Effect of iodine supplementation in Indian pregnant women on maternal and newborn thyroid function and cognitive development

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"Your self identity is something that you cannot rid yourselves of, no matter how old you are or what you do. Be it a retired person or a home-maker; we all want to get to those days when we would introduce ourselves to the world with an identity that can be called as our own"

Dedicated to my beloved father, who instilled in me this need to have self identity; you will always remain in my heart supporting and encouraging me

ABSTRACT

Background: Iodine is a key nutrient in neurodevelopment, and the fetus is entirely dependent on the iodine intake of the mother to fulfill this important requirement for proper brain function. While this is clearly known, it is uncertain if maternal iodine nutrition should be monitored separately against what is in current practice in public health programs to control iodine deficiency. Also, it is unclear whether it is beneficial to supplement pregnant women with iodine in mild-to-moderately iodine deficient and also iodine sufficient areas. Finally, the role of thyroid dysfunction in depression during pregnancy is uncertain.

Objectives: 1) to determine whether iodine supplementation to pregnant women improves maternal and newborn thyroid function, pregnancy outcome, birth weight, infant growth and cognitive performance; 2) to assess iodine intake and potential determinants of intake, in pregnant women and their children who were sharing all meals; 3) to measure thyroid status during pregnancy and assess potential determinants of maternal thyroid function including iodine status, thyroid autoimmunity, body weight and anemia; 4) to assess the association of maternal depression, and thyroid function during pregnancy.

Methods: 1) In a randomized placebo controlled trial (RCT), the MITCH (Maternal Iodine Supplementation and its Effects on Thyroid function and CHild Development) study, pregnant women, gestational age ≤14 weeks, in Bangalore, India, were randomized to receive either a daily supplement of 200 µg oral iodine or placebo from enrolment until delivery. Women were followed through delivery, and then with postnatal follow-up of their infants at 6 weeks, 1 and 2 year. Early neonatal development was assessed using the Neonatal Behavioral Assessment Scale (NBAS) at 6 weeks of age; neurocognitive assessment was done using the Bayley Scales of Infant Development (BSID III) at 1 and 2 years, and BRIEF-P (Behavior Rating Inventory of Executive Function) at 2 years; 2) A cross-sectional study comparing iodine status of pregnant women and their children, who were sharing all meals in Bangalore, India; 3) A cross-sectional study among 334 pregnant women ≤14 weeks of gestation, in Bangalore, India, who were screened for the RCT; 4)

Secondary analysis of the longitudinal data on 318 pregnant women in the RCT.

Results: 1) In the RCT, there were no significant differences between groups in maternal thyroid function tests or thyroid volume during gestation. The prevalence of thyroid dysfunction or anti-TPO antibodies did not differ significantly during gestation and postpartum. Postpartum, there were no significant differences between the maternal and infant groups in thyroid function, birth outcomes or UIC. Neonates whose mothers received iodine supplementation during pregnancy had better orientation scores at 6 weeks of age and lower scores of inhibition suggesting better executive function at 2 years of age although neurocognitive development on the BSID III were not significantly different between groups; 2) In the pilot study, a) median UIC in pregnant women was 172 µg/L, b) the median UIC was >150 µg/L in all trimesters and c) thyroid size was not significantly different across trimesters; the median UIC in children was 220µg/L, indicating 'more than adequate' iodine intake at this age. Median UIC was significantly higher in children than in their mothers (p=0.008). 3) In the cross-sectional study, 21% women were vegetarian, 19% were anemic and 23% were overweight or obese. Iodized salt was used by 98% of women and they were iodine sufficient, median UIC was 184.2 µg/L and all had normal thyroid volume. However, 18% of women had thyroid insufficiency: 3.7% had overt hypothyroidism (83% with positive TPO-Ab), 9.2% had subclinical hypothyroidism and 5.2% had hypothyroxinemia. Women consuming vegetarian diets did not have significantly lower iodine intakes or higher risk of hypothyroidism than those consuming mixed diets, but overweight/obesity and anemia predicted thyroid insufficiency; 4) In the longitudinal study, there was no significant difference in depressive symptoms between the iodine intervention and placebo groups. Women with depressive symptoms had significantly lower serum TSH compared to women without depressive symptoms in the first trimester. Pregnant women with prenatal depressive symptoms had a significantly higher number of medical symptoms.

Conclusion: 1) Iodine supplementation in mildly iodine deficient and in iodine sufficient pregnant women was well-accepted and safe and did not increase the risk of excess iodine intake, hyper- or hypothyroidism, or thyroid autoimmunity. Though there were no

significant effects of iodine supplementation on neonatal and maternal thyroid function and birth outcomes, there were modest effects on neurocognitive development of children as assessed by executive function of children at 2 years. Thus, additional follow-up of these children for neurocognitive testing at a later age when development and cognitive testing is more reliable would provide valuable add on information; 2) The iodized salt program in Bangalore, India was providing adequate iodine to women throughout pregnancy, at the expense of higher iodine intake in their children, suggesting that the current WHO/UNICEF/ICCIDD cut-off for median UIC in children indicating more-than-adequate intake may need to be reconsidered; 3) Despite iodine sufficiency, many pregnant women had thyroid insufficiency predicted by low hemoglobin and higher BMI. The prevalence of overt hypothyroidism was >5-fold higher than reported in other iodine-sufficient populations of pregnant women, thus, screening of maternal thyroid function should be considered in antenatal care at hospitals in Bangalore, India; 4) Although iodine supplementation did not affect maternal depression, we highlighted the need for systemic screening for prenatal depression during antenatal visits as it is an independent risk factor for later development of clinical depression.

CONTENTS

Chapter 1

INTRODUCTION

Background

In 2007, WHO recommended that pregnant women should consume 250 μg iodine daily and in 2011, the American Thyroid Association (ATA) suggested that the strategies to implement this recommendation might differ between countries. While iodine supplementation during pregnancy is clearly beneficial in severely iodine deficient areas, it was unclear whether pregnant women in mild-to-moderately iodine deficient and also iodine sufficient areas should receive supplementation. Countries with successful USI programmes tend not to depend so much on extra iodine supplementation to their pregnant population, while most of the other countries work with the advice of professional societies like the American and European thyroid association and endocrine societies where the onus of such advice rests on the clinical wisdom of the consulting gynecologists. Recently, Thailand became the first country in the world to have introduced maternal iodine supplementation as policy in their national program. However, there is no consensus on what aspects of safety should be evaluated during the course of such supplementation.

Among all the other effects that iodine nutrition incurs on the general metabolism and homeostasis, its effect on brain development is the most pertinent and widely studied. However a recent systematic review of randomized controlled trials of iodine highlighted a lack of quality evidence of the effect of prenatal or periconceptional iodine supplementation on growth and cognitive function of children (1). We therefore set up a multicentre randomized controlled trial of iodine supplementation in pregnant women to determine whether the daily oral administration of iodine (200 μg) to pregnant women in areas of mild-to-moderate iodine deficiency improves maternal and newborn thyroid function, pregnancy outcome, birth weight, infant growth and cognitive performance. The studies belonging to this thesis were conducted at the Indian arm of the trial in Bangalore, one of the districts in the Indian state of Karnataka. The other arm of the trial

was conducted at Thailand, in a region with mild to moderate iodine deficiency.

Pregnancy, nutrition, and brain development

Pregnancy is a pivotal life event which marks the beginning of a life deeply ingrained to the maternal environment. The formation of the most complex organ of the highly organized human system, the brain, starts very early in pregnancy. Approximately 22 days after conception, the neural plate begins to fold inward, forming the neural tube, which eventually becomes the brain and the spinal cord (2). Seven weeks after conception, cell division begins within the neural tube, creating nerve cells (neurons) and glial cells (cells that support neurons). After a neuron is created, it migrates to its place in the brain, where it then grows axons and dendrites projecting out from its cell body. These branching projections make connections with other cells, called synapses, through which nerve signals travel from one cell to another. These neurodevelopmental processes begin during gestation and continue throughout infancy (3). Nutrition is especially important during pregnancy and infancy, which are crucial periods in brain development, laying the foundation for the development of cognitive, motor, and socio-emotional skills throughout childhood and adulthood (3). There is evidence for the effect of specific nutrient deficiencies during early development on five key neuronal processes viz neuron proliferation; axon and dendrite growth; synapse formation, pruning, and function; myelination, and neuron apoptosis (programmed cell death). Most of the neural processes linked to these nutrients (protein-energy malnutrition, fatty acids, iron, iodine and thyroid hormones, zinc, choline, B-vitamins) have been studied in human as well as animal studies while other nutrients, such as copper, which is also important for some of these neurodevelopmental processes, have not been rigorously studied in human populations (3). Severe iodine deficiency, which causes both maternal and fetal hypothyroidism, is, worldwide, the most common cause of mental retardation (4-6). More recent evidence suggests that even mild reductions in maternal

thyroid hormone levels in early pregnancy are associated with reduced IQ in offspring (7, 8). Iodine, being an essential component of the thyroid hormones, is undisputedly the most important nutrient studied for its role in proper brain function.

Brain development and thyroid hormones

Iodine plays a central role in thyroid physiology, being both a major constituent of thyroid hormones and a regulator of thyroid gland function (14). The biologically active hormone interacting with nuclear thyroid receptors (3,5,3'-triiodo-L-thyronine or T3), is synthesized by the deiodination of its precursor thyroxine (T4). It also regulates its own steady-state level by exerting negative feedback regulation on thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone secretion, and by regulating deiodinases in liver and brain (9). The brain is an important target of thyroid hormone not only during development but also in adult animals. Brain growth is characterized by two periods of maximal growth velocity (10). In the first phase (between the third and fifth months of gestation), the supply of thyroid hormones to the growing foetus is almost exclusively of maternal origin while during the second phase (from the third trimester onwards up to the second and third years postnatally), the supply of thyroid hormones to the foetus is essentially of foetal origin (11, 12). Thyroid hormone deficiency during the fetal and postnatal periods in humans may cause irreversible mental retardation and neurological deficits (13). Thyroid hormone deficiency occurring early in pregnancy shows problems in visual attention, visual processing and gross motor skills in the offspring. If it occurs later in pregnancy children are at additional risk of subnormal visual and visuospatial skills as well as slower response speeds and fine motor deficits (14). **Fig 1** shows the timeline of human thyroid system and brain development from conception to birth (15).

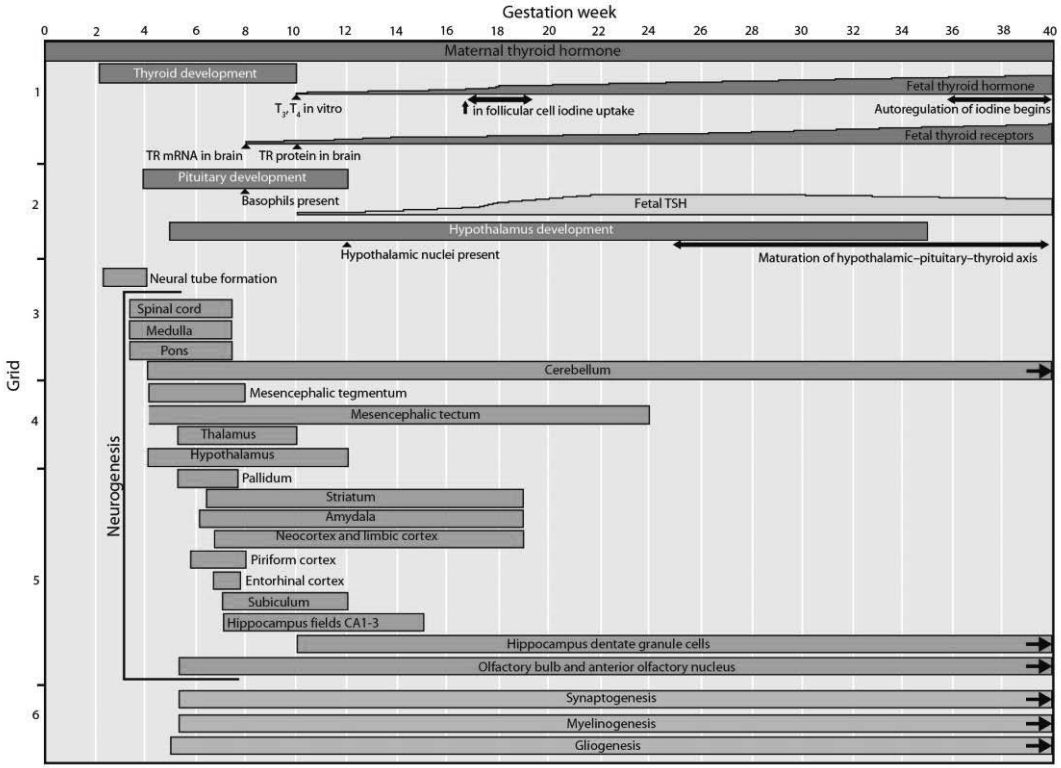

Figure 1: Timeline of human thyroid system and brain development from conception to birth. Reproduced with permission from Environmental Health Perspectives (15).

Superimposing the development of the thyroid system onto the chronology of brain development, it is evident that thyroid hormone may influence brain structure and function during the earliest stages of development in humans and rodents (15). Most critical for brain development is the fact that T3 is produced by the fetus from maternal T4 and this is the only source of T4 for the fetus during the first trimester of pregnancy (5, 12, 14, 16, 17). During this critical period, fundamental processes occur in the development of the fetal central nervous system. In particular, the cerebral vesicles, from which the cerebral cortex develops, become recognizable in the developing central nervous system by embryonic day 35 and the neocortical development begins by embryonic day 46 (18). The concentration of T3 nuclear receptors is low at the beginning of neocortical development but it progressively increases 10-fold by the 16th week of gestation (19), in parallel to neuroblast proliferation and neuronal migration (18). This makes the human brain especially vulnerable during development to deficiencies of iodine and thyroid hormones, not only because of their action through nuclear receptors (19, 20), but also for their possible non-genomic effects that might take place even earlier.

Maternal depression and thyroid function during pregnancy

Maternal depression is considered a risk factor for the socio-emotional and cognitive development of children and stress factors such as negative life events, poor marital relationship, lack of social support, and personal and family psychopathology have been associated with depression. Stress or anxiety during pregnancy, are risk factors for premature birth and growth restrictions within the womb, both of which are risk factors for behavioural problems in the child. Stress/anxiety during pregnancy doubles the risk of a range of emotional, behavioural and cognitive problems (21). Several recent reviews suggest a higher prevalence of depression during pregnancy in women from developing countries compared to pregnant women

from the West (22, 23). While in the past the focus has been on post partum depression (24), more recently there have been a considerable research interest in antenatal depression and its causal factors and outcomes (25). Subjects with thyroid insufficiency seem especially susceptible to the development of depression (26). Some studies have found that antenatal thyroid dysfunction is associated with postnatal depression and that presence of thyroid antibodies are related to depressive mood (27, 28) both during pregnancy and the postnatal period. A recent review found that the evidence base of associations of TSH and thyroid hormone levels was good for cardiovascular, metabolic, bone, and pregnancy outcomes; however, there is paucity of high-quality data for neurological and psychological outcomes (29). In addition, findings from few studies that have looked at the association between thyroid function during pregnancy and pre natal depression are largely inconsistent.

Iodine, thyroid and pregnancy

Maintaining a pregnant woman in a euthyroid state is a challenge for the thyroid gland during gestation. During pregnancy, there is an increased demand for thyroid hormone and decreased iodine availability due to iodine transfer to the fetus and intensified iodine urinary losses induced by the increased renal glomerular filtration (30, 31). Physiological adaptations take place when iodine intake is sufficient and when there is no underlying thyroid pathology: the increase in estrogens induces an increase in thyroxine (T4)-binding globulin, which alters the ratio between free and bound thyroid hormones; the increase in human chorionic gonadotropin hormone, which has thyrotrophic activity, induces a slight increase in free thyroxine (FT4) with a peak at the end of the first trimester, and this causes a transient decrease in thyrotropin (TSH) through negative feedback on pituitary thyrotrophs (30, 32-34); the placental deiodinase III alters metabolism, distribution, and availability of T4 for the mother and for the fetus in the second half of gestation (34, 35). TSH rises over the second and third trimesters, moderately in areas where iodine is sufficient and more markedly in iodine deficient

areas (36, 37). Where iodine intake is adequate, the TSH levels are only slightly higher in the second and third trimesters than 1 year after delivery (38). However, it has been variously reported that the highest TSH level was in the second trimester (39), that the TSH level was similar during pregnancy and after delivery (40), and that the TSH level remained the same throughout pregnancy (41). As for levels of fT4 and fT3, the free thyroid hormones, the highest levels occur in the first trimester, and gradually decrease over the remainder of the pregnancy (36, 38, 41,42). In iodine-deficient areas the decrease is sharper (37). In a study performed in borderline iodine deficiency, fT4 and fT3 levels were significantly lower during pregnancy than 12 months after delivery (43). However, except TSH for which trimester specific cut-offs are available for pregnancy, other thyroid parameters have to fall back on the manufacturer's recommended non-pregnant range for defining cut-offs which are method specific and creates additional difficulties for comparison of studies.

Thyroid dysfunction in pregnancy

Thyroid dysfunction is the most frequent endocrine disorder in pregnant women. Overt hypothyroidism and even subclinical hypothyroidism increases the risk of obstetric complications: miscarriage, fetal death, gestational hypertension, preterm birth, and low birth weight (44-48). Thyroid autoantibodies in pregnancy are associated with recurrent miscarriage (49) and with maternal morbidity later in life (50). When iodine intake is mildly to moderately deficient, there may not be enough T4 available to the fetus, and this may not be recognized because TSH does not necessarily increase because of stable or slightly increased triiodothyronine (T3) levels (34). Severe iodine deficiency can result in overt hypothyroxinemia, goiter, and the spectrum of iodine deficiency disorders (33).

The prevalence of overt and subclinical hypothyroidism in pregnancy is estimated at 0.3-0.5% and 2-3% respectively (51). Endemic iodine deficiency is the most common cause of hypothyroidism seen in

pregnant women worldwide. In areas of iodine sufficiency, the prevalence of overt and subclinical hypothyroidism during pregnancy is 0.2-0.4% and 3-5%, respectively, but varies by trimester of pregnancy and the diagnostic criteria (particularly the TSH upper limit) used for classification (52). Over 90% of thyroid disorders in pregnancy are thought to be of autoimmune etiology (53) and chronic autoimmune thyroiditis is the main cause of hypothyroidism during pregnancy in iodine-sufficient regions (54). Other causes include post-surgical, post-radioiodine ablation and hypothyroidism secondary to pituitary disease which, although rare, can include lymphocytic hypophysitis occurring during pregnancy or postpartum (55). Thyroid dysfunction and antibodies during pregnancy seem to predict later thyroid disease warranting routine assay of thyroid hormones and especially antibodies during pregnancy (50). India, because of its large population, high birth rate and iodine deficient soils, has until recently had a large number of infants potentially exposed to in utero iodine deficiency (56) ; recent studies highlight the presence of iodine insufficiency among Indian women (57). Two additional variables that may affect the maternal thyroid axis and contribute to thyroid insufficiency during pregnancy are iron deficiency anemia (58) and overweight/obesity (59-63) and both are common among women in urban areas of India (64-66). Better understanding of the pattern and prevalence of maternal thyroid dysfunction and associated risk factors could improve screening and treatment in the Indian population. Improved detection of maternal hypothyroidism may be particularly valuable in the first trimester, because thyroid function should be normalized as quickly as possible, early in gestation (67).

Iodine metabolism

Dietary iodine is absorbed efficiently in the gastrointestinal tract. Iodine in organic form is converted mostly to iodide before absorption. Plasma iodide exchanges rapidly with iodide in red blood cells and with both rapid and slow extracellular compartments. The thyroid gland concentrates iodide (I-) against an electrochemical

gradient by a carrier-mediated mechanism driven by ATP. Besides the thyroid, other organs that concentrate iodide include the salivary glands, gastric mucosa, choroid plexus, mammary glands, and the placenta, but only in the thyroid does TSH regulate the process. The enteric phase consists of iodide secreted into saliva and gastric juice, which moves into the small intestine for reabsorption. The kidneys account for about two thirds of the iodide cleared from the plasma and more than 90% of iodide excreted from the body. Sweat and breast milk account for variable fractions of iodide loss. However, the fecal route contributes only about 1 % of total body iodide clearance (68). **Fig.2** shows iodine pathway in the thyroid cell (69). The follicular cell in situ displays functional and structural polarity; the iodide transporter resides in the basolateral plasma membrane, which also contains ATPases and various channels and receptors, including the TSH receptor. At the apical surface, thyroglobulin, thyroid peroxidase, hydrogen peroxide, and iodide all come together. Oxidation and organification occur at or near this cell-colloid interface.

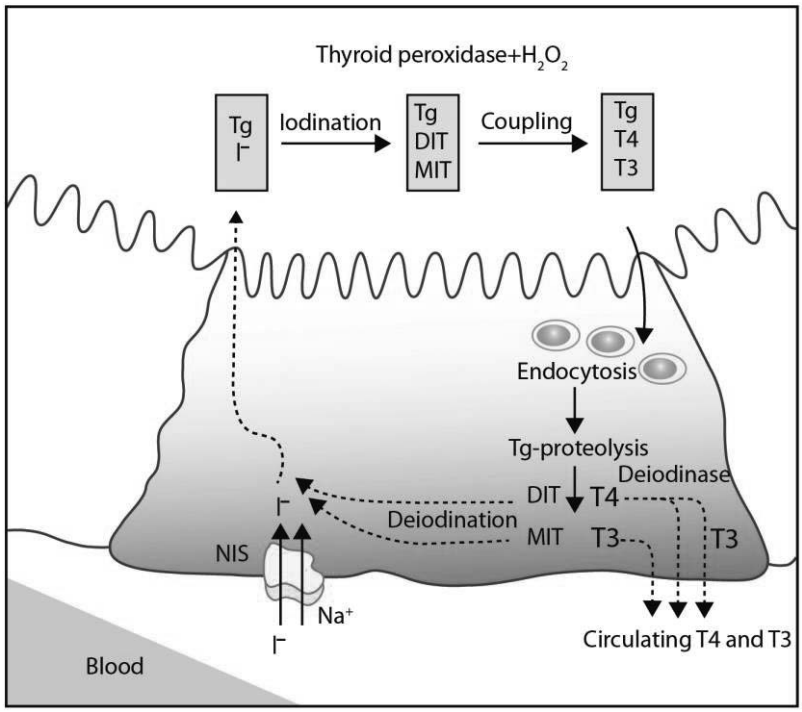

Figure 2: Iodine pathway in the thyroid cell. Iodine (I-) is transported into the thyrocyte by the sodium/iodide symporter (NIS) at the basal membrane and migrates to the apical membrane. I- is oxidized by the enzymes thyroperoxidase (TPO) and hydrogen peroxidase (H_2O_2) and attached to tyrosyl residues in thyroglobulin (Tg) to produce the hormone precursors iodotyrosine (MIT) and di-idotyrosine (DIT). Residues then couple to from thyroxine (T4) and tri-iodothyronine (T3) within the Tg molecule in the follicular lumen. Tg enters the cell by endocytosis and is digested. T4 and T3 are released into the circulation, and iodine on MIT and DIT is recycled with the throcyte. Reproduced with permission from Zimmermann et al. (69).

Hormone secretion involves, first, pinocytosis of colloid-containing iodinated thyroglobulin, then fusion of colloid droplets with lysosomes, followed by proteolysis, which liberates free monoiodotyrosine (MIT), diiodotyrosine (DIT), T4, and T3. Iodotyrosine dehalogenase regenerates iodide from MIT and DIT for reuse within the thyroid or release into the blood, accounting for the iodide leak in the chronic state of iodine excess and in certain

thyroid disorders. Type I iodothyronine deiodinase converts some of the free T4 into T3. Both hormones are released into the circulation by a process that is not well understood. The thyroid also releases thyroglobulin, of which some is iodinated and some uniodinated newly synthesized protein. All of the steps in TH biosynthesis, from oxidation and organification of iodide to the secretion of T4 and T3 into the circulation, are stimulated by TSH and inhibited by excess iodine (68). Iodine comprises 65 and 59% of the weights of T4 and T3, respectively. In the thyroid, mature thyroglobulin (Tg), containing 0.1 to 1.0% of its weight as iodine, is stored extracellularly in the luminal colloid of the thyroid follicle (70, 71).

Assessment of iodine status

Iodine intake is a key determinant of iodine status but is difficult to assess. FFQs (used to assess the type, quantity, and frequency of foods and supplements consumed) exist, but food composition tables for iodine are not readily available (72). UI excretion is an accurate indicator of dietary iodine intake as >90% of ingested iodine is excreted in the urine and UI is highly sensitive to recent changes in iodine intake. For population estimates, daily iodine intake can be extrapolated from UI by assuming 90% of ingested iodine is found in urine and a 24-h urine volume of 1.5 L (73). Using this estimation, a UI of approx 140 μg/L would correspond to a daily intake of 200 μg iodine (74). During pregnancy this extrapolation may be less valid due to an increase in renal iodine clearance (75). WHO/UNICEF/ICCIDD has set the criteria of UIC assessment in school age children to be used to define the prevalence of iodine deficiency in populations. The population UIC cutoff values expressed as μg/L for school-age children are: <20 (severe deficiency), 20–49 (moderate deficiency), 50–99 (mild deficiency), 100–199 (optimum), 200–299 (risk of iodine-induced hypothyroidism), and ≥300 (risk of adverse health). The corresponding values for pregnancy are: <150 (insufficient), 150–249 (adequate), 250–499 (more than adequate), and ≥ 500 (in excess of amount to control deficiency) (72). Adequate iodine intake in school

children does not necessarily indicate iodine adequacy in pregnant women, and pregnant women should be directly monitored in countries with iodized salt programs. Recent evidence suggests that using the median UIC value from a population of school-age children as surrogate for other population groups might put pregnant women at risk of iodine deficiency.

What are the consequences of not getting enough iodine during pregnancy?

Delay in restoring euthyroid status in the developing neonate can lead to irreversible neurodevelopmental morbidity, which may be prevented by prompt thyroid hormone supplementation (76). Even with optimal replacement therapy, however, there are often residual and subtle neurodevelopmental sequelae persisting into childhood (77). At least 200 million children aged under 5 years fail to reach their potential in cognitive and socio-emotional development because of several causes, iodine deficiency being one of the most important factors (78). Maternal malnutrition during gestation impairs embryonic and fetal growth and development, resulting in deleterious outcomes, including iodine deficiency disorders (IDD) (79); the severity of IDD depending on the intakes. Cretinism, dwarfism, abortions, stillbirths, deaf mutism, spastic diplegia, squint are some of the common manifestations. In children, severe iodine deficiency may lead to mental retardation however mild-to-moderate iodine deficiency may also affect the language and hearing development. Children born in iodine deficient areas are at risk of neurological disorders and mental retardation because of the combined effects of maternal, fetal, and neonatal hypothyroxinaemia (80). The Copenhagen Consensus Conference 2008 ranked elimination of IDD as no. 3 in the 10 most cost effective development challenges in our world.

In 1994, a meta-analysis of 18 studies of children and adolescents concluded that IQ scores averaged 13.5 points lower with iodine

deficiency (81). In severely iodine deficient rural areas of Bangladesh, children with low T_4 levels performed less well than those with normal levels, in tests of reading, spelling and general cognitive ability, after controlling for a number of other factors that affected performance (82). A comparison of learning ability and motivation was carried out among 100 children, aged 9 to 15 years, in severely and mildly iodine deficient communities in Uttar Pradesh, India (83). It was found that the children from the severely iodine deficient villages were slow learners and also scored significantly lower on the achievement motivation scale as compared with children from mildly iodine deficient villages. Even lesser degrees of iodine deficiency, which affect many more individuals, can impair mental and motor function. In Papua New Guinea in 1976, the rate of fetal loss in late pregnancy or postnatal death in pregnant women with very low serum T_4 concentrations was twice that of those with higher concentrations (84), perhaps because thyroid hormones have a strong modulating effect on the immune system. However there is no data on mortality or cognition from the mild to moderate iodine deficient and iodine sufficient regions. Longitudinal studies measuring TSH levels and levels of both free thyroid hormones in pregnant and non-pregnant women from areas where iodine intake is adequate are scarce, usually include a small number of participants, and they seldom include data on UIC (85).

What are the consequences of getting too much iodine during pregnancy?

Most people who are iodine sufficient are remarkably tolerant to high dietary intakes of iodine, and intakes up to 1000 µg/d are well tolerated by healthy adults (73). There are concerns that more than adequate iodine intake could increase thyroid autoimmunity in adults (86) but findings are equivocal. There is no clear evidence to define “how much more iodine may become too much iodine” during pregnancy. A recommendation was adopted to indicate that there is no proven further benefit in providing pregnant women with more

than twice the daily RNI (recommended nutritional intake) (87). The safe upper limit in pregnancy is uncertain because the fetal thyroid is vulnerable to iodine excess (88). The fetal thyroid gland is exquisitely sensitive to the inhibitory effects of high iodine concentrations, and a recent study showed that inhibitory effects of high iodine loads could lead to opposite variations in maternal and neonatal thyroid function, i.e. with facilitation of thyroid function in the mother but aggravation in the neonate (89). Congenital hypothyroidism in newborns has been reported in mothers who had an excessive dietary intake of iodine during pregnancy (90, 91). Routine iodine supplementation in pregnancy may not be without risk (1). A recent study reported that iodine supplementation ≥150 μg/d in pregnancy was associated with poorer mental and psychomotor achievements of infants (92). However, the view that iodine supplements at the dose recommended for pregnancy (150–200 μg/d) are safe stems from the fact that these doses are well below the recommended upper intake limit of 600–1100 μg/d (73, 93).

What are the results from iodine supplementation during pregnancy?

It has been shown that iodine supplementation during pregnancy may result in a substantial reduction in foetal and neonatal deaths. In the Democratic Republic of Congo, injection of iodized oil in the second half of pregnancy reduced perinatal and infant mortality and improved birth weight (94). A randomized, placebo-controlled trial of oral iodized oil (100 mg iodine) in West Java, Indonesia, at about 6 weeks of age, on infant mortality up to 6 months of age showed a 72% reduction in risk of mortality in the iodized oil group during the first 2 months, and a delay in the mean time to death: 48 days compared to 17 days (95). A meta-analysis of studies conducted in China in 2005 showed that the average IQ loss from severe iodine deficiency was 12.5 IQ points in children and they recovered 8.7 IQ points with iodine supplementation or IS before and during

pregnancy (96). Controlled trials in which mothers were injected with iodine or placebo before or during pregnancy in areas where iodine deficiency was prevalent showed benefits on infant cognitive development in the Democratic Republic of Congo (97) and motor ability at age 10-11 years in Papua New Guinea (98). In an iodine deficient region in China, 4-7 year old children whose mothers were given iodine during pregnancy performed better on a psychomotor test than those who were supplemented beginning at 2 years of age (99). A Spanish trial found improved scores on both gross and fine motor coordination at 18 months if the supplements were given in early compared to late pregnancy (100). A recent review evaluated the efficacy and safety of iodine supplementation during pregnancy or the periconceptional period on the development and growth of children. Secondary outcomes included pregnancy outcome and thyroid function (1). The review highlighted that iodine supplementation during pregnancy or the periconceptional period in regions of severe iodine deficiency reduced risk of cretinism, but there were no improvements in childhood intelligence, development, growth, or pregnancy outcomes, although there was an improvement in some motor functions. None of the RCTs conducted in regions of mild to moderate iodine deficiency reported childhood development or growth or pregnancy outcomes. Effects of iodine supplementation on the thyroid function of mothers and their children were inconsistent (1). To date, there are no data from randomized controlled trials on the effect of maternal iodine supplementation on birth outcomes, and no data on long-term outcomes, such as maternal goiter, thyroid autoimmunity, or child development.

What measures can be taken to guarantee sufficient iodine intake during pregnancy?

Universal salt iodization (USI) is a mass fortification approach that is intended to cover the iodine requirements of all individuals in the population, placing greater emphasis on ensuring the increased needs of pregnant women. WHO/UNICEF/ICCIDD have estimated

that the iodine requirement during pregnancy is increased >50% compared to non-pregnancy in order to compensate for the increased need for thyroid hormones in the mother and fetus (101) and that an established USI program with adequate salt iodine levels and good population coverage can meet the high iodine requirement of pregnant women. In some countries, both dietary intakes of foods with high native iodine content and iodine supplements contribute to iodine intakes during pregnancy. For example, many Japanese eat seaweed and make soup stock from kelp on a daily basis, and in fact it is widely believed in the Japanese society that seaweed intake is good for pregnancy (102). In the U.S. diet, the common sources of iodine are iodized salt, dairy products, breads and seafood (103). American Thyroid Association (ATA) recommends that pregnant women in North America should supplement their diet with a daily oral supplement that contains 150 μg of iodine and also recommends that in areas of the world outside of North America, strategies for ensuring adequate iodine intake during pregnancy will vary according to regional dietary patterns and availability of iodized salt (104). In Australia, mandatory use of iodized salt (25-65 mg/kg) by bread manufacturers and a daily supplement intake of 150 μg of iodine by pregnant women are recommended by the National Health and Medical Research Council as the two strategies to achieve optimal iodine intakes in this group (105). Using predictive modelling, pregnant women in New Zealand were expected to achieve adequate iodine intakes when 150 μg of supplemental iodine was taken daily, taking into account the contribution of iodized salt in bread (106). Salt iodisation has been recognised as the most effective and cost-efficient strategy to prevent IDD in China and since 2012, China has adopted a new iodized salt standard of 25 or 30 mg/kg according to the actual situation of IDD in each province (107). Over 50 countries on the African continent have salt iodization programs, and 70% of all Africans have regular access to iodized salt (108). Published data in Germany suggest that during pregnancy more than half of the women practice supplementation of iodine (109).

Iodine nutrition scenario in India Vis-a-vis global prevalence of IDD

The global iodine status has improved markedly during the past decade (110, 111), but still 1.88 billion people of the global population are estimated to have insufficient iodine intake (110). About 41 million newborns a year remain unprotected from the enduring consequences of brain damage associated with iodine deficiency (112). **Fig 3** shows the latest global data on the prevalence of iodine deficiency. However, only a limited number of countries have completed UIC surveys in pregnant women and women of reproductive age at the national or sub-national level and thus, there are insufficient data to directly estimate the regional or global prevalence of low iodine intake in these important target groups (113). Southeast Asia has the largest population with inadequate iodine intake globally and 76 million school-aged children in the region have low iodine intake. In India, every year 8.1 million newborns and 8.9 million pregnant mothers are at risk of IDD due to insufficient iodine intake (114). USI remains the single most important source of dietary iodine for the Indian population (115). In the global prevalence data of IDD, India shows adequate iodine nutrition with a subnational UIC of 100-299 μg/L. However, data from subnational studies may not reflect regional differences very well. A review of available studies from India showed significant iodine deficiency in pregnant women (57) suggesting that UI monitoring of pregnant women be included as a vital component of the National program on control of IDD in India (116). It also highlighted the need to conduct national level representative surveys to better quantify the iodine nutrition of pregnant women in India (116). There are some previous data from the Bangalore region on iodine intake. The WHO Global database on iodine deficiency, 2007 reported that the median UIC in 11-18 y-old children in the Bangalore urban district was 185 μg/l (117). However, there are few data on the median UIC of pregnant women from Bangalore region.

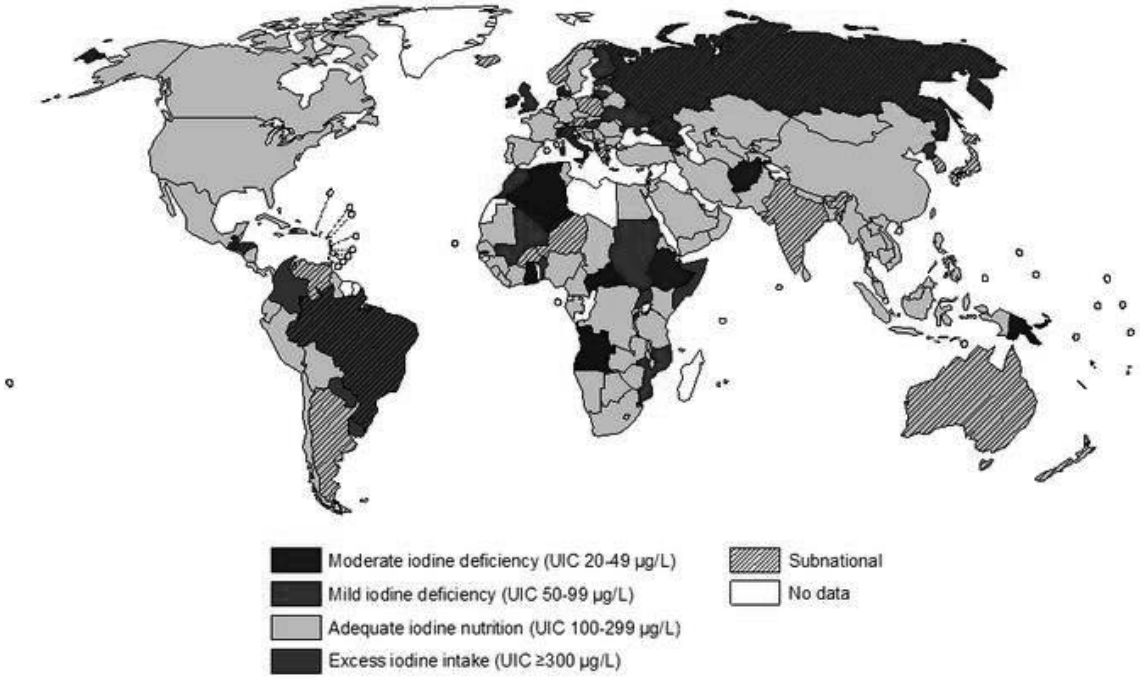

Figure 3: Nation iodine status based on median UIC in school-age children. Source: www.ign.org .

