Selenium inadequacy hampers thyroid response of young children after iodine repletion

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ABSTRACT
Selenium (Se) is an integral component of iodothyronine deiodinase, glutathione peroxidase and thioredoxin reductase enzymes and thus is important for normal thyroid function. This study investigated the influence of Se inadequacy on thyroid response of iodine-replete young children. Serum thyroxine (T4), triiodothyronine (T3), thyroglobulin (Tg), thyroid stimulating hormone (TSH), and Se were analyzed in 54–60 mo old children (n = 628) from the Amhara region of Ethiopia before salt iodization was commenced; analyses were repeated (n = 555) 15 mo after iodized salt became available. Iodized salt coverage increased from 12.2% to 91.6% of households. Median urinary iodine concentration (UIC) among children increased from 9 μg/l to 167 μg/l (p < 0.001). In addition, all thyroid indices except T3 showed significant improvement (p < 0.05). Nearly, half of the study children (49.1%) had Se inadequacy (serum Se < 70 μg/l). Serum Se was significantly correlated with T3 (r = 0.38, p < 0.001), T4 (r = 0.15, p < 0.001), TSH (r = -0.205, p < 0.001) and Tg (r = -0.11, p < 0.01) concentrations 15 mo after iodine repletion; baseline serum Se and T4 (r = -0.22, p < 0.01) were inversely correlated. Despite adequate iodine status, children with low serum Se had lower serum T4 (p = 0.003) and T3 (p < 0.001) but higher TSH concentration (p = 0.003). In the partial least square regression model, Se was among the latent variables significantly explaining T4 and T3. Results of the present study suggest that Se inadequacy negatively affects the thyroid metabolism of iodine-replete children and may present a substantial public health concern thus emphasize the need to consider correction of Se status for normal thyroid function as well as for benefits from its diverse biological roles.

1. Introduction

Selenium (Se) plays an important role in human physiology mainly through its presence in selenocysteine containing proteins. There are 25 human selenoproteins with diverse functions [1]; the iodothyronine deiodinases (IDIs), glutathione peroxidases (GPXs), and thioredoxin reductases (TRxRs) are among the selenoproteins with their function explicitly characterized [2]. Glutathione peroxidase 3 (GPX3) provides antioxidant protection to thyrocytes from attack by hydrogen peroxide (H2O2) produced during thyroid hormone synthesis. In addition, IDI isoforms (D1, D2, and D3) play crucial roles in thyroid hormone synthesis, underlying the important relationship between Se nutrition and thyroid metabolism [3]. The IDI enzymes, D1 and D2 convert the biologically inactive T4 to the bioactive T3 (3, 5, 3′-triiodothyronine). In addition, D1 and D3 enzymes convert T3 to the bioinactive rT3 (3, 5′, 3′-triiodothyronine) [4]. The thyroid is among the glands or organs that retain the most Se per mass. Thus, it can maintain deiodinase activity even in a state of Se deficiency [5]. In addition, it is a priority organ in the hierarchy of Se supply [6]. However, results of several studies show that severe or extended Se deprivation reduces the IDI enzyme activity and alters thyroid metabolism even when iodine nutrition is adequate [7–14]. We previously reported Se deficiency as a public health problem in a severely iodine deficient area of the Amhara region, Ethiopia [15]. Compared to children with normal serum Se concentration, study children with Se inadequacy had significantly higher serum T4 concentration suggesting a reduced activity of IDI enzymes converting T4 to the biologically active T3 [15]. The current study investigated the influence of Se status on the thyroid response of children, 15 mo after iodine repletion.
2. Materials and methods

