BMI = body mass index

Table 2. BMIC, UIC and thyroid hormone markers of mothers and UIC of infants at baseline and at 26 weeks in subjects who received iodine capsule vs iodized salt

	Baseline	**26 weeks**	**p- value**
Mothers			
TSH (μIU/mL)			
- Capsule group	2.6 (1.1, 4.4)	1.2 (0.9, 2.4)	0.009
- I-salt group	1.9 (0.8, 3.2)	1.1 (0.7, 1.8)	0.006
T_4 (μg/dL)			
- Capsule group	7.0 (5.9, 8.1)	6.7 (5.5, 8.0)	0.450
- I-salt group	6.8 (5.4, 8.0)	5.4 (4.8, 6.9)	< 0.001
Subclinical hypothyroidism			
- Capsule group	32% (16/50)	2% (1/50)	0.001
- I-salt group	14% (7/51)	0%	0.016
Hypothyroxinemia			
- Capsule group	8% (4/50)	16% (8/50)	0.180
- I-salt group	8% (4/51)	31% (16/51)	0.004
T_3 (ng/mL)			
- Capsule group	1.3 (0.5)	1.1 (0.4)	0.019
- I-salt group	1.5 (0.4)	1.2 (0.4)	0.002
Tg (ng/mL)			
- Capsule group	4.2 (1.4, 10.0)	4.2 (1.8, 9.0)	0.495
- I-salt group	4.5 (2.3, 11.9)	4.2 (2.1, 8.1)	0.07

	Baseline	26 weeks	p- value
Mothers			
UIC (µg/L)			
- Capsule group	136 (76, 173)	150 (86, 220)	0.082
- I-salt group	95 (64, 142)	110 (73, 191)	0.066
BMIC (µg/L)			
- Capsule group	149 (46, 266)	104 (39, 197)	0.036
- I-salt group	157 (28, 243)	111 (34, 202)	0.127
Infants			
UIC (µg/L)			
- Capsule group	234 (121, 379)	254 (130, 400)	0.340
- I-salt group	193 (107, 331)	195 (108, 352)	0.967

Data are median, mean and percentages. Continuous data are analyzed using paired samples T-test or Wilcoxon, proportions (percentages) were analyzed using McNemar. Subclinical hypothyroidism defined as increased TSH and normal T_4 (TSH > 3.7 µIU/mL and normal T_4) and hypothyroxinemia defined as T_4 less than age-specific cut-off (T_4 < 5.05 µg/dL) [35].

Table 3. Thyroid hormone markers, UIC and BMIC of mothers, and anthropometry and visual information processing tests of infants at 26 weeks (n = 152)

	Capsule group (50)	I-salt group (51)	Control group (51)	p - value
Mothers				
TSH (µIU/mL)	1.2 (0.9, 2.4)[a]	1.1 (0.7, 1.8)[ab]	0.8 (0.5, 1.7)[b]	0.05
T_4 (µg/dL)	6.7 (5.5, 8)[a]	5.4 (4.8, 6.9)[b]	6.0 (5.1, 7.4)[ab]	0.002
Subclinical hypothyroidism	2% (1/50)	0	2% (1/51)	0.599
Overt hypothyroidism	2% (1/50)	2% (1/50)	0	0.605
Hypothyroxinemia	16% (8/51)	31% (16/51)	22% (11/51)	0.203
Subclinical hyperthyroidism	0	2% (1/51)	2% (1/51)	0.241
T_3 (ng/mL)	1.14 (0.4)[ab]	1.25 (0.4)[a]	1.03 (0.4)[b]	0.015
Tg (ng/mL)	4.2 (1.8, 9)	4.2 (2, 8)	3.8 (2, 8)	0.89
UIC (µg/L)	174 (85, 220)	110 (73, 191)	157 (102, 243)	0.31
BMIC (µg/L)	104 (39, 197)	111 (34, 202)	114 (43, 172)	0.99
Goiter				
- Grade 0	35 (70%)	29 (57%)	4 (8%)	< 0.001
- Grade 1	15 (30%)	21 (41%)	17 (33%)	
- Grade 2	0	1 (2%)	30 (59%)	
Infants				
TSH (µIU/mL)	0.56 (0.1, 1.6)	0.5 (0.1, 1.3)	0.1 (0.1, 1.3)	0.96
T_4 (µg/dL)	10.8 (8.7, 13.6)[c]	13.9 (10.6, 17.6)[b]	19.6 (16.4, 25.9)[a]	< 0.001
UIC (µg/L)	253 (130, 400)	195 (108, 351)	200 (113, 329)	0.49
Subclinical hypothyroidism	2% (1/50)	8% (4/51)	10% (5/51)	0.259