Rationale of this thesis

Pregnant women living in areas of mild to moderate iodine deficiency and iodine sufficiency may need extra iodine, but we do not know exactly the benefits and risks involved in such supplementation. There are no randomized controlled trials on iodine supplementation with long term follow up that have been conducted in this important group. This thesis therefore aimed to fill the important gap that exists in the current literature regarding the effect of iodine supplementation during pregnancy on birth outcomes, maternal goiter, thyroid autoimmunity, infant growth and cognitive development in an RCT. The current study is the first multicenter randomized controlled trial of iodine supplementation to pregnant women in areas of mild to moderate iodine deficiency (India and Thailand) to see the effects of Maternal Iodine supplementation (200 μg) or placebo on Thyroid function and Child development (MITCH study). In this thesis, only data from the Indian site are presented. We conducted the study at the antenatal clinic of the Obstetrics and Gynecology Department of St Martha's Hospital in Bangalore, India in accordance with the Declaration of Helsinki. Institutional ethical review boards at St. John's National Academy of Health Sciences, St Martha's Hospital, Bangalore, India and the Wageningen University, The Netherlands approved the study. We recruited pregnant women for the study between November 2008 to March 2011 and completed the data collection in November 2013. We recruited all women presenting to the antenatal clinic for potential inclusion in the study if they had a positive pregnancy test and were:

a) ≥18 and ≤40 years old

b) ≤14 weeks gestational age

c) Planning to reside in the study area for the duration of the study (3 years)

Exclusion criteria were:

a) Chronic diseases including diabetes, heart, kidney and thyroid disease, cancer, hypertension, tuberculosis, asthma, epilepsy, jaundice

b) A positive test for HIV, HbSAg or venereal diseases
c) TSH value outside the normal range (0.3- 7.5 mIU/l)
d) Treatment for infertility
e) Previous repeated spontaneous abortions (4 or more)
f) Current multiple pregnancy as detected by ultrasound
g) Currently breastfeeding.
We randomized the subjects into groups to receive either a daily iodine supplement of 200µg iodine (as potassium iodide tablets, donated by Merck KGsA, Darmstadt, Germany) until delivery or an identical placebo (Merck KGsA, Darmstadt, Germany) until delivery. We kept the codes in a sealed envelope with one of the members of the Data Safety and Monitoring Board; the codes were broken after the data analysis was completed.

We followed pregnant women at each trimester as per their antenatal visits at the hospital, obtained information on morbidity, performed anthropometry and thyroid gland size measurement and collected biological samples. Post-delivery, we followed mother-child pairs at 72 hrs, 6 weeks, 6 months, 1 and 2 years.

In **Chapter 2**, we report the results of the randomized controlled trial of iodine supplementation in pregnant women to determine whether the daily oral administration of iodine (200 µg) to pregnant women improves maternal and newborn thyroid function, pregnancy outcome, birth weight, infant growth and cognitive performance. We also investigate new indicators for assessing iodine status in pregnancy and infancy: neonatal TSH and newborn median urinary iodine concentration.

In **Chapter 3**, we report a pilot study which aimed to assess iodine intake (based on UIC) and potential determinants of intake, in Indian pregnant women and their school aged children who were sharing all meals. Our hypotheses were: 1) effective USI can ensure adequate iodine intake in pregnant women; 2) but this may lead to more-than-adequate or excessive iodine intake in their school aged children.

In C**hapter 4**, we aim to understand the pattern and prevalence of maternal thyroid dysfunction and its associated risk factors in the Indian pregnant population in a cross-sectional study, using the screening data of the randomized controlled trial. The aims were to a) measure thyroid status in first trimester pregnant women in southern India; and b) assess potential determinants of thyroid function in this population, including iodine status, thyroid autoimmunity, body weight and anemia.

In **Chapter 5**, we aim to assess the association of maternal depression, and thyroid function during pregnancy. We hypothesized that thyroid dysfunction would be associated with a higher prevalence of depressive symptoms.

In the concluding **Chapter 6,** we summarise the main findings of this thesis, and their implications for public health. We also discuss the methodological limitations and suggest directions for future research.

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Chapter 2

Effect of iodine supplementation during pregnancy on thyroid function and cognitive development of offspring

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Will be submitted for publication together with results from Bangkok trial

Abstract

Background: Children born to pregnant women in severely iodine deficient areas clearly benefit from maternal iodine supplementation, but there are no data from controlled trials in mildly iodine deficient pregnant women that have measured the effect of iodine supplementation on infant or child development.

Methods: In a randomized placebo controlled trial we recruited 318 healthy pregnant women (gestational age ≤14 weeks) in Bangalore, India and randomized them to either a daily supplement of 200 μg oral iodine or placebo from enrollment until delivery. Women were followed through delivery and followed up postnatal with their infants at 6 weeks, 1 and 2 year. Early neonatal development was assessed using the Neonatal Behavioral Assessment Scale (NBAS) at 6 weeks of age; neurocognitive assessment was done using the Bayley Scales of Infant Development (BSID III) at 1 and 2 years, and BRIEF-P (Behavior Rating Inventory of Executive Function) at 2 years.

Results: Median UIC at the third trimester was significantly higher in the intervention group (279 vs. 180 μg/L; p=0.0032). There were no significant differences between groups in maternal thyroid function tests, thyroid volume, subtypes of thyroid dysfunction, or anti-TPO antibodies during gestation and postpartum. There were also no significant differences between the infant groups in thyroid function, birth outcomes or UIC at any time point. Infants in the intervention group scored significantly better on the NBAS orientation cluster (8.1 vs. 7.1; p=0.018). There were no group differences in the BSID III unadjusted sub scales of cognitive, receptive and expressive communication, fine and gross motor development at 2 years. The BRIEF-P showed higher problem scores of inhibition in the placebo group (median (IQR): 21.0 (19.0, 24.0) as compared to the intervention group (median (IQR): 20.0 (18.0, 21.0) (p=0.028).

Conclusion: Iodine supplementation in iodine sufficient pregnant women is safe and does not increase the risk of thyroid autoimmunity. Although there were no significant beneficial effects of iodine supplementation on thyroid function and birth outcomes, neonates whose mothers received iodine supplementation tended to have better executive function at 2 years of age. Additional follow-up of these children for neurocognitive testing at a later age, when development and cognitive testing is more reliable, would provide valuable add on information.

Introduction

The sharp increase in the requirement for thyroid hormone and iodine during pregnancy constitutes a stimulus for both the maternal and fetal thyroid (1). By 10 weeks of gestation, the fetal thyroid is well-developed and begins concentrating iodine and producing thyroid hormone (2). In areas with a sufficient iodine intake, the maternal and fetal thyroid machinery adjusts to maintain euthyroidism in the mother and fetus (1). In contrast, in areas with severe iodine deficiency, goitre and hypothyroidism can occur in mother and newborn as a result of the inability of the thyroid gland to achieve euthyroidism due to low availability of iodine (3, 4). Mild degrees of iodine deficiency during pregnancy have been linked to maternal goitre and, in observational studies, to reduced intellectual function in children (5, 6). A recent observational study found that mild iodine deficiency during pregnancy has long-term adverse impacts on childhood neurocognition (7). In a population-based iodine sufficient cohort, maternal hypothyroxinemia in early pregnancy was associated with cognitive delay in children at 3 years of age (8). WHO/UNICEF/ICCIDD have recommended iodine intake for pregnant women to be 250 µg/ day in order to compensate for the increased need for thyroid hormones in the mother and fetus which can be achieved with an established salt iodization program (9). Expert organizations like the American Thyroid Association (ATA), European Thyroid Association (ETA), National Health and Medical Research Council (NHMRC) of Australia, The Endocrine Society, Asia and Oceania Thyroid Association, and the Latin American Thyroid Society recommend that pregnant women should take a daily oral supplement of 150 µg of iodine (10-12). However, iodine supplementation to pregnant women should be an interim measure in areas where universal salt iodization was not effective (13).

There is a wealth of literature showing the association between severe iodine nutrition and cognitive capacity in animal and human studies however this evidence base is not from the randomized controlled trials. Till date, 6RCTs have been conducted in regions of

mild to moderate iodine deficiency but none of them reported on childhood development or growth, or on long-term pregnancy outcomes. The effects of iodine supplementation on the thyroid function of mothers and their neonates were inconsistent (14). Thus, there are no long-term data from randomized controlled trials on the effect of iodine supplementation during pregnancy on birth outcomes or infant development in areas of mild deficiency (15). An observational study in the UK found associations between mild maternal iodine deficiency and impaired cognition in childhood (16). Maternal iodine supplementation may not be without risk: excessive maternal iodine intake impairs neurodevelopment and cognitive function in rat offspring (17) and iodine supplementation during pregnancy has been linked to poorer cognitive performance in Spanish infants (18, 19).

Clearly, more data are needed on the effects of iodine supplementation during pregnancy on infant development especially in regions with mild-to-moderate iodine deficiency. Therefore, we conducted a randomized controlled trial of iodine supplementation in pregnant women from Bangalore, India. The objectives were to determine whether the daily oral administration of 200 μg iodine to pregnant women in areas of mild-to-moderate iodine deficiency improves maternal and newborn thyroid function, pregnancy outcome, birth weight, infant growth and cognitive performance.

Methods

Study design and subjects

The MITCH (Maternal Iodine supplementation and its effects on Thyroid function and CHild Development) study with the identifier NCT00791466 at Clinicaltrials.gov employed a randomized, double blind, placebo-controlled design. We conducted the study at the antenatal clinic of the Obstetrics and Gynaecology Department of St Martha's Hospital in Bangalore, India. We recruited pregnant women for the study between November 2008 and March 2011 and

completed the data collection in November 2013. We recruited all women presenting to the antenatal clinic for potential inclusion in the study if they had a positive pregnancy test and were: a) ≥ 18 and ≤ 40 years old; b) ≤ 14 weeks gestational age and c) planning to reside in the study area for the duration of the study (3 years). Exclusion criteria were: a) chronic diseases including diabetes, heart, kidney and thyroid disease, cancer, hypertension, tuberculosis, asthma, epilepsy, jaundice; b) a positive test for HIV, hepatitis B or venereal diseases; c) TSH value outside the normal range (0.3- 7.5 mIU/l); d) treatment for infertility; e) previous repeated spontaneous abortions (4 or more); f) current multiple pregnancy as detected by ultrasound; or g) currently breastfeeding. Even though we stipulated the use of iodine supplements as exclusion criteria, there were no women who were using the only available iodine supplement in the market containing 150µg iodine during the recruitment period. At recruitment, we obtained data from a subset of pregnant women (n=218) on consumption of iodine containing multi-micronutrient powders, including quantity and length of time these supplements had been consumed. The pregnant women enrolled in the present trial were counselled by the obstetrician to discontinue the use of iodine containing multi-micronutrient powder and were prescribed another multi-micronutrient powder not containing iodine.

Ethics

We conducted the study in accordance with the Declaration of Helsinki. Institutional ethical review boards at St. John's National Academy of Health Sciences, St Martha's Hospital, Bangalore, India and Wageningen University, The Netherlands approved the study. We explained the study in detail, in the mother tongue of the participating women and one member of their family, and obtained written, witnessed informed consent. We prepared the informed consent form in English language and back translated the translated versions to check with the original form for any mismatch. The study participants were free to refrain from the study at any time. We reported study related serious adverse events (SAE) within 24 hours

to the Data and Safety Monitoring Board (DSMB). In addition, we reported periodically, the number of dropouts and SAEs both to the DSMB and the IERB of St John's National Academy of Health Sciences.

Recruitment, randomization and enrolment

We present the recruitment details in Fig 1. We enrolled 318 pregnant women and randomized them into groups of iodine intervention, (200µg iodine as potassium iodide (KI) or an identical placebo (donated by Merck KGsA, Darmstadt, Germany) using coding generated from a table of random numbers by the study statistician. The codes were kept in a sealed envelope by one of the members of the Data Safety and Monitoring Board and were broken after the data analysis was completed.

At recruitment, we carried out routine antenatal tests at the clinic where folic acid, iron, calcium supplements and tetanus toxoid injection were provided to the subjects as part of standard antenatal care. We handed over a box of 40 tablets (30 for the month and 10 extra to ensure that the regimen was not missed in case the next appointment was not exactly scheduled in 30 days) and a calendar to the pregnant women, counselled them on how to use the supplements, record the days that the supplements were consumed and to bring back the supplement box along with the calendar at monthly visits. Subjects received a reminder telephone call a day before the scheduled visit. We assessed the compliance with supplement use in 3 ways: (a) direct questioning; (b) capsule counting and c) measurement of urinary iodine as a marker of compliance with the iodine supplement. Compliance was calculated as the percentage of tablets consumed out of the total number of tablets expected to be consumed until each visit. We followed up women monthly during the pregnancy and stopped supplementation at delivery. At baseline and at each trimester as per their antenatal visits to the hospital, we obtained information on morbidity, measured weight and thyroid gland size and collected urine and blood samples. Post-delivery, we

followed mother-child pairs at 72 hrs, 6 weeks, 6 months, 1 and 2 years.

Socio-demography data

At baseline prior to randomization, we obtained socio-demographic information on household composition, education, occupation and income, on a structured multiple choice questionnaire, as well as consanguinity and obstetric history, including parity. We calculated gestational age from the reported first day of the last menstrual period and confirmed this by using ultrasonography in 65% of women. There was a strong positive correlation between the gestational age ascertained by the two methods ($r=0.858$; $p<0.001$).

Anthropometric measurements

We recorded anthropometric measurements in duplicate using standard techniques (20). We recorded maternal weight to the nearest 0.1 kg by using a digital weighing scale (Salter's 9016, Tonbridge, Kent, UK) and maternal height to the nearest 0.1 cm by using a stadiometer (Biorad, Chennai, India). We calculated BMI as weight in kilograms divided by the square of height in metres (kg/m^2). We obtained birth weight, length and head circumference from the hospital records. We measured infant weight to the nearest 0.01 kg by using an electronic baby and toddler scale (Salter's 914, Tonbridge, Kent, UK). We measured infant length to the nearest 0.1 cm by using a stadiometer and head circumference by a flexible non-stretchable measuring table. Low birth weight (LBW) was defined as birth weight <2500 g, and small-for-gestational age (SGA) was defined as birth weight less than the 10th percentile of gender norms for gestational age (20). We calculated Rohrer's ponderal index, as 100 times the birth weight (in grams) divided by the cube of the birth length (cm^3) (20).

Biological specimens

We collected a spot casual spot non-fasting urine samples that were transported on ice, divided into aliquots and stored at -20^0 C until analysis. We determined UIC by using the Pino modification of the Sandell-Kolthoff reaction (21) at the Human Nutrition Laboratory, ETH, Zurich, Switzerland. The laboratory participates successfully in the CDC EQUIP procedures. At a mean UIC of 68.8 ± 2.3 µg/L (acceptable range: 56-73 µg/L), the intra and inter-assay CV was 3.5 % and 3.3 % respectively. At a mean UIC of 200.7 ± 3.6 µg/L (acceptable range: 180-216 µg/L), the intra and inter-assay CV was 1.3 % and 1.8% respectively. We used the UIC data to classify iodine status according to the recommended WHO/UNICEF/ICCIDD criteria for pregnancy, lactation and infancy (9). We collected non-fasting whole blood samples by venipuncture into plain vacutainers (BD diagnostics, Franklin Lakes, New Jersey, USA) that were transported on ice and centrifuged to obtain serum. This was stored at -80^{0}C until analysis by immunoassay of thyroid stimulating hormone (TSH), total triiodothyronine (TT3), total thyroxine (TT4), free T3 (fT3), free T4 (fT4), thyroglobulin (Tg), and anti-thyroid peroxidise antibodies (TPO-Ab). We previously described in detail the immunoassay method used to measure the TFT's, including %CVs, reference range, analytical measurement range and analytical sensitivity (23). With the exception of TSH and TT4, we used the manufacturer's recommended reference range for TFT's. For TSH, we applied a reference range of 0.1-2.5 mIU/L for the first trimester, 0.2-3.0 mIU/L for the second trimester and 0.3-3.0 mIU/L for the third trimester (10, 24). For TT4, for all 3 trimesters, as per the CDC/ATA recommendation, we adjusted the non-pregnant reference range by a factor of 1.5 to arrive at a new reference range (87-241.5 nmol/L) (10, 25). We used these reference ranges for TSH and TT4 to identify thyroid dysfunction in pregnancy. Haemoglobin (Hb) was measured on an automated Coulter counter (ABX Pentra 60C+, Horriba Ltd, Kyoto, Japan). Anaemia was defined as Hb <11.0 g/dL.

We collected cord blood at delivery that was transported on ice and centrifuged to obtain serum, which was stored at -80^{0}C until analysis for TSH. We measured TSH by using a third generation solid phase,

two-site chemiluminescent assay (IMMULITE 1000, Siemens Healthcare diagnostics, USA) and applied a reference range as supplied by the manufacturer: >37wk, 2.3-13.2 mU/L. We collected heel prick sample from the infants at 72 hrs of birth into filter paper cards, dried them at room temperature, placed them into airtight plastic ziplock bags and stored them at -80^{0}C until analysis for TSH. We measured TSH and T4 concentrations using an automated time-resolved fluoroimmunoassay (TSH [DELFIA NeoTSH, PerkinElmer Life Sciences, Turku, Finland] and T4 [Delfia Neonatal T4 kit, PerkinElmer Life Sciences) respectively (26). We applied age-specific reference ranges for TSH and T4 as provided by the Children's Hospital, Zurich, Switzerland: Normal value range TSH: 0-7 d, 0.1-10.5 mU/L ; 7-21 d, 0.1-5.3 mU/L ; 21-60 d, 0.1-5.0 mU/L ; 60-155 d, 0.1-4.5 mU/L ; 155-365 d, 0.1-3.7 mU/L ; 1-99 y, 0.1-3.7 mU/L. Normal value range T4: 0-7 d, 114-245 nmol/L ; 7-21 d, 123-170 nmol/L ; 21-60 d, 84-170 nmol/L ; 60-155 d, 80-165 nmol/L;155-365d, 65-165 nmol/L ; 1-99 y, 65-165nmol/L.

Thyroid gland measurement

We measured thyroid gland volume by using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a 7.5MHz linear transducer. We calculated thyroid volume of each lobe using the formula for a prolate ellipsoid where thyroid volume (ml) = 0.479*length*breadth*depth (cm) and summed the volumes of both lobes to obtain total thyroid volume (9).

Neonatal behavioural assessment scale

We used Neonatal Behavioral Assessment Scale (NBAS) to assess the impact of maternal iodine supplementation on early neonatal development (27). A certified psychologist trained in the administration of NBAS followed standard procedures for scoring as described by Brazelton and Nugent (27). Scores were reduced to seven clusters as follows: habituation, orientation, motor performance, range of state, regulation of state, autonomic stability

and abnormal reflexes, as per the data reduction scheme described by Brazelton and Nugent (27). The missing values in a cluster were replaced by the individual mean cluster score; cases where >3 items were missing in a cluster were not included in the analysis.

Neurocognitive assessment

We administered Bayley's Scale for Infant Development (BSID III) to obtain standard scores for cognitive, language (receptive and expressive subtests) and motor (gross and fine subtests) scales (28). BSID III uses standardized administration and scoring procedures to provide the infant and toddler with situations and tasks that capture his or her interest and provide an observable set of behavioural responses. The cognitive scale assesses play skills, information processing, information processing speed, problem solving, and number concepts. The language scale assesses both receptive (ability to hear, understand, and respond) and expressive (ability to communicate) language skills and is important for identifying critical delays. The motor sub-test assesses quality of movement, sensory and perceptual motor integration, and basic locomotion milestones. Testing was performed by a trained master's level psychologist experienced in child developmental testing.

Assessment of executive functioning

Whereas cognition provides global insight of brain functioning, executive functioning represents different structures and functions of the brain involved in the cognitive regulation of behaviour (29). Executive function is defined as a group of processes, e.g., inhibition, working memory, and the ability to plan and organize, that are dependent on and influence more basic cognitive abilities, such as attention, language, and perception (30). We measured impairment of executive functioning in children at 2 years of age by using the BRIEF-P (31). The BRIEF-P is a standardized rating scale developed to provide a window into behaviours associated with specific domains of executive functioning in children aged 2 to 5 y. The BRIEF-P

consists of a single rating form, completed by parents or other caregivers, with 63 items in 5 scales: inhibition (to stop own behaviour), shifting (to make a transition and change focus from one mind set to another), emotional control (to modulate emotional responses), working memory (to hold information in mind for the purpose of completing a task), and planning/organization (to manage current and future-oriented task demands within the situational context). The scales can be combined into the global executive composite (GEC) but in our cohort of children, items on working memory were not applicable at 2 years of age hence GEC could not be computed. We present only the raw scores on the items of inhibition, shift, emotional control and planning/organization. Higher scores indicate more problems with executive functioning.

Data analysis

All study forms were checked for inconsistencies and the data were double entered into a structured query language database by two data entry operators. Discrepancies or mismatches in the data entry were corrected by a data entry supervisor. The matched data was locked, held by the study statistician and made available for analysis. Statistical analyses were carried out with SPSS (version18, SPSS, Chicago, IL, USA).

Primary outcome measures were maternal thyroid function. Secondary outcome measures were birth outcome, infant thyroid function, infant cognitive and motor development and maternal and infant urinary iodine. We estimated the sample size on an anticipated decrease in the prevalence of elevated newborn TSH (>10 mU/L at postnatal days 2-4) with iodine supplementation. The calculation indicated a sample size of 250 pregnant women (randomized into two groups of 125 women) would be needed to have 80% power to detect a decrease of 8% (from an anticipated incidence of 10% in the control to 2% with supplementation) in the proportion of elevated newborn TSH values with a significance level of 0.05 (two tailed). Before the study began, we anticipated a 10% loss to follow-up and thus the

sample size considered was 275; however as the study was progressing the loss to follow-up was identified to be ˜25% and therefore 318 pregnant women were recruited.

We report normally distributed data as arithmetic means ± SD, non-normally distributed data as medians (quartiles) and categorical data as numbers (%). We examined normal distribution of the data using Q-Q plots and Kolmogrov-Smirnov test. All the thyroid function parameters except total T4 were not normally distributed and were log-transformed except fT3 which was square transformed, and the transformed data were used for further statistical analysis. The normal distribution of the transformed data was verified. We compared basic socio-demographic variables and all thyroid function parameters at all three trimesters between the treatment groups (intervention vs placebo) using t-test for normally distributed data, Mann-Whitney U test for non-normally distributed data and chi-square test for categorical data. Mixed linear analyses were performed for each maternal thyroid function parameter to examine the effect of the intervention on change in these measures during pregnancy across the three trimesters. These were intention to treat analyses. Initially, we created a simple model which only considered treatment group. In the next step, we considered the gestational age at entry into the study as an important covariate since the mean gestational age at entry was early, at 10 weeks (range 5.0-14.6 weeks) and it is known that the earlier supplementation starts, the better. We created a variable “duration of treatment” (Gestational age at trimester 1, 2, or 3 minus gestational age at trimester 1) to quantify the duration (in weeks) the subjects were in the study at the time of each blood draw for thyroid function test. This model considered “duration of treatment” as a covariate and the difference in slope of thyroid function parameters with “duration of treatment” between the intervention groups was considered as a measure of efficacy of the intervention obtained by the interaction with treatment group, henceforth referred to as “interaction term” which is guiding the interpretation of the study outcomes. Further, we considered percentage compliance percentage compliance based on pill counts

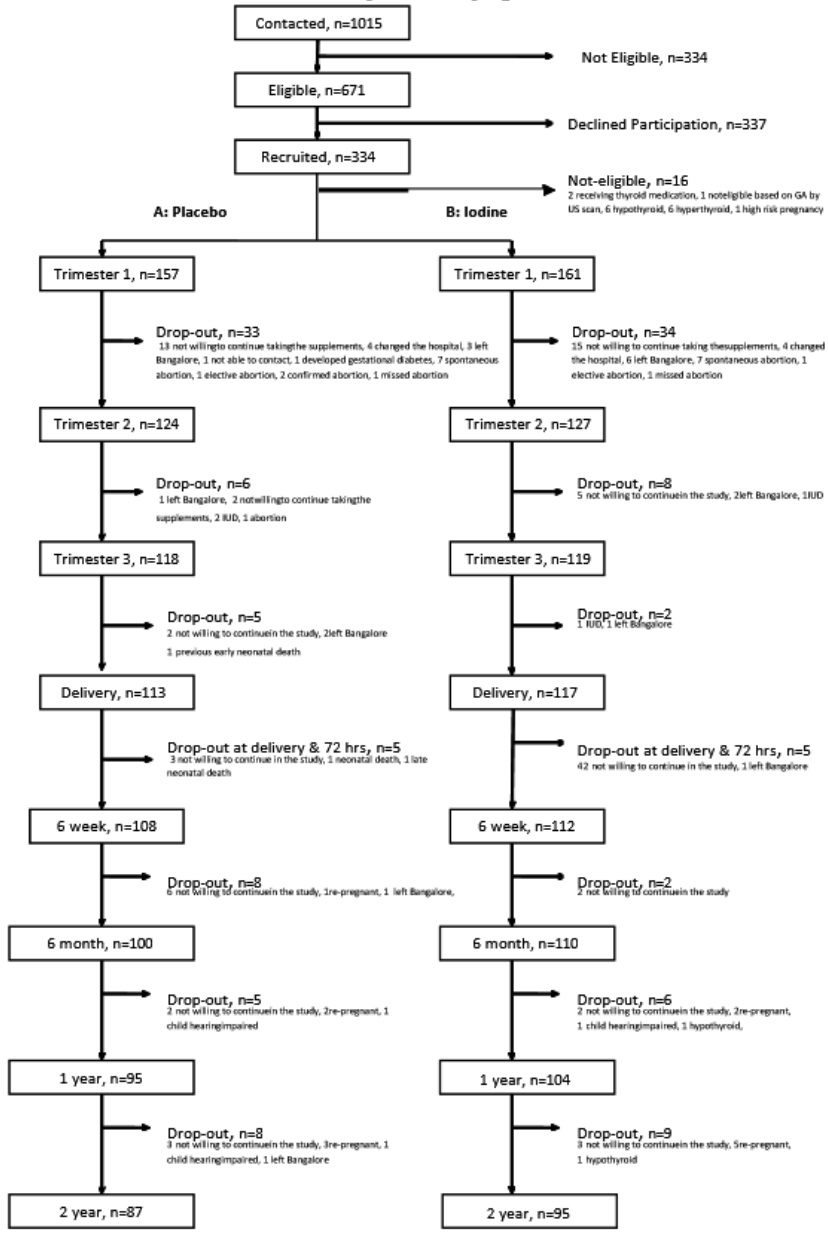
as a covariate in addition to the factors in the second model to adjust for the effect of compliance to intervention/placebo. Compliance to intervention was significantly associated with most thyroid function parameters, but did not modify the interaction between duration of treatment and treatment group. All these models included additional covariates of BMI, parity, baseline gestational age and maternal age. These possible confounders were identified in separate bivariate analyses with the thyroid function parameters. We repeated these analyses in a per-protocol population for thyroid function tests. The per-protocol population was defined as those subjects who were part of the study throughout their pregnancy with their thyroid hormones and antibodies and thyroid gland volume measured. The intervention group was considered as fixed effects and subject as random effect in all the models. The appropriateness of the models was examined using residual plots. Sensitivity analyses were performed by excluding outliers. However results from the original analyses did not change and therefore the analyses including outliers are presented. At delivery, we assessed the difference in TFT measures, UICs, neonatal characteristics and developmental data between the two groups using linear regression analysis with treatment group as independent variable, adjusted for possible confounding effects of maternal characteristics, such as gestational age at delivery, gender, parity, and maternal height. At postnatal maternal time points (6 weeks, 6 months, 1 and 2 year), we assessed the difference in TFT measures by mixed linear model analysis and present the p values for the treatment group. We compared all children's thyroid function parameters, anthropometry data and neurocognitive assessment data between codes using t-test for normally distributed data, and Mann-Whitney U test for non-normally distributed data. Statistical significance was considered at $p < 0.05$ for all analyses.

Results

There were no statistically significant differences in age, gestational age, education, occupation, monthly household income, height, weight or BMI between those who declined participation and those

who agreed to participate in the study (data not shown). Figure 1 is a flow diagram of recruitment, enrolment and follow-up of pregnant women in the RCT. There was a 21% dropout each in the intervention and placebo group from the study entry to second trimester. This dropout rate increased to 26.1 and 24.8% at the third trimester, 27.3 and 28.0% until delivery, 30.4% (n=112) and 31.2% (n=108) at delivery and 72 hrs in the intervention and placebo group respectively thus 30.4% and 31.2% dropout at 6 weeks. At 6 months, 1 year and 2 year, the dropout rate increased to 31.7%, 35.2%, 40.9% and 36.3%, 39.4% and 44.6% in the intervention and placebo group respectively. Thus, there was an overall 43% dropout in the number of participants who were available for assessments at 2 years (n=95 and n=87 in the intervention and placebo group respectively)

Figure 1: Recruitment, enrolment and follow up of Indian pregnant women in the MITCH study.



The baseline characteristics of 318 pregnant women recruited in the study are in Table 1. There were no significant differences between the two groups in any of the baseline characteristics. The gestational age at recruitment was 10.4±2.5 weeks (range 5.0-14.6 weeks) and the prevalence of anemia was 19% (Hb <11 g/dL).

Thirty four percent of pregnant women were consuming multi-micronutrient powders containing iodine in the range of 73-294 µg/100 g. But the mean (range) iodine intake from these powders was low (5-10g/day). The iodine content in these powders ranged from 73-294 µg/100 g. In the intervention group, the mean (range) iodine intake was: 12.4 (3.6-41.7) µg/day for mean (range) duration of 9.5 (0-180) months. This was not statistically significantly different in the placebo group; the mean (range) iodine intake was: 11.5 (3.5-29.8) µg/day for a mean (range) duration of 11.3 (0-180) months. The median (IQR) UIC values between pregnant women using multi-micronutrient powders containing iodine (n=70) was 180.5 (92.1, 303.8) vs 216.5 (110.9, 388.1) in non-users (n=140) at study entry and did not differ significantly (p=0.102).

Table1: Baseline demographic, anthropometric and biochemical characteristics of Indian pregnant women given 200 µg iodine daily or placebo[1]

Parameters	**n**	***Intervention***	**n**	***Placebo***	***p*** [4]
Age, years[1]	161	24.8 ± 4.1	157	24.6 ± 4.1	0.751
Gestational age, weeks[1]	161	10.5 ± 2.4	157	10.2 ± 2.5	0.376
Total monthly household income, INR[2]	161	12000 (7125, 20000)	157	12000 (8000, 24500)	0.943
Level of education[3]	160		155		0.459
No formal education		-		1 (0.6)	
Finished high school		72 (45.0)		60 (38.7)	
Post high school		35 (21.9)		43 (27.7)	
University degree & above		53 (33.2)		51 (32.9)	
Consanguinity[3]	160		155		0.665
Yes		23 (14.4)		25 (16.1)	
No		137 (85.6)		130 (83.9)	
Parity[3]					
0		105 (69.1)		99 (65.1)	0.726
1		45 (29.6)		50 (32.9)	
2		2 (1.3)		3 (2.0)	
History of abortion[3]	152		152		0.450
0		118 (77.6)		126 (82.9)	
≥1		34 (22.3)		26 (17.1)	
Use of I supplements[3]	112		106		0.887
Yes		38 (33.9)		35 (33.0)	
No		74 (66.1)		71 (67.0)	
Weight, kg[1]	160	52.2 ± 9.0	156	53.8 ± 9.2	0.125
Height,cm[1]	160	153.8 ± 5.8	154	154.4 ± 5.6	0.354
BMI, kg/m^2 [1]	160	22.1 ± 3.9	154	22.6 ± 3.9	0.302
Haemoglobin, g/dL [1]	156	11.9 ± 1.4	154	11.9 ± 1.4	0.887
Anemia	156	30 (19.2)	154	29 (18.8)	0.929

[1] Data are mean ± S.D., [2] median (Quartile 1, Quartile 3) and [3] n (%), [4] p values are from t-test for normally distributed data, Mann-Whitney U-test for non-normally distributed data, and chi-square test for categorical data.

Table 2 presents the urinary iodine concentration values and their distribution between the two groups at first, second and third trimester by intention-to-treat analysis. We present p values for treatment group, duration of treatment and interaction of treatment group with duration of treatment. The median (range) UIC of all the women in first trimester was 187.5 (8.1-1152.2) μg/L and was not significantly different between the intervention and placebo groups (p=0.603). In the intervention group, UIC increased by 18.8% and 35.5% in the second and third trimester of pregnancy while in the placebo group, there was no significant change in UIC at the second or third trimester (Figure 2). The median UIC at the second and third trimester was significantly higher in the intervention group (p=0.048 and 0.003, respectively). In a mixed linear model analysis, the overall UIC tended to be 3.2% higher in the intervention group (p=0.750), while there was no significant effect of duration of treatment (p=0.892) and a borderline significant effect of the interaction between duration of supplementation and treatment group (Table 4). The results from the per-protocol analysis (Supplementary Table 1) were comparable to the intention-to-treat analysis.