2.1. Study participants

This study was conducted within a large cluster-randomized trial entitled ‘Effect of Iodized Salt on Child Development in Amhara Region, Ethiopia’; a rural area characterized by the presence of moderate iodine deficiency [16]. The trial (NCT01349634) was registered with www.clinicaltrials.gov. Details of study site selection, sample size calculation, and recruitment of participants for the larger trial are reported elsewhere [15,17]. Briefly, 60 out of 75 districts from six zones of the Amhara region (West Gojjam, East Gojjam, South Gonder, North Wollo, South Wollo and Wagehmera) were randomly selected. From each of the 60 districts, one rural village was randomly selected. At baseline, all households with children aged 6–60 mo were registered and further categorized into 6–11 mo, 18–22mo, and 54–60 mo. The present study was nested within the larger study. A subset of 26 of the 60 districts were selected randomly and the analysis included only children from the 54–60 mo age group. Data for 624 children were analyzed at baseline; and 15 mo later samples from 555 of the children were available for analysis. The main study was a cluster randomized control trial in which the intervention districts received iodized salt approximately 4 mo earlier than it became available through normal market forces in the control districts. As the group difference in access to iodized salt was small and the thyroid markers indicated short-term iodine nutrition, the present study used a pre-post analysis approach to test the changes in thyroid profile of the children.

2.2. Blood collection and analysis

Protocols for blood collection, analysis and quality control were reported previously [15]. Briefly, blood was drawn by venipuncture into trace-element-free tubes, allowed to clot and centrifuged in the field. The serum was transported in vials to Bahir Dar in an icebox and kept at 20°C until transferred to the Ethiopian Public Health Institute (EPHI) at Addis Ababa and stored at 80°C. Duplicates of frozen serum samples were shipped on dry ice to Oklahoma State University for Se analysis. Needles, blood tubes, vials, pipette tips and gloves recommended for trace mineral research were used throughout the work.

Serum Se was analyzed by Inductively Coupled Plasma Mass Spectrometer (PerkinElmer, ELAN9000, Norwalk, CT, USA) following dilution in 0.1% double distilled nitric acid (GFS Chemicals, Inc.). Calibrating standards were prepared by diluting a Se stock solution (Perkin Elmer Life and Analytical Sciences) in 0.1% nitric acid (GFS Chemicals, Inc.) and Triton- X 100 (Perkin Elmer Life and Analytical Sciences). Gallium was used as an internal standard. For quality control purposes, a serum trace elements certified reference sample (#66816, UTAK Laboratories, Inc; Valencia, CA) was analyzed at least every ten samples. The accuracy and precision of the method were 96% and 3.0%, respectively (certified value = 111 μg/l and the value obtained by the laboratory was 114.8 μg/l). Serum Se < 70 μg/l was used to define Se inadequacy [18].

An automated electro-chemiluminescence immuno assay using an Elecsys2010 clinical analyzer (Cobas e411, Roche Diagnostics GmbH, Mannheim, Germany) was used to analyze thyroid markers (T₄, T₃, TSH, and Tg) in serum. Elecsys reagents were used to calibrate the instrument every time thyroid markers were analyzed or new batches of reagents were used. Moreover, Preci Control Clin Chem Multi 1 and Preci Control Clin Chem Multi 2 reagents for each thyroid marker (T₄, T₃, TSH and Tg) were analyzed for quality control purpose and the readings were in the acceptable level. The laboratory was participating in the ‘One World Accuracy’ external quality assurance program.

2.3. Collection and analysis of urinary and salt iodine

Samples of table salt (approximately 10 g) were collected from the study households at both time points. Salt samples were assessed qualitatively on-site using a rapid test kit (RTK). The iodine content of salt samples found positive for the RTK were analyzed quantitatively by titration [19].

Spot urine samples for iodine analysis were collected into clean cups, and transferred to tubes. The tubes were wrapped in paraffin to avoid evaporation, transported in a cooled storage container, and kept at -20°C at EPHI until analysis. Urinary iodine was determined by the colorimetric ceric ion arsenious acid wet ashing method based on the Sandell-Kolthoff reaction [19]. The detection limit of the instrument was 5 μg/l.

2.4. Statistical analysis

Descriptive statistics (proportions, mean, standard deviation, median, range) have been used to describe the data. The distribution of measured variables was tested using the Kolmogorov–Smirnov test. Non-normally distributed data were log-transformed for statistical tests that assume normality of data distribution. Pearson correlation to study the relationships between serum Se, iron status, and thyroid markers, and independent Student’s t-test to compare thyroid markers by Se status were used. Predictor parameters displayed collinearity thus partial least squares (PLS) regression was used to identify parameters significantly predicting serum T₃ and T₄ concentrations. The predictor parameters tested in the model were UIC, T₃ (if T₄ was the response parameter), T₄ (if T₃ was the response parameter), TSH, Tg, ferritin, soluble transferrin receptor (sFTR), and Se concentration. SPSS for Windows (v18, Chicago, IL, USA) was used for statistical analyses and p < 0.05 was considered significant.