Infants	Capsule group (50)	I-salt group (51)	Control group (51)	p - value
Subclinical hyperthyroidism	30% (15/50)	39% (20/51)	51% (26/51)	0.098
WAZ score	-0.35 (1.3)	-0.4 (1.1)	-0.5 (1.3)	0.73
WLZ score	0.38 (1.1)	0.46 (1.2)	0.13 (1.2)	0.36
LAZ score	-0.8 (1.3)	-1.1 (1.1)	-0.8 (1.2)	0.57
HCAZ score (cm)	0.73 (0.24, 1.62)	0.78 (-0.24, 1.60)	0.74 (0.09, 1.63)	0.74
VIP tests of infants				
Total shift	4 (2, 6)	4 (2, 6)	4 (2, 6)	0.92
No. of looks	6 (5, 7)	6 (5, 8)	6 (5, 8)	0.34
Average look	9.6 (5.8)	8.0 (5.2)	8.9 (5.7)	0.38
Longest look	22.5 (17.8)	19.5 (19.1)	21.3 (17.4)	0.41
Total novelty quotient (> 0.55)				
- Yes	13 (26%)	26 (51%)	24 (47%)	0.024
- No	37 (74%)	25 (49%)	27 (53%)	

Data are median, mean and percent. Continuous data were analyzed using Tukey's HSD post hoc test, ordinal data was analyzed using Ordered Logistic Regression and categorical data were analyzed using Chi-square test. Subclinical hypothyroidism defined as increased TSH and normal T_4 (TSH > 3.7 µIU/mL and normal T_4), overt hypothyroidism defined as increased TSH and low T_4 (TSH > 3.7 µIU/mL and T_4 < 5.05 µg/dL), hypothyroxinemia defined as T_4 less than age-specific cut-off (T_4 < 5.05 µg/dL) and subclinical hyperthyroidism is defined as increased TSH (TSH > 3.7 µIU/mL and normal T_3 and T_4 [34,35].

Table 4. Multiple regression predicting VIP tests using stepwise criteria (n = 101)

Dependent variables	Predictors	Beta	p
Total shift	Infants UIC/Cre at 26 weeks	0.24	0.02
	Maternal T_3 at 26 weeks	-0.21	0.043
	Maternal T_4 at baseline	0.226	0.029
	Adjusted R square = 0.12		
Average looks	Maternal T_3 at baseline	0.262	0.008
	Maternal Tg at baseline	-0.24	0.015
	Adjusted R square = 0.104		
Longest look	Maternal Tg at baseline	-0.249	0.012
	Maternal T_3 at baseline	0.238	0.016
	Adjusted R square = 0.10		

Table 5. Maternal thyroid hormones, infants nutritional status and gender as associates of infants total novelty quotient (n = 152)

	Total Novelty Quotient > 0.55		OR (95% CI)	p
Variables	Yes	No		
Child sex				
- Male	24	26	1.00	
- Female	14	37	0.350 (0.139, 0.907)	0.030
Maternal T_3 at 26 wks				
- < 0.8 ng/mL	9	7	1.00	
- 0.8 – 1.9 ng/mL	25	54	0.287 (0.022, 3.716)	0.339
- > 1.9 ng/mL	4	1	0.076 (0.007, 0.799)	0.032
Maternal TSH at 26 wks				
- < 3.7 µIU/mL	37	61	1.00	
- > 3.7 µIU/mL	1	2	0.613 (0.045, 8.350)	0.714
Maternal T_4 at 26 wks				
- < 5.05 µg/dL	10	14	1.00	
- > 5.05 µg/dL	28	48	1.185 (0.393, 3.581)	0.763
WAZ score of infants at baseline				
- < -1 Z score	13	11	1.00	
- > -1 Z score	25	52	0.390 (0.118, 1.180)	0.093
LAZ score of infants at 26 wks				
- < -1 Z score	23	25	1.00	
- > -1 Z score	15	38	0.570 (0.208, 1.535)	0.263