Table 2: Urinary iodine concentrations (µg/L) of Indian pregnant women given 200 µg iodine daily or placebo[1,2,3,4] (intention-to treat analysis)

UIC	***Baseline (10.4 ± 2.5 weeks)***		***Trim 2 (24.4 ± 1.5 weeks)***		***Trim 3 (32.9 ± 1.3 weeks)***			
	Intervention	***Placebo***	***Intervention***	***Placebo***	***Intervention***	***Placebo***	***% DCT***	***p***
	(n=161)	(n=157)	(n=127)	(n=124)	(n=119)	(n=118)		
	206.2	173.2	245.0	209.7	279.5	180.4	3.18 ↑	0.750[2]
	(97.3, 336.6)	(96.9, 349.8)	(120.7, 439)	(96.6, 323)	(131.7, 426.6)	(98.1, 315.0)	0.06 ↑	0.892[3]
							1.21 ↑	0.072[4]
> 500	13 (8.3)	12 (8.1)	26 (21.0)	10 (8.2)	21 (19.3)	12 (10.5)		
150 - 500	83 (53.2)	71 (47.7)	58 (46.8)	71 (58.2)	58 (53.2)	55 (48.2)		
100 - 149	21 (13.5)	26 (17.4)	17 (13.7)	10 (8.2)	12 (11.0)	18 (15.8)		
50 -99	23 (14.7)	27 (18.1)	15 (12.1)	18 (14.8)	13 (11.9)	18 (15.8)		
< 50	16 (10.3)	13 (8.7)	8 (6.5)	13 (10.7)	5 (4.6)	11 (9.6)		

[1]median (Quartile 1, Quartile 3) or n (%), % DCT: % difference in change over time between the slopes of two groups, p value per intention-to-treat using linear mixed models of log transformed UIC adjusted for code, BMI, parity, baseline gestational age, maternal age, time from start, interaction effect of treatment and time from start. p values are for code [2], time from start [3], interaction effect of treatment and time from start [4]

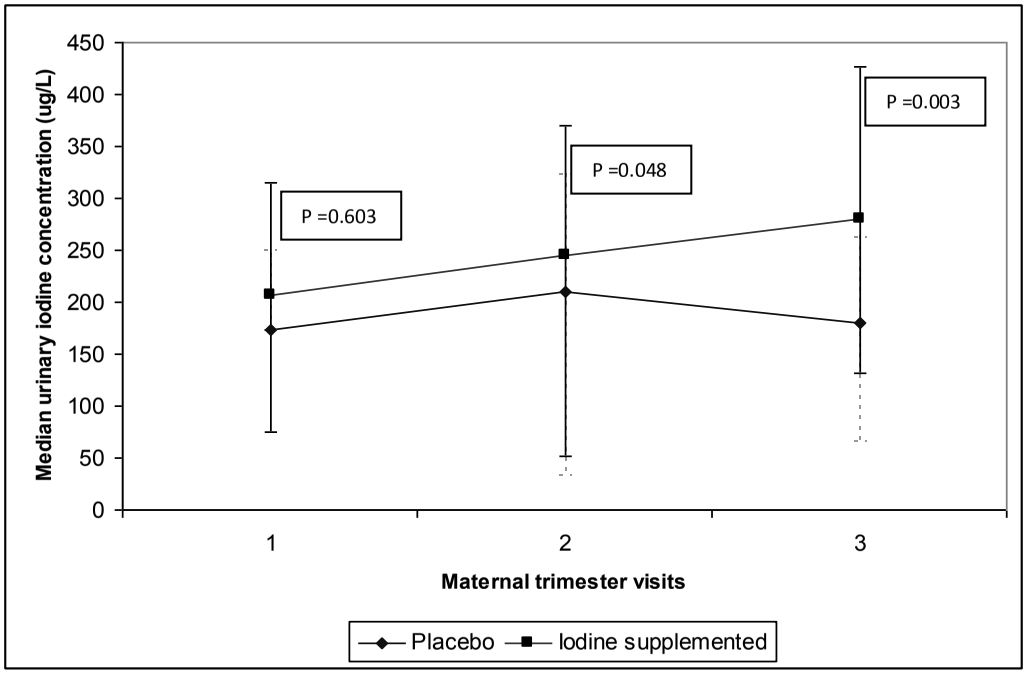


Figure 2: Effects of iodine supplementation (200µg) vs placebo in Indian pregnant women on the median urinary iodine concentration (µg/L). Data are median and the solid and dashed error bars are median ± lower/upper quartile for the intervention and placebo group respectively

Supplementary Table 1: Urinary iodine concentrations (µg/L) of Indian pregnant women given 200 µg iodine daily or placebo[1,2,3] (per protocol analysis)

UIC	***Baseline (10.4 ± 2.5 weeks)***			***Trim 2 (24.4 ± 1.5 weeks)***			***Trim 3 (32.9 ± 1.3 weeks)***		
	Intervention	***Placebo***	***p***	***Intervention***	***Placebo***	***p***	***Intervention***	***Placebo***	***p***
	(n=91)	(n=93)		(n=94)	(n=98)		(n=92)	(n=97)	
	211.7 (101.4, 339.6)	170.8 (91.9, 356.4)	0.488[2]	265.6 (128.7, 501)	209.7 (94.5, 333.1)	**0.019**[2]	284.3 (145.4, 474.8)	174.5 (89.9, 320.6)	**0.002**[2]
> 500	5 (5.5)	9 (9.7)	0.523[3]	24 (25.5)	10 (10.2)	**0.024**[3]	20 (21.7)	11 (11.3)	0.076[3]
150 - 500	52 (57.1)	43 (46.2)		43 (45.7)	55 (56.1)		48 (52.2)	44 (45.4)	
100 - 149	12 (13.2)	13 (14.0)		12 (12.8)	8 (8.2)		8 (8.7)	16 (16.5)	
50 -99	12 (13.2)	18 (19.4)		10 (10.6)	13 (13.3)		12 (13.0)	16 (16.5)	
< 50	10 (10.5)	10 (10.8)		5 (5.3)	12 (12.2)		4 (4.3)	10 (10.3)	

[1]median (Quartile 1, Quartile 3) or n (%), P[2] value for treatment groups by Mann Whitney U test, P [3] value for treatment groups by categories by Pearson's chi-square

The thyroid function and thyroid volume of the two groups at baseline, second and third trimesters is shown in Table 3, analysed by intention-to-treat analysis. We present p values for treatment group, duration of treatment and interaction of treatment group with duration of treatment. The thyroid function parameters were not significantly different between the intervention and placebo groups except baseline TSH (p=0.015) and fT4 (p=0.021). In a mixed linear model analysis, the overall TSH was 5.7% lower in the intervention group but not significant (p=0.159). There was a significant 0.5% increase in TSH for every week in the study (p<0.001) in both groups. However this increase was not different between the groups ($p_{interaction}$=0.986). The percentage of women with elevated TSH levels decreased over pregnancy in both the groups. At baseline, fT4 was significantly higher in the intervention group and was comparable between the groups in the second and third trimesters. In a mixed linear model analysis, fT4 and TT4 were 3% higher in the intervention group (p=0.006 and p=0.049 respectively). Overall, fT4 decreased from baseline by 0.34% for every week in the study but there was no significant difference in this decline between the groups ($p_{interaction}$=0.070). ($p_{interaction}$ =0.070). The percentage of women with low fT4 concentrations increased 5 and 8 fold in the second and third trimester from baseline in the intervention group. A similar trend was also seen in the placebo group. However, prevalence of low titres of TT4 was quite low for TT4 (0.8-2.9%) in the second and third trimester for both the groups. FT3 and TT3 increased significantly every week (0.3%,p=0.011); 1.32%, p<0.001) respectively during pregnancy but there were no significant differences between groups and interaction effect. While a 5 fold increase in the higher TT3 reference group from baseline was observed in both the groups at the second and third trimester, fT3 did not show similar change. Thyroglobulin was 10.6% lower in the intervention group after treatment though not significant (p=0.367). There was a significant 0.6% increase in thyroglobulin for every week in the study (p=0.009) however this increase was not different between the groups ($p_{interaction}$=0.739). TT3/TT4 ratio and FT3/TT3 ratio increased significantly each week (0.01%, p<0.001); 1.1%, p<0.001) respectively

during pregnancy but there was no significant difference between the treatment group and time from start*group interaction. The thyroid volume of the pregnant women increased systematically through the second and third trimester in the intervention group (5.0 vs 5.2 vs 5.8) and placebo group (5.0 vs 5.3 vs 5.6) There was a significant 0.5% increase in the thyroid gland volume for every week in the study ($p<0.001$) without treatment or interaction effects. In the per-protocol analysis (Supplementary Table 2), all the results were comparable to the intention-to-treat analysis except that TSH and fT4 were not significantly different between the two groups at baseline.

Table 3: Thyroid function and thyroid volume of Indian pregnant women given 200µg iodine daily or placebo[1,2,3,4] (intention-to-treat analysis).

TFT	*Baseline (10.4 ± 2.5 weeks)*		*Trimester 2 (24.4 ± 1.5 weeks)*		*Trimester 3 (32.9 ± 1.3 weeks)*			
	Intervention	***Placebo***	***Intervention***	***Placebo***	***Intervention***	***Placebo***	***% DCT***	***p***
	(n=161)	(n=157)	(n=127)	(n=124)	(n=119)	(n=118)		
TSH, mIU/L	1.1 (0.7, 1.6)	1.3 (0.9, 1.8)	1.5 (1.1, 2.0)	1.6 (1.1, 2.2)	1.6 (1.0, 2.0)	1.6 (1.2, 2.2)	5.7 ↓	0.159[2]
Low[2]	4 (2.5)	3 (1.9)	-	1 (0.8)	-	-	0.51 ↑	<0.001[3]
Elevated[2]	18 (11.2)	19 (12.1)	7 (6.2)	9 (7.6)	5 (4.8)	10 (9.7)	0.01 ↑	0.986[4]
fT4, ng/dL	1.13 (1.03, 1.24)	1.09 (0.98, 1.19)	0.94 (0.88, 1.01)	0.93 (0.84, 1.0)	0.93 (0.86, 1.03)	0.95 (0.86, 1.04)	2.98 ↑	0.006[2]
<0.89	8 (5.1)	13 (8.5)	30 (26.3)	48 (40.3)	42 (40.4)	34 (33.0)	0.34 ↓	<0.001[3]
>1.76	3 (1.9)	-	-	-	-	-	0.09 ↓	0.070[4]
TT4, nmol/L	148.0 (120,174)	142.8 (118, 164)	159.6 (142, 180)	158.3 (134, 180)	161.5 (139,184)	160.9 (139,180.2)	3.0 ↑	0.049[2]
<87	3 (1.9)	9 (6.0)	2 (1.8)	1 (0.8)	3 (2.9)	2 (1.9)		<0.001[3]
>241.5	1 (0.6)	-	-	-	-	2 (1.9)		0.243[4]
fT3, pg/mL	3.81 (3.26, 4.41)	3.68 (3.23, 4.22)	3.97 (3.51, 4.35)	4.04 (3.47,4.58)	4.0 (3.49, 4.47)	3.95 (3.32,4.53)	3.3 ↑	0.204[2]
<2.6	9 (5.9)	11 (7.5)	6 (5.3)	6 (5.0)	3 (3.0)	5 (5.0)	0.30 ↑	0.011[3]
> 4.8	18 (11.8)	13 (8.8)	14 (12.3)	23 (19.3)	13 (12.9)	14 (13.9)	0.11 ↓	0.508[4]

TFT	Baseline (10.4 ± 2.5 weeks)		Trimester 2 (24.4 ± 1.5 weeks)		Trimester 3 (32.9 ± 1.3 weeks)			
	Intervention	Placebo	Intervention	Placebo	Intervention	Placebo	% DCT	p
TT3, nmol/L	2.2 (1.9, 2.7)	2.1 (1.8, 2.6)	2.7 (2.4, 3.3)	2.7 (2.4, 3.2)	2.8 (2.4, 3.2)	2.9 (2.4, 3.2)	4.23 ↑	0.114[2]
< 1.1	-	-	-	1 (0.8)	2 (2.0)	1 (1.0)	1.32 ↑	0.000[3]
> 3.1	10 (6.7)	9 (6.1)	40 (35.4)	41 (34.7)	30 (30.6)	32 (31.4)	0.09 ↓	0.635[4]
Tg, µg/L	6.2 (2.6, 10.8)	7.1 (3.0, 12.4)	6.0 (3.1, 11.2)	7.3(3.7, 11.6)	6.9(3.6, 10.2)	7.0 (4,11.4)	10.58 ↓	0.367[2]
> 55.6	-	2 (1.3)	1 (0.9)	3 (2.5)	1 (1.0)	3 (3.0)	0.57 ↑	0.009[3]
TT3/TT4 ratio	0.0157 (0.0134, 0.0184)	0.0158 (0.0135, 0.0178)	0.0173 (0.0153, 0.0198)	0.0175 (0.0155, 0.0199)	0.0161 (0.0068, 0.0195)	0.0168 (0.0133, 0.0199)	0.02 ↑	0.615[2]
							0.01 ↑	<0.001[3]
							0.01 ↓	0.431[4]
fT3/fT4 ratio	3.4014 (2.7203, 4.0073)	3.4020 (2.8913, 3.9909)	4.1301 (3.5806, 4.8038)	4.4552 (3.6836, 5.1706)	4.1554 (3.4770, 4.8828)	4.1299 (3.4796, 4.8613)	3.69 ↓	0.297[2]
							1.07 ↑	<0.001[3]
							0.15 ↓	0.470[4]
Thyroid vol, ml	5.0 (4.3, 5.9)	5.0 (4.3, 6.0)	5.2 (4.5, 6.3)	5.3 (4.4, 6.5)	5.8 (4.7, 6.9)	5.6 (4.8, 7.0)	0.94 ↑	0.761[2]
							0.54 ↑	<0.001[3]
							0.03 ↓	0.806[4]

[1] median (Quartile 1, Quartile 3) or n (%), [2] trimester-specific reference ranges (1st=0.1-2.5 mIU/L, 2nd=0.2-3.0 mIU/L, 3rd=0.3-3.0 mIU/L), % DCT: % difference in change over time between the slopes of two groups, p value per intention-to-treat using linear mixed models of log transformed thyroid function adjusted for code, BMI, parity, baseline gestational age, maternal age, time from start, interaction effect of treatment and time from start. p values are for code [2], time from start [3], interaction effect of treatment and time from start [4]

Supplementary Table 2: Thyroid function and thyroid volume of Indian pregnant women given 200µg iodine daily or placebo[1,2,3,4,5] (per protocol analysis)

TFT	*Baseline (10.4 ± 2.5 weeks)*			*Trimester 2 (24.4 ± 1.5 weeks)*			*Trimester 3 (32.9 ± 1.3 weeks)*		
	Intervention (n=95)	***Placebo (n=98)***	***p***	***Intervention (n=95)***	***Placebo (n=98)***	***p***	***Intervention (n=95)***	***Placebo (n=98)***	***p***
TSH, mIU/L	1.1 (0.7, 1.7)	1.3 (0.8, 1.7)	0.079[3]	1.5 (1.2, 2.0)	1.5 (1.1, .2)	0.741[3]	1.6 (1.0, 2.0)	1.6 1.2,2.3)	0.198[3]
Low[2]	2 (2.1)	3 (3.1)	0.932[5]	-	1 (1.0)	0.439[5]	-	-	NC[5]
Elevated[2]	8 (8.4)	11 (11.2)	0.729[4]	5 (5.3)	8 (8.2)	0.447[4]	5 (5.3)	10 (10.2)	0.200[4]
fT4, ng/dL	1.12 (1.03, 1.25)	1.12 (0.9, 1.2)	0.610[3]	0.94 (0.89, 1.0)	0.94 (0.85, 1.0)	0.486[3]	0.93 (0.86, 1.04)	0.95 (0.87, 1.04)	0.576[3]
<0.89	5 (5.4)	7 (7.4)	0.517[4]	26 (27.4)	36 (36.7)	0.164[4]	37 (39.4)	30 (30.6)	0.204[4]
>1.76	1 (1.1)	-	0.261[5]	-	-	NC[5]	-	-	NC[5]
TT4, nmol/L	149.9 (123,172)	149.3 (124, 169)	0.789[3]	158.3 (140, 180)	156.4 (129, 177)	0.418[3]	160.9 (137,184)	160.9 (143, 80.2)	0.875[3]
<87	1 (1.1)	4 (4.3)	0.247[4]	1 (1.1)	1 (1.0)	0.982[4]	3 (3.2)	2 (2.0)	0.339[4]
>241.5	1 (1.1)	-	0.121[5]	-	-	NC[5]	-	2 (2.0)	0.147[5]
fT3, pg/mL	3.68 (3.1, 4.4)	3.8 (3.2, 4.2)	0.890[3]	4.0 (3.6, 4.4)	3.9 (3.4, 4.5)	0.942[3]	4.0 (3.5, 4.5)	3.95 (3.3, 4.5)	0.933[3]
<2.6	4 (4.3)	5 (5.4)	0.919[4]	5 (5.3)	6 (6.1)	0.710[4]	3 (3.3)	5 (5.2)	0.804[4]
>4.8	9 (9.8)	8 (8.7)	0.680[5]	11 (11.6)	15 (15.3)	0.860[5]	11 (12.1)	12 (12.5)	0.613[5]
TT3, nmol/L	2.3 (1.9, 2.7)	2.2 (1.9, 2.7)	0.464[3]	2.8 (2.4, 3.3)	2.7 (2.3,3.2)	0.530[3]	2.8 (2.4, 3.2)	2.9(2.4,3.2)	0.745[3]
<1.1	-	-	0.504[4]	-	1 (1.0)	0.524[4]	1 (1.1)	1 (1.0)	0.967[4]
>3.1	6 (6.6)	4 (4.3)	NC[5]	34 (36.2)	31 (32.0)	0.299[5]	26 (29.2)	30 (30.9)	0.921[5]
Tg, µg/L	5.4 (2.6,10.5)	6.7 (3.2,11.1)	0.297[3]	5.9 (3.0, 9.1)	7.2 (3.8,11.0)	0.196[3]	6.9 (3.6,10.3)	7.2 (4.2, 11.6)	0.259[3]

TFT	Baseline (10.4 ± 2.5 weeks)			Trimester 2 (24.4 ± 1.5 weeks)			Trimester 3 (32.9 ± 1.3 weeks)		
	Intervention (n=95)	Placebo (n=98)	p	Intervention (n=95)	Placebo (n=98)	p	Intervention (n=95)	Placebo (n=98)	p
Tg, µg/L									
>55.6	-	1 (1.1)	0.324[5]	1 (1.1)	2 (2.0)	0.579[5]	1 (1.0)	3 (3.1)	0.333[5]
TT3/TT4 ratio	0.0158 (0.0137, 0.0184)	0.0158 (0.0136, 0.0175)	0.500[3]	0.0173 (0.0154, 0.0196)	0.0172 (0.0155, 0.0198)	0.777[3]	0.0174 (0.0154, 0.0205)	0.0174 (0.0152, 0.0203)	0.726[3]
fT3/fT4 ratio	3.2017 (2.6358, 3.9783)	3.3333 (2.8228, 3.9666)	0.574[3]	4.1365 (3.5875, 4.8038)	4.1888 (3.5986, 5.1479)	0.459[3]	4.0732 (3.4237, 4.8255)	4.0333 (3.4646, 4.7405)	0.680[3]
Thyroid vol, ml	4.98 (4.3, 5.9)	5.14 (4.2, 6.1)	0.763[3]	5.3 (4.6, 6.3)	5.1 (4.3, 6.5)	0.415[3]	5.9 (4.7, 6.9)	5.6 (4.6, 6.9)	0.562[3]
TPO-Ab (IU/mL)									
≤35	83 (88.3)	83 (85.6)	0.576[5]	83 (87.4)	89 (90.8)	0.442[5]	82 (86.3)	89 (90.8)	0.325[5]
≥35	11 (11.7)	14 (14.4)		12 (12.6)	9 (9.2)		13 (13.7)	9 (9.2)	

[1] median (Quartile 1, Quartile 3) or n (%), [2] trimester-specific reference ranges (1st=0.1-2.5 mIU/L, 2nd=0.2-3.0 mIU/L, 3rd=0.3-3.0 mIU/L), P value for treatment groups by Mann Whitney U test [3], by categories of elevated, normal and low TFT (Pearson's chi-square) [4], by categories of elevated and low TFT (Pearson's chi-square) [5]

Fig 3 presents the prevalence of thyroid dysfunction in the two groups across the antenatal and postnatal time points. There were no cases of overt hyperthyroidism during pregnancy and at 1 and 2 years after delivery. Some cases were reported at 6 wks: 3 (3.2%) vs 2 (2.4%) and 6 months: 3 (3.9%) vs 1 (1.3%) in the intervention and placebo group respectively. In the intervention group, the prevalence of subclinical hyperthyroidism was 2.6%, in the first trimester, absent in the second and third trimester, 4.3% at 6 wks, 5.3% at 6 months and then absent in year 1 and 2. In the placebo group, the prevalence of subclinical hyperthyroidism was 1.3% in the first trimester, 0.8% in the second trimester, absent in the third trimester and 6 weeks, 3.9% at 6 months and then absent in year 1 and 2. The prevalence of overt hypothyroidism was 1.93 vs 2.65 % at baseline, 1.8 vs 0% at second trimester, absent in the third trimester and 6 wks, 3.9 vs 3.9% at 6 months, 1.4 vs 1.6% at 1 year and absent at 2 year in the intervention and placebo group respectively. The prevalence of subclinical hypothyroidism was 7.7 vs 9.9% in the first trimester, 4.4 vs 7.6% in the second trimester, 4.8 vs 8.7% in the third trimester, absent at 6 wks, 10.5 vs 2.6% at 6 months, 7.0 vs 4.8% at 1 year and 10.3 vs 12.8% at 2 year in the intervention and placebo group respectively. In the intervention and placebo group respectively, the prevalence of isolated hypothyroxinemia was 0 vs 3.3% in the first trimester, 0.9 vs 0.8% in the second trimester, 2.9 vs 1.9% in the third trimester, 8.5 vs 17.1% at 6 wks, 1.3 vs 9.1 % at 6 months, 15.5 vs 11.1% at 1 year and 2.6 vs 2.6% at 2 year. During pregnancy, there was no significant difference between groups in prevalence of overt or subclinical hypothyroidism or isolated hypothyroxinemia. There were no differences when a summary variable of these three conditions was created and compared among groups.

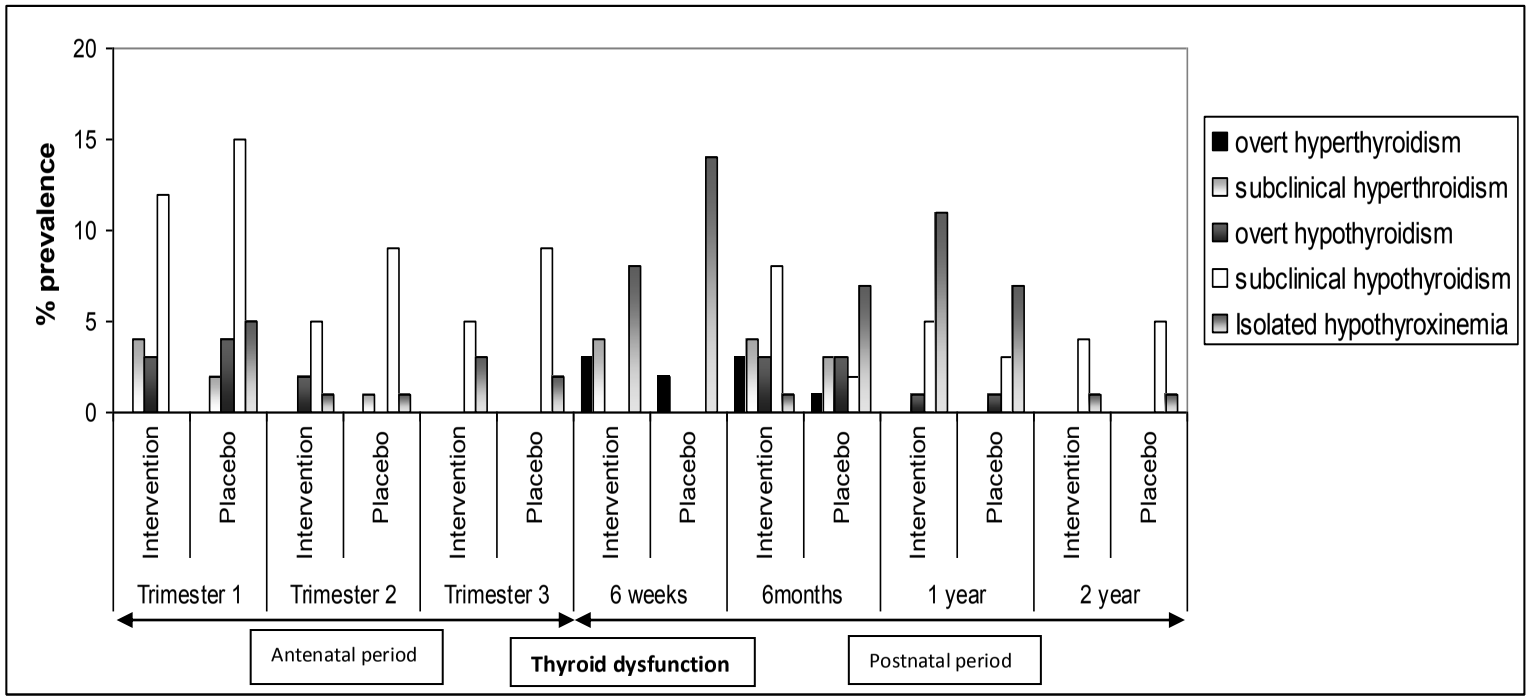


Figure 3: Prevalence of thyroid dysfunction among Indian women given 200 µg iodine daily or placebo during pregnancy. Thyroid dysfunction is defined using trimester specific reference range for TSH and TT4 as per CDC/ATA recommendation in the antenatal period and non-pregnant reference range for TSH and fT4 in the postnatal period

Table 4 presents the continuous and categorical data of thyroid function, thyroid volume and UICs of women at postnatal time points 6 weeks, 6 months, 1 and 2 year, by the two groups. There were no significant differences in thyroid function measures between the two groups except TT3 and TT3/TT4 ratio which showed a 6.5% and 0.09% difference in the intervention group with respect to placebo group (p=0.017 both). In both the groups, TSH levels decreased at 6 wks and thereafter remained at the levels that were achieved by the end of pregnancy. TT4, TT3 fT3, fT3/fT4 ratio were lower in the postpartum period compared to their pregnancy levels in both the groups of women while fT4 levels decreased in the second and third trimester but returned to first trimester levels in the postpartum period in both the groups. Tg and TT3/TT4 ratio remained more or less in the same range of values throughout pregnancy and postpartum period in both the groups. In both the groups, thyroid volume at 6 wks was same as the third trimester gland size and then it continued to increase at 6 months, 1 and 2 year. In both the groups, UIC was sharply lower from the pregnancy levels but it was still >100 μg/L indicating iodine sufficiency.

Fig 4 shows the percentage of normal and high TPO-Antibody (TPO-Ab) titres in the women at antenatal and postnatal time points. The prevalence of high ATPO-Ab titres was 12.7 vs 12.3% in the first trimester, 12.1 vs 8.3% in the second trimester, 13.2 vs 9.7 % in the third trimester, 8.5 vs 17.1% at 6 wks, 16.7 vs 10.3% at 6 months, 11.3 vs 12.1% at 1 year and 10.3 vs 17.9% at 2 year in the intervention and placebo group respectively. In a generalised estimated equation analysis, there were no statistically significant differences in the prevalence of high ATPO-Ab titres between the intervention and placebo group (p=0.851 for treatment group, p=0.394 for duration of treatment, p=0.192 for interaction effect).

Table 4: Thyroid function of Indian women given 200µg iodine daily or placebo during pregnancy[1,2]

TFT	*6 weeks*		*6 months*		*1 year*		*2 year*			
	Intervention (n=97)	***Placebo (n=92)***	***Intervention (n=76)***	***Placebo (n=76)***	***Intervention (n=72)***	***Placebo (n=66)***	***Intervention (n=75)***	***Placebo (n=68)***	***% diff***	***P[2]***
TSH, mIU/L	1.3(0.9, 1.8)	1.2(0.9, 1.9)	1.6 (0.9, 2.6)	1.5 (0.9, 2.4)	1. 7(1.1, 3.0)	1.8 (1.3, 2.6)	1.7 (1.3, 2.7)	2.5 (1.3, 3.3)	1.1 ↓	0.786
<0.4	7 (7.2)	4 (4.8)	9 (11.8)	4 (5.1)	1 (1.4)	0 (0)	0 (0)	0 (0)		
>4.2	0 (0)	1 (1.2)	9 (11.8)	6 (7.7)	6 (8.3)	5 (7.6)	4 (10.3)	5 (12.8)		
fT4, ng/dL	1.0 (0.9, 1.2)	1.1 (0.9,1.2)	1.1 (0.9, 1.2)	1.1 (0.9, 1.2)	1.1 (0.9, 1.2)	1.0 (0.9, 1.1)	1.3 (1.2, 1.4)	1.2 (1.1,1.3)	0.6 3↑	0.545
< 0.89	8 (8.5)	14 (16.9)	5 (6.6)	11 (14.3)	13 (18.3)	10 (15.6)	1 (2.6)	1 (2.6)		
>1.76	4 (4.3)	6 (7.2)	3 (3.9)	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)		
TT4, nmol/L	100.2 (87.8, 115.3)	103.0 (86, 119.8)	96.6 (79.3, 110.8)	97.3 (84.8, 111.6)	96.5 (83.2, 111.8)	98.3 (85.7, 109.3)	104.9 (90.8, 115.2)	97.0 (87.5, 117.1)	1.5 ↓	0.574
< 58	2 (2.1)	2 (2.4)	6 (8.0)	4 (5.2)	2 (2.9)	2 (3.2)	0 (0)	1 (2.6)		
>161	3 (3.2)	3 (3.6)	1 (1.3)	2 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)		
fT3, pg/mL	2.8 (2.3, 3.2)	2.8 (2.5,3.2)	3.3 (2.9, 3.6)	3.2 (2.7, 3.6)	3.1 (2.7, 3.2)	3.2 (2.8, 3.6)	2.9 (2.5, 3.4)	3.2 (2.7, 3.6)	3.1 ↓	0.211
<2.6	36 (38.7)	27 (34.2)	8 (11.0)	16 (21.1)	10 (14.7)	12 (19.4)	12 (31.6)	9 (23.1)		
>4.8	1 (1.1)	2 (2.5)	4 (5.5)	3 (3.9)	0 (0)	2 (3.2)	0 (0)	0 (0)		
TT3, nmol/L	1.7 (1.4, 1.8)	1.8 (1.5,2.1)	1.7 (1.4, 1.9)	1.6 (1.5, 1.9)	1.6 (1.4, 1.8)	1.7 (1.5, 1.9)	1.7 (1.5, 1.9)	1.8 (1.5, 2.0)	6.5 ↓	**0.017**
<1.1	3 (3.3)	1 (1.3)	5 (7.4)	1 (1.4)	2 (3.3)	3 (5.1)	1 (2.7)	1 (2.8)		
>3.1	1 (1.1)	2 (2.6)	0 (0)	1 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)		
Tg,µg/L	7.7 (3.2,12.2)	6.8 (4.4,12.9)	6.7 (3.5,10.8)	6.1 (3.4, 12.3)	7.2 (3.8, 10.2)	6.1 (2.6,13.1)	6.1 (3.1, 11.8)	5.6 (3.6,11.6)	7.9 ↓	0.522
>55.6	3 (3.2)	2 (2.4)	1 (1.4)	2 (2.6)	0 (0)	0 (0)	2 (5.1)	0 (0)		

TFT	*6 weeks*		*6 months*		*1 year*		*2 year*			
	Intervention	***Placebo***	***Intervention***	***Placebo***	***Intervention***	***Placebo***	***Intervention***	***Placebo***	***% diff***	***P[2]***
	(n=97)	***(n=92)***	***(n=76)***	***(n=76)***	***(n=72)***	***(n=66)***	***(n=75)***	***(n=68)***		
TT3/TT4 ratio	0.0162 (0.0145, 0.0186)	0.0179 (0.0155, 0.0200)	0.0173 (0.0146, 0.0207)	0.0173 (0.0150, 0.0206)	0.0167 (0.0145, 0.0191)	0.0167 (0.0148, 0.0199)	0.0167 (0.0153, 0.0179)	0.0171 (0.0154, 0.0206)	0.09↓	**0.017**
fT3/fT4 ratio	2.5962 (2.2217, 3.0998)	2.6904 (2.1583, 3.1478)	2.9118 (2.5595, 3.4180)	2.8859 (2.5065, 3.4265)	2.8091 (2.4045, 3.2019)	3.0485 (2.5301, 3.7680)	2.2586 (2.0810, 2.6798)	2.7266 (2.2901, 2.9478)	3.0 ↓	0.306
Thyroid volume	5.8 (4.9, 6.9)	5.8 (5.0,6.8)	5.9 (5.12, .3)	5.9 (4.9, 6.9)	6.4 (5.6, 7.2)	6.7 (5.7, 7.2)	6.7 (5.9, 7.4)	6.9 (6.3, 7.9)	1.9 ↓	0.422
UIC,µg/L	126.34 (68.26, 235.61)	135.36 (80.97, 221.54)	184.29 (106.31, 291.29)	195.39 (125.53, 330.52)	212.29 (134.47, 338.28)	212.31 (130.79, 344.61)	181.93 (105.09, 327.06)	173.98 (102.68, 309.09)	3.86↑	0.623
≥100	56 (60.2)	62 (67.4)	61 (78.2)	72 (84.7)	67 (87.0)	66 (80.5)	59 (78.7)	52 (76.5)		
50 – 99	20 (21.5)	11 (12.0)	11 (14.1)	7 (8.2)	5 (6.5)	14 (17.1)	10 (13.3)	9 (13.2)		
20 – 49	12 (12.9)	17 (18.5)	4 (5.1)	6 (7.1)	2 (2.6)	2 (2.4)	5 (6.7)	6 (8.8)		
<20	5 (5.4)	2 (2.2)	2 (2.6)	0 (0)	3 (3.9)	0 (0)	1 (1.3)	1 (1.5)		

[1] median (Quartile 1, Quartile 3) or n (%), % diff: % difference in intervention group with respect to placebo group s; p value for treatment code using linear mixed models of log transformed thyroid function [2]

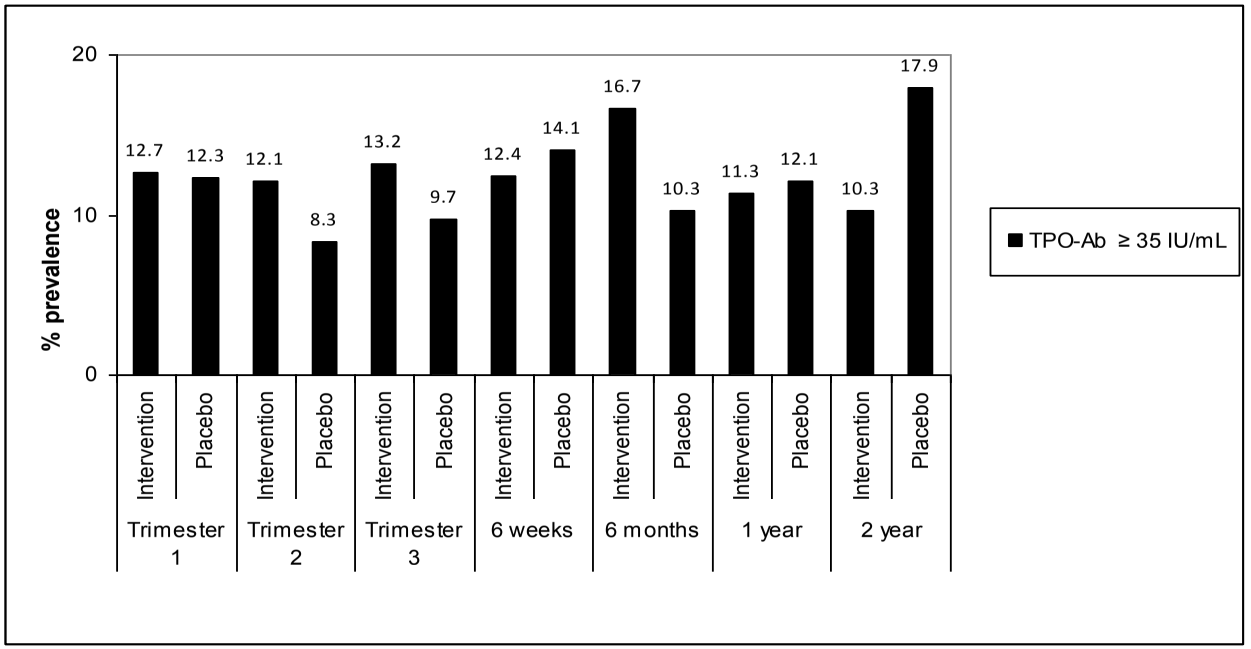


Figure 4: Percentage of normal and high TPO-Ab titers in Indian women given 200 µg iodine daily or placebo during pregnancy

The neonatal characteristics, thyroid function, thyroid volume, UIC and NBAS score in newborns in the treated and placebo groups are shown in Table 5. There was no difference in the length of gestation between the intervention and placebo groups (38.6 vs 38.7 wks). The placebo group had a higher percentage of female to male (48 vs 65) when compared to the intervention group (56 vs 52) but this difference in gender was not significant between the two groups (p=0.163). Infant birth weight in the placebo group was 174 g higher compared to intervention group but this difference was not statistically significant when birth weight was adjusted for gestational age at delivery, gender, parity and maternal height. Between intervention and placebo group, the prevalence of low birth weight (22.8 vs 17.4%), small-for-gestational-age (35.4 vs 24.8%) and pre-term (8.0 vs 8.6%) were not significantly different. The transient neonatal hypothyroxinemia (neonatal TSH>5 mIU/L) observed in both the groups at delivery (73.4 vs 76.7) resolved at 72 hrs post delivery with no cases of neonatal TSH >5 mIU/L. T4 levels decreased from delivery to 72 hrs post delivery in both the groups. The percentage of infants with UIC <100 µg/L was also not different between the two groups. There was no difference in any of the domains of the Neonatal behavioural assessment scale at 1 week postpartum but at 6 weeks, infants in the intervention group scored significantly higher in the orientation cluster (8.1 vs 7.1) (p=0.018).

The thyroid function and anthropometry of children at ages 6 weeks, 6 months, 1 and 2 year are shown in Table 6. There was no difference in the measures of thyroid function (TSH, T4, UIC) or anthropometry (weight, height, head circumference) in both the groups of children at any of the time point.

