

Serum Zinc, Iron and Urinary Iodine Levels and their Relationship to Other Indices of Malnutrition among Lactating Mothers in Two Agro-Ecological Zones of Rural Ethiopia

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Abstract

There are limited studies on the magnitude and severity of zinc, iron and iodine deficiency, in addition to inadequate serum ferritin levels and anaemia, among lactating mothers across different agro-ecological zones of rural Ethiopia. A cross-sectional study examining socio-demographic parameters, anthropometry, dietary diversity, household salt usage, urinary iodine and blood tests for zinc, ferritin and anaemia was conducted in 202 mothers among eight rural villages from lowland and midland agro-ecological zones of Ethiopia. Goitre was determined by palpation. The prevalence of low status of serum zinc, ferritin, iron deficiency anaemia (IDA), and total goitre rates among lactating mothers irrespective of their agro-ecological zones were 71.8%, 60.4%, 10.9%, and 35.2%, respectively. The median urinary iodine was 120 µg/L. Low level of serum zinc, iron and IDA was significantly higher in lowland mothers (96.2%, 89.4% and 20.2%) than in midland agro-ecological zone (45.9%, 29.9%, and 1.0%). Prevalence of goitre among lactating mothers was not significantly different across agro-ecological zones. Adequately iodised salt was available in 36.6% of households. Of the mothers, 37.5% in lowland were deficient in both biomarkers (zinc and ferritin) while only 2.0% in midland agro-ecological zone were deficient. None of the lactating mothers were aware of iodine deficiency diseases or the importance of using iodised salt. Haemoglobin levels were significantly correlated with maternal body mass index (BMI), mid-upper arm circumference (MUAC) and dietary diversity. Lactating mothers in rural Ethiopia are at high risk of micronutrient deficiencies. The prevalence of micronutrient deficiencies was significantly influenced by agro-ecological zone.

Keywords: Zinc; Ferritin; Iodine; Ethiopia; Lactating mothers

Introduction

Micronutrient deficiencies are a major public health problem in many developing countries, particularly in infants and pregnant women. In 2011, 116,000 deaths in children under 5 years were attributable to zinc deficiency (1.7% of mortalities in this group) [1]. Approximately, 2 billion people (over 30% of world population) are anaemic, many due to iron deficiency [2]. Additionally, about 1.88 billion (28%) of the world's population are considered to have insufficient iodine intake and hence are at risk of iodine deficiency, of whom more than 321 million are Africans [3].

Zinc is a micronutrient with diverse physiologic and metabolic functions [4]. It is involved in all the main biochemical pathways and plays multiple roles in cellular proliferation and differentiation. It affects physical growth, immunity, reproductive function and neuro-behavioural development [5]. The amount of zinc required for non-pregnant adult women (19+ years) is 9 mg per day. This amount increases to 11 mg zinc/d in pregnancy and in lactation to 12 mg/d [6].

Iron deficiency occurs predominantly due to a deficiency of bioavailable dietary iron and/or increased demands such as during childhood, pregnancy and lactation [7]. High demands for iron due to menstrual blood loss, pregnancy, lactation and nutritional deficiencies are the most common causes of iron deficiency anaemia in reproductive-age women [8]. Nutritional requirements during lactation are greater than during pregnancy [9]. The quantity of milk produced by mothers depends very much on the mother's diet [9]. Severely malnourished mothers have reduced lactation performance contributing to increased risk of child mortality [10].

Lactating mothers from low-income settings are considered as a nutritionally vulnerable group [11]. Anaemia was estimated to be an underlying factor for 22% of maternal deaths around the world, of which severe anaemia is a major contributor [12,13]. The few studies conducted among Ethiopian mothers on prevalence of zinc and iron deficiency suggest there is very high level of prevalence. A study conducted in Gondar (Northwest Ethiopia) among pregnant and non-pregnant mothers reported prevalence of low zinc status was 74% and 55%, respectively [14]. Similarly, another study evaluating zinc deficiency among pregnant women in rural Sidam, South Ethiopia, reported prevalence of 53% [15]. According to a study in nine different regions of Ethiopia conducted by Umeta, *et al.* [16], the prevalence of anaemia, ID and iron deficiency anaemia (IDA) were 30.4, 49.7 and 17.0%, respectively. The single study in Ethiopia documenting the prevalence of low serum ferritin among lactating mothers in a slum of Addis Ababa reported that the prevalence was 22.3% [17]. Similarly, iodine deficiency in eastern Ethiopia has a prevalence of 82% among pregnant women [18]. In Ethiopia, goitre rates are reported to range from 34.5% to 37.0% among childbearing women [19].