CHAPTER SIX

MANUSCRIPT THREE

Title: High variability of iodine in salt and of urinary iodine concentrations (UIC) from rural households in Sidama zone, southern Ethiopia

Abstract

Background: Iodine is a chemical element essential for the synthesis of thyroid hormones. Iodine deficiency is one of the most common nutritional problems in the world. The study population had a long history of severe iodine deficiency.
Objective: To assess urinary iodine concentration, rate of goiter in mothers and school-age children and household salt iodine concentration in a small sample of households 10 months after launch of the national salt iodization program.
Design: Cross-sectional study on a randomly selected sample of women and school children. Goiter was assessed by palpation. Concentrations of iodine in salt, urine and water were analyzed by inductively coupled plasma mass spectrometery (ICP-MS).
Setting: Sidama zone, southern Ethiopia.
Subjects: Mothers and school children age 5 to 16 years.
Results: The study included 193 mothers and 76 children. The median UIC was 143 (84, 202) µg/L in the mothers and 187 (102, 278) µg/L in the children. Mother's UIC ranged from 16.7 to 767.2 µg/L and children's UIC ranged from 18.7 to 738.7 µg/L. Rate of goiter was high with 79% in the mothers and 71% in the children. The median salt iodine concentration (SIC) was 8 (4.3, 13.4) ppm (mg/kg) with a range from 0 to 42 ppm. Despite the launch of salt iodization program in Ethiopia, 94% of the study participants

said they did not use iodized salt and 88% did not know the benefits of iodized salt. Of the 24 mothers who reported knowing the benefits of iodized salt, 23 said it prevents goiter but none mentioned cognitive effects. Iodine content of their drinking water was below the detection limit of 1 μg/L.

Conclusions: The only apparent source of iodine for this population was iodized salt; however, the salt contained minimal but variable amounts of iodine. The high variability of salt iodine concentration (SIC) was also reflected in the UIC of the mothers and children.

Introduction

Iodine is a chemical element found in trace amounts in the human body and is primarily obtained through the diet [1]. Iodine is essential for the synthesis of thyroid hormones which regulate the metabolic processes of most cells and play important roles in human growth and development [2].

Iodine deficiency is one of the most common nutritional problems of the world. Globally, it is estimated that 2 billion people, most of them in developing countries, suffer inadequate intakes of iodine [3]. Due to its multiple effects on human health, iodine deficiency is referred to as iodine deficiency disorders (IDDs) [4]. Enormous progress towards eliminating iodine deficiency has been made, however iodine deficiency is reappearing even in developed countries [5].

In Ethiopia the goiter rate among 15 to 49 years old women was 35.8% and in school age children was 40% in a survey conducted in 2005. In the southern region of the country, of 1702 women examined for goiter, 43.2% had palpable and 17.7% had visible goiter, which is a total goiter rate of 60.9%. In school children in the same region, the prevalence of goiter was 56.2%. These data show that Ethiopia, particularly the southern region, was severely affected by iodine deficiency [6,7]. However, recently Ethiopia has been identified as one of the few African countries where iodine deficiency was a moderate problem [5].

Iodine is widely distributed in the environment as iodide. Iodine found in the soil can be washed away by leaching, flooding and erosion, which leaves the soil and drinking water depleted of iodine. Plants grown in this soil will be low in iodine content and hence animals and humans that rely on these plants will most likely become iodine

deficient [8]. For people who reside in iodine-deficient areas, the best ways to alleviate iodine deficiency are using iodized salt and diversifying local food with foods from iodine-sufficient areas. Iodine containing compounds such as fertilizers, livestock feed, and compounds used in irrigation and milk processing can increase iodine content in food as well as dairy products [9].

A review in 30 developing countries showed that urinary iodine concentration (UIC) was significantly correlated with household iodized salt availability [10]. In a prospective study in Denmark 4 years after a mandatory salt iodization program (13 ppm iodine) a lower median thyroid volume was observed in women age 18 to 65 years old. And a large relative decline of thyroid volume was observed in younger females from places where iodine deficiency was moderate [11]. A national survey on iodine deficiency was conducted in Tanzania after twelve years of a salt iodization program. The iodine status of school children, aged 6 to 18 years, was assessed by goiter rate (n = 140,358) and UIC (n = 4523). Total goiter rate significantly decreased from 61% in the 1980s to 12.3% in 2004 and the median UIC was found to be 204 µg/L [12].