Table 5: Neonatal characteristics, thyroid function, thyroid volume, urinary iodine concentration and Neonatal Behavioural Assessment Scales score in Indian newborns born to women who were given 200 µg iodine daily or placebo during pregnancy

	Intervention	***n***	***Placebo***	***n***	***β***	***P*[3,4]**	***R*²**
At Delivery							
Characteristics							
Gender; Female: Male, n	56:52	108	48:65	112		0.163	
Birth weight, gram[2]	2733 ± 428.8	101	2907 ± 518.1	109	-0.102	0.074	0.399
<2500, n (%)	23 (22.8)	101	19 (17.4)	109		0.334	
Preterm, n (%)	9 (8.0)	113	10 (8.6)	116		0.857	
Gestational age (weeks) [2]	38.6 ± 1.5	113	38.7 ± 1.8	116	0.068	0.763	0.002
Head circumference, cm[2]	33.3 ± 1.5	106	33.6 ± 1.8	100	-0.138	0.499	0.223
Ponderal index[2]	2.5 ± 0.5	81	2.6 ± 0.5	81	-0.084	0.276	0.011
Small-for-gestational age, n (%)	35 (35.4)	99	27 (24.8)	109		0.096	
Thyroid function							
Cord blood TSH[1] (mIU/L)	7.9 (4.8, 11.5)	64	7.4 (5.4, 10.5)	64	0.039	0.742	0.016
>5, n (%)	47 (73.4)	64	49 (76.6)	64		0.683	
DBS-T4[1], nmol/L	70.5 (50.9, 87.7)	66	72.7 (52.2, 92.2)	68	0.012	0.863	0.013

	Intervention	*n*	*Placebo*	*n*	*β*	*P*[3,4]	*R*[2]
Post Delivery (within 72 hrs of delivery)							
DBS-TSH[1], mIU/L	0.9 (0.5, 1.5)	92	0.8 (0.5, 1.3)	93	-0.039	0.425	0.013
>5, n (%)	0 (0)		0 (0)				
DBS-T4[1], nmol/L	112.0 (86.1, 144.0)	92	112.0 (71.1, 149.0)	93	-0.034	0.637	0.015
UIC[1] (µg/L)	376.6 (183.48, 566.9)	76	347.4 (219.6, 560.6)	65	0.109	0.464	0.075
<100, n (%)	5 (6.6)	76	6 (9.2)	65		0.558	
Week 1 postpartum							
NBAS score[1]						*P*[4]	
Habituation	4.9 (2.6, 5.8)	50	4.5 (3.0, 5.9)	45		0.881	
Orientation	5.4 (4.2, 6.5)	61	5.3 (4.1, 6.7)	59		0.725	
Motor system	4.6 (4.0, 5.4)	61	4.6 (4.2, 5.2)	56		0.791	
Range of state	3.9 (3.5, 4.2)	58	4.0 (3.5, 4.3)	54		0.762	
State regulation	5.2 (4.5, 6.1)	58	5.5 (4.7, 6.5)	54		0.355	
Autonomic system	4.3 (3.7, 5.4)	58	4.3 (3.0, 5.3)	54		0.695	
Abnormal reflex score	4.0 (3.0, 5.0)	57	4.0 (3.0, 5.0)	55		0.927	

	Intervention	*n*	*Placebo*	*n*	*β*	*P*[3,4]	*R*[2]
Week 6 postpartum							
UIC[1] (µg/L)	286.89 (142.8, 564.6)	91	241.46 (139.1, 396.6)	84	0.193	0.171	0.016
<100, n (%)	11 (12.1)	91	12 (14.3)	84		0.667	
NBAS score[1]						*P*[4]	
Habituation	3.8 (2.4, 5.4)	34	4.1 (2.6, 5.7)	28		0.955	
Orientation	8.1 (5.6, 8.6)	83	7.1 (4.8, 8.3)	78		**0.018**	
Motor system	5.2 (4.8, 5.8)	81	5.4 (4.8, 5.8)	77		0.475	
Range of state	3.5 (2.5, 4.0)	80	3.7 (3.2, 4.0)	77		0.134	
State regulation	6.2 (5.2, 7.4)	80	6.0 (5, 6.9)	77		0.134	
Autonomic system	4.3 (3.1, 5.6)	80	4.7 (3.5, 5.7)	76		0.419	
Abnormal reflex score	3.0 (2.0, 4.0)	80	3.0 (2.0, 4.0)	78		0.262	

[1]median (Quartile 1, Quartile 3); [2] mean ± SD; P[3] p value from linear regression (adjusted for gestational age at delivery, gender), birth weight adjusted for gestational age at delivery, gender, parity, maternal height; P[4] p value from Mann Whitney U test

Table 6: Thyroid function and anthropometry of children born to pregnant women given 200µg iodine daily or placebo during pregnancy

Parameters	*6 weeks*		*6 months*		*1 year*		*2 year*		
	Intervention	*Placebo*	*Intervention*	*Placebo*	*Intervention*	*Placebo*	*Intervention*	*Placebo*	*P*
Thyroid function	**(n=93)**	**(n=84)**	**(n=70)**	**(n=68)**	**(n=63)**	**(n=59)**	**(n=78)**	**(n=74)**	
TSH (mIU/L) [1]	0.8 (0.6, 1.0)	0.7 (0.5, 1.1)	0.6 (0.4, 0.8)	0.6 (0.4, 0.9)	0.6 (0.5, 0.8)	0.6 (0.5, 0.8)	0.4 (0.3, 0.6)	0.4 (0.2, 0.5)	0.910
TT4 (nmol/L) [1]	76.6 (57.4, 109)	82.8 (56.9, 115)	62.9 (48.8, 82.7)	68.8 (51.7, 89.4)	64.5 (51.0, 80.3)	63.0 (46.3, 80.5)	67.3 (49.3, 76.1)	63.1 (55.3, 73.9)	0.412
UIC (µg/L) [1]	286.9 (142.7, 564.6)	241.5 (139, 396.6)	282.63 (169.01, 655.23)	279.60 (146.75, 435.43)	251.76 (138.31, 565.43)	230.74 (130.5, 509.67)	226.08 (86.46, 449.52)	208.59 (87.89, 431.62)	0.339
Anthropometry	**(n=105)**	**(n=98)**	**(n=87)**	**(n=89)**	**(n=98)**	**(n=89)**	**(n=97)**	**(n=89)**	
Weight (kg) [2]	4.3 ± 0.8	4.4 ± 0.9	7.1 ± 1.2	7.3 ± 1.3	8.4 ± 1.0	8.6 ± 1.3	10.5 ± 1.5	10.7 ± 1.6	0.821
Height (cm) [2]	56.4 ± 4.5	57.1 ± 4.1	67.5 ± 4.6	67.1 ± 4.0	72.8 ± 3.9	73.3 ± 3.3	83.4 ± 3.9	83.9 ± 4.1	0.326
Head circumference(cm) [2]	37.2 ± 1.7	37.7 ± 1.8	42.4 ± 1.9	42.7 ± 1.8	44.3 ± 1.9	44.6 ± 1.7	45.8 ± 2.1	46.2 ± 1.9	0.094

[1] Data are median (Quartile 1, Quartile 3), [2]mean ± S.D; *p value for treatment code using linear mixed models*

Table 7 presents the raw scores of Bayleys scales of infant development (BSID-III) assessed in children at 1 and 2 years of age. Between the two groups of children, there was no difference in the unadjusted sub scales of cognitive, receptive and expressive communication, fine and gross motor development at 2 years. At 1 year, unadjusted expressive communication was statistically significantly different between the two groups. Table 8 presents the raw scores of executive function as assessed by Brief P in both the groups of children at 2 years of age. The unadjusted scale on inhibition showed lower problem scores of inhibition in the children of mothers who received iodine during pregnancy (median (IQR): 20.0 (18.0, 21.0) compared to those in the placebo group, (median (IQR): 21.0 (19.0, 24.0) and this difference was statistically significant (p=0.028).

Table 7: BSID raw scores of children born to women given 200 µg iodine daily or placebo during pregnancy

Mean BSID (raw scores)	***1 year***			***2 year***		
	Intervention (n=96)	***Placebo (n=88)***	***P***	***Intervention (n=98)***	***Placebo (n=89)***	***P***
Cognitive	46.7 ± 4.7	47.1 ± 4.8	0.511	64.3 ± 5.9	64.4 ± 6.7	0.964
Receptive	16.2 ± 2.9	16.7 ± 3.0	0.338	26.9 ± 3.8	26.6 ± 3.8	0.457
Expressive	16.3 ± 2.9	17.2 ± 2.9	**0.047**	28.6 ± 4.6	27.9 ± 4.2	0.328
Fine motor	30.2 ± 2.9	30.4 ± 2.8	0.673	40.6 ± 3.6	40.4 ± 3.5	0.650
Gross motor	46.8 ± 4.0	46.1 ± 4.3	0.240	60.7 ± 3.9	59.9 ± 3.8	0.144

Mean ± SD, P value from Independent sample t- test

Table 8: Brief-P raw scores of 2 year old children born to women given 200 µg iodine daily or placebo during pregnancy.

Brief-P raw scores	***Intervention (n=86)***	***Placebo (n=86)***	***P***
Inhibition	20.0 (18.0, 21.0)	21.0 (19.0, 24.0)	**0.028**
Shift	12.0 (10.0, 13.2)	13.0 (11.0, 15.0)	0.123
Emotional control	13.0 (11.0, 14.0)	13.0 (11.0, 15.0)	0.764
Planning and Organisation	12.0 (11.0, 13.0)	12.0 (10.5, 14.0)	0.077

Median (Quartile1, Quartile3), P value from Mann Whitney U test

Discussion

This study has two distinctions: this is the first study to measure child development at two years as an outcome of iodine supplementation during pregnancy in a randomised controlled trial. The second distinction, though not anticipated, is that the pregnant women were iodine sufficient, not mildly iodine deficient, likely due to recent improvements in the coverage and quality of iodized salt in the area. This was confirmed by maternal UIC >150 µg/L throughout pregnancy, UIC >100 µg/L in the postpartum period and a less than 3% frequency of neonatal TSH concentrations >5mU/L on samples collected 3–4 days after birth (32).

Iodine supplementation brought about an 18.8% and 35.5% increase in the UIC in the second and third trimester of pregnancy in the intervention group. In this study, the significant difference in UIC values was observed in the third trimester between the intervention and placebo group (p=0.003). There was a difference of 35 and 99 µg/L UIC between the two groups at second and third trimester respectively while this difference should have been ~147 µg/L between the intervention and treatment groups considering >80% compliance with supplement use (200 µg iodine) and 92% dietary iodine bioavailability. It could be that the renal handling of UI excretion is efficiently targeted at providing iodine until the fetal thyroid matures around second trimester. So irrespective of additional supplementation, this mechanism is efficient in pregnant women with sufficient intra-thyroidal stores at the beginning of pregnancy. There are conflicting reports of gestational changes in UI excretion; mean UIC did not change with progression of pregnancy in France (33), Sweden and Sudan (34), whereas data from the United Kingdom (35), Spain (36), and Hong Kong (37) showed an increasing level of UIC with advancing gestational age. In cross-sectional studies assessing iodine supplement use (140-150 µg/dose) and thyroid function, median UICs were 111 Vs 61 µg/L in UK (38); 60 Vs 35 µg/L at term in Denmark (39) in women taking iodine supplement and women not taking a supplement respectively. Results from

randomised trials of iodine supplementation (n=35-120; 50-230 µg iodine/day from 11/14/17/18 wk to term) in mild-to-moderately iodine deficient pregnant women showed significantly higher median UI in the intervention group compared to controls (1, 40-43). In a prospective, randomized, open-label trial, Antonangeli et al. (44) supplemented mildly iodine-deficient pregnant women (n=67; median UI 74 µg/g cr) with 50 µg or 200 µg iodine/day from 18-26 wk to 29-33 wk. Median UI was significantly higher in the 200 µg group than in the 50 µg group (230 vs. 128 µg/g cr). Our results compare with those from Japan where overall median UICs throughout pregnancy and in the postpartum period were 224.0 and 135.0µg/L, respectively, suggesting sufficient iodine nutrition (45). In Ireland, UI excretion in the immediate antenatal and early postpartum periods showed a precipitous fall in median values from 93 µg/L antenatally to 36 µg/L at delivery subsequently rising to 49 µg/L and 63 µg/L at days 3 and 10 postpartum respectively (46). In a longitudinal observational study conducted in Slovenia, an iodine sufficient area, UIC was significantly higher during pregnancy than after delivery (P=0.044) (47). The fact that the maternal UIC levels at the end of pregnancy drops considerably at 6 weeks postpartum indicates normalization of thyroid hormone production and renal function for UI excretion, and also reflects iodine losses into breast milk in lactating women (45). It is also interesting to note that the maternal UIC levels reached back to those of pregnancy values from 6 months onwards in both the groups indicating sufficient iodine nutrition.

In this RCT in both the groups of women, TT4 levels were higher during pregnancy than in the postpartum period. In a longitudinal study done in 20 pregnant women from a borderline iodine deficient area, TT4 levels were increased during pregnancy when compared to postpartum levels (48). In a cross-sectional study of healthy, iodine-sufficient Swedish pregnant women across trimesters and postpartum, T4 concentrations were both higher during pregnancy than in the postpartum period. T4 levels remained stable throughout pregnancy (49). Results from randomised trials of iodine supplementation (n=54,108; 200, 230 µg iodine/day from 11/17 wk

to term) in mild-to-moderately iodine deficient pregnant women showed no significant effect of intervention on T4 (41, 42). The results from this study showed that in both the groups of women, fT4 levels decreased in the second and third trimester but returned to first trimester levels in the postpartum period. Even though there was a borderline significant change in the interaction term, this was not clinically significant (with a 0.009% difference between the slopes of the two groups). It has been reported that the highest levels of fT4 occur in the first trimester, and gradually decrease over the remainder of the pregnancy (49-52). A gradual decrease of the fT4 level during pregnancy, with its lowest level in the third trimester, has been reported in iodine-sufficient areas (34, 51, 53, 54). A study carried out in Japan showed that 3 to 4 days after delivery the values of both free thyroid hormones were already significantly higher than in the third trimester of pregnancy (53). In a cross-sectional study in Denmark, iodine supplement use (150 μg/day) showed significantly higher fT4 compared to the non-supplement group at term (39). Results from randomised trials of iodine supplementation (n=66,54; 150, 200 μg iodine/day from 11/17 wk to term) in mild-to-moderately iodine deficient pregnant women showed no significant differences in maternal fT4 between groups (40, 42). In a prospective, randomized, open-label trial, Antonangeli et al. (44) supplemented MID pregnant women (n=67; median UI 74 μg/g cr) with 50 μg or 200 μg iodine/day from 18-26 wk to 29-33 wk. There were no differences in maternal fT4 between groups.

In our study, TSH increased in both groups of women from first trimester through pregnancy, decreased at 6 wks postpartum and then increased again until 2 years after delivery; TT3 levels were higher during pregnancy than in the postpartum period ; fT3 levels were lower in the postpartum period compared to their pregnancy levels; fT3/fT4 ratio were lower in the postpartum period compared to their pregnancy levels; Tg and TT3/TT4 ratio remained more or less in the same range of values throughout pregnancy and postpartum period. Our findings suggest that the changes in maternal thyroid

hormone levels during pregnancy reflect physiological changes and occur in pregnant women irrespective of their iodine nutrition.

One of the concerns about iodine intake in pregnancy and postpartum has been the risk of exacerbating thyroid autoimmunity (55). In this study, the prevalence of elevated TPO-Ab titers was similar in the intervention and placebo groups at baseline (12.7 vs 12.3%). In the intervention group, the prevalence did not change during the second (12.1%) and third trimesters (13.2%) until the postpartum period; 6 wks (12.4%), 6 months (16.7%), 1 year (11.3%), 2 year (10.3%). The placebo group showed a decrease in prevalence at subsequent trimesters (8.3 and 9.7%) with an increase in the postpartum period; 6 wks (14.1%), 6 months (10.3%), 1 year (12.1%), 2 year (17.9%). In previous maternal iodine supplementation studies in areas of mild-to-moderate iodine deficiency, supplementation did not induce or worsen postpartum thyroiditis (40) or induce autoimmunity (41). From this longitudinal study, we have shown that iodine supplementation even in iodine sufficient pregnant women is safe and does not increase the risk of thyroid autoimmunity.

There was no difference in any of the clusters of the NBAS at 1 week postpartum but at 6 weeks, infants in the intervention group scored significantly higher in the orientation cluster (8.1 vs 7.1) ($p=0.018$). Our results are the first from an iodine intervention trial but similar to an earlier case-control study where infants of women with hypothyroxinemia at 12 weeks had significantly lower scores on NBAS orientation index compared with controls (56). We also found that in the cognitive assessment at 1 year unadjusted expressive communication was statistically significantly different between the two groups with children born to mothers who received placebo performing better on BSID-III. However, this difference might be a transient effect since it did not sustain at 2 yrs of age. On the BRIEF-P at 2 years, inhibition scores were significantly higher in the children of mothers in the placebo group compared to those whose mothers received iodine during pregnancy, indicative of executive

dysfunction. In a population-based cohort that examined the association between maternal urinary iodine during early pregnancy and executive functioning in children at 4 y of age, it was found that the children of mothers with low urinary iodine showed higher scores on the problem scales of inhibition (β =0.05 (95% CI: 0.01, 0.10), P = 0.03) and working memory (β = 0.07 (95% CI: 0.02, 0.12), P = 0.003) (63). Mildly-deficient maternal iodine intake during pregnancy may have different effects in the mother and fetus. It has been suggested that their response varies, with the mother more likely to maintain euthyroidism while the fetus may become hypothyroid (57). This might explain why we found modest impairments in newborn infant development despite the lack of a measurable intervention effect on maternal thyroid function. Increasingly, studies are using measures of executive function as they have been linked to future development of disorders of childhood psychopathology such as attention deficit and hyperactivity disorder. Future studies are needed to show if these children develop later differences in cognitive development.

In this RCT, in both the groups of women, thyroid volume increased systematically through the pregnancy and postpartum period with a similar pattern observed also for TSH. At 2 year postpartum, thyroid volume had increased by 34% and 38% from baseline in the intervention and placebo group respectively. Our study suggests that the goitrogenic effect of pregnancy may be mediated via increased TSH secretion and that the thyroid volume increase during pregnancy is primarily the result of increased vasculature of the gland, independent of the iodine supply. A longitudinal observational study done in 20 pregnant women from a borderline iodine deficient area also showed a goitrogenic effect of pregnancy which was unexplained by alterations in thyroid function variables (48). In that study, thyroid volume increased during pregnancy but not in the postpartum period while TSH level was unaltered during pregnancy when compared with postpartum levels, although TSH levels at 2 years showed a 33% increase from the baseline level at 18 weeks of pregnancy (48). Berghout and Wiersinga (50) reviewed that in countries affected by mild or moderate iodine deficiency (Ireland,

Germany, Belgium, Italy, Denmark), thyroid volume increases 15-31% during pregnancy, while in iodine-sufficient countries (Finland, USA, the Netherlands), there is little or no increase in thyroid volume during pregnancy (50). Results from randomised trials of iodine supplementation (n=35-120; 50-230 µg iodine/day from 11/14/17/18 wk to term) in mild-to-moderately iodine deficient pregnant women have shown varied results. There was no significant effect of intervention on maternal thyroid volume (41, 44), treated women had smaller thyroid volumes compared to controls (1), maternal thyroid volume increased 16% in the treated group vs. 30% in controls (42), thyroid volume did not change in the treated group while in the controls, there was a 16% increase in thyroid volume (43).

In this study, median cord blood TSH, median dried blood spot TSH and T4, and prevalence of TSH >5mIU/L and UIC <100 µg/l was not different between neonates in both the groups. Earlier RCT's of iodine supplementation (n= 54-120; 100-230 µg iodine/day from 11/14/17 wk to term) conducted in mild-to-moderately iodine deficient pregnant women, reported no effect of intervention on newborn TSH (41), maternal or cord T3, FT4, and T3/T4 ratio (1), maternal or cord T4, T3, and FT4 (42). Newborns of the treated group had significantly higher UI, smaller thyroid volumes (1) and lower Tg concentrations compared to controls (1, 42). Our findings suggest that maternal iodine supplementation in iodine sufficient regions does not result impact significantly on neonatal thyroid function.

In this RCT, maternal iodine supplementation did not seem to affect infant birth weight. Between newborns born to mothers who received 200 µg iodine daily and placebo during pregnancy, the prevalence of low birth weight (22.8 vs 17.4%), small-for-gestational-age (35.4 vs 24.8%) and pre-term (8.0 vs 8.6%) was comparable and not statistically significant between the two groups. Iodine repletion of pregnant women improved head circumference in a severely iodine-deficient area of western China (82) while significantly increased placental and birth weights were reported in a region of endemic

goiter area in Algeria (58). In a prospective cohort study nested in a Bangladeshi population-based randomized supplementation trial in pregnant women, maternal iodine status at an average gestational age of 8 weeks, was positively associated with weight, length, and head circumference in male newborns up to ~1 mg/L maternal UIC. But in this study, ~60% of these measurements were done mostly within 72 hr of birth and therefore may not truly reflect the association with birth weight (59). An earlier study on Spanish infants looking at associations of thyroid function with birth weight or SGA suggested that iodine status during pregnancy may be related to prenatal growth (36) but to date there is no conclusive evidence for this.

Measurement of FT4 by isotope dilution tandem mass spectrometry provides accurate and reliable results during pregnancy, but these assays are not broadly available. In contrast, automated immunoassays are currently the most widely used systems for measuring fT4, but they are variously biased by several factors, which are responsible for significant method-dependent variations in FT4 measurement in pregnancy (60). A recent review did not recommend using fT4 as a screening tool since there are considerable method-dependent variations in free T4 assessment during pregnancy (61). According to CDC/ATA recommendation, FT4 estimate methods are sensitive to abnormal binding-protein states such as pregnancy and there is no absolute FT4 value that will define hypothyroxinemia across methods. While TT4 changes in pregnancy are predictable and not method-specific, TT4 <100 nmol/L was indicative of hypothyroxinemia in pregnancy (25). We therefore used these recommendations and adjusted the non-pregnant reference range by a factor of 1.5 to derive at a new reference range (87-241.5 nmol/L), the lower limit of which fell close to 100 nmol/L (CDC/ATA recommendation)(10, 25) . This was useful in interpreting the data on fT4 and TT4. In this iodine sufficient pregnant population, irrespective of iodine supplementation, fT4 decreased and TT4 increased during pregnancy. While the prevalence of low titres of these hormones was quite low for TT4 (0.8-2.9%), this prevalence

was quite high for fT4 (26.3-40.4%) in the second and third trimester respectively. Currently, using TSH along with fT4 is considered to be the best screening measure for women at risk of thyroid dysfunction during pregnancy. Considering serum TSH may be a less sensitive measure of mild thyroid hormone deficiency in early pregnancy, use of TT4 and not fT4 might improve clinical screening performance for thyroid disorders in pregnancy.

The overall dropout rate was 30.4% and 31.2% at delivery and 72 hrs in the intervention and placebo group respectively, which was close to the anticipated dropout rate of ~25% and the number of women (n=125) that were required in each group and thus the statistical power of the study appeared to be adequate. However, we had only 64 neonatal TSH from blood spots at delivery (cord blood) each in the intervention and placebo group which increased to 92 and 93 respectively at 72 hrs. But since there were no differences in the prevalence of elevated neonatal TSH at delivery and 72 hrs (which was the basis of our sample size calculation) we are confident that addition of more numbers to the existing data would have not made any difference in the study findings. Moreover, throughout the study, women who dropped out were equally divided between the two groups for various reasons: adverse events, women/family not willing to continue using the supplements, moved out of the study area, re-pregnant. Thus we feel that the exposure itself was not the reason for the dropout rate and hence the findings of the study are inferential.

In conclusion, our findings indicate that the changes in maternal thyroid hormone levels during pregnancy reflected physiological changes and occurred irrespective of iodine supplementation. However, additional iodine supplemented to iodine sufficient pregnant women is safe and does not increase thyroid autoimmunity, which is reassuring. Though there were no significant effects of iodine supplementation on neonatal and maternal thyroid function and birth outcomes, there were modest effects on neurocognitive development of children as assessed by executive function of children at 2 years. However, there are concerns over reliability of

neurocognitive assessments in children less than 4 yrs of age and thus follow-up of these children would provide further insights. Although salt iodization is likely to be a more effective and equitable strategy to increase iodine intakes in populations, iodine supplementation to pregnant women should be considered as an important interim measure of ensuring optimal iodine nutrition until universal salt iodization reaches all strata of the population.

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Conflicts of interest: None.

Ethics of human subject participation: The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving pregnant women and their children were approved by the institutional ethical review boards at St John's National Academy of Health Sciences and St Martha's Hospital, Bangalore, India. The study was explained in detail to the

participating women and written informed consent from each participant was obtained at recruitment.

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Chapter 3

The iodized salt programme in Bangalore, India provides adequate iodine intakes in pregnant women and more-than-adequate iodine intakes in their children

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Abstract

Objective: To compare the iodine status of pregnant women and their children who were sharing all meals in Bangalore, India.

Design: A cross-sectional study evaluating demographic characteristics, household salt iodine concentration and salt usage patterns, urinary iodine concentrations (UIC) in women and children, and maternal thyroid volume (ultrasound).

Setting: Antenatal clinic of an urban tertiary-care hospital, which serves a low-income population.

Subjects: Healthy pregnant women in all trimesters, aged 18–35 years, who had healthy children aged 3–15 years.

Results: Median (range) iodine concentrations of household powdered and crystal salt were 55·9 (17·2–65·9) ppm and 18·9 (2·2–68·2) ppm, respectively. The contribution of iodine-containing supplements and multi-micronutrient powders to iodine intake in the families was negligible. Adequately iodized salt, together with small amounts of iodine in local foods, were providing adequate iodine during pregnancy: (i) the overall median (range) UIC in women was 172 (5–1024) μg/l; (ii) the median UIC was >150 μg/l in all trimesters; and (iii) thyroid size was not significantly different across trimesters. At the same time, the median (range) UIC in children was 220 (10–782) μg/l, indicating more-than-adequate iodine intake at this age. Median UIC was significantly higher in children than in their mothers (P =0·008).

Conclusions: In this selected urban population of southern India, the iodized salt programme provides adequate iodine to women throughout pregnancy, at the expense of higher iodine intake in their children. Thus we suggest that the current cut-off for median UIC in children indicating more-than-adequate intake, recommended by the WHO/UNICEF/International Council for the Control of Iodine Deficiency Disorders may, need to be reconsidered.

Introduction

Universal salt iodization (USI) is a mass fortification approach that is intended to cover the iodine requirements of all individuals in the population; it is estimated that 128 countries have established iodized salt programmes [1]. As USI programmes mature in many countries, greater emphasis is being placed on ensuring that USI meets the increased needs of pregnant women because of the risk of irreversible fetal brain damage due to iodine deficiency [2, 3]. Another focus of mature programmes is the need for careful monitoring to avoid not only iodine deficiency but also iodine excess[1]. Median urinary iodine concentration (UIC) in school-aged children (SAC) and household access to adequately iodized salt based on national standards are routinely used as the primary indicator of the impact of USI programmes. However, the iodine requirement of SAC is disproportionately lower than that of pregnant women, which leaves only a narrow intake range to meet the needs of pregnant women without leading to more-than-adequate intake in children according to the current UIC cut-off of 200µg/l[4]. The WHO/UNICEF/International Council for the Control of Iodine Deficiency Disorders (ICCIDD) have estimated that the iodine requirement during pregnancy is increased by >50% compared with non-pregnancy in order to compensate for the increased need for thyroid hormones in the mother and fetus [4]. The WHO/UNICEF/ICCIDD have also stated that an established USI programme with adequate salt iodine levels and good population coverage can meet the high iodine requirement of pregnant women. However, there are concerns that this may be possible only at the expense of more-than-adequate and excessive intakes, reflected by UIC in the range of 200–299 µg/l and >300 µg/l, respectively, in SAC.

There are several reasons for this concern. The daily requirement for iodine during pregnancy is estimated to be 250 µg, more than twice the 120 µg requirement, for instance, in SAC [4]. In populations consuming iodized salt, iodine intake during pregnancy increases

due to higher energy intake, but does not fully compensate for the higher demand for iodine. The daily energy requirement of a pregnant woman is 10 460 kJ/d (2500 kcal/d) in the second and third trimester (+1255 kJ/d (+300 kcal/d) compared with a non-pregnant woman), as compared with the daily energy requirement of 8368–10 460 kJ/d (2000–2500 kcal) for SAC [5]. Thus, the difference in energy requirement in pregnant women is only 0–25% compared with SAC, while the difference in iodine requirement is >100 %. The relatively small increase in food intake during pregnancy is therefore unlikely per se to provide the additional iodine requirement unless iodine-rich foods are preferentially selected.

Adequately iodized salt at the household level is defined by WHO as salt containing 15–40 mg iodine/ kg[4,6], and many USI programme managers, aware of the irreversible effects of iodine deficiency on fetal development, feel justified in setting salt iodization levels near the upper end of the recommended range in order to ensure adequate iodine intake during pregnancy. However, only a limited number of countries have completed UIC surveys in pregnant women and women of reproductive age on the national or sub-national level, and thus there are insufficient data to directly estimate the regional or global prevalence of low iodine intake in these important target groups[7]. Indian salt legislation stipulates that the iodine content in salt at consumption level should be at least 15 ppm[8] and USI remains the single most important source of dietary iodine for the Indian population[9].

There has been a remarkable improvement in the consumption of adequately iodized salt in India, with the national coverage reaching 51% in 2005–2006 and 71% in 2009[10,11]. Access to iodized salt was higher in urban (83·2%) than rural households (66·1%)(12); however, nearly 20% of households were found to be consuming inadequately iodized salt and 9% were using salt that was not iodized[11]. A recent review recommended the mandatory use of adequately iodized salt in the mid-day meal programme for SAC in order to reach the last 30%

of households that are likely to be least accessible and most socio-economically disadvantaged [13].

Studies have assessed concurrent iodine intakes in SAC and pregnant women, and reported that USI provides adequate iodine to pregnant women only when iodine intakes for children are more than adequate (median UIC >200 μg/l) [14,15]. However, these studies did not assess pregnant women and children from the same household sharing all meals together, which limits comparisons between the groups. Therefore, the aim of the present study was to assess iodine intake (based on UIC), and potential determinants of intake, in Indian pregnant women and their children who are sharing all meals. Our hypotheses were: (i) effective USI can ensure adequate iodine intake in pregnant women; but (ii) this may lead to more-than-adequate or excessive iodine intake in their children.

Methods

The present study was carried out in southern India in Bangalore, one of the districts in the Indian state of Karnataka. Bangalore is India's third most populous city with 9·6 million inhabitants and the fifth-most populous urban agglomeration [16,17]. The average literacy rate in Karnataka is 75 %, being 69% and 88% in rural and urban Karnataka, respectively [18]. The average literacy rate of Bangalore district is 88% [16] and the city is among the top ten preferred entrepreneurial locations in the world [19]. The study site was the antenatal clinic (morning and evening) of the Obstetrics and Gynecology Department of St Martha's Hospital, which serves Bangalore's less-affluent population. The morning antenatal clinic operates between 08.30 and 12:30 hours and the evening antenatal clinic operates between 16.00 and 18.00 hours. We aimed to recruit 200 pairs of pregnant women and their children who were sharing all meals in the same household. Our inclusion criteria were healthy pregnant women across all trimesters, aged 18–35 years, who had healthy children, aged 3–15 years. It is a common practice in India for a working mother to leave her children at their grandparents'

home during the day. In such a scenario, sharing of meals from a common household basket does not hold. We thus excluded from the study such mother–child pairs. In addition, the Government of India runs a Mid-Day Meal Programme, one of the largest school lunch programmes in the world [20]. This flagship programme for achievement of universalization of elementary education is being implemented in partnership with the State Government [21]. The programme sources double-fortified salt from the Tamil Nadu Salt Corporation. The double fortified salt premix produces a salt with 50 ppm iodine (as KI) and 1000 ppm Fe at production level [22]. For the purpose of the current study, we ensured that the children we enrolled were not participating in this programme. Thus, the schoolchildren in the present study were those who were carrying food from home, which is a common practice in Indian settings, and thus sharing all meals including lunch with their mothers. We excluded women with a multiple pregnancy and those who were still breast-feeding. Data were collected from May 2008 to September 2011.

Information was obtained by a structured questionnaire and included: (i) age of the mother and her child; (ii) sex of the child; (iii) parity as per the antenatal record; (iv) number of members (adults and children) in the household; (v) education and occupation of the pregnant woman and her husband; (vi) monthly income of the household; (vii) household usage of iodized or noniodized salt including brand and type of salt; (viii) cooking practices using salt; (ix) average household consumption of salt; (x) use and frequency of seafood consumption; and (xi) knowledge of iodine and opinion on salt type preferred with its reason, this information was collected on a subset of the sample. Data on iodine intake from supplements and multi-micronutrient powders available in the market were collected from a subset of pregnant women and their children. This subset was a convenience sample. Crude average per capita consumption of powdered and crystal salt was estimated by dividing the reported monthly consumption of salt (using single recall) by the number of members in the household.

Pregnant women were classified into three trimesters according to their gestational age. This was based on their reported first day of the last menstrual period but, where available, first-trimester ultrasound scan was used. Body weight and height were recorded using standard anthropometric techniques [23]. Weight was recorded to the nearest 0·1 kg using a digital weighing scale (Salters 9016, Tonbridge, UK). Height was recorded to the nearest 0·1 cm using a locally made stadiometer (Biorad, Chennai, India).

A single spot urine sample, obtained throughout the day, was collected from all mothers and children within a week of each other. Samples were transported on ice, divided into aliquots and stored at − 20°C until analysis. UIC was determined using the Pino modification of the Sandell–Kolthoff reaction [24] with external reference standards (C. Zeder, ETH Zurich, Switzerland). The intra-assay CV at a mean UIC of 74, 162 and 282 μg/l (n 21 each) was 11, 9 and 13 %, respectively. The iodine nutrition status in pregnant women and their children was evaluated according to the recommended WHO/UNICEF/ICCIDD criteria [4]. Salt samples of all the types used by the household were collected in clean plastic containers and stored at − 80°C until analysis. Composite samples from each recognized commercial brand were analysed for iodine content by using iodometric titration; those from an unknown source were analysed only qualitatively (iodine presence: yes/no) using a test kit (MBI Chemicals, Chennai, India). Twenty-two duplicate titrations of brand specific composite samples (made by mixing 10 g of salt from five different households and sampling 2 × 10 g of this homogenized mixture for analysis) were done. It would have required fifty-seven more duplicate titrations to also analyse the samples from unknown brands individually, which was not possible within the resources available for this project.

Thyroid gland volume of the pregnant women was measured using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a 7·5MHz linear transducer and ultrasound coupling gel. The thyroid volume of

each lobe was calculated using the formula for a prolate ellipsoid [4], where Thyroid volume (ml) = 0·479×length (cm)×breadth (cm)×depth (cm). The thyroid volume was calculated as the sum of the volumes of both lobes and did not include the isthmus.

Statistical analyses were performed using the statistical software package SPSS version 14·0 and Microsoft® Excel (MS Office 2003). Weight-for-age Z-scores (WAZ), heightfor-age Z-scores (HAZ) and BMI-for-age Z-scores (BAZ) were computed from data on height, weight, age and gender using the WHO Anthro and AnthroPlus software. Data are presented as mean and standard deviation when normally distributed. For non-normal distributions, data are presented as median (range) or median (25th, 75th percentile). Mann–Whitney and Kruskal–Wallis tests were used for comparisons and P values <0·05 were considered significant. Proportions were compared using x2 tests. Spearman correlations were performed for associations.

Results

We recruited 226 pregnant women and their children for the study, and 194 complete sets of data for mother–child pairs were obtained. Maternal indicators are shown in Table 1; 78·3% (*n* 177) of the study women attended the outpatient clinic in the morning while 21·7% (n 49) attended the evening clinic. Although the UIC of pregnant women from the morning clinic was higher than for pregnant women from the evening clinic (187 *v.* 128 μg/l), this difference was not statistically significant. Data on intake of iodine-containing supplements and multi-micronutrient powders were collected from a subset of pregnant women and their children (*n* 105). Of these, 50·5% (*n* 53) of the pregnant women and 46·7% (*n* 49) of the children were consuming iodine-containing multi-micronutrient powders, averaging 18·5 and 15·9 μg/d, respectively. There was no significant difference in median UIC between mothers or children consuming iodine containing multi-micronutrient powders as compared with

those not consuming them (192 *v.* 163 µg/l in mothers and 228 *v.* 218 µg/l in children, respectively).