Although low serum level of zinc and iron, and iodine deficiency is increasingly being recognized as a widespread problem in Ethiopia, there are very limited studies on the prevalence and severity of these deficiencies among lactating mothers across different agro-ecological zones in Africa, particularly in Ethiopia. This study was conducted in two agro-ecological zones of rural Ethiopia with the aim of assessing the prevalence and variations of maternal zinc, iron and iodine deficiency across these zones and their relationship to other indices of malnutrition.

Materials and Methods

Participants and study design

The study was conducted in the Babile, Enderta and Hintalo Wajirat districts of Ethiopia. These districts were selected due to their proximity to the two Ethiopian universities who were partner institutions in this project funded by Irish Aid. Babile District (Woreda), which is 560 km away from Addis Ababa in the eastern part of Ethiopia and adjacent to Haramaya University, represented a lowland agro-ecological zone. The altitude of Babile Woreda ranges from 950 to 2000 meters above sea level and data were collected from 1000-1500 meters above sea level. The major agricultural product for consumption is sorghum, and oil seeds and groundnuts are used as cash crops. Hintalo Wajirat and Endreta districts (683 km and 773 km away from Addis Ababa in the Northern part of Ethiopia, respectively and adjacent to Mekelle University) represent midland agro-ecological zones. Data were collected from altitude of greater than 2000 meters above sea level where the majority produce cereals (Teff and barley) and are involved in animal husbandry.

A community based cross sectional study was conducted in four kebeles randomly selected from each geographical area. The study was conducted from January to February 2014 which is the post-harvest season. Data on socio-demographic status, anthropometry and feeding practices were generated. Blood and urine samples were collected from the mothers. Out of the 208 mothers who gave samples of blood for test of ferritin, haemoglobin and zinc, 202 (97%) samples were analysed, while the remaining samples were discarded due to insufficiency (3 samples) and haemolyses (3 samples). Urinary iodine levels and salt iodine concentration were measured and goitre rates were assessed by palpation.

Socio-demographic status and anthropometric measures

Socio-demographic characteristics of the participants were assessed using a pre-tested questionnaire that included questions on education levels, health services utilization and water and sanitary facilities. Weights of the lactating women were measured to the nearest 0.1 kg on a battery powered digital scale (Seca 770, Hanover Germany) with a weighing capacity of 0 to 140 kg and heights were measured to the nearest 0.1 cm using a wooden height-measuring board with a sliding head bar following standard anthropometric techniques [20]. Mid upper arm circumference (MUAC) was also measured using a non-stretchable MUAC tape (MUAC measuring tape/PAC-50) [20] on the left upper arm of the mothers. To measure weight and height, study subjects removed their shoes, jackets and wore light clothing. To avoid variability among the data collectors, all the anthropometric measurements were taken by two different data collectors and compared. In case of variation among the data collectors, the researcher (the first author) took the measurement again for validation. The Body Mass Index (BMI) [weight/height^2 (kg/m^2)] was calculated and the threshold of 18.5 kg/m^2 was used to identify underweight women.

Dietary intake assessment

Dietary intake was assessed by simple questionnaire that allowed all types of foods consumed during each of the 24 previous hours to be noted. Each woman involved in the study was asked to recall all the communal dishes she had eaten in the compound during this period. The recall was randomly made on weekdays or on weekend days, since weekends do not have any special significance with respect to dietary intake in the context of our study. We took care to not include atypical days (local feasts or celebrations) in the recall. The protocol of Women Dietary Diversity Score (WDDS) was standardized by FANTA [21].

Blood collection, serum separation and micronutrient measurements

Five millilitres of venous blood were collected aseptically in the morning [22] from the antecubital vein of the participants and was aliquoted into tubes without anticoagulants by a trained health professional. Samples of serum were collected in to zinc/metal free vacutainers and gloves were free of talcum powder. A sample was allowed to clot and centrifuged to separate serum. Serum

was stored frozen at -20°C and transported by plane to the Ethiopian Public Health Institution (EPHI <http://www.ephi.gov.et>), Addis Ababa. Serum ferritin, which reflects body iron stores, was analysed using an enzyme-linked immunosorbent assay (ELISA) with a fully automated Elecsys 1020 using commercial kits purchased from Boehringer Mannheim, Germany by a senior medical technologist. Serum ferritin (SF) levels were defined as: iron deficiency if SF $<15\ \mu\text{g}/\text{litre}$ as recommended by WHO [23].