In Ethiopia, the salt iodization program was launched early 2012. Ten months after we collected our data for this study in 2013, the Ethiopian Public Health Institute reported that the national iodized salt coverage (above 0 ppm) was 95.2% with 42.7% of the households having salt with iodine greater than 15 ppm and 23.2% falling within the national standard (20 to 40 ppm) [13]. However, to the best of our knowledge no study has reported the impact of the salt iodization program on urinary iodine and rate of goiter in any part of the country following the launch of the salt iodization program.

The purpose of the present study was to assess urinary iodine concentration, rate of goiter in mothers and school-age children and household salt iodine concentration in a small sample of households 10 months after launch of the national salt iodization program. Moreover, knowledge of mothers about IDDs as well as the practices and utilization of iodized salt were assessed.

Materials and methods

The study was conducted in Sidama zone, southern Ethiopia where iodine deficiency was highly prevalent prior to the national salt iodization program [14-16]. Sidama zone has a variety of climate conditions and its elevation ranges from 500 m to 1500 m above sea level. The major staple foods are corn and enset *(Enset ventricosum)* [17].

The study participants were recruited from eight kebeles (the smallest administrative unit) from randomly selected households which included 40 mothers and 76 school age children and 153 lactating women recruited for another study.

Urine samples were collected from mothers and children for analysis of UIC. Because 90% of dietary iodine is excreted in the urine, UIC is a commonly used biochemical indicator for iodine status [18]. Urine samples were collected in a cup from each participant and transferred to tightly sealed vials. Water samples from eight communal water tap points and from nearby Lake Awassa were collected for iodine analysis. A 10 g salt sample was collected from each household for iodine content analysis.

The mother's knowledge, attitude and practice about iodized salt, iodine deficiency, goiter and other related factors were assessed using open-ended questions.

Open-ended questions were re-coded for analysis. Socio-demographic data were collected using structured interview.

Laboratory analysis

Urinary and drinking water iodine concentrations were analyzed by inductively coupled plasma mass spectrometery (ICP-MS Elan 9000, Perkin Elmer, Norwalk, CT). Salt iodine concentration (SIC) was analyzed with a portable digital electronic iodine checker (WYD, UNICEF) and re-analyzed using inductively-coupled-plasma mass spectrometry. In measuring SIC using the two methods a reasonably good correlation was obtained ($r = 0.745$, $p < 0.001$). Total goiter of mothers and children was determined by palpation based on the following grades: grade 0, no palpable or visible goiter; grade 1, palpable goiter but not visible when neck is in the normal position; grade 2, visible goiter when neck is in the normal position [19].

Statistical analysis

Data were analyzed using SPSS statistics for Windows v. 20.0 (Armonk, NY: IBM Corp.). Frequency, percentage and median (IQR) were used to present the data.

Results

Demographic and socio-economic characteristics of study participants are presented in Table 1. The mean age of the mothers was 25.6 (8.1) and the children was 9.6 (4.4) years. The mean household size was 5.6 (2.1) and mean number of children was 3.2 (1.8). Among the children who participated in this study, 60% were males. Of the respondents, 55% had some formal education. Household food insecurity was high in the study participants with 14% severely, 24% moderately and 15% mildly food insecure.

The median UIC of mothers was 143 and of the children was 187 µg/L, which is above the cutoff for an iodine deficient area [18] (Table 2). As indicated in Figure 1, UICs of both mothers and children were above the cutoff (100 µg/L) for iodine sufficiency. Only one mother and one child had UIC below 20 µg/L. Overall 67.5% of the mothers and 77% of the children had adequate, more than adequate or excessive iodine intakes.

However, the rate of goiter was highly prevalent in both mothers and children. Most (76%) of the women and 71% of the children had either palpable or visible goiter. The high rate of goiter indicates goiter was far above what would be called a public health problem [18] (Table 2).

A markedly high variation was observed in SIC. Nearly 79% of the salt samples contained low iodine concentration, below the minimum requirement (15 ppm) for adequately iodized salt (Table 2). Only 21% of the salt samples analyzed met the requirement for adequate iodized salt (15 – 40 ppm). Overall the SIC ranged between 0 ppm to 42 ppm. We also analyzed water iodine concentration from eight communal tap water points and from the lake but none of the water samples contained iodine above the detection limit of 1 µg/L (data not shown).