Table 1: Maternal indicators among the pregnant women aged 18–35 years, Bangalore, India, May 2008 to September 2011

Parameter	n	%
Level of Education of subject		
No formal education	4	1.8
Finished high school (10th Grade)	98	43.4
Post high school	58	25.7
University degree & above	66	29.2
Level of Education of subject's husband		
No formal education	2	0.9
Finished high school (10th Grade)	102	45.1
Post high school	53	23.5
University degree & above	69	30.5
Occupation of subject		
Unemployed	203	89.8
Unskilled worker	2	0.9
Skilled worker	1	0.4
Others (secretarial job, teacher, shop owner, business)	20	8.8
Occupation of subject's husband		
Unemployed	1	0.4
Unskilled worker	11	4.9
Skilled worker	49	21.7
Others (secretarial job, teacher, shop owner, business)	165	72.9
Parity		
0	10	4.4
1	163	72.4
≥2	52	23.1
Trimester of pregnancy		
1	33	14.6
2	79	35.0
3	114	50.4

Opinion on salt preference		
Powder salt	108	61.7
Crystal salt	46	26.3
Other	21	12
Reason for salt type preference		
Taste	41	23.4
Tradition	36	20.6
Quantity	39	22.3
Health	32	18.3
Others	27	15.5
Household seafood consumption per week		
Never	61	27.0
< 1 time	124	54.9
1-2 times	35	15.5
>2 times	6	2.7
Cooking practices with salt		
Salt added during cooking	210	92.9
Salt added at the end of cooking	16	7.1
Knowledge of iodine		
Yes	53	30.6
No	120	69.4
	Median	25^{th},75^{th} percentile
Total monthly household income (INR)	10000	(6500, 20000)

We collected samples of all available salt types from the study households. Some households were using both powdered and crystal salt. In total, 275 salt samples were obtained, and 86% of the powdered salt samples (*n* 164) and 71% of the crystal salt samples (*n* 106) were from a known brand; 79% (*n* 216) of the salt samples were analysed for their iodine content by titration. The median (range) iodine concentration of household powdered and crystal salt was 55·9 (17·2–65·9) ppm and 18·9 (2·2–68·2) ppm, respectively. All of the remaining powdered salt samples (n 23) tested positive for iodine content by the kit method, whereas 75% of the remaining crystal salt samples (*n* 31) were iodized as determined by the kit method. Overall,

inadequately iodized crystal salt (<15 ppm) was found in 3·1% (*n* 8) of the households, but in each case except one with crystal salt iodine content of 12·7 ppm and no powdered salt use, the households had adequately iodized powdered salt. In the households that used both types of salts, crystal salt contributed 57% to the monthly salt usage. Regarding salt preference, 62% (*n* 108) of the pregnant women preferred powdered salt, 26% (*n* 46) preferred crystal salt, and 12% (*n* 21) preferred both types of salts. There was a significant difference in the median UIC of pregnant women and children among these three groups of preferred salt use: powdered salt v. crystal salt *v.* both types of salt (P=0·006; 188 *v.* 133 *v.* 96μg/l in mothers and 248 *v.* 151 *v.* 218 μg/l in children, respectively). Figure 1 shows the UIC of pregnant mothers and their children by usage of powdered and crystal salt and the salt iodine content (<45 ppm and >45 ppm). There was no statistically significant difference in the median UIC of pregnant mothers and their children between the salt iodine content categories among the salt type groups. The estimated average daily consumption of salt per capita based on dietary recall was 13 (SD 6·8) g/d; calculated from the median iodine content in the salt samples, the estimated median (range) per capita iodine intake from salt was ≈301 (0–2283) μg/d.

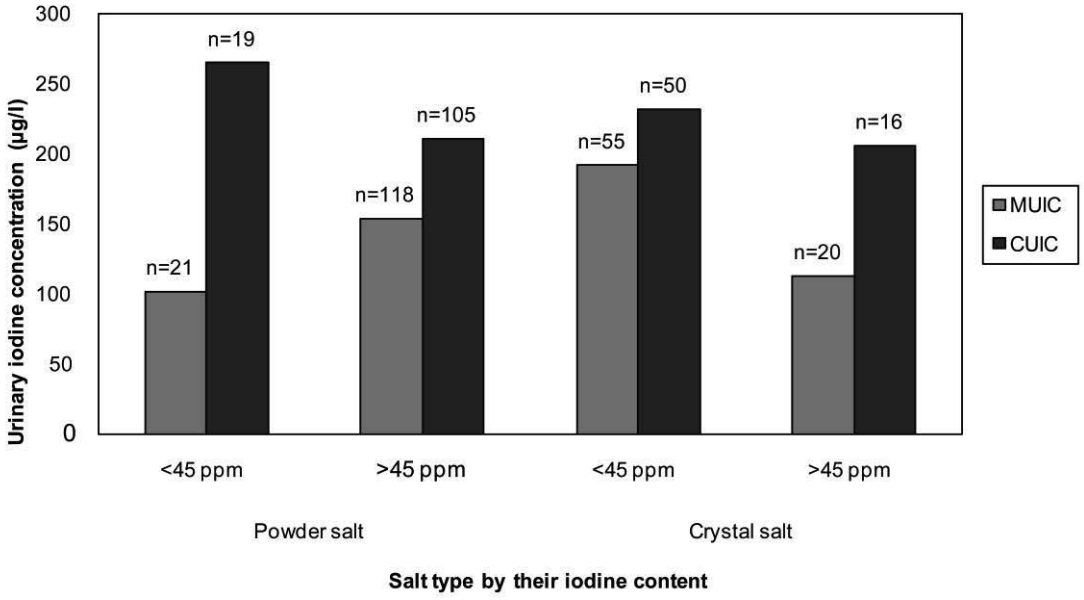

Figure 1: Median urinary iodine concentrations in matched pairs of pregnant women and their children by the salt type and their iodine content.

Maternal gestational age, anthropometrics, thyroid volume and UIC by trimester are shown in Table 2. Gestational age calculated from the date of the last menstrual period correlated highly with that from the uterine ultrasound scan (r=0·99, P = 0·001). Mean thyroid volume and median UIC did not differ significantly among trimesters. There was no correlation between the median UIC and thyroid volume in these pregnant women. The median (range) UIC in the pregnant women was 172 (5–1024) μg/l and indicates iodine sufficiency; the distribution of UIC is shown in Fig. 2(a). Overall, the estimated median (range) daily iodine intake as calculated from UIC in pregnant women was ≈278 (0–1670) μg/d assuming a daily urine volume of 1·5 litres and 92% dietary iodine bioavailability [5].

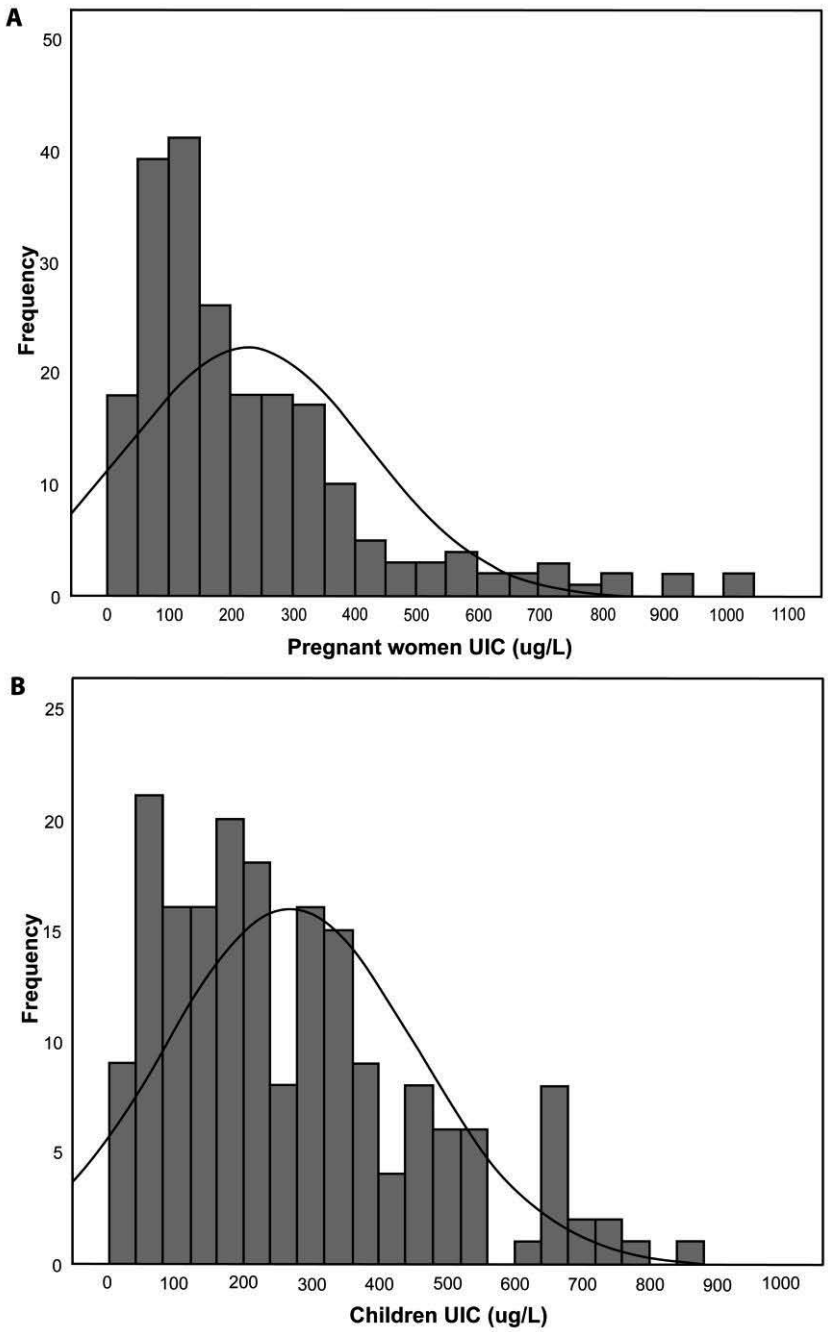

Figure 2: Distributions of urinary iodine concentration among (A) pregnant women and (B) their children.

Table 2: Characteristics of pregnant women aged 18–35 years, Bangalore, India, May 2008 to September 2011

		Overall		Trimester 1		Trimester 2		Trimester 3	
Parameters	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	226	28.0	3.9	27.0	3.6	28.3	4.2	28.0	3.7
Gestational age by LMP (weeks)	226	23.7	9.8	9.2	1.8	17.4	3.3	32.3	4.2
Gestational age by US (weeks)	178	24.2	9.3	9.4	1.9	17.2	3.6	32.0	4.0
Weight (kg)	226	62.2	11.2	56.8	8.8	60.7	12.6	64.9	9.9
Height (cm)	226	153.9	5.7	154.9	3.8	153.7	5.9	153.9	6.1
Thyroid gland size(ml)	218	7.0	2.0	7.1	2.6	7.0	2.7	7.0	1.9
	n	Median	Range	Median	Range	Median	Range	Median	Range
UIC (µg/l)	216	172.0	5-1024	169.9	16-690	158.7	17-1024	191.8	5.3-1020

LMP, last menstrual period; US, ultrasound; UIC, urinary iodine concentration

Table 3: Characteristics of children of the children aged 3–15 years, Bangalore, India, May 2008 to September 2011

	Total			<6 years			6-15 years		
Parameters	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Age (years)	194	5.4	1.9	141	4.9	0.9	53	7.8	1.7
Weight (kg)	194	17.8	5.9	141	15.3	3.1	53	24.2	6.8
Height (cm)	194	106.5	14.0	141	100.3	8.9	53	123.0	11.4
WAZ	186	-0.7	1.2	141	-0.7	1.2	45	-0.5	1.2
HAZ	193	-0.8	1.1	140	-0.8	1.1	53	-0.6	1.2
BAZ	193	-0.3	1.2	140	-0.3	1.1	53	-0.3	1.3
	n	Median	Range	*n*	Median	Range	*n*	Median	Range
UIC (µg/l)	187	220.0	10-867	135	220.0	10-867	52	220.0	10-867

WAZ, weight-for-age Z-score; HAZ, height-for-age Z-score; BAZ, BMI-for-age Z-score; UIC, urinary iodine concentration

Age, anthropometrics and UIC of the children (preschoolers and SAC) are shown in Table 3; 45·2% of them were boys. The median (range) UIC in the children was 220 (10–782) μg/l, significantly higher than the median UIC in their mothers (P=0·008) and indicating more-than adequate iodine intake in this group. The distribution of UIC among children is shown in Fig. 2(b). Using the formula of the US Institute of Medicine (5) to estimate iodine intakes from UIC and body weight in children, a median UIC of 220 μg/l in 6–15-year-old children (*n* 52) with a mean age of 7·8 years and a body weight of 24·2 kg would correspond to a median iodine intake of ≈140 (range 9–413) μg/d. As shown in Fig. 3, there was a weak but significant positive correlation between the UIC of the children and that of their pregnant mothers (*r*=0·160; P=0·03). By trimester, this correlation was significant only in the first trimester (*r*=0·423; P=0·035), not in the second (*r*=0·162; P=0·21) or the third (*r*=0·063; P=0·54).

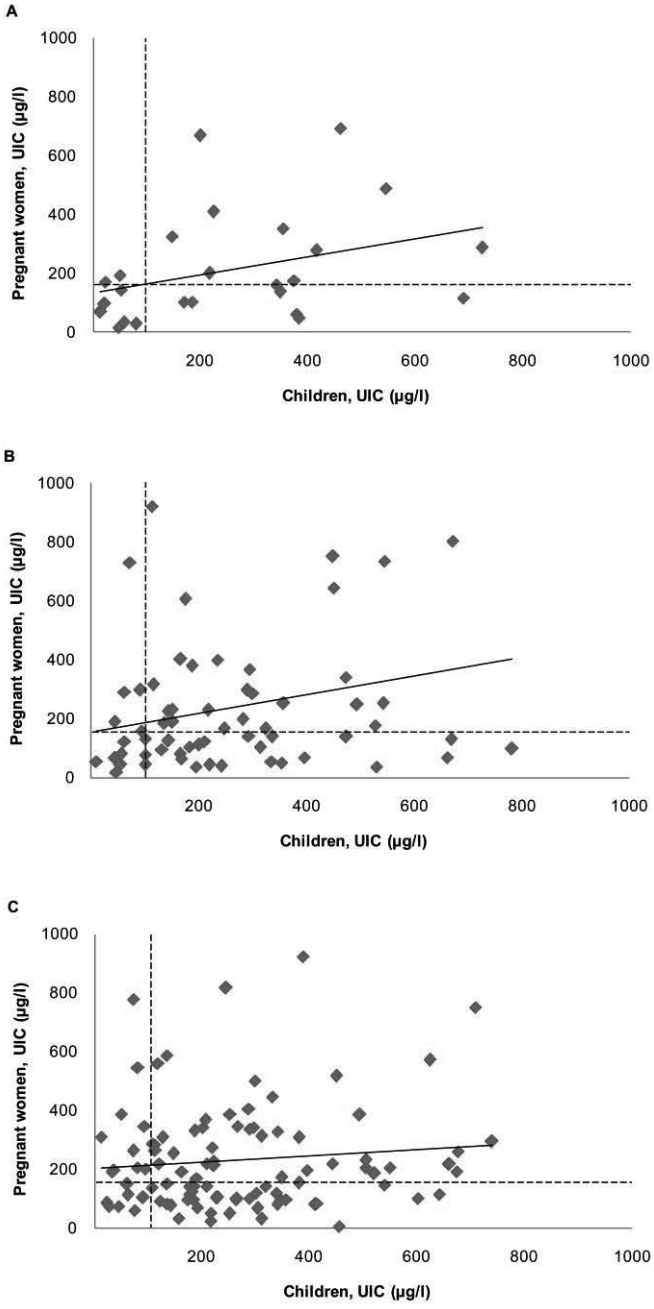

Figure 3: Median urinary iodine concentrations in matched pairs of pregnant women and their children by Trimester 1 (A) y=0.305x+133.6; R2=0.114, Trimester 2 (B) y=0.314x+154.9; R2=0.065 and Trimester 3 (C) y=0.107+202.6; R2=0.011. The dotted lines on each axis indicate the cut-off for iodine adequacy for the groups.

Discussion

The current cross-sectional study is the first to report iodine intakes in pregnant women and their children sharing a common household food basket and all meals. Based on median UIC, pregnant women were iodine sufficient but children had more-than-adequate iodine intake. A recent review concluded that when the median UIC in SAC or non-pregnant women indicated iodine intake was adequate or above requirements in a region, about half the time pregnant women in that same region had inadequate iodine intake [15]. Multiple studies have reported that when SAC have adequate iodine status (median UIC=100–199 μg/l), pregnant women are deficient (median UIC <150 μg/l), and that iodine sufficiency in pregnancy is attained only when the SAC have more-than-adequate (median UIC=200–299 μg/l) iodine status [14,15, 25–28]. In an earlier Thai study using similar methodology [25] but in families where not all meals were consumed within the household, despite a median UIC of 200 μg/l in SAC, iodine intakes in pregnant women were inadequate, with a median UIC of only 108 μg/l. In Tasmania, iodine status of pregnant women before and after iodine fortification of bread was compared with concurrent data for schoolchildren; the data showed pregnant women to be iodine deficient after supplementation, while children were sufficient [29]. Recent studies from Belgium [30] and China [31] also suggest that median UIC in SAC may not be a good proxy of iodine status in the entire population. In our data, there was a modest positive correlation between UIC in pregnant mothers and their children, and this correlation was strongest in the first trimester, consistent with an earlier study from Thailand [25]. In a survey in Kyrgyzstan where children and pregnant women did not live together but were from the same settlements with access to the same salt supplies, their median UIC values were also positively correlated ($r=0{\cdot}63$; $P<0{\cdot}001$) [26].

Our findings form a valuable contribution to the discussion on whether salt iodization programmes can adequately cover the iodine requirements of all population groups including that of pregnant

women. In our study population, the salt iodization programme was adequately covering the iodine requirement of pregnant women. A systematic review of iodine nutrition status in Indian pregnant women found that median UIC were in the range of 95–178 μg/l with 60–95% of pregnant women consuming adequately iodized salt [32]. A recent study in rural and urban settings in the Maharashtra region of India reported median UIC of 203 and 211 μg/l at 17 and 34 weeks of pregnancy, suggesting adequate dietary provision at both gestational stages studied and implying that iodine deficiency was unlikely to be a frequent problem in that population[33]. However, studies from rural India, especially in tribal populations, report significant iodine deficiency in pregnant women and areas where only 19% of salt is adequately iodized [34,35]. Thus, our results should not be generalized to less-affluent, rural areas of India where adequately iodized salt is not available and where pregnant women are likely to still have inadequate iodine intakes. Because there are wide variations in iodized salt quality and coverage across India, additional population-based studies in other regions of India are needed to confirm these findings.

There are some previous data from the Bangalore region on iodine intake. The WHO Global database on iodine deficiency 2007 reported that the median UIC in 11–18-year-old children in the Bangalore urban district was 185 μg/l[36]. The National Family Health Survey (NFHS-3) for the state of Karnataka, India (2005–2006) found 66·5 and 27·6% of households in urban and rural areas, respectively, were using salt iodized at >15 ppm [10]. The 2009 Coverage Evaluation Survey conducted by UNICEF reported that the use of non-iodized salt seemed more common in the state of Karnataka compared with other states, with 40·1, 23·9 and 35·9% of the households using non-iodized salt (0 ppm), inadequately iodized salt (<15 ppm) and adequately iodized salt (≥15 ppm), respectively [12]. The Iodized Salt Coverage Study in 2010 found only a marginal improvement from NFHS-3 in the consumption of adequately iodized salt in rural Karnataka (35·4%) and attributed this to the continued preference for crystal salt that tends to be less well iodized[37], which is confirmed by

our study. Currently, in Karnataka, iodized crystal salt is distributed through the public distribution system at 3·00 Indian rupees/kg [38] and 64·2% of the pregnant women in our study preferred powdered to crystal salt; this likely explains why non-iodized crystal salt was present in only 4% of households (but in each case, adequately iodized powdered salt was present) and the median UIC in pregnant women indicated sufficient intake. However, two-thirds of households (64·3%) were using both types of salt and in these households crystal salt contributed 57% of the monthly salt usage. Therefore, it is important that crystal salt, along with powdered salt, continues to be adequately iodized.

A better understanding of the iodine sources, planned and unplanned, is crucial for the design and monitoring of national iodine nutrition programmes [1]. Accounting for iodized salt intake obtained from processed foods is becoming increasingly important, as well as iodine containing products such as iodine supplements, multi micronutrient supplements, home fortification products such as micronutrient powders, ready-to-use supplementary foods and, in some specific cases, iodine in the natural environment[1]. In our study, the contribution of iodine containing supplements and multi-micronutrient powders to iodine intakes was negligible; the only significant source of iodine was household salt, and the mean per capita consumption of household salt was estimated to be high, at 13 (SD 6·8) g/d. This was a crude estimate derived by dividing the monthly consumption of salt by the number of members in the household that included both adults and children. Although our data provide only a rough estimate of actual salt consumption, it is in general agreement with the mean per capita salt intake of 11·3 (SD 5·1) g/d calculated using 3 d weighed food records in a recent study in Bangalore[39]. The estimated daily iodine intake in pregnant women and 6–15-year-old children (n 52) in the present study was ≈278 and 140 μg/d, respectively [5]. While we did not collect dietary data for iodine intakes in the present study, recent 24 h dietary recall intake data from a comparable group of pregnant women in this area found that these women consume a traditional South Indian meal with

dairy products but no bread and a minimum amount of processed foods (N Jaiswal, unpublished data). Thus, iodized salt is most likely the most important source of iodine and a secondary source could be dairy products in these households. However, it is important that native dietary sources of iodine and processed foods be identified and accounted for iodine intake in populations. The findings of the current study can likely be generalized to other households in Bangalore. The lunch meal the children shared with their pregnant mothers in the present study would be similar to that provided in the noon meal programme in Karnataka. The suggested menu for the meals in this programme are meals of rice and lentils, semolina with vegetables and rice, and lentils with vegetables, all cooked with double fortified salt containing Fe and 50 ppm iodine[40]. The median iodine concentration of powdered salt in our study was 55 ppm.

Although iodine intake in our study appeared mainly due to iodized salt, in other countries, both dietary intake of foods with high native iodine content and iodine supplements contribute to iodine intakes during pregnancy. For example, many Japanese eat seaweed and make soup stock from kelp on a daily basis; and in fact it is widely believed in Japanese society that seaweed intake is good for pregnancy [41]. In the US diet, the common sources of iodine are iodized salt, dairy products, breads and seafood [42]. To achieve a total of 250 µg iodine ingestion daily in North America, the American Thyroid Association recommends that pregnant women should supplement their diet with a daily oral supplement that contains 150 µg iodine [43]. However, iodine intake in the USA continues to fall and pregnant women are iodine deficient; despite recommendations for iodine supplementation by experts, iodine supplements are used by only 22% of US pregnant women [44]. The American Thyroid Association also recommends that in areas of the world outside North America, strategies for ensuring adequate iodine intake during pregnancy will vary according to regional dietary patterns and availability of iodized salt [43]. In the UK, a recent study called for iodine deficiency in pregnant women to be treated as an important public health issue, particularly considering there is no national salt

iodization programme and dietary guidelines from the UK government are outdated [45]. In Australia, mandatory use of iodized salt (25–65 mg/kg) by bread manufacturers and a daily supplement intake of 150 μg iodine by pregnant women are recommended by the National Health and Medical Research Council as the two strategies to achieve optimal iodine intakes in this group [46]. Using predictive modelling, pregnant women in New Zealand were expected to achieve adequate but not excessive iodine intakes when 150 μg of supplemental iodine was taken daily, taking into account the contribution of iodized salt in bread [47]. However, disparities in supplement use by New Zealand pregnant women highlight the need for further efforts towards USI, such as the mandatory fortification of additional processed foods with iodized salt [48].

A limitation of our study is the use of a single spot urine sample collected from pregnant mothers and their children within a week of each other. Considering that UIC in spot samples varies substantially between days and seasons [49–51], as a consequence of a circadian rhythm of iodine excretion [52] and due to differences in fluid intake[53], it would have been preferable to obtain samples at the same time. Although we did not attempt to estimate the proportion of our population with high and low intakes of iodine, a sub-sample collection would have removed the within-person variance thus permitting a correction to the population distribution. In a population with a median UIC in the sufficient range, this correction of the tails would lead to a decrease in the proportion at either extreme of the distribution[54]. However in the present study, we found a good significant correlation (r=0·432; P=0·035) between the UIC values of the children and their pregnant mothers in their first trimester.

We are aware of no published data that show iodine intakes by SAC in the more-than-adequate range indicated by a median UIC of 200–299 μg/l have adverse effects. On the contrary, a recent study including data from twelve countries and more than 2500 children concluded that chronic iodine intakes from iodized salt resulting in

more-than-adequate UIC values do not cause thyroid dysfunction in 6–12-year-old children [55]. That study recommended that the more-than-adequate iodine intake range (200–299 µg/l) should be reconsidered and merged with the adequate iodine intake range resulting in a widened range of 100–299 µg/l indicating sufficient intake in children [55]. Earlier, it had been shown that UIC up to 500 µg/l is not associated with thyroid volume in a large international sample of 6–12-year-old children with iodine intakes ranging from adequate to excessive [56]. Most people who are iodine sufficient are remarkably tolerant to high dietary intakes of iodine, and intakes up to 1100 µg/d are tolerated well by healthy adults [5]. There are concerns that more-than-adequate iodine intake could increase thyroid autoimmunity in adults [57] but findings are equivocal. Therefore, considering the available evidence, we feel the iodine intake level of the children in the current study is very likely to be safe.

It could be argued that the current Indian salt legislation with a cut-off value for adequacy at 15 ppm needs a revision in the current scenario to incorporate a range with an upper limit for salt iodization, as is done in many national programme standards. According to WHO/UNICEF/ ICCIDD, an adequate iodine level in household salt is defined as salt containing 15–40 mg iodine/kg [4,6]. We found a median (range) iodine concentration of 55·0 (17·2–65·9) µg/g in powdered salt and 18·9 (2·2–68·2) µg/g in crystal salt in the present study. Studies from Rajasthan, India and China showed that optimal iodine status of both children and pregnant women was attained when the salt iodine content was approximately 30 mg/kg [58,59]. In the Rajasthan study, it was proposed that the iodization of household salt should be increased from the current level in order to provide optimal iodine intakes in the population [58]. In a recent cross-sectional study from Shanghai, China where the median salt iodine concentration was 29·5 mg/kg and 91·5% of households were using adequately iodized salt, pregnant women were still iodine deficient while the general population had adequate iodine nutrition [31]. In Kyrgyzstan, pregnant women had adequate iodine intakes only in

those households where salt iodine content was ≥25 mg/kg [26]. In these studies, salt was the main source of iodine intake in the diet. In populations where a substantial proportion of the total iodine intake comes from milk, UIC in SAC may overestimate the iodine status of the general adult population because milk consumption usually is higher in children [7]. However, in most countries where salt is the primary source of iodine in the diet, the differences between children and adults is smaller and the median UIC in SAC may be used to represent iodine status of the population at large [60].

Conclusion

In conclusion, our data indicate that in this selected urban population of southern India, salt iodization (including iodization of crystal salt) ensures adequate iodine intake in pregnant women, although iodine intake in their children is in the more-than-adequate range according to current cut-off criteria. Although the study suggests that a well functioning iodized salt programme can provide adequate iodine to pregnant women, continuous monitoring of this critical target group is required and in populations where iodized salt is either not available or not adequately iodized, iodine supplementation during pregnancy should be considered. In addition, evaluation of the current recommendation to use median UIC in SAC as a proxy for the iodine status in the general population and population groups most vulnerable to iodine deficiency, such as young children and pregnant women, was recently recognized as a research priority [61]. We suggest that the current WHO/UNICEF/ICCIDD cut-off for median UIC in children indicating more-than-adequate intake may need to be reconsidered to allow the iodized salt programme to cover the increased needs of pregnant women.

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Chapter 4

High prevalence of maternal hypothyroidism despite adequate iodine status in Indian pregnant women in the first trimester

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Abstract

***Background**:* Iodine requirements are increased during pregnancy to maintain maternal and fetal euthyroidism. There have been recent improvements in iodized salt coverage in India but whether iodized salt is sufficient to sustain iodine requirements during pregnancy remains uncertain. Our aims were to measure thyroid status in first trimester pregnant women in southern India and assess potential determinants of thyroid function, including iodine status, thyroid autoimmunity, dietary patterns, body weight and anemia.

Methods: Cross-sectional study among 334 pregnant women ≤14 weeks of gestation, in Bangalore, India. We measured anthropometrics, urinary iodine concentration (UIC), maternal thyroid volume (by ultrasound) and thyroid function. We applied a thyrotropin (TSH) upper limit of 2.5 mIU/L to classify thyroid insufficiency. Using a questionnaire, we obtained socio-demographic and dietary data, obstetric history, and use of iodised salt and iodine supplements.

Results: Among the women, the mean (standard deviation) gestational age was 10.3 (2.5) weeks, 67% were nulliparous, 21% were vegetarian, 19% were anemic and 23% were overweight or obese. Iodized salt was used by 98% of women, and they were iodine sufficient: median UIC (range) was 184.2 (8.1-1152) μg/L and all had a normal thyroid volume. However, 18% of the women had thyroid insufficiency: 3.7% had overt hypothyroidism (83% with positive TPO-Ab), 9.2% had subclinical hypothyroidism and 5.2% had hypothyroxinemia. Women consuming vegetarian diets did not have significantly lower iodine intakes or higher risk of hypothyroidism than those consuming mixed diets, but overweight/obesity and anemia predicted thyroid insufficiency.

Conclusion: In this urban population of southern India, pregnant women have adequate iodine status in the first trimester. Despite this, many have thyroid insufficiency, and the prevalence of overt

hypothyroidism is more than fivefold higher than reported in other iodine sufficient populations of pregnant women.

Introduction

Iodine requirements during pregnancy sharply increase to maintain maternal and fetal euthyroidism (1). In the first trimester, the mother requires additional iodine to produce and transfer thyroid hormone across the placenta while the fetal thyroid matures. In the second half of pregnancy, the fetus begins producing thyroid hormone, which requires additional iodine transfer from the maternal circulation (2). Worldwide, iodine deficiency during pregnancy is a leading cause of maternal thyroid insufficiency, which can irreversibly impair fetal brain development, and the first trimester is a particularly vulnerable period (1). Thus, in iodine deficient populations, prevention of hypothyroidism and cretinism is most effective when iodine is supplied to women before or very early after conception (3).

The World Health Organization states that an established universal salt iodization program with adequate salt iodine levels and good population coverage can meet the increased iodine requirement of pregnant women (4). However, maternal iodine insufficiency is prevalent even in countries with established programs (5-9). India, because of its large population, high birth rate and iodine deficient soils, has until recently had a large number of infants potentially exposed to *in utero* iodine deficiency (10). However, household coverage of iodized salt has increased, and it is now estimated that >70% of households in India have access to iodized salt (10). Nevertheless, recent studies highlight the presence of iodine insufficiency among Indian women (11).

In areas of iodine sufficiency, the prevalence of overt and subclinical hypothyroidism during pregnancy is 0.2-0.4% and 3-5% respectively, but varies by trimester of pregnancy and the diagnostic criteria (particularly the thyrotropin [TSH] upper limit) used for classification (12). More than 90% of thyroid disorders in pregnancy are thought to be of autoimmune etiology (13), and chronic autoimmune thyroiditis is the main cause of hypothyroidism during pregnancy in iodine

sufficient regions (14). Two additional variables that may affect the maternal thyroid axis and contribute to thyroid insufficiency during pregnancy are iron deficiency anemia (15) and overweight/obesity (7, 16-19), and both are common among women in urban areas of India (20-22). Better understanding of the pattern and prevalence of maternal thyroid dysfunction and associated risk factors could improve screening and treatment in the Indian population. Improved detection of maternal hypothyroidism may be particularly valuable in the first trimester, because thyroid function should be normalized as quickly as possible early in gestation (12).

Therefore, the aims of this cross-sectional study were to: a) to measure thyroid status in first trimester pregnant women in southern India; and b) to assess potential determinants of thyroid function in this population, including iodine status, thyroid autoimmunity, body weight, and anemia.

Subjects and methods

This cross-sectional study consisted of a cohort of pregnant women who were screened for the Maternal Iodine supplementation and its effects on Thyroid function and CHild development (MITCH) study (Clinicaltrials.gov with the identifier NCT00791466). The parent study investigates the effects of oral iodine supplementation during pregnancy on pregnancy outcome, infant growth and offspring cognitive development. The study was conducted at the antenatal clinic of the Obstetrics and Gynecology Department of St. Martha's Hospital in Bangalore, India. Pregnant women were recruited between December 2008 and March 2011. We conducted the study in accordance with the Declaration of Helsinki. Institutional ethical review boards at St. John's National Academy of Health Sciences, St Martha's Hospital, Bangalore, India and Wageningen University, The Netherlands approved the study. We explained the study in detail to the participating women and one member of their family, and obtained written, witnessed informed consent.

Study population

We screened all women presenting to the antenatal clinic for potential inclusion in the study if they had a positive pregnancy test and were: a) ≥ 18 and ≤ 40 years old; and b) ≤ 14 weeks gestational age. Exclusion criteria were: a) chronic diseases, including diabetes, heart, kidney and thyroid disease, cancer, hypertension, tuberculosis, asthma, epilepsy, jaundice; b) a positive test for HIV, HbSAg or venereal diseases; c) treatment for infertility; d) previous repeated spontaneous abortions (four or more); e) current multiple pregnancy as detected by ultrasound; and e) currently breastfeeding. Of the 1058 pregnant women who presented at the antenatal clinic at the start of their pregnancy during the study period, 1015 women were contacted for the study; 43 women were not contacted because, based on their hospital record, they did not meet the inclusion criteria. Of the 1015 women contacted, 344 women were not eligible for the study because they did not meet the inclusion criteria. Of the remaining 671 pregnant women who were considered eligible, 337 declined to participate in the study. A total of 334 pregnant women were eligible and consented for their participation in this study. There were no statistically significant differences in age, gestational age, education, occupation, monthly household income, height, weight or body mass index (BMI) between those who declined participation and those who agreed to participate in the study (data not shown).

Socio-demographic and anthropometric data

We used a structured multiple-choice questionnaire to obtain sociodemographic information on household composition, education, occupation, and income, as well as consanguinity and obstetric history, including parity. We did not collect information on family history of thyroid disease. We calculated gestational age from reported first day of the last menstrual period and confirmed this by using ultrasonography in 63% of the women. We recorded anthropometric measurements in duplicate using standard

techniques (23). We recorded weight to the nearest 0.1 kg by using a digital weighing scale (Salter's 9016, Tonbridge, Kent, UK), and height to the nearest 0.1 cm by using a stadiometer (Biorad, Chennai, India). We calculated BMI as weight in kilograms divided by the square of height in metres (kg/m^2); we defined overweight as BMI of 25.0-29.9 kg/m^2 and obesity as ≥30 kg/m^2 (23).

Data on diet, salt and iodine supplement use

By using a self reported written questionnaire, we obtained data on dietary habits including: a) whether the subject was a vegan, a lacto-ovo vegetarian or nonvegetarian; b) household usage of iodized or noniodized salt, including brand and type of salt; c) cooking practices using salt; d) use and frequency of seafood and egg consumption; and e) knowledge of iodine nutrition and reason for iodized salt consumption, data on consumption of nutritional supplements containing iodine or iodine containing multi-micronutrient powders, including quantity and length of time these supplements had been consumed, these data were collected from a subset of pregnant women. This subset was a convenience sample.

Laboratory analysis

We collected a casual spot nonfasting urine sample that was transported on ice, divided into aliquots, and stored at -20^0C until analysis. We determined UIC by using the modification of Pino *et al.* the Sandell-Kolthoff reaction (24) with external reference standards (C. Zeder, ETH Zurich, Switzerland). At a mean UIC of 82.6 μg/L, the intra and inter-assay CV was 9.5% and 7.6% respectively, and at a mean UIC of 206.4 μg/L, the intra and inter-assay CV was 3.2% and 5.3% respectively. We collected a nonfasting whole blood sample by venipuncture into plain vacutainers (BD diagnostics, Franklin Lakes, NJ) that was transported on ice and centrifuged to obtain serum, which was stored frozen at -80^0C until analysis for TSH, total triiodothyronine (TT3), total thyroxine (TT4), free T3 (fT3), free T4 (fT4), thyroglobulin (Tg), thyroid binding globulin (TBG), anti-thyroid

peroxidise antibodies (TPO-Ab) and anti-Tg antibodies (Tg-Ab). With the exception of TSH, for which trimester-specific ranges are available, we used the manufacturer's recommended reference range for thyroid function tests. We measured TSH by using a third generation solid phase, two-site chemiluminescent assay (IMMULITE 1000, Siemens Healthcare Diagnostics, Llanberis, Gwynedd, United Kingdom) and applied a reference range of 0.1-2.5 mIU/L for the first trimester (25, 26). The percent CVs at a TSH of 0.44 and 4.75 were 3.86% and 7.16% respectively. The analytical measurement range of the TSH assay was up to 75 mIU/L and its analytical sensitivity was 0.004 mIU/L. We measured TT4, fT4, TT3, fT3, Tg, Tg-Ab, TPO-Ab and TBG by using immunoassays (IMMULITE 1000). The reference range for TT4 was 4.5-12.5 µg/dL, 58-161 nmol/L, the precent CVs at 4.3 and 11.5µg/dL were 6.5% and 7.6% respectively; the analytical measurement range was 1-24 µg/dL; and analytical sensitivity was 0.4 g/dL. The reference range for TT3 was 70-204 ng/dL, 1.1-3.1 nmol/L; the percent CVs at a TT3 of 61.7 and 192.5ng/dL were 9.0% and 7.26% respectively; the analytical measurement range was 40-600 ng/dL and analytical sensitivity was 35ng/dL. The reference range for TBG was 15-34 µg/mL, 0.3-0.6 µmol/L, the percent CV at a TBG of 15.09 was 7.58%; the analytical measurement range was 3.5-80 µg/mL; and analytical sensitivity was 1.1µg/mL. The reference range for fT4 was 0.89-1.76 ng/dL, 11.5-22.7 pmol/L; the percent CVs at a fT4 of 0.95 and 2.5 ng/dL were 5.14% and 4.91% respectively; the analytical measurement range was 0.3-6.0 ng/dL; and the analytical sensitivity was 0.13 ng/dL. The reference range for fT3 was 2.6-4.8 pg/mL, 4.0-7.4 pmol/L; the percent CVs at a fT3 of 2.39 and 5.92 pg/mL were 4.24% and 4.13% respectively; the analytical measurement range was 1-40 pg/mL; and the analytical sensitivity was 1 pg/mL. The reference range for Tg was 1.7-55.6 ng/mL; the percent CV at a Tg of 9.69 ng/mL was 3.64%; the analytical measurement range was up to 300 ng/mL; and the analytical sensitivity was 0.2 ng/mL. The reference range for Tg-Ab was 0-40 IU/mL, the percent CV at 25.21 IU/mL was 5.29%; the analytical measurement range was 20-3000 IU/mL; and the analytical sensitivity was 10 IU/mL. We applied a cut off value for

TPO-Ab of <35 IU/mL; the percent CV at 50.35 IU/mL was 6.97%; the analytical measurement range was 10-1000 IU/mL; and the analytical sensitivity was 7 IU/mL. Haemoglobin (Hb) was measured on an automated Coulter counter (ABX Pentra 60C+, Horriba Ltd, Kyoto, Japan). Anaemia was defined as Hb < 11.0 g/dL.