A small portion of whole blood from the syringe was used to test haemoglobin level of the mothers immediately on site by using portable HemoCue analyser (HemoCue[®] Hb 301) which is considered to be a gold standard for field work in resource poor settings [24]. The cut-off point for anaemia was based on WHO (2011) recommendation for non-pregnant mother. The threshold criteria used to determine the severity of anaemia as a mild, moderate or severe public health problem were as follows: prevalence rate of anaemia was mild 5.0-19.9%, moderate was 20-39.9% and severe was $>40\%$, respectively. Level of anaemia was classified as: mild anaemia (Hgb 10-11.9 g/dl), moderate anaemia (Hgb 7-9.9 g/dl) and severe anaemia (Hgb less than 7 g/dl). Haemoglobin level was adjusted for altitude as follow: altitude of less than 1000 meter above sea level had no adjustment for haemoglobin, for 1000 meter subtract 0.2 g/dl, from 1000-1500 meter, subtract 0.5 g/dl, from 1500-2000 meter, subtract 0.8 g/dl and 2000 to 2500 subtract 1.3 g/dl from haemoglobin reading. Iron deficiency anaemia was defined as serum ferritin less than $15\ \mu\text{g}/\text{litre}$ and haemoglobin less than 12 g/dl [25].

For the determination of zinc, frozen sera were shipped to EPHI on dry ice in polystyrene packaging material. The concentration of zinc in serum was determined at the National Food, Medicines, Health Service Administration and Control Authority (FMHACA) laboratory by using Shimadzu Flame Atomic Absorption Spectroscopy (AA 6800 model, Japan). Serum samples (200 μL) were added to a trace metal-free plastic test tube and diluted by addition of 6% butanol in 1:5 ratios. Calibration of the Atomic Absorption Spectrophotometer was carried out using a series of standards of zinc, 0, 0.1, 0.2, 0.3 and 0.4 ppm by dilution from stock of 1000 ppm AAS zinc standards. Each series of standards was diluted with 5% glycerol to equate with viscosity of serum. Zinc concentration was measured using an air-acetylene flame at a wavelength of 213.9 nm and a slit width of 0.7 nm. The results were calculated from two runs [26,27]. To minimize the risk of contamination, all glassware and plastic tubes used were immersed in 10% (v/v) solution of nitric acid for 24 h, washed with distilled water and rinsed with deionized water before use. Because blood samples in this survey were not necessarily obtained fasting but collected in the morning between 8-10 a.m., we used a cut-off value of 66 $\mu\text{g}/\text{dl}$ for mothers and 65 $\mu\text{g}/\text{dl}$ for children, as recommended by the International Zinc Nutrition Consultative Group (IZiNCG) [22].

Urinary iodine

To determine the presence of iodine in the household salt, data collectors asked households to provide a teaspoon of the salt used for cooking on the night prior to data collection. The salt was immediately tested on site for iodine using the iodine rapid test kit (MBI Kits International).

Five to ten millilitres of spot urine samples were collected from all mothers to examine urinary iodine concentration (UIC). Samples were tightly sealed in plastic tubes that were free from iodine or any other chemical to avoid leakage and cross-contaminations with iodine from other sources. The urine samples were kept in a cold box until analyses were performed in duplicate in the iodine laboratory of the EPHI using the method based on the Sandell-Kolthoff reaction [27]. UIC was expressed as the median micrograms of iodine per liter ($\mu\text{g}/\text{litre}$) of urine. Urinary iodine status of the lactating mothers was classified using WHO/UNICEF/ICCIDD recommended cut-off points for urinary iodine concentration for populations [29]. That is $\geq 100\ \mu\text{g}/\text{litre}$ is categorized as adequate and $<100\ \mu\text{g}/\text{litre}$ insufficient.

Goitre rates

All mothers were clinically examined by trained nurses for goitre using palpation of the thyroid. Five day practical training was given on how to palpate neck for goitre and demonstration and evaluation was given during pretesting of the tools. Classification of goitre rate was made as recommended by WHO/UNICEF/ICCIDD [30].