Despite the launching of the salt iodization program in Ethiopia, nearly 94% of the women interviewed did not know whether or not the salt they were using was iodized. Except one woman, all of the women said they use rock (non-iodized) salt for cooking and only 24 (12%) women knew the benefits of iodized salt. Among those, 23 women said iodized salt prevents goiter (Table 3). Of the 39 (20%) women who said they know the causes of goiter. Only 10 of the respondents related goiter to lack of iodine in salt or iodine itself (Table 3).

As indicated in table 4, the majority of the women add salt to food near the end of cooking and 38% said they cook the salt together with the food. They add variable amounts of salt to food and the amount of salt was estimated by teaspoon (tsp). Most of the women stored their salt in a water bottle.

Discussion

The best way to alleviate iodine deficiency disorders is to implement universal salt iodization [3]. The results of the present study showed a significant improvement in urinary iodine and salt iodine concentration following the salt iodization program launched in 2012 in Ethiopia. However, a high prevalence of goiter with 76% in mothers and 71% in children remained a serious problem [18].

Compared to previous studies the pattern of UIC distribution in both mothers and children is shifting towards iodine adequacy. In consecutive years from 2007 to 2010 in similar study areas, the proportion of study participants with UIC below 100 µg/L (an indicator for iodine inadequacy) was nearly 100%. In 2007, 99% of the children involved in the study had UIC below 20 µg/L [20]. In 2009, 89% of the pregnant women had UIC below 50 µg/L with 60% below 20 µg/L [15]. Similarly in 2010, the proportion of women with UIC below 100 µg/L was 96% [16]. In the present study the proportion of mothers and children with UIC below 20 µg/L was almost zero. Except for 8.5% of mothers and 7% of children, the rest had UIC above 100 µg/L. However, the high variability observed in UIC is of a great concern. Mother's UIC ranged from 16.7 to 767.2 µg/L and children's UIC ranged from 18.7 to 738.7 µg/L.

The study population has poor dietary sources of iodine [16]. The iodine concentration of their drinking water was also extremely low. Hence the high variability in UIC could be explained from the high variability observed in SIC. Salt iodine concentration ranged from 0 ppm to 42 ppm. The variability in SIC is again a great concern because it is highly likely that one can get non-iodized salt one day and excessively iodized salt another day because the mothers normally buy small amounts of

salt that they could use for one to three days. The ultimate goal of universal salt iodization program or salt iodization program is to alleviate iodine deficiency but if it is not seriously monitored, excessive iodine intake could also be of great concern [5]. In Ethiopia is iodized manually using Knapsack sprayers and small scale iodization machines which make it difficult to homogeneously iodized salt [13].

Nonetheless, although early awareness is necessary for a quick improvement and better product, it could be too early to comment on the Ethiopia salt iodization program at this time. In Saudi Arabia, 20 years after a universal salt iodization program was implemented SIC showed variation from 0 to 112 ppm [21]. A drastic decrease over time in the iodine content of salt was observed in Cambodia despite a mandatory salt iodization program. The median iodine content of salt dropped from 22 ppm in 2011 to 0 ppm in 2014. It was assumed that this drastic drop in salt iodine content could be due to infiltration of non-iodized salt from the production site to the market and/or due to inappropriately packaging of iodized salt [22]. Long term experience from different countries showed that salt iodization programs can only be effective and sustainable with strong monitoring strategies and commitment from government, donors, producers, distributors and consumers [23].

Maintaining the sustainability of a salt iodization program requires its own multidimensional commitment, and retaining the iodine in the salt in turn requires careful storage, handling and proper utilization methods. Iodine is a volatile trace mineral and hence it can easily be lost from improper storage, handling and cooking process. It was estimated that 20% of the iodine will be lost during the cooking process [18]. A study in Wukro, Northern Ethiopia, confirmed that 57% of the salt iodine content that was

available at the production level was lost by the time it reached the household [24]. The stability of iodine in salt can be affected by impurities and alkalinity or acidity and moisture content of salt, heat, light and humidity [25]. In the present study except for two mothers who added salt to food after cooking, the rest cooked the salt with food. Most stored the salt in containers likely to contain moisture which could cause iodine loss. Only five mothers purchased salt from retail shops; the remainder of the mothers (97%) purchased salt from the open market. From our observation the salt in the open market is highly exposed to impurities, warm temperature and humidity (Data not shown).