Thyroid gland measurement

We measured thyroid gland volume by using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a 7.5MHz linear transducer. We calculated thyroid volume of each lobe using the formula for a prolate ellipsoid where thyroid volume (ml) = 0.479 ×length× breadth ×depth (cm), and summed the volumes of both lobes to obtain total thyroid volume (4).

Statistical analysis

All the thyroid function parameters except total T4 were non-normally distributed, and these were log-transformed before analysis. We confirmed the normality of the log-transformed data using the Kolmogrov-Smirnov test. We report normally distributed data as arithmetic means ± standard deviation (SD), non-normally distributed data as medians (quartiles) and categorical data as numbers (%). We examined correlations among thyroid hormones by using Pearson's correlation, the association of thyroid function parameters with maternal characteristics using linear regression analysis, and present the R^2 for each characteristic in the univariate analysis. All characteristics with $p < 0.20$ in the univariate analyses were considered in multiple variable model to adjust for possible confounding effects of maternal characteristics and the results presented with R^2 for multivariate model. The regression coefficient from the linear regression analysis of log transformed data was interpreted as percent change for one unit increase in independent variable (27). The percentage change for TT4 was calculated by dividing the regression coefficient by the reference group mean and multiplied by 100. Regression of TSH, fT4, TT4, and Tg considered

maternal age, gestational age (<10 wks vs. >10 wks), haemoglobin, BMI (normal vs. underweight, overweight, obese), consanguinity, parity (0 vs. ≥1), maternal education, maternal employment, food habits (vegetarian vs. non-vegetarian, lacto-ovo-vegetarian), reason for salt use (health vs. taste, tradition, quantity, others), seafood intake, egg intake, use of iodine containing multi-micronutrient powders and presence of Tg-Ab and TPO-Ab above the cut off values. Statistical analyses were carried out with SPSS v 18.0 (SPSS, Inc., Chicago, IL).

Results

The demographic, anthropometric and dietary characteristics of the women are shown in Table 1. Mean gestational age was 10.3 weeks and two-thirds of the women were nulliparous. Notably, 23% were overweight or obese, 18.3% were anemic (Hb <11.0 g/dL), 20.7% were vegetarian, and 56.1% and 36.9% of the women were not consuming seafood and eggs respectively. Although nearly all were using iodized salt at home, only about half were aware of iodine in their diet, and only 10% specifically chose iodised salt for health reasons. One third of women were consuming multi-micronutrient powders (5-10g/day) containing iodine in the range of 73-294 µg/100 g. But the mean (range) iodine intake from these powders was low: 12.4 (4-59) µg/day for a mean (range) duration of 10 (0-180) months.

Thyroid parameters and UICs in the women are shown in Table 2. The median UIC (range) was 184.2 (8.1-1152) µg/L and none of the women had an elevated thyroid volume or an elevated thyroglobulin; all of these indicators suggest iodine sufficiency (28). The distribution of UIC is shown in Figure 1.

Table 1: Demographic, anthropometric and dietary characteristics of first trimester pregnant women in Bangalore, India

Parameters	*Values*
Age, years (n= 334)[1]	24.7 ± 4.2
Gestational age, weeks (n=334)[1]	10.3 ± 2.5
Total monthly household income, INR (n=318)[1]	14000 (8000, 24625)
Level of Education (n=331)[1]	
No formal education	1 (0.3)
Finished high school (10th grade)	136 (41.1)
Post high school	85 (25.7)
University degree and above	109 (32.9)
Level of husband's education (n=330)[1]	
No formal education	5 (1.5)
Finished high school (10th grade)	138 (41.8)
Post high school	96 (29.1)
University degree and above	91 (27.6)
Occupation (n=330)[1]	
Unemployed	272 (82.4)
Unskilled worker	2 (0.6)
Skilled worker	11 (3.3)
Others (secretarial jobs, teachers, shop owners)	45 (13.6)
Husband's occupation (n=327)[1]	
Unemployed	0
Unskilled worker	26 (7.9)
Skilled worker	86 (26.3)
Others (secretarial jobs, teachers, shop owners)	215 (65.7)
Consanguinity (n = 331)[1]	
Yes	51 (15.4)
No	280 (84.6)
Parity (n=319)[2]	

0	213 (66.8)
≥ 1	106 (33.2)
History of Abortion (n=319) [2]	
0	255 (79.9)
≥ 1	64 (20.1)
Haemoglobin, g/dL (n=322)	11.9 ± 1.4
Weight, kg (n=331)[3]	52.8 ± 9.1
Height, cm (n=329)[3]	154.1 ± 5.6
BMI, kg/m^2 (n=329)[3]	22.3 ± 3.9
BMI category (n=329)[3]	
Underweight	50 (15.2)
Normal	204 (62.0)
Overweight	60 (18.2)
Obese	15 (4.6)
Food habits (n = 323)[4]	
Vegetarian	57 (17.6)
Lacto-ovo-vegetarian	10 (3.1)
Non-vegetarian	256 (79.3)
Salt used (n=272) [4]	
Iodised powder salt	64 (23.5)
Iodised crystal salt	2 (0.7)
Both (iodised powder and crystal salt)	203 (74.6)
Loose crystal and iodised powder salt	3 (1.1)
Reason for salt choice (n=275) [4]	
Taste	110 (40.0)
Tradition	71 (25.8)
Quantity	48 (17.5)
Health	28 (10.2)
Others	18 (6.5)
Knowledge of iodine (n=273) [4]	

Yes	125 (45.8)
No	148 (54.2)
Salt use practice (n = 277) [4]	
Salt added during cooking	237 (85.6)
Salt added at the end of cooking	40 (14.4)
Frequency of seafood consumption per week (n=320) [4]	
Never	180 (56.3)
<1	93 (29.1)
>1	47 (14.7)
Frequency of egg consumption per week (n =289) [4]	
Never	118 (40.8)
<5	112 (38.8)
>5	59 (20.4)
Use of nutritional iodine supplements (n = 231) [4]	
Yes	78 (33.8)
No	153 (66.2)

Data are mean ± SD, median with quartiles in parantheses, and numbers with percentages in parantheses

Socio-demography[1], obstetrics history[2], anthropometry [3], dietary characteristics [4]

Parity was defined as the number of times a woman has given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or was stillborn. Abortion was defined as termination of pregnancy, whether spontaneous or induced

Table 2: Thyroid parameters and urinary iodine concentrations in first trimester pregnant women in Bangalore, India

Parameters	*n*	*Median (range)*
Thyrotropin (TSH), mIU/L	334	1.2 (0.02-32.8)
Total thyroxine (TT4), nmol/L	322	146.1 (55.9-242)
Free T4, pmol/L	325	14.3 (8.9-78.5)
Total triiodothyronine (TT3), nmol/L	313	2.2 (0.6-4.0)
Free T3, pmol/L	316	5.7 (0.5-47.5)
Thyroglobulin, μg/L	318	6.6 (0.1-87.1)
Thyroxine binding globulin, μmol/L	313	0.7 (0.1-1.5)
Thyroid gland volume, ml	332	5.0 (1.5-10.6)
Urinary iodine concentration, μg/l	321	184.2 (8.1-1152)
Thyroid dysfunction[1]		*Percent (%)*
Overt hyperthyroidism	1	0.3
Subclinical Hyperthyroidism	6	1.8
Overt hypothyroidism	12	3.7
Subclinical Hypothyroidism	30	9.2
Hypothyroxinemia	17	5.2

[1]overt hyperthyroidism, low TSH + high free T4; subclinical hyperthyroidism, low TSH + normal free T4; overt hypothyroidism, high TSH+ low free T4; subclinical hypothyroidism, high TSH+ normal free T4; isolated hypothyroxinemia, normal TSH + low free T.

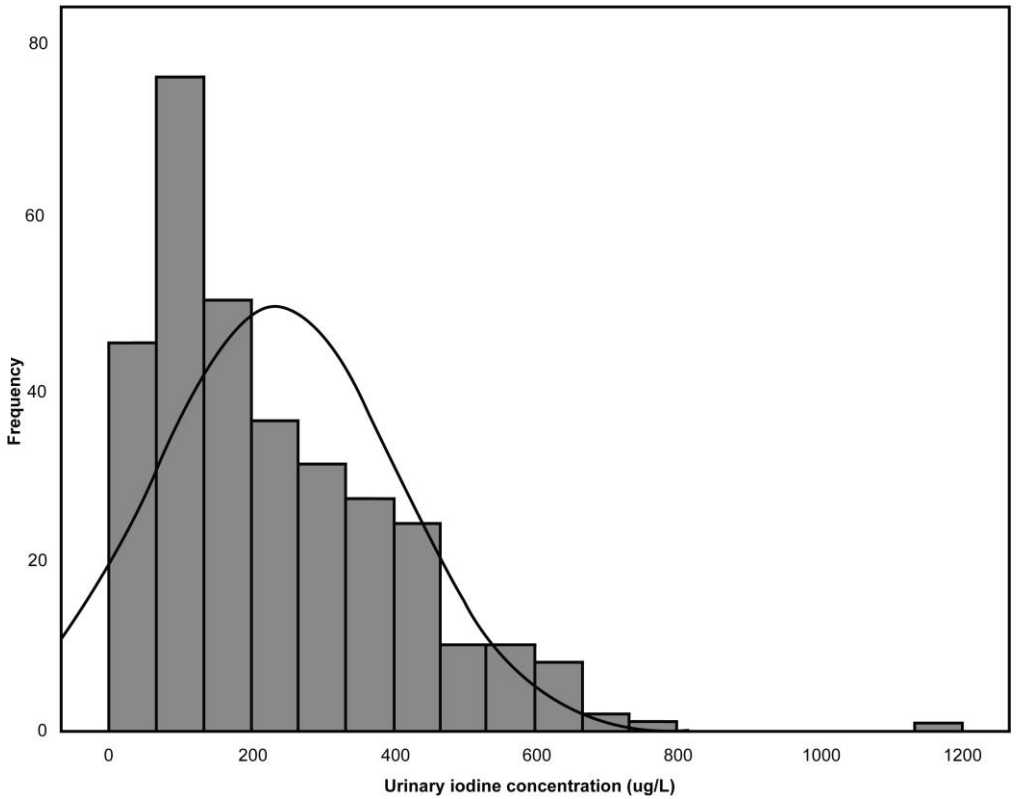

Figure 1: Distribution of UICs in first trimester pregnant women from Bangalore, India (n = 321)

The prevalence of thyroid function tests outside reference ranges are shown in Figure 2. Only seven (2.1%) women were hyperthyroid, six of whom had subclinical hyperthyroidism. In contrast, 42 (12.9%) were hypothyroid, 30 of whom had subclinical hypothyroidism; and 17 (5.2%) had isolated hypothyroxinemia with normal serum TSH. Thirty-two (9.7%) and 46(14.0%) of the women had elevated TPO-Abs and Tg-Abs, respectively.

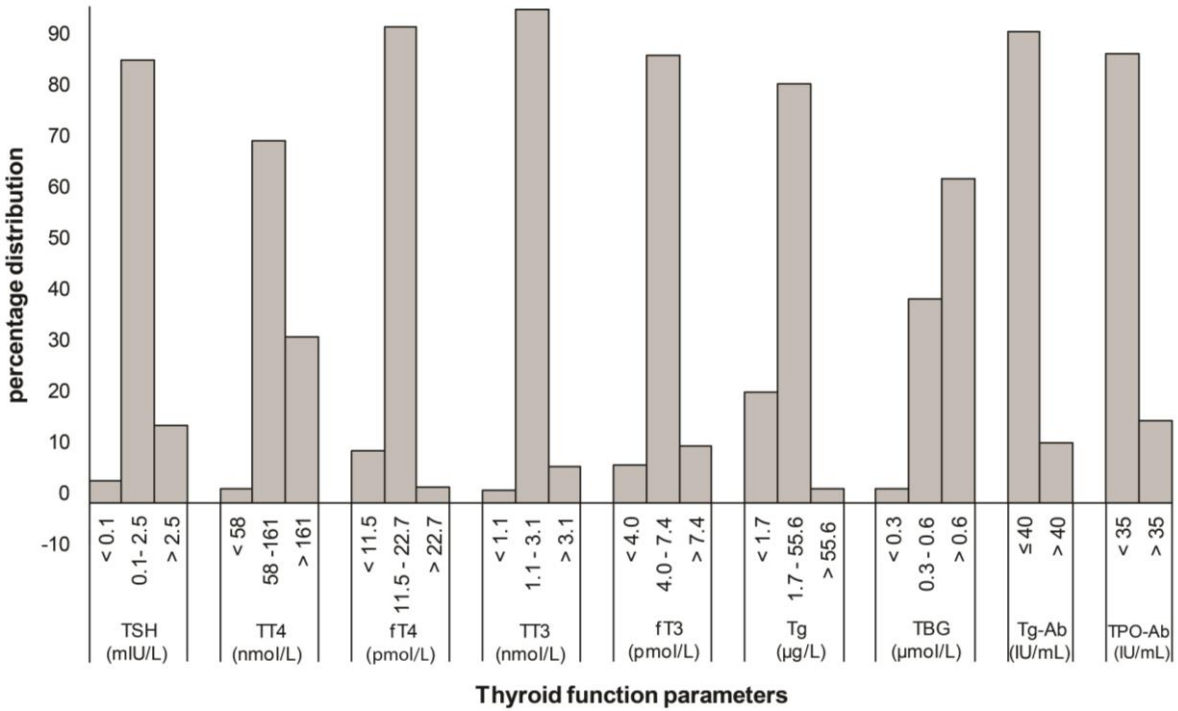

Figure 2: Prevalence of thyroid function tests outside reference ranges in first trimester pregnant women from Bangalore, India. Ech set of bars on the x axis indicate the distribution of each thyroid function parameter; y axis represent percentages

Table 3 shows the comparisons of characteristics of women with thyroid hypofunction (hypothyroidism or hypothyroxinemia) to those who were euthyroid. In women with thyroid hypofunction, hemoglobin was lower (p=0.005), and there was a significantly increased prevalence of TPO-Ab (34.5% vs. 9.2%) and Tg-Ab (22.4% vs. 6.5%; p=<0.001) compared to euthyroid women. Hemoglobin levels did not differ between pregnant women with overt and subclinical hypothyroidism (p=0.825). The prevalence of elevated TPO-Ab was 12.5%, 26.7% and 83.3% in women with hypothyroxinaemia, subclinical hypothyroidism, and overt hypothyroidism respectively, and the median UICs in these three subgroups were 146.8, 166.1 and 195.9 μg/L respectively.

Table 3: Comparison of characteristics between euthyroid and hypothyroid first trimester pregnant women in Bangalore, India

Parameters[1]	*Overt or subclinical hypothyroid, hypothyroxinaemic (n=58)*	*Euthyroid (n=260)*	*p-Value*[2]
Gestational age, weeks	10.4 ± 2.6 (5.2-14.5)	10.3 ± 2.4 (5.0-14.6)	0.91
BMI, kg/m^2	22.7 ± 3.8 (16.1-32.6)	22.1 ± 3.9 (14.5-36.3)	0.32
Hemoglobin, g/dL	11.4 ± 1.6 (6.7-14.4)	12.0 ± 1.4 (5.9 -15.7)	0.005
Thyroglobulin, µg/L	5.9 (0.1, 12.5)	6.7 (3.3, 11.7)	0.074
Thyroxine-binding globulin, µmol/L	0.6 (0.5, 0.8)	0.7 (0.5, 0.8)	0.235
Thyroid gland volume, ml	4.9 (4.4, 5.9)	5.1 (4.3, 5.9)	0.9
UIC, µg/l	173.0 (92.8, 368.3)	185.9 (95.9, 337.6)	0.6
Tg-Ab, IU/mL			
≤ 40	45 (77.6)	243 (93.5)	<0.001
> 40	13 (22.4)	17 (6.5)	
TPO-Ab, IU/mL			
<35	38 (65.5)	236 (90.8)	<0.001
>35	20 (34.5)	24 (9.2)	
Anaemia	14 (25.9)	45 (17.8)	0.169
BMI category			
Underweight	8 (14.0)	40 (15.6)	0.6
Normal	35 (61.4)	161 (62.6)	
Overweight	11 (19.3)	47 (18.3)	
Obese	3 (5.3)	9 (3.5)	

Parameters[1]	*Overt or subclinical hypothyroid, hypothyroxinaemic (n=58)*	*Euthyroid (n=260)*	*p-Value*[2]
Age, years			
<20	4 (6.9)	27 (10.4)	0.83
20-29	48 (82.8)	201 (77.3)	
>30	6 (10.3)	32 (12.3)	
Level of education			
No formal education	1 (1.7)	-	0.45
Finished high school	22 (37.9)	106 (41.2)	
Post high school	13 (22.4)	71 (27.6)	
≥University degree	22 (38.0)	80 (31.2)	
Parity			
Primiparous	38 (70.4)	164 (65.9)	0.525
Multiparous	16 (29.6)	85 (34.1)	
Consanguinity	10 (17.2)	39 (15.2)	0.695
Knowledge of iodine	19 (44.2)	95 (44.2)	0.98

[1]Data are mean ± SD with min-max in parantheses, median with quartiles in parantheses and numbers with percentages in parantheses

[2] p values are from t-test for normally distributed data, Mann Whitney U test for non-normally distributed data and chi-square test for categorical data

Table 4 presents the significant univariate and multivariate regression models with TSH, fT4, TT4 and Tg as dependent variables. Vegetarianism was not associated with any of the thyroid function tests. In the multivariate regression, presence of elevated TPO-Ab was associated with higher TSH (β= 0.238; p=0.006), and lower fT4 (β= -0.094; p=0.032) and lower Tg (β= -0.96; p=<0.001). Use of iodine supplements was associated with lower Tg (β= -0.286; p=0.028) and higher fT4 (β= 0.055; p=0.045). Overweight and obesity were associated with lower fT4 (β= -0.08; p=0.018) and TT4 (β= -20.9; p=0.023) respectively, with fT4 and TT4 concentrations 8.2% and 14.2 % lower in overweight/obese women compared to normal weight women. As fewer data were available for iodine supplement use (n=231) and salt use reason (n=275), the multiple variable linear regression analyses were repeated excluding these two variables in order to examine the associations in the complete sample (Supplementary Table S1). All the previously noted associations remained significant and additionally, Hb was associated with lower TSH (β= -0.041; p=0.010) and higher fT4 (β = 0.015; p=0.011). In addition, obesity was associated with lower fT4 (β= -0.090; p= 0.028), with fT4 concentrations 9.4% lower in obese women compared to normal weight women. In addition, a sensitivity analysis was performed for fT4 by removing one outlier and the significant associations remained the same.

Table 4: Factors associated with TSH, FT4, TT4 and TG in first trimester pregnant women in Bangalore, India

		Univariate analysis				Multivariate model [1]			
Outcome	Predictors	R^2	β	P	% change	R^2	β	P	% change
TSH	Hb, g/dL	0.023	-0.045	**0.007**	4.6 ↓ (8, 1.3)	0.128	-0.032	0.094	3.2↓
	TPO-Ab, <35 vs >35 IU/mL	0.133	0.464	**<0.001**	59 ↑ (39.6, 80.9)		0.238	**0.006**	26.9 ↑ (7.1, 50)
	Tg-Ab, <40 vs >40 IU/mL	0.096	0.463	**<0.001**	58.9 ↑(36.1, 85.3)		0.237	**0.019**	26.7 ↑(4.1, 54.3)
	Iodine supplement use, Yes vs No	0.020	-0.122	**0.031**	13 ↓ (26.2, 1.2)		-0.104	0.051	11 ↓
fT4	Hb, g/dL	0.018	0.016	**0.018**	1.6 ↑ (0.3, 3.0)	0.216	0.015	0.126	1.5 ↑ (0.4, 3.4)
	TPO-Ab, <35 vs >35 IU/mL	0.014	-0.062	**0.030**	6.4 ↓ (0.6, 12.5)		-0.094	**0.032**	9.9 ↓(0.8, 19.6)
	Tg-Ab, <40 vs >40 IU/mL	0.038	-0.118	**0.01**	12.5 ↓ (5.4, 20.1)		-0.174	**0.001**	19.0 ↓(8.0, 31.2)
	Iodine supplement use, Yes vs No	0.010	0.040	**0.129**	4.1 ↑ (1.2, 9.5)		0.055	**0.045**	5.7 ↑ (0.1, 11.4)
	Gestational Age, <10 vs >10 wks	0.026	-0.058	**0.003**	6 ↓ (1.9, 10.2)		-0.067	**0.011**	6.9 ↓(1.5, 12.5)
	BMI category								
	ref: normal	0.039							
	underweight		0.029	0.296	2.9 ↑ (2.6, 8.8)		0.035	0.327	3.6 ↑(3.7, 11.2)
	overweight		-0.066	**0.012**	6.8 ↓ (1.5, 12.4)		-.079	**0.018**	8.2 ↓ (1.4, 15.6)
	obese		-0.104	**0.040**	11 ↓ (0.5, 22.5)		-.085	0.169	8.9 ↓ (3.7, 22.9)
	Salt use reason								
	ref: health	0.049							
	taste		-0.078	**0.046**	8.1 ↓(0.1, 16.6)		-.082	0.099	8.5 ↓(1.5, 19.6)
	tradition		-0.062	**0.130**	6.4 ↓ (1.8, 15.4)		-.064	0.204	6.6 ↓(3.6, 17.7)
	quantity		-0.142	**0.001**	15.2 ↓ (5.7, 25.7)		-.136	**0.014**	14.6 ↓ (2.8, 27.8)
	other		-0.012	0.837	1.2 ↓ (10.4, 13)		-.054	0.383	5.5 ↓(7, 19.4)
	Egg intake								
	ref: >5 time/wk	0.007							
	< 5		0.039	0.232	4 ↑ (2.5, 10.8)		0.044	0.235	4.5 ↑ (2.9, 12.4)
	never		0.049	**0.143**	5 ↑ (1.7, 12.3)		0.038	0.340	3.9 ↑ (4.1, 12.3)
TT4	TPO-Ab, <35 vs >35 IU/mL	0.016	-12.41	**0.022**	8.5 ↓ (1.2, 15.7)	0.174	-5.78	0.344	3.9 ↓
	Tg-Ab, <40 vs >40 IU/mL	0.035	-21.42	**0.001**	14.6 ↓ (6.1, 23.0)		-19.6	**0.006**	13.3 ↓ (3.9, 22.7)
	Age,yrs								

		Univariate analysis				Multivariate model [1]			
Outcome	Predictors	R^2	β	P	% change	R^2	β	P	% change
	ref: >30	0.021							
	<20		16.033	**.047**	11.1 ↑ (0.1, 22.1)		6.468	0.430	4.5 ↑
	20-29		-0.641	0.912	0.4 ↓		-4.25	0.465	3 ↓
	Gestational age, <10 Vs >10 wks	0.059	16.36	**0.01**	12.0 ↑ (6.8, 17.3)		15.58	**0.01**	11.5 ↑(5.9, 17.0)
	BMI category								
	ref: normal	0.026							
	underweight		-0.661	0.900	0.4 ↓		-3.24	0.531	2.2 ↓
	overweight		-9.49	**0.057**	6.4 ↓ (0.2, 13.1)		-3.93	0.432	2.7 ↓
	obese		-22.06	**0.021**	15 ↓ (2.3, 27.7)		-20.4	**0.027**	13.9 ↓ (1.6, 26.1)
	Education								
	ref: graduate	0.013							
	high school		8.728	**0.048**	6.2 ↑ (0.05, 12.4)		8.43	**0.055**	6.0 ↑(0.1, 12.2)
	Post high school		5.964	0.224	4.3 ↑		5.06	0.293	3.6 ↑
	Seafood intake								
	ref: >1 time/wk	0.026							
	< 1		14.85	**0.014**	11.2 ↑ (2.2, 20.2)		14.02	**0.022**	10.6 ↑ (1.6, 19.6)
	never		15.40	**0.006**	11.6↑ (2.2, 20.0)		8.06	0.171	6.1 ↑
	Egg intake								
	ref: >5 time/wk	0.028							
	< 5		10.97	**0.069**	8.2↑(0.6, 17.1)		4.031	0.512	3.0 ↑
	never		17.87	**0.004**	13.4↑ (4.2, 22.7)		10.37	0.124	7.8 ↑
Tg	TPO-Ab, <35 vs >35 IU/mL	0.081	--0.825	**<0.001**	128 ↓ (67.9, 210)	0.149	-0.957	**<0.001**	160 ↓ (81.1, 274)
	Age,yrs								
	ref: >30	0.017							
	<20		0.567	**0.021**	76.3 ↑ (8.9, 185)		0.594	**0.048**	81.1 ↑ (0.4, 226)
	20-29		0.220	0.211	24.6 ↑		0.262	0.202	29.9 ↑
	Iodine supplement use, Yes vs No	0.016	-.260	**0.062**	29.7 ↓ (1.3, 70.2)		-0.286	**0.028**	33.1 ↓ (3.1, 71.9)

[1]For description of the regression models, please see statistical methods.

Supplementary table S1: Factors associated with TSH, FT4, TT4 and TG in first trimester pregnant women in Bangalore, India

		Univariate analysis				Multivariate model [1]			
Outcome	Predictors	R^2	β	P	% change	R^2	β	P	% change
TSH	Hb, g/dL	0.023	-0.045	**0.007**	4.6 ↓ (8, 1.3)	0.141	-0.041	**0.010**	4.2 ↓ (1, 7.3)
	TPO-Ab, <35 vs >35 IU/mL	0.133	0.464	<**0.001**	59 ↑(39.6, 80.9)		0.322	<**0.001**	38.0 (19.2, 59.5)
	Tg-Ab, <40 vs >40 IU/mL	0.096	0.463	<**0.001**	58.9 ↑(36.1, 85.3)		0.221	**0.012**	24.7 ↑ (5.0, 48.3)
fT4	Hb, g/dL	0.019	0.014	**0.014**	1.4 ↑ (0.3, 2.6)	0.116	0.015	**0.011**	1.5 ↑ (0.3, 2.6)
	TPO-Ab, <35 vs >35 IU/mL	0.054	-0.102	<**0.001**	10.7 ↓ (5.6, 16.1)		-0.065	**0.010**	6.7 ↓ (1.6, 12.1)
	Tg-Ab, <40 vs >40 IU/mL	0.048	-0.112	<**0.001**	11.8 ↓ (5.9, 18.2)		-0.119	0.056	12.6 ↓
	Gestational Age, <10 vs >10 wks	0.023	-0.046	**0.007**	4.7 ↓ (1.3, 8.2)		-0.051	**0.003**	5.2 ↓ (1.7, 8.8)
	BMI category								
	ref: normal	0.049							
	underweight		0.038	0.110	3.9 ↑ (0.9, 8.8)		0.105	0.928	11.1 ↑
	overweight		-0.057	**0.010**	5.9 ↓(1.4, 10.6)		-0.063	**0.005**	6.5 ↓(1.9, 11.2)
	obese		-0.095	**0.025**	10 ↓(1.2, 19.6)		-0.090	**0.028**	9.4 ↓ (1.0, 18.6)
	Egg intake								
	ref: >5 time/wk	0.011							
	< 5		0.028	0.299	2.8 ↑ (2.5, 8.5)		0.043	0.441	4.4 ↑
	never		0.049	**0.082**	5 ↑ (0.6, 11.1)		0.086	0.122	9.0 ↑
TT4	TPO-Ab, <35 vs >35 IU/mL	0.016	-12.41	**0.022**	8.5 ↓ (1.2, 15.7)	0.174	-5.78	0.344	3.9 ↓
	Tg-Ab, <40 vs >40 IU/mL	0.035	-21.42	**0.001**	14.6 ↓(6.1, 23.0)		-19.6	**0.006**	13.3 ↓ (3.9, 22.7)
	Age, yrs								
	ref: >30	0.021							
	<20		16.033	**.047**	11.1 ↑(0.1, 22.1)		6.468	0.430	4.5 ↑
	20-29		-0.641	0.912	0.4 ↓		-4.25	0.465	3 ↓
	Gestational age, <10 Vs >10 wks	0.059	16.36	**0.01**	12.0 ↑ (6.8, 17.3)		15.58	**0.01**	11.5 ↑ (5.9, 17.0)
	BMI category								
	ref: normal	0.026							
	underweight		-0.661	0.900	0.4 ↓		-3.24	0.531	2.2 ↓
	overweight		-9.49	**0.057**	6.4 ↓ (0.2, 13.1)		-3.93	0.432	2.7 ↓
	obese		-22.06	**0.021**	15 ↓ (2.3, 27.7)		-20.4	**0.027**	13.9 ↓ (1.6, 26.1)
	Education								
	ref: graduate	0.013							
	high school		8.728	**0.048**	6.2 ↑ (0.05, 12.4)		8.43	**0.055**	6.0 ↑ (0.1, 12.2)

		Univariate analysis				Multivariate model [1]			
Outcome	Predictors	R^2	β	P	% change	R^2	β	P	% change
TT4									
	Post high school		5.964	0.224	4.3 ↑		5.06	0.293	3.6 ↑
	Seafood intake								
	ref: >1 time/wk	0.026							
	< 1		14.85	**0.014**	11.2 ↑(2.2, 20.2)		14.02	**0.022**	10.6 ↑ (1.6, 19.6)
	never		15.40	**0.006**	11.6↑ (2.2, 20.0)		8.06	0.171	6.1 ↑
	Egg intake								
	ref: >5 time/wk	0.028							
	< 5		10.97	**0.069**	8.2↑ (0.6, 17.1)		4.031	0.512	3.0 ↑
	never		17.87	**0.004**	13.4↑(4.2, 22.7)		10.37	0.124	7.8 ↑
Tg	TPO-Ab, <35 vs >35 IU/mL	0.081	-0.825	**<0.001**	128 ↓ (67.9, 210)	0.081	-0.825	**<0.001**	128 ↓ (67.9, 210)
	Age,yrs								
	ref: >30	0.017							
	<20		0.567	**0.021**	76.3 ↑ (8.9, 185)		0.083	0.127	8.6 ↑
	20-29		0.220	0.211	24.6 ↑		0.006	0.914	0.6 ↑

[1]For description of the regression models, please see statistical methods.

Discussion

The major findings of this study are that first trimester pregnant women in Bangalore have sufficient iodine status, as indicated by an adequate median UIC and normal thyroid volumes and serum Tg concentrations. But despite this b) nearly one in five women had thyroid insufficiency and in these women, more than one-third had had signs of autoimmune thyroiditis, and there was a high prevalence of overt hypothyroidism (3.7%), with 83% of these women having positive TPO-Ab. A potential explanation for the high prevalence of hypothyroidism in our sample could be thyroidal disruptors, from environmental or dietary sources (29). We have no data on potential exposure to environmental thyroid disruptors, such as perchlorate, in our sample. Dietary goitrogens have been described in selected plant foods of Indian origin (30), and include thiocyanates (metabolites of cyagenic glucosides present in plant foods such as cabbage, cauliflower, bamboo shoot, cassava, mustard, turnip and radish) and isoflavones. The consumption of isoflavones (found in soy products, peas, bean, nuts, grain products, coffee and tea) is high in some Asian populations (31). A study looking for associations between isoflavone intake and thyroid function and autoimmunity in menopausal Indian women reported a modest reduction in serum fT_3 levels (32). A limitation of the present study is that we did not collect data on intake of goitrogenic foods, although we did collect single 24 hour dietary recall data and frequency of consumption of seafood and eggs in the pregnant population. However, it is unlikely that the quantity and frequency of consumption of these potentially goitrogenic foods in the rice-based diet of south India would be high enough to produce hypothyroidism.

The prevalence of overt hypothyroidism in our study population is more than fivefold higher than reported from other iodine-sufficient women early in pregnancy (12). The prevalence of thyroid insufficiency in iodine-sufficient women in pregnancy varies and is in large part dependent on gestational age and the definition of the upper reference limit for TSH used. We used the widely accepted

criteria of the American Thyroid Association, that is, a TSH upper limit of 2.5 mIU/L in the first trimester (25). In women in the United Kingdom and Italy <15 weeks' gestation (using a TSH upper limit of 3.65 mIU/L in the United Kingdom and 3.5 mIU/L in Italy), the overall prevalence of subclinical and overt hypothyroidism was 2.3% and 0.25%, respectively (33). In women in the Czech Republic at 9-12 weeks' gestation, using a TSH upper limit of 3.67 mIU/L, the respective prevalences were 3.9% and 0.4 (34), and in U.S. women at <20 weeks of gestation, using a TSH upper limit of between 2.74 and 5.09 mIU/L, the respective prevalences were 2.3% and 0.2% (35). A recent retrospective study of U.S. women in all three trimesters, using the same TSH reference range in the first trimester that we used in this study (2.5 mIU/L), reported much higher prevalences of subclinical hypothyroidism (15.1%) but a low prevalence (<0.5%) of overt hypothyroidism (36). A recent study from China found a 27.8% prevalence of subclinical hypothyroidism using the diagnostic criteria of TSH >2.5 mIU/L and 4.0% using the laboratory TSH reference interval of 0.14–4.87 mIU/L (37). A recent study from the Netherlands suggested there may be ethnic differences in reference ranges to diagnose thyroid disease (38). However, if we apply a TSH upper limit of 3.5mIU/L in our population to draw better comparison with studies from the available literature, the prevalence of subclinical hypothyroidism drops to 3.1%, but the prevalence of overt hypothyroidism remains the same at 3.7%, more than fivefold higher than reported in other iodine-sufficient populations of pregnant women.

In a cross-sectional study among healthy pregnant women without a family history of thyroid illness conducted in northern India in 2008, the prevalence of overt and subclinical hypothyroidism was 1.3% and 14.2% respectively, using a TSH reference range of 0.27–4.2 mIU/L) (39). In another study among pregnant women from northern India, the prevalence of overt and subclinical hypothyroidism was 4.6% and 6.5% respectively, using a TSH reference range of 0.5–5.5 mIU/L (40). In iodine-sufficient areas, the main cause of hypothyroidism during pregnancy is chronic autoimmune thyroiditis (14) and this

also appears to be the primary cause in our study population. In women early in pregnancy in Europe and the United States, positive TPO-Ab are present in 10% of women with hypothyroxinemia, 30-60% with subclinical hypothyroidism, and 70-90% with overt hypothyroidism (41). These prevalences are similar to the ones in our subjects: TPO-Ab was elevated in 12.5%, 26.7% and 83.3% of women with hypothyroxinemia, subclinical hypothyroidism and overt hypothyroidism respectively. Advancing parity may increase risk for thyroid autoimmunity (42) but in our data, the prevalence of TPO-Ab did not differ significantly between nulliparous and multiparous pregnant women.

Worldwide, iodine deficiency is a common cause of maternal thyroid insufficiency (1). Moderate to severe iodine deficiency can result in both maternal and fetal hypothyroxinemia (43, 44). In less severely affected areas, isolated maternal hypothyroxinemia is more common (12). A review of iodine status in Indian pregnant women reported a range of median UICs of 95-178 μg/l, with 60-95% consuming adequately iodized salt (11). In a recent cross-sectional study from the Bangalore region, we found a median UIC of 172 μg/L in pregnant women consuming adequate amounts of iodized salt (45). In large populations of pregnant women, although the overall median UIC may indicate adequate iodine status, subgroups with different dietary patterns may remain at risk of low intakes. For example, among pregnant women in Maharashtra, the median UIC was 203 and 211 μg/L at 17 and 34 weeks of gestation, but a subgroup of women were hypothyroxinemic and borderline iodine deficient with a median UIC of only 147 μg/L (46). However, in our study, the subgroup of women with thyroid insufficiency had a sufficient median UIC that was not significantly different from women who were euthyroid. In addition, women consuming vegetarian diets did not have lower iodine intakes than those consuming mixed diets, nor did those consuming less fish (the food group richest in native iodine). Thus, it appears that poor iodine intake in a subgroup of pregnant women is not the cause of the high prevalence of thyroid dysfunction in our sample.