Variables

Dependent variables were micronutrients and biomarkers of micronutrient status (serum zinc, ferritin, iron deficiency anaemia, urinary iodine) among lactating mothers. The independent variables were the socio-demographic and economic status, health status of mothers, water, sanitation, health services utilization and cultural/social characteristics related to feeding style of the mother. Nutritional knowledge of mother, WDDS and meal frequency, and health seeking behavior of the family was also assessed.

Data processing and analysis

The data were double entered by separate data clerks into EPI Data version 3.1. Data cleaning and editing were undertaken before analysis. Data were checked for normality by using the Kolmogorov-Smirnov test. Data were transferred to SPSS (v 16.0) statistical packages and Stata (v.11) for analysis. Descriptive statistics were used to show the magnitude of each variable. Cross tabulations and linear regression were used to see the difference across the agro-ecological regions and associations of different variables. For WDDS, BMI, anthropometric data, haemoglobin and serum ferritin levels, mean and standard deviation were analysed. Multiple binary and linear logistic regressions were applied to control for confounding after testing binary linear regression.

Ethical consideration

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by University College Cork, Ireland and Haramaya University College of Health and Medical Sciences Institution, Ethiopia, Research Ethics Review Committees. Subsequently, the final approval of the protocol was granted by the Ethiopian National Ministry of Science and Technology Ethical Review Committee. Informed consent was obtained from the mothers and they were informed that they had the right to refuse or exit from the study at any time and refusing to participate in the study would not have any negative implications for them. Women who were found to be undernourished during assessment were referred to the nearest health institution for health care services.

Results

The majority 137 (67.8%) of the mothers were illiterate. Health service utilization was low as 138 (68.3%) of the mothers reported that they gave birth at home. The majority 145 (71.8%) of the mothers wash their hands after toilet but only 113 (55.9%) households have toilet facility (Table 1).

Concerning feeding practices, 56.9% of the households never consumed meat and 47% never consumed egg at least one time in the previous month. The majority (60.9%) of mothers reported that they drink coffee or tea more than four times in a week, and 86.6% drank coffee the day preceding the survey. Only 27.7% drink milk or milk with coffee. About 78.7% of the households reported that they cook salt with food and 18.3% of households reported washing their salt before use as majority of the households in rural Ethiopia use crystal salt (Table 1).

Variables	Category	Total n (%)	Lowland* n (%)	Midland** n (%)	P-value
Age (y)	15-24	48 (23.8)	22 (21.2)	26 (26.5)	1.00
	25-34	114 (56.4)	63 (60.6)	51 (52.0)	0.52
	35-49	40 (19.8)	19 (18.2)	21 (21.5)	0.93
Education	Illiterate	137 (67.8)	78 (75.0)	59 (60.2)	0.19
	Attend school	65 (32.2)	29 (25.0)	39 (39.8)	
Parity	3 and below	71 (35.1)	38 (36.5)	33 (33.7)	0.80
	>=4	131 (64.9)	66 (63.5)	65 (66.3)	
Took deworming tablets during pregnancy	No	132 (65.3)	65 (62.3)	67 (68.4)	0.60
	Yes	70 (34.7)	39 (37.7)	31 (31.6)	
Family size	1-5	100(49.5)	54 (51.9)	48(46.9)	0.80
	>5	102(50.5)	50 (48.1)	52(53.1)	
Number of children <5 y	1 child	79 (39.1)	30 (28.8)	49 (50)	1.00
	2 children	110 (54.5)	61 (58.7)	49 (50)	0.19
	3 children	13 (6.4)	13 (12.5)	0	-----
Household with toilet	Yes	113 (55.9)	52 (50.0)	61 (62.2)	0.30
	No	89 (44.1)	52 (50.0)	37 (37.8)	
Hand washing after toilet	Yes	145 (71.8)	76 (73.1)	69 (70.4)	0.80
	No	57 (28.2)	28 (26.9)	29 (29.6)	0.70
Birth interval	First birth	27 (13.4)	11 (10.6)	16 (16.3)	1.00
	1-2 y	89 (44.1)	59 (56.7)	30 (30.6)	0.14
	>2 y	86 (42.5)	34 (32.7)	52 (53.1)	0.96
Attend antenatal care	No	32 (15.8)	22 (21.2)	10 (10.2)	0.20
	Yes	170 (84.2)	82 (78.8)	88 (89.8)	
Place of delivery of index child	Home	138 (68.3)	86 (82.7)	52 (53.1)	0.01
	Health institution	64 (31.7)	18 (17.3)	46 (46.9)	
Meat consumption in a month	Never	115 (56.9)	84 (80.8)	31 (31.6)	0.00
	1-2 times	87 (43.1)	20 (19.2)	67 (68.4)	
Egg consumption in a month	Never	95 (47.0)	73 (70.2)	22 (22.4)	<.001
	1-2 times	67 (33.2)	13 (12.5)	54 (55.2)	0.23
	3-6 times	40 (19.8)	18 (17.3)	22 (22.4)	1.00
Eat legumes yesterday	No	60 (29.7)	59 (56.7)	1 (1)	<0.001
	Yes	142 (70.3)	45 (43.3)	97 (99)	
Consumption of tea/ coffee in a week	<4 times	79 (39.1)	25 (24.0)	54 (55.1)	0.009
	>4 times	123 (60.9)	79 (76.0)	44 (44.9)	
Drink coffee yesterday	No	27 (13.4)	18 (17.3)	9 (9.2)	0.30
	Yes	175 (86.6)	86 (82.7)	89 (90.8)	
Drink milk yesterday	No	146 (72.3)	51 (49.0)	95 (96.9)	<0.001
	Yes	56 (27.7)	53 (51.0)	3 (3.1)	