Since the launch of salt iodization program in Ethiopia, it was assumed that all the salt in the market was iodized. However, of the participants, 94% said they did not use iodized salt and 88% did not know the benefits of iodized salt. Of the 24 mothers who reported knowing the benefits of iodized salt, 23 said it prevents goiter but none mentioned cognitive effects. The mothers suggested causes of goiter to be drinking dirty water, drinking tap water, drinking rain water, but only 10 mothers said lack of iodine or lack of iodized salt. Although prevalence of goiter is high and visible in the community, knowledge of causes and prevention of iodine deficiency disorders (IDD) is minimal.

Because the majority of the mothers said they did not use iodized salt does not indicate the salt was not iodized, but that they do not even know the difference between iodized and non-iodized salt. From our observation we have understood that the iodized salt was packed in 50 kg bags. But rural people purchase small amounts of salt enough for one to three days. Therefore, there is no way for them to get the information labeled in the 50 kg bags. For effective transfer of information and minimizing the loss as a result of poor storage conditions, packaging of iodized salt in small amounts might help.

In conclusion the only apparent source of iodine for this population was iodized salt; however, the salt contained minimal but variable amounts of iodine. Lack of iodized salt may be further compounded by storage, handling and cooking techniques. Until machinery based salt iodization techniques and effective monitoring strategies are materialized it is difficult to sustain a good quality salt iodization program. Moreover, mechanisms should be devised to control the infiltration of non-iodized salt to the local market. In order to enable the community to say 'No!' for non-iodized salt it is indispensable to create awareness about IDDs and causes, consequences and prevention methods of IDDs.

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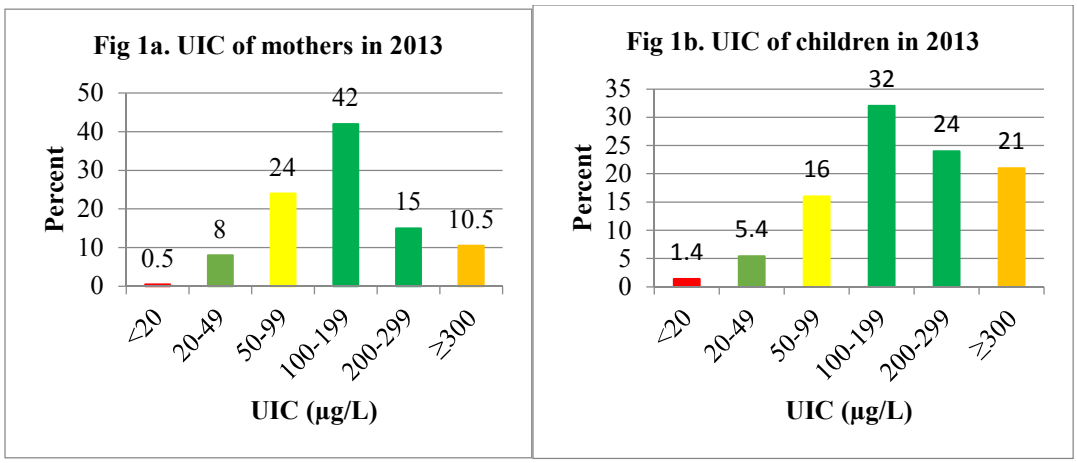

Figure 1. Urinary iodine concentration of mothers and children 10 months after salt iodization program in Sidama zone, southern Ethiopia.

Table 1. Socio-demographic characteristics of study participants

	Mean (SD)	Percent
Mothers (n = 193)		
Mothers age (years)	25.6 (8.1)	
Household size	5.7 (2.1)	
Number of children	3.2 (1.8)	
Mothers education (%)		
- No formal education		45
- Some education		55
Children (n = 76)		
Children's age (years)	9.6 (4.4)	
Children's gender (%)		
- Male		60
- Female		40

Table 2. Urinary iodine concentration of mothers and children and salt iodine concentration from Sidama zone, southern Ethiopia