2.5. Ethical considerations

The study was approved by the National Health Research Ethics Review Committee at the Ethiopian Science and Technology Commission and the Institutional Review Boards at McGill University, Canada, and Oklahoma State University, USA. Written informed consent was obtained from all parents or guardians of the study children.

3. Result

The mean age of children at baseline was 56.9 ± 1.8 mo (54–60 mo) and 72.0 ± 1.9 mo (69–78 mo) at the second collection period. The iodized salt coverage increased from 12.2% to 91.6% of households. The median iodine concentration (by titration) of salt samples increased from below limit of detection during baseline to 14 ppm 15 mo after iodized salt became available. The median UIC after 15 mo of iodized salt availability was 167 μg/l (IQR 97, 287 μg/l) representing a substantial and clinically significant increase from baseline (9 μg/l; p < 0.001). Based on the WHO epidemiologic criteria for assessing iodine nutrition using median UIC, the study population had adequate iodine nutrition. However, despite the improvement, 26.4% of the study population had UIC classified as deficient (UIC < 100 μg/l) of whom 2.3% had UIC in the severely iodine deficient range (UIC < 20 μg/l), 7.1% had UIC in the moderately iodine deficient range (20–49 μg/l), and 17.0% had UIC in the mild iodine deficiency range (50–99 μg/l).

Compared to the cut-off for normal levels of thyroid markers, 2.9% of children had low serum T₃ (< 1.2 nmol/l), 2.7% had elevated serum Tg (> 78 μg/l), and 35.1% had elevated serum TSH concentration (> 4.2 mU/l). As expected, there was a significant change in the concentration of thyroid markers 15 mo after salt iodization (Table 1). However, there was a significant decrease in T₄ concentration in response to the consumption of iodized salt though it remained in the
normal range.

At final blood collection, the median serum Se concentration was 70.6 μg/l (IQR 48.2, 96.6). This value was not statistically different (p = 0.07) from the median baseline value (61.4 μg/l). Selenium inadequacy was prevalent in 49.1% of children at the final phase of blood collection. According to the epidemiological criteria to assess the adequacy of Se status, about 40% of participants had lower serum Se compared to the amount required to maximize IDI activity (> 0.82 μmol/l) and 42.2% lacked the amount of Se for optimal GPX and selenoprotein P activity (> 1.00–1.20 μmol/l) [20].

Selenium concentration was significantly correlated with T4 (r = −0.22; p < 0.01) at baseline and with T3 (r = 0.38; p < 0.001), T4 (r = 0.15; p < 0.001), TSH (r = 0.205; p < 0.001) and Tg (r = −0.11; p < 0.01) 15 mo after iodine repletion. In addition, serum Se was positively correlated with T3/T4 ratio (r = 0.29; p < 0.001). After the intervention, compared to children with normal Se status, children with Se inadequacy had significantly lower T4 (p < 0.003), and T3 (p < 0.001), but higher TSH (p = 0.003) (Table 2). In addition, considering only iodine adequate subjects (UIC ≥ 100 μg/l), children with serum Se lower than the optimal cutoff for IDI enzyme activity had significantly lower serum T3 (1.6 ± 0.2 vs 1.8 ± 0.3; p < 0.001) but higher TSH (4.0 ± 0.2 vs 3.5 ± 2.4; p = 0.002) compared to those children with normal Se level for IDI activity.

The final partial least square regression model has three significant latent variables (serum Se, UIC and Tg) explaining 66.7% of the variance for T3 and four latent variables (serum Se, UIC, T3, and Tg) in the case of T4 as a response parameter explaining 73.4% of the variance (Table 3).

### 4. Discussion

Selenium is important for normal thyroid metabolism. A deficiency of Se affects normal function of the thyroid gland even with iodine repletion. The present study investigated the influence of Se inadequacy on thyroid response of young children 15 mo after iodized salt became available. There was a significant improvement in children’s median UIC and thyroid markers (except serum T3) in response to the availability of iodized salt. Based on the median UIC, the present study children had adequate iodine status. However, nearly half (49.1%) of the children had low serum Se indicating deficiency. Despite correcting iodine nutrition, Se inadequacy in these young children was associated with lower serum T4, T3, and Tg but higher TSH concentrations.