Variables	Category	Total n (%)	Lowland* n (%)	Midland** n (%)	P-value
Wash salt before use	No	165 (81.7)	87 (83.7)	78 (79.6)	0.66
	Yes	37 (18.3)	17 (16.3)	20 (20.4)	
Practice of using salt	Add after cooking	43 (21.3)	9 (8.7)	34 (34.7)	0.01
	Cook with food	159 (78.7)	95 (91.3)	64 (65.3)	

*Babile District (Woreda) which is 560 km away from Addis Ababa in the eastern part of Ethiopia is representing a lowland agro-ecological zone. **Hintalo Wajirat and Endreta districts are 683 km and 773 km away from Addis Ababa in the northern part of Ethiopia, respectively and represent midland agro-ecological zones

Table 1: Distribution of selected socio-demographic and maternal characteristics of lactating mothers in two agro-ecological zones of rural Ethiopia (N = 202)

Comparison of maternal characteristic between the two agro-ecological zones

Among 202 participants included in the survey, mean and standard deviation of age were 28.5 ± 5.9 years. Comparing maternal characteristics of lowland and midland, midland had significantly ($p < 0.05$) higher rate of focused antenatal care, haemoglobin, serum zinc, and serum ferritin concentrations than their lowland counterparts, while the latter had a statistically significant ($p < 0.05$) higher number of children aged less than five and low birth interval (Table 2).

Parameters	Lowland# (n=104)	Midland## (n=98)	P-Value
Age (y)	28.3 ± 5.2	28.6 ± 6.6	0.751
BMI	19.2 ± 2.3	18.9 ± 1.8	0.365
Parity	4.4 ± 2.2	4.3 ± 2.5	0.813
Children <5 y	1.8 ± 0.6	1.5 ± 0.5	0.000**
Attended antenatal care	2.7 ± 1.2	3.8 ± 1.5	0.000**
Haemoglobin (g/dl)	12.9 ± 2.0	13.4 ± 0.8	0.023**
Serum zinc ($\mu\text{g/l}$)	45.0 ± 14.0	68.8 ± 24.0	0.000**
Serum ferritin ($\mu\text{g/l}$)	26.8 ± 23.6	80.2 ± 51.0	0.000**
Median urinary iodine ($\mu\text{g/l}$)	122.1 ± 108.8	118.2 ± 83.6	0.970
Family size (total in home)	5.8 ± 2.0	5.7 ± 2.0	0.906
MUAC (cm)	22.0 ± 1.6	21.7 ± 1.4	0.930
Height of mothers (cm)	159.7 ± 5.8	159.2 ± 5.4	0.597
Birth interval (y)	2.2 ± 1.5	2.9 ± 2.0	0.014**
Weight of mothers (kg)	49.1 ± 6.8	48.1 ± 4.9	0.225
WDDS	3.0 ± 1.1	3.1 ± 0.4	0.767
Mean duration of lactation (months)	13.3 ± 4.4	13.3 ± 5.3	0.988

Values are expressed as mean \pm standard deviation; BMI: Body Mass Index calculated as weight in kg/height in meter squared (kg/m^2); MUAC: Mid Upper Arm Circumference; WDDS: Women Dietary Diversity Score