	Median (IQR)	Percent
Maternal UIC (µg/L)	143 (84, 202)	
Maternal goiter		
- No goiter		24
- Palpable goiter		26
- Visible goiter		50
Children's UIC(µg/L)	187 (102, 278)	
Children's goiter		
- No goiter		29
- Palpable goiter		60
- Visible goiter		11
Household SIC (ppm)	8 (4.3, 13.4)	
- < 5 ppm		28.6
- 5 – 9.9 ppm		31.8
- 10 – 15 ppm		18.2
- > 15 ppm		21.4
- Range	0 to 42 ppm	

Table 3. Mother's knowledge of iodized salt and causes of goiter

Questions to the women	Frequency	Percent
What kind of salt do you use?		
- Rock salt	192	99.5
- Packed iodized salt	1	0.5
Do you use iodized salt?		
- Yes	12	6.2
- No	181	93.8
Do you know the benefits of iodized salt?		
- Yes	24	12.4
- No	169	87.6
What is the benefit of iodized salt?		
- Prevents goiter	23	95.8
- Makes one strong	1	4.2
Do you know the causes of goiter?		
- Yes	39	20.2
- No	154	79.8
What are the causes of goiter?		
- Drinking dirty water	14	35.9
- Lack of iodine in salt	8	20.5
- Drinking tap water	4	10.3
- Drinking rain water	4	10.3
- Lack of food	2	5.1

Questions to the women	Frequency	Percent
- Lack of salt	2	5.1
- Lack of iodine	2	5.1
- Drinking untreated water	1	2.6
- Drinking river water	1	2.6
- Hereditary	1	2.6

Table 4. Utilization and handling of salt (n = 193)

Questions to the women	Frequency	Percent
When do you add salt to food when cooking?		
- Cook together	73	37.8
- At the end	118	61.2
- After cooking	2	1
How much salt (tsp) do you add to each food that you cook?		
- One	130	67.4
- Two	59	30.6
- Three	4	2
How do you store your salt?		
- In a tight bottle	21	10.9
- In a water bottle	133	68.9
- In a plastic bag	38	19.7
- Cover with paper	1	0.5

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Appendices

Annex 1. Table A. Correlation coefficients of maternal thyroid function markers and infants iodine status at baseline and infants VIP tests

Variables	M_Tg	M_T_3	M_T_4	M_TSH	C_UIC	C_UIC/ Cre	Total shift	Average look	Longest look
M_Tg	1.00								
M_T_3	0.05	1.00							
M_T_4	0.09	0.36**	1.00						
M_TSH	-0.13	0.08	-0.06	1.00					
C_UIC	0.12	0.29**	0.18	-0.01	1.00				
C_UIC/Cre	0.02	-0.032	0.01	-0.18	0.53**	1.00			
Total shift	0.28**	0.09	0.10	0.07	0.16	0.01	1.00		
Average look	-0.20*	0.24*	0.13	0.16	0.05	0.01	-0.11	1.00	
Longest look	-0.22*	0.22*	0.14	0.15	0.05	0.02	-0.13	0.96***	1.00

Note: M_Tg – Maternal thyroglobulin, M_T_3 – Maternal triiodothyroinie, M_T_4 – Maternal thyroxine, M_TSH – Maternal thyroid stimulating hormone, C_UIC – Children UIC, C_UIC/Cre – Children UIC/Creatinine

Annex 2. Table B. Correlati**on** coefficients of maternal thyroid function markers and infants iodine status at six months and infants VIP tests

Variables	M_Tg	M_T_3	M_T_4	M_TSH	C_UIC	C_UIC/ Cre	C_TSH	C_T_4	Total shift	Average look	Longest look
M_Tg	1.00										
M_T_3	0.13	1.00									
M_T_4	0.21*	0.37**	1.00								
M_TSH	-0.25*	-0.37**	-0.05	1.00							
C_UIC	0.12	-0.09	0.08	0.01	1.00						
C_UIC/Cre	-0.01	-0.09	-0.07	-0.05	0.18	1.00					
C_TSH	0.16	0.05	-0.01	-0.06	0.11	0.01	1.00				
C_T_4	0.01	-0.04	-0.06	-0.08	-0.17	0.03	0.25*	1.00			
Total shift	0.04	-0.12	-0.05	-0.03	0.09	0.17	0.01	-0.01	1.00		
Average look	-0.17	0.11	0.24*	0.26**	-0.04	0.03	0.01	-0.14	-0.11	1.00	
Longest look	-0.18	0.12	0.26**	0.25*	-0.09	-0.01	-0.08	-0.16	-0.13	0.96***	1.00

Note: M_Tg – Maternal thyroglobulin, M_T_3 – Maternal triiodothyronine, M_T_4 – Maternal thyroxine, M_TSH – Maternal TSH, C_UIC – Children UIC, C_UIC/Cre – Children UIC/Creatinine, C_TSH – Children TSH, C_T_4 – Children thyroxine.