Other potential contributing factors to thyroid insufficiency in our women were the high prevalence of anemia (19%) and overweight/obesity (23%). Hemoglobin was significantly lower in women with thyroid insufficiency than in euthyroid women (Table 3), and a lower hemoglobin was associated with higher TSH and lower fT4 (Table 4). Iron deficiency may impair thyroid function during pregnancy because thyroid peroxidase is an iron-dependent enzyme (47). Previous studies in children have shown that iron deficiency impairs correction of thyroid dysfunction during iodine repletion, and in Swiss pregnant women in the second and third trimester, the relative risk of hypothyroxinaemia was more than sevenfold higher in women who were anemic (15). A limitation of our study was that we did not measure other markers of iron status besides hemoglobin.

In the multiple regression analysis, overweight and obesity were significant predictors of a lower fT4 and TT4 (Table 4). Adiposity may increase the risk for thyroid insufficiency, and overweight adults tend to have higher TSH and lower fT4 concentrations than normal weight adults (48). Previous studies have reported an inverse relationship between fT4 concentrations and maternal weight and BMI during early pregnancy (17-19). One study found that high maternal BMI at mid-gestation positively correlated with the fT3/fT4 ratio (16). In mildly iodine-deficient pregnant Thai women, those who were overweight had a 3.6-fold higher risk of hypothyroxinemia in the first trimester compared to normal weight women (7). As India modernizes, overweight and obesity prevalence among adult women is increasing (22). If higher BMI is a risk factor for thyroid insufficiency in pregnant women, this may become increasingly important in India.

Currently, screening of maternal thyroid function is not routinely performed during antenatal care at hospitals in Bangalore. Our data suggest screening should be considered in this setting. The high prevalence of thyroid insufficiency in pregnant women in Bangalore may have important health consequences. Overt maternal

hypothyroidism increases the risk for multiple adverse pregnancy outcomes, including preeclampsia, spontaneous abortion, and postpartum haemorrhage, as well as premature birth and low birth weight (49, 50). Many of these complications have also been associated with subclinical hypothyroidism at lower rates (51), but not all studies have found adverse impacts (18). Mild hypothyroidism may also adversely affect cognitive development of the offspring, but not all studies agree (12). The largest controlled trial showed no benefit of treatment of mild maternal hypothyroidism on offspring IQ at 3.5 years of age (33). In summary, evidence is stronger that detection and treatment of overt and subclinical hypothyroidism will benefit obstetric outcomes rather than offspring cognitive development.

Almost all the salt (both powder and crystal) used in the households of the pregnant women in our study was iodized, illustrating the wide coverage of the Indian iodised salt program in this region. Household coverage of adequately iodized salt in India has increased remarkably over the past decade, with the national coverage reaching 51% in 2005–2006 and 71% in 2009 (10). But there is better coverage of adequately iodized salt in urban areas and higher socioeconomic groups (10). Our subjects were urban women from low-to-middle-income families, and the very high coverage and adequate iodine status reported here might not be generalizable to other areas of India. Use of iodized salt during the reproductive years leading up to pregnancy improves maternal thyroid economy during gestation and reduces the risk of maternal thyroid insufficiency because it contributes to normal intrathyroidal iodine stores (52). In countries with salt iodization programs, if pregnant women restrict their dietary salt intake, they have an increased risk of becoming hypothyroxinemic (53), indicating the importance of iodised salt as a dietary source of iodine. Among our women, fewer than one in five chose iodized salt for health reasons, and almost 50% had no knowledge of iodine nutrition. The low awareness of the health benefits of iodized salt in our study are consistent with findings from a recent survey in Karnataka (the Indian State where this study was

done) that found awareness of the health benefits of iodized salt played a minimal role in salt purchase choice (54). These findings argue that although the program of salt iodization is currently effective, in order to increase consumer awareness and maintain demand for iodized salt in Bangalore, public health strategies such as a consumer education campaign would be valuable for program sustainability.

In conclusion, in this population of urban first trimester pregnant Indian women, the prevalence of overt hypothyroidism is surprisingly high. But iodine intakes appear to be adequate, and there are apparently no subgroups of the population with low iodine intake that predict thyroid insufficiency. The most common factor underlying the high prevalence of thyroid insufficiency is autoimmune thyroiditis. Both a low hemoglobin and higher BMI predict an increased risk. These findings contribute to a better understanding of the pattern and prevalence of maternal thyroid dysfunction and associated risk factors in the Indian population that could improve screening and treatment of gestational thyroid insufficiency.

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Chapter 5

Thyroid function indicators and antenatal depression in South Indian women throughout pregnancy

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Abstract

Purpose: Thyroid dysfunction and depression are common in pregnancy. However, the role of thyroid dysfunction in depression during pregnancy is less well understood.

Aim: The aim of the present study was to examine the relationship of thyroid function with depression in pregnancy across trimesters.

Method: Subjects were 318 pregnant women who were randomized into two groups: a) iodine intervention (200µg oral iodine per day) or b) an identical placebo in a randomized, double blind, placebo-controlled trial. Thyroid hormones and antibodies, and depression using the Kessler's scale for psychological distress, were assessed at each of the three trimesters of pregnancy. Mann Whitney U tests and logistic regression were performed to examine associations between iodine supplementation, indicators of thyroid function and depression.

Results: There was no significant difference in depressive symptoms between the iodine intervention and placebo groups. In the first trimester, women with depressive symptoms had significantly lower serum TSH compared to women without depressive symptoms. Pregnant women with prenatal depressive symptoms had a significantly higher number of medical symptoms.

Conclusion: In early pregnancy, serum TSH is lower in women with depressive symptoms compared to women without depressive symptoms. Medical symptoms were associated with antenatal depressive symptoms

Introduction

Depression in pregnancy is common. Studies from different parts of the world have shown that the prevalence rate of depression ranges from 4.4% (Lee et al. 2004) to 48.4% (Shah et al. 2011). Several recent reviews suggest a higher prevalence of depression during pregnancy in women from developing countries compared to pregnant women from the West (Lee et al. 2004; Shah et al. 2011). While in the past the focus has been on post partum depression (Kitamura et al.1996), more recently there has been a considerable research interest in antenatal depression and its causal factors and outcomes (Highet Gemmill and Milgrom 2011). Depression in pregnancy has been found to be associated with several factors. These include biological/nutritional (Leung and Kaplan 2009), psychosocial (Bowen and Mujaharine 2006) and socio-demographic variables (Bowen et al. 2009). In addition, several studies have highlighted the adverse effects of depression during pregnancy on birth outcomes and child health including early development (Straub et al. 2012; Weinstock 2005).

The association between mood disorders and thyroid dysfunction is well recognized. The prevalence of mood disorders is higher in patients with thyroid dysfunction (Zainal et al. 2010); thyroid status predicts treatment response in major depression, and augmentation with thyroid hormone has therapeutic efficacy in treatment-resistant depression (Cooper-Kazaz and Lerer 2008). Both overactive and underactive thyroid have been found to be related to mood alterations (Hage and Azar 2012, Kirkegaard and Faber 1998). It is also noted that there is a higher prevalence of anti-thyroid antibodies in patients diagnosed with depression when compared to the general population (Hage and Azar 2012). Thyroid dysfunction is common during pregnancy and worldwide iodine insufficiency is a leading cause of maternal thyroid insufficiency (Zimmerman 2009). In iodine sufficient areas autoimmune thyroiditis is the most common cause of maternal hypothyroidism (De Groot et al. 2012). Few studies have examined the association between thyroid function in the antenatal

period and depression. Pederson et al. (2007) found that antenatal thyroid dysfunction, in terms of lower fT4, led to a higher likelihood of postnatal depressive symptoms. Lambrinoudaki et al. (2010) found that lower levels of serum FT3 and FT4 during the antenatal period are associated with mood disturbances in the first week after delivery. Bunevicius et al. (2009) found that women who were depressed in late pregnancy had higher fT4 concentration and higher prevalence of subclinical hyperthyroidism.

In a cross-sectional study of pregnant women who were screened for this intervention trial, there was a high prevalence of hypothyroidism with nearly one in five women having thyroid insufficiency in the first trimester and among them over one-third had autoimmune thyroiditis (Jaiswal et al. 2014).Thus, the aim of the present study was to longitudinally examine the relationship between thyroid hormones and depression in this cohort of urban South Indian pregnant women. We hypothesized that thyroid dysfunction would be associated with depression.

Materials and Method

This longitudinal study consisted of a cohort of pregnant women who were recruited for the Maternal Iodine Supplementation and its Effects on Child Development (MITCH) study (Clinicaltrials.gov with the identifier NCT00791466). The intervention study investigated the effects of oral iodine supplementation of 200µg/day during pregnancy on pregnancy outcome, infant growth and offspring cognitive development. The study was conducted at the antenatal clinic of the Obstetrics and Gynaecology Department of St Martha's Hospital in Bangalore, India. Pregnant women were recruited between December 2008 and March 2011. The study was conducted in accordance with the Declaration of Helsinki. Institutional ethical review boards at St. John's National Academy of Health Sciences, St Martha's Hospital, Bangalore, India and Wageningen University, The Netherlands approved the study. Study details were explained to the

participating women and one member of their family, and written, witnessed informed consents were obtained.

Study population

All pregnant women aged between 18 and 40 years, who were ≤ 14 weeks gestational age presenting to the antenatal clinic were invited to participate in the study, and1058 women were screened for eligibility to participate in the study. Women with major chronic diseases, those with a positive test for HIV, HbSAg or venereal diseases, those undergoing treatment for infertility, those who had previous repeated spontaneous abortions (4 or more), those with current multiple pregnancy as detected by ultrasound and those who were currently breastfeeding were excluded from the study. A total of 318 pregnant women consented to participate.

Socio-demographic data

A structured multiple choice questionnaire was used to obtain socio-demographic information on education, occupation and income, religion and obstetric history, including parity. Gestational age was calculated from the reported first day of the last menstrual period and confirmed by ultrasonography in 65% of women.

Laboratory analysis

A non-fasting whole blood sample was obtained by venipuncture into plain vacutainers (BD diagnostics, Franklin Lakes, New Jersey, USA) that was transported on ice and centrifuged to obtain serum, which was stored at -80^{0}C until analysis for thyroid stimulating hormone (TSH), total triiodothyronine (TT3), total thyroxine (TT4), free T3 (fT3), free T4 (fT4), thyroglobulin (Tg), thyroid binding globulin (TBG), anti-thyroid peroxidase antibodies (TPO-Ab) and anti-Tg antibodies (Tg-Ab). We previously described in detail the immunoassay method used to measure the TFT's, including %CVs, reference range, analytical measurement range and analytical sensitivity (Jaiswal et al.

2014).With the exception of TSH for which trimester-specific range is available, we used the manufacturer's recommended reference range for TFT's. For TSH, we applied a reference range of 0.1-2.5 mIU/L for the first trimester, 0.2-3.0 mIU/L for the third second trimester and 0.3-3.0 mIU/L for the third trimester (Stagnaro-Green et al, 2011; Mannisto et al, 2011). Assessments were carried out at ≤14 weeks, 24 weeks and 33 weeks of pregnancy.

Assessment of Maternal depression

Depressive symptoms were assessed on the Kessler's psychological distress scale (K-10). The K-10 has 10 items based on a 4 week recall period, each item has five response categories and is scored from 0 to 4 ('0'='None of the time', '1'= A little of the time', '2'='Some of the time', '3'= 'Most of the time', '4'= All of the time') yielding a sum of score (range 0-40) (Kessler et al. 2003). In a study of 194 women in their third trimester we found that the K-10 compared well with the Edinburgh postnatal depression scale (EPDS) (Cox et al. 1987) in detecting prenatal depression and a receiver operating characteristic analysis showed K-10 to be a good screening measure of prenatal depression in South Indian pregnant women at a cut-off of ≥6 (sensitivity=100%, specificity=81.3%, and area under the curve=0.95) (Fernandes et al. 2011). In addition, K-10 is simpler to score as it has uniform response options for all the 10 questions as opposed to EPDS, where items have different response options. We have since used the K-10 in studies of assessing depression in south Indian pregnant women (Lukose et al. 2014). In the present study, maternal depression was assessed at three time points, at ≤14 weeks, 24 weeks and 33 weeks of pregnancy and we used a K -10 score ≥6 to define pregnant women with prenatal depressive symptoms.

Medical morbidity

We captured medical morbidity through a questionnaire that had a checklist of common medical symptoms associated with pregnancy, administered at baseline and subsequently every trimester. The

check list of medical symptoms was related to respiratory, gastrointestinal, central nervous system and dermatological illnesses and the responses were recorded in a binary yes/no format. A medical morbidity score was computed by totalling the number of medical symptoms endorsed by subjects at baseline and in subsequent trimesters. The maximum score was 23.

Statistical analyses

Data are described as number (%) or median (25^{th}, 75^{th} percentile). The K-10 scores were compared between the intervention and placebo groups using Mann Whitney U test. Subjects with prenatal depressive symptoms were compared with those without depressive symptoms (K-10 score ≥6, Depression group) in each trimester on socio-demographic variables, biochemical indices of thyroid function and medical morbidity. Most of the biochemical indices of thyroid functions were not normally distributed and hence Mann Whitney U Test was used. Categorical data were compared using Chi square tests. The variables that showed a statistically significant group difference ($p<0.1$) in these analyses were considered in multiple variable logistic regression analysis of the dichotomous variable representing presence of antenatal depressive symptoms. The adjusted odds ratio (OR) and 95% Confidence interval (95% CI) are reported. SPSS version 18 (SPSS Inc. Chicago, Ill.) was used for statistical analyses and $p<0.05$ was considered statistically significant.

Results

Socio-demographic characteristics

Mean±SD age of the pregnant women was 24.7±4.1 years. The majority of the women were primiparous, had finished high school education, belonged to Hindu religion and were unemployed with a median (25^{th}, 75^{th} percentile) household income of INR 14,000 (8000, 25000) (Table 1). Prevalence of pr natal depressive symptoms among

pregnant women in the first trimester (10.4 ± 2.5 weeks) was 32% (n=102), 15% (n=38) in the second trimester (24.4 ± 1.5 weeks) and 20% (n=42) in the third trimester (32.9 ± 1.3 weeks). 10 women had depressive symptoms (Score: ≥6 on K10 scale) in all three trimesters.

Table 1: Socio-demographic characteristics of the sample

Parameters (N=318)	***Values***
Age, years [1]	24.7±4.1
Total monthly household income, INR [2]	14000 (8000, 25000)
Level of Education [3](n=315)	
Illiterate	1 (0.3)
Primary School	6 (1.9)
Middle school	21(6.7)
High school	105 (33.3)
Post high school	78 (24.8)
Graduate	90 (28.6)
Post Graduate	14(4.4)
Subject's occupation [3](n=314)	
Unemployed	260(82.8)
Unskilled worker	1(0.3)
Skilled worker	11(3.5)
Secretarial staff/primary school teacher	31(9.9)
Semi professional	7(2.2)
Professional	4(1.3)
Subject's religion[3](n=315)	
Hindu	254(80.6)
Christian	31(9.8)
Islam	30(9.5)
Parity [3](n=304)	
0	204(67.1)
≥ 1	100(32.9)

Mean ± SD[1], Median (25th, 75th percentile) [2], Numbers (%) [3]

There were no differences in the prevalence of depressive symptoms between the iodine intervention group (n=157) (median K-10 score

(25th percentile, 75th percentile): 4 (2,6) in first trimester, 3(1,5) in the second trimester and 3(2,5) in the third trimester) and placebo group (n=161): 4(2,6) in first trimester, 2(1,4) in the second trimester and 3(2,5) in the third trimester) group. Hence both the intervention and the control group are considered together for the remaining analyses. Socio-demographic characteristics were comparable between women with and without prenatal depressive symptoms.

Thyroid functions and depression

The overall prevalence of overt and subclinical hypothyroidism was 2.2% (n=7) and 8.5% (n=27) respectively, in the first trimester; 0.6% (n=2) and 4.4% (n=14) respectively, in the second trimester; 0% and 4.4% (n=14) respectively, in the third trimester. There were no cases of overt hyperthyroidism across all trimesters. The overall prevalence of subclinical hyperthyroidism in the sample was 1.9% (n=6), 0.3% (n=1) and 0% in the first, second and third trimester respectively. Isolated hypothyroxinemia was prevalent in 1.6% (n=5), 0.6% (n=2) and 1.6% (n=5) of pregnant women in the first, second and third trimester respectively. The prevalence of thyroid function disorders in the depression and no depression group in each trimester is presented in Figure 1.

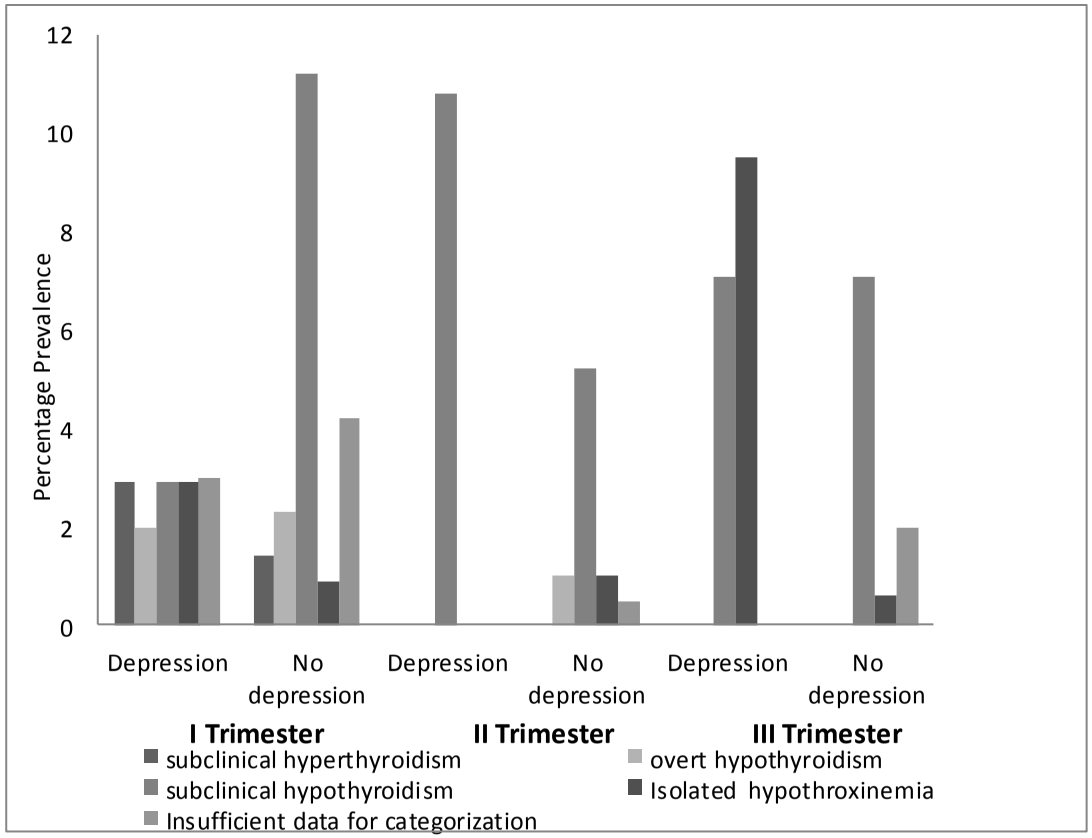

Figure 1: Percentage prevalence of thyroid function disorders in depressed and non-depressed groups of pregnant women across three trimesters 1, II and III

Median (25th, 75th percentile) TSH was significantly lower in the group of pregnant women who had depressive symptoms: 1.01(0.64, 1.71) compared to pregnant women who had no depression: (1.27 (0.81, 1.80) in the first trimester (p= 0.030) and the second trimester: 1.17(0.80, 1.81) vs 1.62(1.16, 2.13) (p=0.014).This was comparable in the third trimester. In the third trimester, median (25th, 75th percentile) fT4 was lower in the group with depressive symptoms compared to those without depressive symptoms: 11.4(10.6, 13.2) vs 12.3(11.1, 13.4) respectively, p= 0.032) (Table 2).

All the other thyroid function parameters were comparable between the groups including the percentage distribution of women with thyroid antibodies (TPO-Ab >35 IU/mL): 12.8% (n=27) in no depression group and 12% (n=12) in the depression group in the first trimester, 11.3% (n=22) in no depression group and 5.3% (n=2) in the depression group in the second trimester,10.8% (n=17) in no depression group and 9.5% (n=4) in the depression group in the third trimester) and Tg-Ab> 40 IU/mL:(8.5% (n=18) in no depression group and 8% (n=8) in the depression group in the first trimester, 4.1% (n=8) in no depression group and 2.6 % (n=1) in the depression group in the second trimester,3.9% (n=6) in no depression group and 2.4% (n=1) in the depression group in the third trimester).

Table 2: Comparison of thyroid function between depressed and non-depressed groups of pregnant women across three trimesters

Trimester	*Group*	*TSH (µIU/ml)*	*TT3 (nmol/L)*	*fT3 (pmol/L)*	*TT4 (nmol/L)*	*fT4 (pmol/L)*	*TBG (µmol/L)*	*Tg(ng/mL)*
I	Depression (n=102)	1.01 (.64,1.71)	2.21 (1.89,2.70)	5.74 (4.8,6.58)	145 (118,167)	14.6 (12.8,16.1)	0.65 (0.52, 0.78)	6.35 (2.91,10.05)
	No depression (n=211)	1.27 (.81, 1.80)	2.22 (1.85,2.68)	5.72 (5.01,6.72)	146 (128,161)	14.1 (12.8,15.3)	0.67 (0.54, 0.81)	6.68 (2.85,12.4)
	P value	0.**030**	0.804	0.511	0.786	0.120	0.778	0.384
II	Depression (n=38)	1.17 (.80, 1.81)	2.81 (2.45, 3.35)	6.51 (5.52,7.44)	158 (132,186)	12.0 (11.1,13.0)	1.03 (.83,1.16)	6.30 (4.28,10.5)
	No depression (n=194)	1.62 (1.16,2.13)	2.71 (2.38,3.24)	6.09 (5.31,6.81)	159 (139,180)	11.9 (11.0,12.8)	0.95 (.82,1.10)	6.75 (3.75,11.67)
	P value	0.**014**	0.340	0.066	0.531	0.622	0.064	0.950
III	Depression (n=42)	1.56(1.11-2.03)	2.87 (2.27-3.14)	6.00 (5.12,6.72)	153 (131,176)	11.4 (10.6,13.2)	1.00 (0.81,1.15)	7.11 (4.9,11.82)
	No depression (n=156)	1.61(1.10, 2.00)	2.85 (2.47,3.23)	6.15 (5.3,6.97)	163 (139,185)	12.3 (11.1,13.4)	0.96 (0.81,1.15)	6.53 (3.62,10.95)
	P value	0.886	0.498	0.472	0.167	**0.032**	0.264	0.465

Values are median *(25th, 75th percentile); p value from Mann Whitney U test*

Pregnant women with prenatal depressive symptoms had a significantly higher number of medical symptoms compared to women without depressive symptoms across all three trimesters (Table 3). The medical symptoms of diarrhoea, nausea, vomiting, cough, sore throat, fever, constipation, giddiness, tiredness, heartburn and painful urination were more common in depressed women, out of which sore throat, giddiness and tiredness were reported in all three trimesters.

In all the three trimesters a larger number of medical symptoms were associated with depressive symptoms (Table 4). After adjusting for this, low TSH was associated with the presence of depressive symptoms in first trimester alone (OR=0.70, 95% CI: 0.52 - 0.93) and not in the second trimester. The association of low fT4 to depressive symptoms in the third trimester was also not associated with presence of depressive symptoms after adjusting for number of medical symptoms in the logistic regression.

Table 3 Difference in medical symptoms between depressed and non-depressed groups of pregnant women across three trimesters

Trimester	***Group***	***n***	***No. of medical symptoms***
I	Depression	208	6 (5,8)
	No depression	100	5 (3,6)
	P value		<**0.001**
II	Depression	209	4 (3,7)
	No depression	38	3 (2,5)
	P value		**0.002**
III	Depression	171	5 (3,8)
	No depression	45	3 (2,5)
	P value		<**0.001**

Values are median (25th, 75th percentile); p value from Mann Whitney U test

Table 4 Logistic regression analysis of depressive symptoms in three trimesters of pregnancy

Trimester	***Factors***	***Odds Ratio****	***95% CI***
I	TSH	0.70	0.52-0.93
	Medical symptoms	1.37	1.22-1.53
II	TSH	0.89	0.61-1.29
	Medical symptoms	1.25	1.08-1.44
III	FT4	0.89	0.75-1.06
	Medical symptoms	1.38	1.21-1.58

* Adjusted Odds Ratio in multiple variable logistic regression

Discussion and Conclusion

In this cohort of pregnant urban south Indian women, we found a high prevalence of depressive symptoms with the highest prevalence in the first trimester of pregnancy (32%). Among the various biochemical indices of thyroid function, lower levels of TSH were predictive of prenatal depressive symptoms in the first trimester. Medical symptoms reported by pregnant women were independently associated with prenatal depression across the three trimesters.

Several studies from South East Asia have noted a high prevalence of depressive symptoms during pregnancy (Imran & Haider 2010; Gausia et al. 2009). Our findings of a 32% prevalence rate of depressive symptoms among pregnant South Indian women are consistent with these studies and similar to the reported prevalence in one of our earlier studies (Lukose et al. 2014). Such a high prevalence of depressive symptoms with its associated disability and impairment is a source of considerable burden for pregnant women (Karatas et al. 2009; Mariam & Srinivasan 2009). In agreement with earlier longitudinal studies of prenatal depression, the prevalence rates tend to be lower at later time points in pregnancy (Bunevicius et al. 2009; Lee at al. 2007).

Few studies have examined the association between indicators of thyroid function and maternal depression during pregnancy. In an

early prospective study of depression and thyroid function in 1017 pregnant women, high titres of TPO-Ab at 12 weeks gestation (OR 2.1; 95% CI: 1.1-5.8) and at 24 weeks gestation (OR 2.8; 95% CI: 1.9-7.1) were independently associated with a syndromal diagnosis of depression (Pop et al. 2006). The same study also reported that pregnant women with decreased TSH in the first trimester had 2.8 fold greater risk for depression. In a study of 199 pregnant women from Lithuania, with a diagnosis of major depression or dysthymia found that depressed women had higher fT4 values and a trend towards lower levels of TSH, only in the third trimester (Bunevicius et al. 2009). While it is difficult to compare our study with these two reports as the participants were pregnant women with diagnosable depression as opposed to only having depressive symptoms in our study, nevertheless one of the consistent finding is the association between lower levels of TSH, indicative of sub-clinical hyperthyroidism, with prenatal depression. This association between lower TSH and prenatal depressive symptoms in our cohort of women is all the more remarkable given the high prevalence of autoimmune thyroiditis and sub-clinical hypothyroidism in the screened population of pregnant women (Jaiswal et al. 2014). An association between sub-clinical hyperthyroidism and depression has been reported in the general population (Scholte et al. 1992) and in patients with Graves' disease (Bunevicius & Prange 2006). It is important to further explore the association between subclinical hyperthyroidism and prenatal depression as subclinical hyperthyroidism can have several negative long term health outcomes that include osteoporosis and cardiovascular vulnerability (Casey et al. 2006).

Findings on the association between thyroid autoimmunity and prenatal depression have been inconsistent. The studies that examined this have yielded conflicting results. Two studies from the Netherlands (Kuipjens et al.2001; Pop et al. 2006) reported an association between elevated levels of TPO antibodies and prenatal depression independent of thyroid function in early pregnancy. However, a study from the UK (Oretti et al.1997) and Lithuania

(Bunevicius et al. 2009) did not find an association between thyroid immunity and prenatal depression.

Our findings indicate that common medical symptoms such as nausea, tiredness, giddiness and abdominal pain were predictive of depression across trimesters. Most of the earlier studies found severity of medical symptoms as one of the risk factors associated with prenatal depression in early pregnancy (Kitamura et al. 1996; Reeves et al. 1991). Among the various medical symptoms, nausea and vomiting in the first trimester are particularly associated with a higher risk of prenatal depression (Chou et al. 2003; Lukose et al. 2014). Most studies report an association between medical symptoms and prenatal depression in early pregnancy, but in the present study this association persists across trimesters. While it is difficult to ascribe a causal relationship between medical symptoms and maternal depressive symptoms, the presence of medical symptoms must alert the physician to the possibility of prenatal depression.

To our knowledge, this is the first study in India to examine the association between thyroid function and prenatal depression. This report is based on a prospective longitudinal design with multiple assessments at different time points and adds to the small but expanding literature on identifying correlates of prenatal depression. However, this study has some limitations. We screened for the presence of depressive symptoms but did not assess for the presence of syndromal depression. However, several studies have shown that sub-clinical depression is associated with considerable disability and impairment (Karatas et al. 2009; Mariam & Srinivasan 2009). We did not assess the severity of medical symptoms though we found medical symptoms to be positively associated with prenatal depression across trimesters. 67 (21.1%) women dropped out from the study in the second trimester, and 14 (4.4 %) women dropped out in the third trimester. However, there was no difference in the prevalence of depression in those who continued in the study and those who dropped out (data not provided).

In conclusion, we found a high prevalence of depressive symptoms among urban south Indian pregnant women from a low socioeconomic background. There was an association between low levels of TSH and prenatal depression in early pregnancy. Medical symptoms were associated with prenatal depression. Depression and thyroid dysfunction being common in pregnancy and more importantly having an influence on birth outcomes and post natal child development (Straub et al. 2012; Weinstock 2005), future studies with larger sample size are needed. We are currently following up the mother-child dyad through 2 years of age with periodic assessment of maternal depression, child development and thyroid function. This study, in addition, highlights the need for systemic screening for prenatal depression during antenatal visits as it is an independent risk factor for later development of clinical depression (Satyanarayana et al. 2011; Mariam & Srinivasan 2009; Davey et al. 2011).

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Conflicts of interest: None.

Ethics of human subject participation: The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving pregnant women and their children were approved by the institutional ethical review boards at St John's National Academy of Health Sciences and St Martha's

Hospital, Bangalore, India. The study was explained in detail to the participating women and written informed consent from each participant was obtained at recruitment.

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Chapter 6

GENERAL DISCUSSION

Globally, in 2011, 32 countries and 1.88 billion people remained iodine deficient, including 241 million schoolchildren, who had insufficient dietary iodine intake (3). A recent review estimated that 350 million Indians are at risk of iodine deficiency disorders (IDD) as they consume salt with inadequate iodine and every year nine million pregnant women and eight million newborns are at risk of IDD in India. In sub-national IDD surveys (2001-2006) in seven States of India, household level consumption of adequately iodized salt (≥15 ppm) ranged from 18.2-91.9% and median UIE ranged from 76-173.2 µg/l (1); 91% households had access to iodized salt, 71% consumed adequately iodized salt and 9% consumed salt with no iodine; wide variations in coverage were seen in urban (83.2%) and rural (66.1%) areas and States, with Karnataka being a low coverage state (35.5%) (2).

Iodine deficiency has far reaching adverse effects in the general population, and particularly during pregnancy as the fetus is entirely dependent on the mother for its iodine nutrition. Recent evidence suggests that using the median UIC value from a population of school children as surrogate for pregnant women may place them at risk of iodine deficiency. It is also uncertain if it is beneficial to supplement mildly iodine deficient pregnant women with iodine. WHO does not recommend supplementation in areas with well-functioning iodized salt programs while many expert medical groups recommend supplementation in areas with mild iodine deficiency. The potential adverse effects of mild iodine deficiency during pregnancy are unclear, as controlled trials of iodine supplementation in mildly iodine deficient pregnant women have not shown that supplementation increases concentrations of maternal or newborn thyroid hormones (4). In this thesis we aimed to address two important questions: 1) whether pregnant women should be monitored separately for iodine nutrition and 2) seek clarity on the issue that persists in the field of iodine nutrition: whether it is beneficial to supplement pregnant women with iodine in mild-to moderately iodine deficient and also iodine sufficient areas.

Therefore, we aimed to determine whether daily oral iodine supplementation in pregnant women with mild iodine deficiency improves cognitive development of their offspring at 2 years.

6.1 Main findings

Table 1 summarises the main findings from this thesis.

In the RCT, there were no significant differences between the iodine supplemented or control group in maternal thyroid function tests or thyroid volume during gestation. The prevalence of all subtypes of thyroid dysfunction, or anti-TPO antibodies, did not differ significantly during gestation and postpartum. Postpartum, there were no significant differences between the maternal and infant groups in thyroid function, birth outcomes or UIC. Thus, iodine supplementation even in iodine sufficient pregnant women was found to be safe without increasing the risk of thyroid autoimmunity. Neonates whose mothers received iodine supplementation during pregnancy had better orientation scores at 6 weeks of age and lower scores of inhibition suggesting better executive function at 2 years of age although neurocognitive development on the BSID III were not significantly different between groups. Thus, additional follow-up of these children for neurocognitive testing at a later age when development and cognitive testing is more reliable would provide valuable add on information (Chapter 2).

In a cross-sectional study (pilot study) comparing iodine status of pregnant women and their 3-15 year old children who were sharing all meals in Bangalore, we found that adequately iodized salt, together with small amounts of iodine in local foods, were providing adequate iodine during pregnancy: a) median UIC in women was 172 µg/L, b) the median UIC was >150 µg/L in all trimesters and c) thyroid size was not significantly different across trimesters. At the same time, the median UIC in children was 220µg/L, indicating 'more than adequate' iodine intake at this age. Median UIC was significantly higher in children than in their mothers (p=0.008). We concluded that in our selected urban population of Southern India, the iodized salt program was providing adequate iodine to women throughout pregnancy, at the expense of higher iodine intake in their children. Thus we suggested that the current WHO/UNICEF/ICCIDD cut-off for median UIC in children, indicating more-than-adequate iodine intake, may need to be reconsidered (Chapter 3).

There have been recent improvements in iodized salt coverage in India but whether iodized salt is enough to sustain iodine requirements

during pregnancy remains uncertain. In a cross-sectional study among 334 pregnant women ≤14 weeks of gestation who were screened for the RCT, we aimed to measure thyroid status in first trimester pregnant women and assess potential determinants of thyroid function, including iodine status, thyroid autoimmunity, dietary patterns, body weight and anemia. We found that 21% women were vegetarian, 19% were anaemic and 23% were overweight or obese. Iodized salt was used by 98% of women and they were iodine sufficient: median UIC was 184.2 μg/L and all had normal thyroid volume. However, 18% of women had thyroid insufficiency: 3.7% had overt hypothyroidism (83% with positive TPO-Ab), 9.2% had subclinical hypothyroidism and 5.2% had hypothyroxinemia. Women consuming vegetarian diets did not have significantly lower iodine intakes or higher risk of hypothyroidism than those consuming mixed diets, but overweight/obesity and anemia predicted thyroid insufficiency. Thus we concluded that in our selected urban population of southern India, pregnant women have adequate iodine status in the first trimester. Despite this, many had thyroid insufficiency, and the prevalence of overt hypothyroidism was >5-fold higher than reported in other iodine-sufficient populations of pregnant women. We suggested that improved screening and treatment of gestational thyroid insufficiency is important in this population (Chapter 4).

While the relationship between thyroid functions and mood symptoms has been extensively studied, the role of thyroid dysfunction in depression during pregnancy is less well understood. In a secondary analysis of the RCT data, we aimed to examine the relationship of thyroid function with depression across three trimesters. We found no significant difference in the prevalence of depressive symptoms between the iodine intervention vs the placebo group. In the first trimester, women with depressive symptoms had significantly lower serum TSH compared to women without depressive symptoms. Pregnant women with prenatal depressive symptoms had a significantly higher number of medical symptoms. Although iodine supplementation did not affect maternal depression, we highlighted the need for systemic screening for prenatal depression during antenatal visits as it is an independent risk factor for later development of clinical depression (chapter 5).