Iodine is a critical component of the thyroid hormone with diverse biological functions. However, iodine deficiency is widespread causing an increased risk of disorders including goiter, physical growth retardation, cognitive impairment, spontaneous abortion, and infant mortality [21]. There has been significant progress towards eliminating iodine deficiency globally mainly through salt iodization. Based on median UIC data available for school-age children from 127 countries in the year 2016, only 15 countries were defined as iodine deficient [22].

In areas of endemic iodine deficiency, little or no change may occur in goiter rate after several months or even years of ensuring iodine nutrition. For example, a study evaluating the effectiveness of a mandatory salt iodization program showed that while salt iodine level, iodized salt coverage, and median UIC increased significantly after one year, the goiter rate remained unchanged [23]. After five to six years of correcting iodine nutrition by the introduction of salt iodization, marked reduction in total goiter rate could be achieved but goiter still remained endemic in the area [24]. Iodized salt prophylaxis may prevent the development of goiter in children born after iodine prophylaxis begins or may control goiter enlargement in older children who were previously iodine deficient but may not be effective in goiter regression in children who were iodine deficient during infancy [25]. In the present study, the significant reduction in T3 concentration in response to greater iodine supply contrasted to results of other research where iodine supply resulted either in an increase or no change in T3 concentration. This reduction might be due to the decline in serum TSH concentration; TSH is among the factors that regulate thyrocyte biology and thyroid hormone secretion by stimulating IDI enzyme activity and deiodination induction [26]. For example, an earlier study evaluating the effect of TSH on iodothyronine 5′ deiodinations on cultured thyroid tissues from patients with Graves’ disease found that addition of TSH stimulated all of IDI activities in a dose dependent manner resulting in an increase in T3 concentration [27].

Selenium deficiency is widespread globally mainly due to low concentrations of plant-available Se in agricultural soils. Nearly half of the present study children had Se inadequacy and the deficiency had a clear geographical pattern (data not shown). Even though not statistically significant, median serum Se concentration had increased from 61.4 μg/l to 70.6 μg/l between the first and second blood collection points (a period of 15 mo), and the proportion of Se deficient children declined from 58.7% to 49.1%. Dietary diversity correlates well with micronutrient status and is used as a proxy indicator of micronutrient nutrition. However, in the present study children, no significant change in mean dietary diversity score was observed (data not shown). Concentration of Se in serum increases with age, yet age-related reference values are not available. This increase is thought to be associated to the normal dietary changes of children as they grow older [28]. The present study however was limited to qualitative assessment of food intakes which was not able to capture changes in the quantity of dietary Se.

Several researches show that Se inadequacy affects normal thyroid metabolism.

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**Table 1**

<table>
<thead>
<tr>
<th>TSH (mU/l)</th>
<th>T4 (nmol/l)</th>
<th>T3 (nmol/l)</th>
<th>Tg (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>3.4 [2.8, 6.3]</td>
<td>9.5 ± 2.8</td>
<td>2.0 [1.7, 2.3]</td>
</tr>
<tr>
<td>Endline</td>
<td>3.1 [2.1, 4.6]</td>
<td>10.4 ± 1.9</td>
<td>1.7 [1.5, 2.0]</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>p = 0.011</td>
</tr>
</tbody>
</table>

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**Table 2**

Comparison of level of thyroid markers by Se status of young children from the Amhara region of Ethiopia, 15 mo after iodine repletion.