Annex 2. Ethical approvals

Oklahoma State University Institutional Review Board

Date:	Monday, December 03, 2012	Protocol Expires: NOT APPROVED
IRB Application No:	HE1156	
Proposal Title:	Community Salt Testing and Relation of Iodine Intake to Visual Information Processing of Ethiopian Infants	
Reviewed and Processed as:	Full Board **Modification**	

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s):

Tafere G. Belay 301 HES Stillwater, OK 74078	David Thomas 116 N. Murray Stillwater, OK 74078	Barbara Stoecker 421 HS Stillwater, OK 74078

The requested modification to this IRB protocol has been approved. Please note that the original expiration date of the protocol has not changed. The IRB office MUST be notified in writing when a project is complete. All approved projects are subject to monitoring by the IRB.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

The reviewer(s) had these comments:

The modification request to add Dr. David Thomas as a co-PI to the protocol is approved.

Signature :

Shelia Kennison, Chair, Institutional Review Board

Monday, December 03, 2012
Date

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FOOD, MEDICINE AND HEALTH CARE
ADMINISTRATION AND CONTROL AUTHORITY OF
ETHIOPIA

ቁጥር 02/1/22/29
Ref. No.
ቀን 30/04/2005
Date:

Tafere G/Egziabher, PI
Hawassa University, Hawassa

Subject: Clinical trial Authorization

It is to be recalled that the protocol for the clinical trial entitled: ''**Community salt testing and relation of iodine intake to visual information processing of Ethiopian infants** '' with reference no 02/06/05/45 dated 24/12/12 has been evaluated and found to be cleared technically but the subject group is prohibited by the proclamation (661/2009 article 15 sub articles 3&4). As a result your application was not approved. However, the authority has approved your application with special consideration after thorough evaluation of the benefit of the trial to Ethiopian lactating mothers and infants.

The trial Authorization is subject to the following conditions:

- The authority shall be informed immediately of any toxic effects or death, which may occur during the clinical trial and any data, received which might cause doubt on the validity of the continuation of the study
- The authority shall be informed of any decision to discontinue the clinical trial. The reason of the cancellation must be stated.

ፋክስ/Fax: 251-1-52 13 92 P.O.Box: 5681 Tel: 251-1-52 41 22/52 41 23 E-mail: daca@telecom.net.et
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IN REPLY REFER TO OUR Ref. No.

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FOOD, MEDICINE AND HEALTH CARE
ADMINISTRATION AND CONTROL AUTHORITY OF
ETHIOPIA

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Ref. No.
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Date:

- The clinical trial must be conducted in accordance with the protocol submitted to the authority. Any amendment to the protocol shall be submitted to the authority for approval.
- The authority shall inspect the trial site at any time for compliance of the conduct of the trial for the Good Clinical trial practice

With best regards,

Tehulu Denekew Alameneh
Director General

Cc:

Product registration & Licensing Directorate
EFMHACA

VITA

Tafere G/Egziabher Belay

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECTS OF MATERNAL IODINE SUPPLEMENTATION ON MATERNAL AND INFANT IODINE STATUS AND THYROID FUNCTION AND ON INFANT VISUAL INFORMATION PROCESSING

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in May, 2016.

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, OK in July, 2010.

Completed the requirements for the Bachelor of Science in Rural Development and Family Sciences at Debub University, Awassa, Southern Region/Ethiopia July, 2006.

Experience:

September 2005 to December 2007: Hawassa University, Faculty of Agriculture, Department of Rural Development and Family Sciences: as an academic staff.

February 1996 to August 1998: Rama Integrated Rural Development Project: as Development Agent and Assistant Manager.

Professional Memberships:

Member of the American Society of Nutrition.
Member of the Academy of Nutrition and Dietetics.