Table 6.1: Main findings of the studies described in this thesis

Chapter	Study design	Population	Objectives	Main findings
2	Randomized controlled trial	318 pregnant, iodine sufficient Indian women, randomized to receive 200µg iodine or placebo during pregnancy	To determine the effects of iodine supplementation on maternal and newborn thyroid function, pregnancy outcome, infant growth and cognitive development.	Iodine supplementation to iodine sufficient pregnant women was safe without increasing thyroid autoimmunity. No beneficial effect was seen on neonatal and maternal thyroid function and cognitive development. However, supplemented children had better orientation at 6 wks & lesser inhibition on test of executive function at 2 yrs.
3	Cross-sectional	Pregnant Indian women and their children aged 3-15 years	To compare the iodine status of pregnant women & their children sharing all meals	Pregnant women were getting adequate iodine (UIC 172µg/L) at the expense of higher iodine intake in their children (UIC 220 µg/L).
4	Cross-sectional	334 pregnant Indian women of ≤14 wks gestation	To assess determinants of thyroid function in first trimester pregnant women	Despite adequate iodine status (UIC 184 µg/L), 18% pregnant women had thyroid insufficiency predicted by anemia and overweight/obesity.
5	Longitudinal	318 pregnant iodine sufficient Indian women followed through pregnancy	To assess the association of maternal depression and thyroid function during pregnancy	In early pregnancy, serum TSH was lower in women with depressive symptoms compared to women without depressive symptoms.

6.2 Methodological considerations

In this part of the thesis, we critically reflect on the methodology adopted in the pilot study that may affect the interpretation of findings. We also highlight the methodological constraints, strengths and limitations, internal and external validity of the RCT that make the findings robust and generalizable to similar settings.

6.2.1 Pilot study

Since there was no data on the iodine status in pregnant women from Southern India, in a pilot study, we aimed to assess the median UIC of pregnant women from Bangalore, in a target population, which was chosen for our RCT. In addition to this objective, we aimed to compare the median UIC of these women to that of their children, 3-15 years old, in pairs that were sharing all meals at home. Thus, all children participating in the school lunch programmes and sharing some meals at their grand parent's homes were excluded. Such comparisons have been made in earlier studies but in those studies pregnant women and children were not sharing all meals together in the same household which limits comparisons between the groups. Our findings from this study showed that pregnant women were adequate in iodine nutrition while their children were "more than adequate" in their iodine status. We also captured information on sources of iodine in this population (consumption of seafood and multi-micronutrient powders containing iodine, salt sample analysis, average household consumption of salt) to arrive at estimated iodine intakes in this population. Thus, we think that our study provides definitive evidence for the inadequacy of using median UIC in children as a surrogate for iodine nutrition in pregnant population. But since our study was hospital based and in an urban setting, additional population-based studies in other regions of India are needed to confirm these findings. We also cannot generalize study results to less-affluent, rural areas of India where adequately iodized salt is not available and where pregnant women are likely to still have inadequate iodine intakes.

6.2.2 Randomized controlled trial (RCT)

Dropouts

There was a 21% dropout each in the intervention and placebo group from the study entry to second trimester. This dropout rate increased to 26.1 and 24.8% at the third trimester, 27.3 and 28.0% until delivery, 30.4% (n=112) and 31.2% (n=108) at delivery and 72 hrs in the intervention and placebo group respectively. Thus the overall dropout rate was close to the anticipated dropout rate of ~25% and the number of women (n=125) that were required in each group and thus the statistical power of the study appeared to be adequate. However, we had only 64 neonatal TSH from blood spots at delivery (cord blood) each in the intervention and placebo group which increased to 92 and 93 respectively at 72 hrs. But since there were no differences in the prevalence of elevated neonatal TSH at delivery and 72 hrs (which was the basis of our sample size calculation) we are confident that addition of more numbers to the existing data would have not made any difference in the study findings.
There was an overall 43% dropout in the number of participants who were available for assessments at 2 years (n=95 and n=87 in the intervention and placebo group respectively). However, throughout the study, women who dropped out were equally divided between the two groups for various reasons: adverse events, women/family not willing to continue using the supplements, moved out of the study area, re-pregnant. Thus we feel that the exposure itself was not the reason for the dropout rate and hence the findings of the study are inferential.

Selection bias

This study being an RCT removes the chances of selection bias considerably. However there were significant differences in baseline TSH and fT4 which cannot be ignored. So apparently there would be some outliers at baseline who moved out of the study later. But these were definitely not because of the treatment itself since those who moved out of the study for various reasons were equally divided between the two groups.

Non-consenters

We collected the information on women excluded/not willing to participate in the RCT. There were no statistically significant differences in age, gestational age, education, occupation, monthly

household income, height, weight or BMI between those who declined participation and those who agreed to participate in the study (data not shown). Thus we are confident that there was no selection bias in our study.

Non response bias- ITT/per protocol

We repeated all analyses in a per-protocol population for thyroid function tests. The results from the per-protocol analysis (Chapter 2; Supplementary Table 1 and 2) were comparable to the intention-to-treat analysis and thus we are confident that there was no non-response bias.

Information or measurement bias

We do not foresee any information bias since the codes were kept in a sealed envelope until the data analysis was complete. We broke the codes in the presence of the study statistician and a former member of the Data Safety Monitoring Board. All through the data collection and analyses, the investigators, study statistician and data collection personnel were blinded to the treatment codes.

All the study forms were checked for inconsistencies by the study supervisor and the data were double entered into a structured query language database by 2 data entry operators. Discrepancies or mismatches in the data entry were corrected by a data entry supervisor. The matched data was locked, held by the study statistician and made available for analysis. Hence we do not feel there are any reporting errors in the data.

All the instruments used in the study like weighing scale and pipettes, were calibrated periodically. We collected all the anthropometry readings in duplicate. We collected 3 readings while measuring the thyroid gland size and a mean of length, width and depth of the right and left lobe was taken to calculate the total volume.

The inter-assay and intra-assay coefficients for UIC and TFTs were all within the acceptable range. The Bangalore laboratory where the biochemical sample analysis was done had College of American Pathologists (CAP) accreditation, National Accreditation Board for

Testing and Calibration Laboratories (NABL) accreditation and was an intercontinental referral lab for Esoterix Inc. Belgium, a LabCorp company, USA. The lab had quality control systems in place through internal QC process & participation in external Proficiency Testing programs; Laboratory Information management system (LIMS) for data management, formal documentation for endorsement and approvals of Work Artifacts for Clinical study with project code no. CLG162/STJ/MITCH/2011. Quality control samples were run before each test groups for the assays and the results of the quality control samples were reviewed for acceptability. Samples were run only after verification that the quality control data is within acceptable limits for a given assay. Calibration of assays was performed in accordance with the calibration Standard Operating Procedures and Instrument operating procedure. The frequency of calibration was in accordance with the manufacturer's recommendations. Since we had an idea that the effects of iodine supplementation, if any, would be subtle in terms of TFT's, we analysed all the data for each participant (all antenatal time points) in one run and thus eliminated the possibility of any inter assay variation that could affect the TFT results.

All the research assistants performing measurements on anthropometry, dietary data collection, and administration of questionnaires were trained before the start of the study and also periodically. For the psychological assessments, proper training and certification was done for Neonatal behavioural assessment scale (NBAS), Bayley's Scales of Infant Development (BSID), Kessler's psychological distress scale (K-10), Bradleys' Home environment and Brief-P measurements.

Confounding

We identified possible confounders in bivariate analyses with the thyroid function parameters and adjusted for covariates of BMI, parity, baseline gestational age and maternal age. We considered the gestational age at entry into the study as an important covariate since the mean gestational age at entry was early, at 10 weeks (range 5.0-14.6 weeks) and it is known that the earlier supplementation starts, the better. We created a variable "time from start" (Gestational age at trimester 1, 2, or 3 minus gestational age at trimester 1) to quantify the duration (in weeks) the subjects were in the study at the time of each blood draw for thyroid function test.

This variable “time from start” was considered as a covariate and the difference in slope of thyroid function parameters with “time from start” between the intervention groups was considered as a measure of efficacy of the intervention obtained by the interaction with intervention. Further, we considered percentage compliance as a covariate in addition to all the factors described above to adjust for the effect of compliance to intervention/placebo. Compliance to intervention was significantly associated with most thyroid function parameters, but did not modify the interaction effect.

Study strengths

One of the major strength of the study was that this was a randomized controlled trial with study subjects carefully randomized so that there were no differences in the maternal characteristics at baseline. However, TSH and fT4 showed significant differences at baseline in the intention to treat analysis but not the per protocol analysis. But since our data analysis using linear mixed models factored in these differences and considered the importance of early gestational age at entry into the study, we are confident that our study findings are generalizable to similar settings. In addition, the contrasting findings between observational studies and randomised controlled trials are not uncommon and highlight that typical statistical adjustments of potential confounders in observation studies are likely to be inadequate to capture the full extent of influences of social and environmental factors (5).

Study limitations

Even though not anticipated, the pregnant women in Bangalore site were found to be iodine sufficient and not mildly iodine deficient. This was most likely due to recent improvements in the coverage and the quality of iodized salt in the area. This is an important limitation of the study since the intervention was not originally designed to be tested in an iodine sufficient population. But this does add a unique aspect to the study: in population-based iodine intervention in a mildly-iodine deficient area, because of the usual wide range of iodine intakes, there will very likely be a portion of the women at the upper end of the distribution who are iodine sufficient. Thus, this study provides important information on the potential effects of iodine supplementation in this group. Even though we aimed to assess the

potential benefits and risks of iodine supplementation in this thesis, the question remains whether this trial was ethical to perform in the first place. This is because of the widespread recommendation for iodine supplements to be taken from preconception through to lactation and for all prenatal supplements to contain iodine. However, antenatal iodine supplementation was (at the start of the study) and continues to be not part of the antenatal prophylaxis programme in India. All the studies within this thesis were conducted in accordance with the Declaration of Helsinki. Institutional ethical review boards at St. John's National Academy of Health Sciences, St Martha's Hospital, Bangalore, India and the Wageningen University, The Netherlands approved the studies.

Other points for discussion

One of the exclusion criteria in our study was TSH > 7.5 IU/mL. When we started the study in 2008, there was no consensus on trimester specific reference ranges for TSH or the thyroid hormones. Although most clinicians recognized the reference range for TSH was likely lower in the 1st trimester compared to non-pregnancy and in later pregnancy, it was not sure how low it should be. And no one was sure of the clinical relevance of this, and how much the values may be altered by differences in iodine status. Considering that we wanted to see the effects of iodine treatment, we did not want to exclude women with marginal thyroid dysfunction, as this may be more common in areas of iodine deficiency. Thus, the upper end of TSH reference range in our local laboratory for non-pregnant adults (0.3-5.5) + 2.0 mU/L was set as cut-off for exclusion from the study.

Public Health Importance

Our finding from the intervention study showed that additional iodine supplementation to iodine sufficient pregnant women is safe and does not increase thyroid autoimmunity. Even though there were no significant effects of iodine supplementation on neonatal and maternal thyroid function and birth outcomes, there were modest effects on the neurocognitive development of young children. This was assessed by testing the executive function of children at 2 years of age. However, there are concerns over the reliability of neurocognitive assessments in children of less than 4 yrs of age and

thus a follow-up of these children could provide further insights. Although salt iodization is likely to be a more effective and equitable strategy to increase iodine intakes in populations, iodine supplementation to pregnant women should be considered as an important interim measure of ensuring optimal iodine nutrition until universal salt iodization reaches all strata of population. It is important that the intrathyroidal stores are adequate when women enter pregnancy and thus continuous monitoring of iodine status of this important target group is recommended.

Our data from the pilot study indicated that salt iodization ensures adequate iodine intake in pregnant women, although iodine intake in their children is in the more-than-adequate range. But we cautioned against our results be generalized to less affluent, rural areas of India where adequately iodised salt is not available so that pregnant women are likely to still have inadequate iodine intakes. Because there are wide variations in iodized salt quality and coverage across India, we recommended that additional population-based studies in other regions of India be done to confirm our study findings.

Based on our screening data of the iodine intervention study, we found the prevalence of overt hypothyroidism surprisingly high in first trimester pregnant women from Bangalore, India. We found their iodine intakes to be adequate; low hemoglobin and higher BMI predicted an increased risk for thyroid insufficiency mainly attributed to autoimmune thyroiditis. Our data suggested that screening of maternal thyroid function be considered in antenatal care at hospitals in Bangalore. We found low awareness of the health benefits of iodized salt in our study. Although the program of salt iodization is currently effective, public health strategies such as a consumer education campaign are critical for program sustainability.

Based on our longitudinal study examining thyroid function and depression in pregnancy across trimesters, we found that although iodine supplementation did not affect maternal depression, there was a need for systemic screening for prenatal depression during antenatal visits as it is an independent risk factor for later development of clinical depression.

The immediate consequence from our research findings would be that countries with successful salt iodization programmes will be

motivated to maintain their efforts in ensuring that all groups of population especially pregnant women receive adequately iodized salt in order to achieve optimal iodine nutrition. This could be ensured by including pregnant women in the systematic surveys of nutrition monitoring which are conducted at the national/sub-national level. The results from our study also provide additional data that would be helpful in increasing awareness of the importance of adequate iodine nutrition and combating anemia in pregnant women. Finally, maintaining an ideal body weight remains at the heart of every public health programme considering the double burden of malnutrition that is currently witnessed in countries in nutrition transition.

Future Research

In the RCT, neurocognitive development at 2 years of age did not differ between children whose mothers received iodine supplementation during pregnancy compared to the placebo group. However, neonates had better orientation scores at 6 weeks of age in the intervention group of children while the scores of inhibition were significantly higher in the children of mothers in the placebo group compared to those whose mothers received iodine during pregnancy indicative of executive dysfunction. However, there are concerns over reliability of neurocognitive assessments in children less than 4 yrs of age. Thus additional follow-up of these children for neurocognitive testing at an age considered satisfactory for assessments would add valuable information. Increasingly, studies are using measures of executive function as they have been linked to future development of disorders of childhood psychopathology such as attention deficit and hyperactivity disorder. Future studies are needed to show if these children develop later differences in cognitive development.

We found a high prevalence of overt hypothyroidism in our screening population; this was despite pregnant women being iodine sufficient. Over 90% of thyroid disorders in pregnancy are thought to be of autoimmune etiology (7) and chronic autoimmune thyroiditis is the main cause of hypothyroidism during pregnancy in iodine-sufficient regions (8). Although approximately 70% of the risk for developing autoimmune thyroid disease is attributable to genetic background, environmental triggers are thought to play a role in the development of autoimmune thyroid disease in susceptible individuals. Understanding the association of environmental agents with thyroid

dysfunction can be utilized to reduce the risk to populations. Knowledge of the specific factors that trigger autoimmune thyroid disease and their mode of action may also inform risk reduction in the individual patient (6).

A potential explanation for the high prevalence of hypothyroidism in our sample could be thyroidal disruptors, from environmental or dietary sources (9). We have no data on potential exposure to environmental thyroid disruptors, such as perchlorate or dietary goitrogens (including thiocyanates (metabolites of cyagenic glucosides present in plant foods such as cabbage, cauliflower, bamboo shoot, cassava, mustard, turnip and radish) and isoflavones). However, it is unlikely that the quantity and frequency of consumption of these potentially goitrogenic foods in the rice-based diet of south India would be high enough to produce hypothyroidism. Also, there was no evaluation of maternal smoking in our dataset. However, national family health survey data (2005-2006) for the state of Karnataka (Bangalore is one of the districts of Karnataka) revealed that less than 1% of women in the age group of 15-49 smoked cigarettes and cigars/pipes. Therefore we do not think that active cigarette smoking could be a potential risk factor for thyroid dysfunction in our population. But, a recent report from Bangalore suggests that the level of pollution in the city is equivalent to smoking 6 cigarettes per day (10). A recent study from Thailand also found that low-level exposure to perchlorate was positively associated with TSH and negatively associated with free T4 in first-trimester pregnant women. Thiocyanate exposure was also positively associated with TSH in a subgroup of pregnant women with low iodine excretion. Thus future research in this area would provide additional information on the reasons of high prevalence of hypothyroidism in this population.

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Summary

Adequate iodine nutrition during the *in utero* thyroid-sensitive periods of brain maturation is what determines how well the brain and ultimately the cognitive functions develop. Children born to mothers from severely iodine deficient areas clearly benefit from maternal iodine supplementation but data are equivocal on its effect in areas that are mildly iodine deficient and iodine sufficient.

India, because of its large population, high birth rate and iodine deficient soils, has until recently had a large number of infants potentially exposed to in utero iodine deficiency and recent studies highlight the presence of iodine insufficiency among Indian women. Iodine status of school aged children (SAC) is often used as a proxy for the iodine nutrition of pregnant women at the population level, even though the difference in energy requirement in pregnant women is only 0-25% compared to SAC, while the difference in iodine requirement is >100%. This means that the relatively small increase in food intake during pregnancy is unlikely per se to provide the additional iodine requirement unless iodine rich foods are preferentially selected which is not the practice currently during pregnancy.

WHO recommends that pregnant women in areas where iodized salt programs are weak consume 250 μg iodine daily; ATA suggests that the strategies to implement this recommendation might differ between countries. While iodine supplementation during pregnancy is clearly beneficial to pregnant women in severely iodine deficient areas, it was unclear whether those in mild-to-moderately iodine deficient or iodine sufficient areas should receive supplementation. There are no long-term data from controlled trials in mildly iodine deficient pregnant women that have measured the effect of iodine supplementation on infant or child development.

This thesis therefore aims to fill the important gap that exists in the current literature regarding the effect of iodine supplementation during pregnancy on birth outcomes, maternal goiter, thyroid autoimmunity, infant growth and cognitive development. Data was collected in a Randomized Controlled Trial (RCT) carried out in Bangalore, India, on pregnant women and their infants from November 2008 to November 2013, the women receiving a supplement of 200 μg iodine per day from November 2008 to March 2011. In addition, potential determinants of thyroid function including iodine status, thyroid autoimmunity, maternal depression, body weight and anemia were assessed. Prior to the RCT, in

a cross-sectional study, we compared the median UIC of these women to that of their children, 3-15 years old, in pairs that were sharing all meals at home. This study started in May 2008, running in parallel with the RCT, and the data collection ended in September 2011.

In the RCT (**Chapter 2)**, we aimed to determine whether the daily oral administration of iodine (200 μg) to pregnant women improves maternal and newborn thyroid function, pregnancy outcome, birth weight, infant growth and cognitive performance. In the intervention and placebo groups of women, there were no significant differences in maternal thyroid function tests or thyroid volume during gestation. The prevalence of all subtypes of thyroid dysfunction, or anti-TPO antibodies, did not differ significantly during gestation and postpartum. Postpartum, there were no significant differences between the maternal and infant groups in thyroid function, birth outcomes or UIC. Neonates whose mothers received iodine supplementation during pregnancy had better orientation scores at 6 weeks of age and lower scores of inhibition suggesting better executive function at 2 years of age although neurocognitive development on the BSID III were not significantly different between groups. Thus we concluded that iodine supplementation even in iodine sufficient pregnant women was safe without increasing the risk of thyroid autoimmunity. We also concluded that additional follow-up of these children for neurocognitive testing at a later age when developmental and cognitive testing is more reliable would provide valuable add on information.

Chapter 3 describes a pilot study that assessed iodine intake (based on UIC) and potential determinants of intake, in Indian pregnant women and their children who were sharing all meals. This study found a) median UIC in pregnant women was 172 μg/L, b) the median UIC was >150 μg/L in all trimesters and c) thyroid size was not significantly different across trimesters; the median UIC in children was 220μg/L, indicating 'more than adequate' iodine intake at this age. Median UIC was significantly higher in children than in their mothers (p=0.008). We concluded that the iodized salt program was providing adequate iodine to women throughout pregnancy, at the expense of higher iodine intake in their children, in Bangalore, India suggesting that the current WHO/UNICEF/ICCIDD cut-off for median UIC in children indicating more-than-adequate intake may need to be reconsidered.

In a cross-sectional study of the pregnant women screened for the RCT (C**hapter 4)**, we aimed to understand the pattern and prevalence of

maternal thyroid dysfunction and its associated risk factors in the Indian pregnant population. We found the prevalence of overt hypothyroidism surprisingly high in the first trimester pregnant women whose iodine intakes were adequate. Both low hemoglobin and higher BMI predicted an increased risk for thyroid insufficiency mainly attributed to autoimmune thyroiditis. We thus suggested that screening of maternal thyroid function be considered in antenatal care at hospitals in Bangalore.

In a secondary analysis of the longitudinal data on pregnant women in the RCT **(Chapter 5)**, we aimed to assess the association of maternal depression, and thyroid function during pregnancy. We found that there were no significant differences in depressive symptoms between the iodine intervention and placebo groups. In the first trimester, women with depressive symptoms had significantly lower serum TSH compared to women without depressive symptoms. Pregnant women with prenatal depressive symptoms had a significantly higher number of medical symptoms. Although iodine supplementation did not affect maternal depression, we highlighted the need for systemic screening for prenatal depression during antenatal visits as it is an independent risk factor for later development of clinical depression.

In the concluding **Chapter 6,** we summarise the main findings of this thesis, and their implications for public health. We also discuss the methodological limitations and suggest directions for future research.

In conclusion, this is the first study to measure child development at two years as an outcome of iodine supplementation during pregnancy in a randomised controlled trial. Even though not anticipated, the pregnant women were found to be iodine sufficient and not mildly iodine deficient. This was most likely due to recent improvements in the coverage and the quality of iodized salt in the area. The results from this RCT indicate that additional iodine supplemented to iodine sufficient pregnant women is safe and does not increase thyroid autoimmunity. Even though there were no significant effects of iodine supplementation on neonatal and maternal thyroid function and birth outcomes, there were modest effects on the neurocognitive development of young children. This was assessed by testing the executive function of children at 2 years of age. However, there are concerns over the reliability of neurocognitive assessments in children of less than 4 yrs of age and thus a follow-up of these children could provide further insights. Although salt iodization is likely to be a

more effective and equitable strategy to increase the iodine intake in a population, iodine supplementation to pregnant women should be considered as an important interim measure for ensuring optimal iodine nutrition until universal salt iodization reaches all strata of the population. In addition, pregnant women should be included in the systematic surveys of nutrition monitoring which are conducted at the national/sub-national level. The results from our study also provide additional data that would be helpful in increasing awareness of the importance of adequate iodine nutrition and combating anemia in pregnant women. Finally, maintaining an ideal body weight remains at the heart of every public health programme considering the double burden of malnutrition that is currently witnessed in countries in nutrition transition.

Samenvatting

Een juiste jodium voeding gedurende de *in utero* schildklier-gevoelige perioden van hersen maturatie bepaalt hoe goed de hersenen en uiteindelijk de cognitieve functies zich ontwikkelen. Kinderen geboren uit moeders in gebieden met een ernstig jodium tekort profiteren duidelijk van maternale jodium aanvulling, maar gegevens zijn dubbelzinnig over effecten voor gebieden met een gering of geen jodium tekort.

Vanwege zijn grote populatie, het hoge geboortecijfer ervan, en de ontoereikendheid van jodium in de bodems, heeft India tot recent een groot aantal zuigelingen die potentieel in de baarmoeder aan jodiumtekort zijn blootgesteld, terwijl recente studies op de aanwezigheid van jodium tekort onder Indiase vrouwen wijzen. De status van jodium bij kinderen in de schoolleeftijd (SAC) wordt vaak als een proxy gebruikt voor de voeding van jodium bij zwangere vrouwen op het bevolkingsniveau. Echter, het verschil in hun energie behoefte is slechts 0-25% ten opzichte van SAC, terwijl het verschil in jodium behoefte >100% is. Dit betekent dat de relatief kleine stijging in voedsel opname tijdens de zwangerschap waarschijnlijk niet per se om de vereiste extra jodium gaat, tenzij jodiumrijk voedsel wordt geselecteerd. In de praktijk gebeurt dit op dit moment niet tijdens de zwangerschap.

De WHO beveelt een dagelijkse consumptie van 250 µg jodium aan bij zwangere vrouwen in gebieden met programma's met zwak gejodeerd zout, en de ATA suggereert dat de strategieën naar de uitvoering van deze aanbeveling tussen landen kunnen verschillen. Terwijl de toevoeging van jodium tijdens de zwangerschap duidelijk gunstig is in gebieden met een ernstig jodium tekort, was het onduidelijk of zwangere vrouwen in gebieden met een zwak-tot-matig jodium tekort, en ook in gebieden met voldoende jodium, toch een suppletie moeten krijgen. Er bestaan geen lange-termijn gegevens uit gecontroleerde proeven van zwangere vrouwen met een gering jodium tekort, waarbij het effect van jodium suppletie werd gemeten op de ontwikkeling van de zuigeling of het kind.

Dit proefschrift wil daarom de belangrijke leemte vullen die er in de huidige literatuur bestaat over het effect van jodium suppletie tijdens de zwangerschap op geboorte resultaten, op struma bij moeders, op schildklier autoimmuniteit, op de groei van jonge kinderen en hun cognitieve ontwikkeling in een RCT. Van November 2008 tot maart 2011, alsook in November 2013 werden bij zwangere vrouwen uit Bangalore, India, gegevens verzameld over suppleties van 200 µg jodium per dag. Bovendien werden potentiële determinanten van de schildklier functie

beoordeeld, zoals jodium status, schildklier autoimmuniteit, maternale depressie, lichaamsgewicht en anemie. In een cross-sectionele studie voorafgaand aan de RCT, wilden we in paren die alle maaltijden samen thuis deelden de mediane UIC waarden van deze vrouwen met die van hun kinderen tussen 3-15 jaar vergelijken. Deze studie begon in mei 2008, parallel aan de RCT, terwijl het verzamelen van de gegevens in september 2011 eindigde.

In de RCT (**Hoofdstuk 2**) willen we bepalen of de dagelijkse orale toediening van jodium (200 µg) aan zwangere vrouwen de schildklier functie van moeder en pasgeboren kind verbetert, alsook het resultaat van de zwangerschap, het geboortegewicht, en de groei en cognitieve prestaties van de kleuter. In de interventie en placebo groepen van vrouwen bestonden er geen significante verschillen in functie of volume van de schildklier van de moeder tijdens de zwangerschap. De prevalentie van alle subtypen van schildklier dysfunctie, of anti-TPO antilichamen, verschilt niet significant tijdens de zwangerschap en postpartum. Er waren geen significante verschillen tussen de groepen van moeders en kinderen in schildklier functie, geboorte resultaten, of UIC. Pasgeborenen waarvan de moeders jodium suppletie tijdens de zwangerschap ontvangen hadden, hadden betere oriëntatie scores wanneer ze 6 weken oud waren en lagere scores van remming. Dit suggereert een betere uitvoeringsfunctie op 2-jarige leeftijd, hoewel de neuro-cognitieve ontwikkeling of de BSID III niet significant tussen de groepen verschillen. We concludeerden dus dat jodium suppletie bij zwangere vrouwen, zelfs wanneer deze voldoende jodium hadden, veilig was zonder het risico van schildklier autoimmuniteit te verhogen. Tevens blijkt dat extra follow-up van deze kinderen voor neuro-cognitieve testen op latere leeftijd, wanneer ontwikkeling en cognitieve testen meer betrouwbaar is, waardevolle toevoegingen aan informatie zou bieden.

Hoofdstuk 3, een pilot-studie, die een op UIC gebaseerde jodium opname en potentiële determinanten van opname bepaalt, beschrijft de jodium opname voor Indiase zwangere vrouwen en hun kinderen die alle maaltijden samen hebben. Deze studie vond dat bij zwangere vrouwen a) de mediane UIC waarde 172 µg/L was, b) dat de mediane waarde van UIC in alle trimesters >150 µg/L was, en c) dat de grootte van de schildklier niet significant anders was over de trimesters. Bij kinderen was de mediane UIC waarde 220µg/L, wat voor deze leeftijd op een 'ruim voldoende' jodium opname wijst. De mediane UIC waarde was significant hoger in kinderen dan die in hun moeders (p = 0,008). Wij concludeerden

dat het programma van gejodeerd zout voldoende jodium geeft aan vrouwen tijdens de zwangerschap, ten koste van hogere jodium opname in hun kinderen. Dit suggereert dat voor Bangalore, India, de huidige WHO/UNICEF/ICCIDD scheidslijn voor de mediane UIC waarde voor kinderen, die op een meer dan voldoende opname wijst, moet worden heroverwogen.

In een cross-sectionele studie van de zwangere vrouwen, gescreend voor de RCT (**Hoofdstuk 4**), wilden we proberen het patroon en de prevalentie van maternale schildklier dysfunctie en de bijbehorende risicofactoren in de Indiase zwangere bevolking te begrijpen. We vonden de prevalentie van opvallende hypothyreoïdie verrassend hoog voor zwangere vrouwen in hun eerste trimester, waarvan de jodium opname voldoende is. Zowel een laag hemoglobine en een hogere BMI voorspelt een verhoogd risico voor schildklier insufficiëntie voornamelijk toegeschreven aan auto-immune thyroiditis, zodat wij voorgesteld hebben dat in Bangalore screening van maternale schildklier functie in prenatale zorg in de ziekenhuizen moet worden overwogen.

In een secundaire analyse van de longitudinale gegevens op zwangere vrouwen in de RCT (**Hoofdstuk 5**), we hebben ons gericht op het bepalen van het verband tussen maternale depressie en de schildklier functie tijdens de zwangerschap. We vonden geen significante verschillen in symptomen van depressie tussen de groepen van jodium interventie en placebo. Vrouwen met depressie symptomen hadden een significant lagere serum TSH ten opzichte van vrouwen zonder depressie symptomen in hun eerste trimester. Zwangere vrouwen met prenatale symptomen van depressie hadden een significant groter aantal medische symptomen. Hoewel jodium suppletie de maternale depressie niet beïnvloedde, hebben we gewezen op de noodzaak voor een systematische screening voor prenatale depressie tijdens prenatale bezoeken, aangezien het een onafhankelijke risicofactor is voor een latere ontwikkeling van klinische depressie.

In het afsluitende **Hoofdstuk 6** vatten we de belangrijkste bevindingen uit deze thesis samen, en bespreken we ook de methodologische beperkingen, alsook de implicaties voor de volksgezondheid en voor richtingen van toekomstig onderzoek.

Concluderend, dit is de eerste studie om de ontwikkeling van kinderen in hun tweede jaar in een gerandomiseerde gecontroleerde trial te meten als

gevolg van jodium suppletie tijdens de zwangerschap. Hoewel niet verwacht, bevatten de zwangere vrouwen voldoende jodium en waren niet zwak jodium arm, waarschijnlijk als gevolg van de recente verbeteringen in de dekking en kwaliteit van gejodeerde zout in het gebied. De resultaten van deze RCT blijkt dat aanvullende jodium, gegeven aan zwangere vrouwen met voldoende jodium, veilig is en dat schildklier autoimmuniteit niet toeneemt. Ook al waren er geen significante effecten van jodium suppletie op schildklier functie en geboorte resultaten van neonatale en maternale vrouwen, waren er geringe effecten op de neuro-cognitieve ontwikkeling van kinderen, zoals bepaald door de executieve functie van kinderen in hun tweede jaar. Echter, er bestaat bezorgdheid over de betrouwbaarheid van neuro-cognitieve evaluaties in kinderen jonger dan 4 jaar, zodat een follow-up van deze kinderen betere inzichten kan bieden. Hoewel zout jodering een meer effectieve en rechtvaardige strategie kan zijn ter vergroting van jodium opname in populaties, moet jodium suppletie aan zwangere vrouwen worden beschouwd als een belangrijke tussentijdse maatregel voor optimale jodium voeding, totdat universele zout jodering alle lagen van de bevolking bereikt. Bovendien moeten zwangere vrouwen worden opgenomen in systematische overzichten van het monitoren van voeding, uitgevoerd op nationaal en sub-nationaal niveau. De resultaten van onze studie bieden ook aanvullende gegevens die nuttig zou kunnen zijn bij het vergroten van het bewustzijn over en de bestrijding van bloedarmoede bij zwangere vrouwen. Tot slot, behoud van een ideaal lichaamsgewicht blijft de kern van elk programma voor volksgezondheid, gegeven de dubbele last van ondervoeding die momenteel wordt gezien in landen in nutritionele overgang.

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About the author

Curriculum vitae

Nidhi Jaikrishna (maiden name Jaiswal) was born on the 25th of August, 1979 in Faizabad district of Uttar Pradesh State, India. In 1994, she completed high school from Bethany Convent School, Allahabad, Uttar Pradesh, India. In 1999, she graduated with a Bachelor's degree in Home Science from the College of Home Science, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India. In 2009, she was nominated for a research fellowship from the Indian Council of Agricultural Research, New Delhi, India, to pursue her post graduate studies. In 2002, she completed her Master of Science degree in Foods and Nutrition from the Post Graduate & Research Centre, A.N.G.R. Agricultural University, Hyderabad, India, with a research project titled "Development of low fat potato products through microwave processing" and MSc. thesis project titled "Antioxidant profile in diabetic subjects".

Following her Master's Degree, she underwent training for a Post Graduate Certificate course in 2003 at the National Institute of Nutrition, Hyderabad, India. After this, she worked in different capacities in nutrition related work over brief periods of time. In 2003, she worked on a research project evaluating the National program 'Integrated Child Development Services' at the Indian Institute of Health and Family Welfare, Hyderabad, India. Later in 2005, she worked as a dietician at Fortis Hospitals, Chandigarh, India. In July 2006, she obtained Junior Research fellowship from the Indian Council of Medical Research, New Delhi, India, to pursue doctorate in Social Sciences following which she joined St. John's Research Institute, Bangalore, India, in February 2007. She worked at the Division of Nutrition in two research studies; one assessing the relationship between maternal nutritional status and pregnancy outcome and another evaluating the effect of physical exercise on B vitamin status of well nourished, non-anaemic girls aged 18-24 years. In September 2007, she was admitted for a sandwich PhD program at the Division of Human Nutrition, Wageningen University supervised by Prof. Michael Zimmermann (Switzerland), Asst. Prof. Alida Melse-Boonstra (the Netherlands) and Prof. Srinivasan K (India). Following this, she finished her PhD research proposal in November 2007 and in May 2008, she started data collection for a

cross-sectional study to assess the median UIC of pregnant women from Bangalore, in a target population, which was chosen for the RCT. This study ran parallel to the RCT of iodine supplementation to pregnant women in areas of mild to moderate iodine deficiency which was conducted from November 2008 to November 2013 at St Martha's Hospital, Bangalore, India.

Publications

Susan Thomas, **Nidhi Jaiswal**, Tinku Thomas, Alida Melse-Boonstra, Michael B Zimmermann, Krishnamachari Srinivasan. Submitted for publication to Archives of Women's Mental Health (under review)

Nidhi Jaiswal, Alida Melse-Boonstra, Tinku Thomas, Chetana Basavaraj, Surjeet Kaur Sharma, Krishnamachari Srinivasan, Michael B Zimmermann. High prevalence of maternal hypothyroidism despite adequate iodine status in Indian pregnant women in the first trimester. Thyroid. 2014. 24(9). DOI: 10.1089/thy.2014.0071

Nidhi Jaiswal, Alida Melse-Boonstra, Surjeet Kaur Sharma, Krishnamachari Srinivasan, Michael B Zimmermann. The iodized salt programme in Bangalore, India provides adequate iodine intakes in pregnant women and more-than-adequate iodine intakes in their children.Public Health Nutr. 2014. Apr 24:1-11. doi: 10.1017/S136898001400055X

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Alida Melse-Boonstra, **Nidhi Jaiswal**. Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development. Best Practice & Research Clinical Endocrinol & Metabolism. 2010 24:29-38.

Nidhi Jaiswal, Anurag Chaturvedi. Development of low fat potato products through microwave processing J. Res. ANGRAU 2002: 30(3): 33-35.

Overview of completed training activities

With the educational activities listed below, the PhD candidate has complied with the educational requirements set by the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

Courses, workshops, conferences	Organiser and location	Year
Discipline specific activities		
NBAS training	Ramathibodi Hospital, Thailand	2009
Training on BSID-III	SJRI, Harvard Medical School,	2009
ILSI Conference on micronutrient fortification of foods	ILSI, New Delhi, India,	2011
IAEA Course on stable isotope techniques in nutrition	SJRI, IAEA	2011
International course in Nutrition research methods	SJRI, Harvard, Tufts	2011
Lab training	Clinigene Intl, Bangalore	2012
Training course on nuclear techniques related to nutrition and non-communicable diseases	SJRI, IAEA	2012
Nutritional epidemiology workshop on analytical approaches to incorporating dietary biomarkers and reducing measurement error	SJRI, Albert Einstein College of Medicine, New York (Group presentation)	2013
IUNS 20th ICN	Granada, Spain, (Poster presentation)	2013
45th Annual Conference of the Nutrition Society of India	NIN, Hyderabad, India (Poster presentation)	2013
General courses		
VLAG PhD week	VLAG	2007
Presentation skills	CENTA	2007
Working with Endnote	WUR Library	2007
Seminar series on Biostatistics	SJRI, Bangalore, India	2008
Basic course in Research	SJMC, Bangalore, India	2011

Methodology		
Erasmus Winter Programme	EMC, Rotterdam	2012
Masterclass for R	HNE	2012
Optional courses and activities		
Preparation of PhD research proposal	HNE	2007
PhD excursion to Mexico and USA	HNE	2011
Seminar (PhD retreat and departmental)	HNE	2007
Seminar (Departmental)	SJRI, Bangalore, India	2011

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