<table>
<thead>
<tr>
<th>T4</th>
<th>T3</th>
<th>TSH</th>
<th>Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se deficient</td>
<td>10.0 ± 2.8</td>
<td>2.0 [1.7, 2.3]</td>
<td>4.4 [2.8, 6.2]</td>
</tr>
<tr>
<td>Baseline  Se adequate</td>
<td>8.7 ± 2.5</td>
<td>2.1 [1.8, 2.3]</td>
<td>4.4 [2.9, 6.5]</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>Se deficient</td>
<td>10.2 ± 1.5</td>
<td>1.7 ± 0.3</td>
<td>3.9 ± 1.8</td>
</tr>
<tr>
<td>Endline  Se adequate</td>
<td>10.7 ± 2.3</td>
<td>1.9 ± 0.3</td>
<td>3.4 ± 2.3</td>
</tr>
<tr>
<td>p-value</td>
<td>p = 0.003</td>
<td>p &lt; 0.001</td>
<td>p = 0.003</td>
</tr>
</tbody>
</table>

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**Table 3**

Interrelationship between response and predictor parameters and weights of PLS regression model between Se and iodine status and thyroid response of young children from the Amhara region of Ethiopia, 15 mo after iodine repletion.

<table>
<thead>
<tr>
<th>Response parameters</th>
<th>Predictor parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>T4</td>
</tr>
<tr>
<td>T3</td>
<td>0.78</td>
</tr>
<tr>
<td>T3</td>
<td>–</td>
</tr>
</tbody>
</table>

NS, not significant.

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1 all such values are median [IQR25, IQR75].

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Selenium inadequacy in children with a double burden of Se and iodine deficiency negatively affected the efficacy of iodine supplementation in restoring normal thyroid function and the degree of severity of Se deficiency significantly predicted thyroid response [13]. Selenium deficiency was significantly associated with elevated T4 concentration [15,29]. A negative association between serum Se concentration and fT4 in Iranian school children was reported [30]. Even though the analysis was based on a very small sample size, Pizzulli and Ranjbar [31], reported that hypothyroidism (elevated TSH) of placebo controlled trial, supplementation of Se-enriched yeast (100, 200, or 300 μg/d) positively and dose dependently affected thyroid function by reducing TSH and fT4 but again without affecting fT3 and fT3/fT4 ratio [36]. Selenium (60 μg/day) supplementation to UK pregnant women with mild to moderate iodine deficiency decreased fT4 concentration [37]. On the other hand, Se supplementation at different doses (100, 200, or 300 μg Se/d) to the UK elderly showed no effect on thyroid function. However, this result may be because the elderly participants had a mean baseline serum Se concentration (90 μg/l) greater than the amount required for optimal IDI activity [38]. Potentially for the same reason, no significant effect on thyroid hormone changes was reported among older New Zealanders of marginal Se deficiency [39]. In subjects with borderline iodine sufficiency, Se deficiency was not an independent risk factor for goiter development [40]. Similarly, in an iodine sufficient area, Se deficiency was associated with neither goiter nor TSH concentration [41].

A literature review of the interaction of Se and thyroid functions in infants, children and adolescents reported that even though Se is important for normal thyroid function, the deficiency has only a modest effect [42]. A study involving a multi-factorial analysis of the effect of iodine and Se on thyroid hormone concentration in a goiter endemic and Se deficient area and control area was implemented in northern Democratic Republic of Congo (formerly Zaire). The authors reported a significant association between UIC and serum T4, fT4, and TSH and between red blood cell-GPX (RBC-GPX) and serum TSH. In addition, both UIC and RBC-GPX were significantly associated with serum T4, fT4 and TSH concentrations. However, the association between serum Se and thyroid function indicators was not significant [43]. In a double-blind, randomized, controlled trial, iodine deficient children were supplemented with Se and iodine or iodine alone. Results show that serum thyroid hormone concentrations were significantly changed to normal reference ranges in both intervention groups compared to children in the placebo group. However, no significant differences in the level of serum thyroid hormones among the two interventions were observed suggesting that iodine but not Se was important for correcting thyroid hormone status [44]. In addition to iodine and Se, iron deficiency has an adverse effect on thyroid metabolism by reducing thyroxine oxidase activity [45]. In the present study, however, none of the iron indicators had a significant association with the thyroid markers, perhaps because only a small portion of the children were iron deficient [46].

In general, nearly half of the study children were Se deficient and about a quarter of children had UC below the recommendation. Selenium deficiency negatively affected thyroid response of iodine-replete children suggesting it could potentially compromise the effectiveness of the salt iodization program. Results of the present study also suggest the need to consider correcting Se nutrition both for increasing effective utilization of iodine and for the biological importance of Se in general.

Declarations of interest

None.

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