**P-Values statistically significant at $P < 0.05$

#Babile District (Woreda) which is 560 km away from Addis Ababa in the eastern part of Ethiopia is representing a lowland agro-ecological zone. ##Hintalo Wajirat and Endreta districts are 683 km and 773 km away from Addis Ababa in the northern part of Ethiopia, respectively and represent midland agro-ecological zones

Table 2: Comparison of maternal characteristics of lactating mothers in two agro-ecological zones of rural Ethiopia (N = 202)

Prevalence of low zinc, iron and iron deficiency anaemia

The mean serum zinc concentration of the study participants was 57.5 ± 23.1 $\mu\text{g/litre}$ (Table 2) with a low serum level of (serum zinc < 66 $\mu\text{g/litre}$) in 145 participants (71.8%) (Table 3). Mean serum ferritin (SF) concentration of the participants was 52.7 ± 4.8 $\mu\text{g/litre}$ (Table 2). The prevalence of iron deficiency (ID) determined by serum ferritin (< 15 $\mu\text{g/litre}$) was 20.8% (Table 3). Iron deficiency anaemia (low haemoglobin + SF) was observed in 22 participants (10.9%). Among 41 (20.3%) mothers with zinc and ID in combination, 39 (95.1%) of them also were from lowland agro-ecological zones (Table 3).

	Responses	Total n (%)	Lowland# n (%)	Midland## n (%)	P-value
Iron deficiency (serum ferritin SF)	Severe (<15 µg/l)	42 (20.8)	39 (37.5)	3 (3.1)	0.000
	Normal ferritin value (>15 µg/l)	160 (79.2)	65(62.5)	95 (96.9)	
IDA (low Hgb +SF)	Yes	22 (10.9)	21 (20.2)	1 (1)	0.000
	No	180 (89.1)	83 (79.8)	97 (99)	
Level of anaemia (hae-moglobin)	Moderate (7-9.9 g/dl)	5 (2.5)	5 (4.8)	0	NA 0.000 0.000
	Mild (10-11.9 g/dl)	37 (17.8)	30 (28.8)	7 (6.1)	
	Overall anaemia (Hgb<12g/dl)	42 (20.3)	35 (33.6)	7 (6.1)	
	Non anaemic mothers	160 (79.7)	69 (66.4)	91 (93.9)	
Serum zinc	<66 µg/l	145 (71.8)	100 (96.2)	45 (45.9)	0.000
	>=66 µg/l	57 (28.2)	4 (3.8)	53 (54.1)	
MUAC	<22 cm	84 (41.6)	51 (49.0)	33 (33.7)	0.027
	>22 cm	118 (58.4)	53 (51.0)	65 (66.3)	
BMI	<18.5 (kg/m ²)	85 (42.1)	42 (40.4)	43 (43.9)	0.702
	>18.5 (kg/m ²)	117 (57.9)	61 (59.6)	56 (56.1)	
Urinary iodine	<100 µg/l	86 (42.6)	47 (45.2)	39 (39.8)	0.440
	>=100 µg/l	116 (57.4)	57 (54.8)	59 (60.2)	
Salt iodine	0 ppm	26 (12.9)	12 (11.5)	14 (14.3)	0.380 0.004
	15ppm	102 (50.5)	65 (62.5)	37 (37.7)	
	>15ppm	74 (36.6)	27 (26.0)	47 (48.0)	
Goitre rate	Grade 2 (Visible)	5 (2.5)	4 (3.8)	1 (1)	0.190 0.880
	Grade 1 (Palpable)	66 (32.7)	34 (32.7)	32 (32.7)	
	Grade 0 (No goitre)	131 (64.9)	66 (63.5)	65 (66.3)	
	Total goitre rate (TGR) (visible + palpable)	71 (35.2)	38 (36.5)	33 (33.7)	
Ferritin and zinc deficient**	Yes	41 (20.0)	39 (37.5)	2 (2.0)	0.000
	No	161 (80.0)	65 (62.5)	96 (98.0)	

BMI: Body Mass Index calculated as weight in kg/height in meter squared (kg/m²); MUAC: Mid Upper Arm Circumference; Hgb: Haemoglobin; SF: Serum ferritin; WDDS: Women Dietary Diversity Score

**Ferritin and zinc deficient is defined as mothers with serum ferritin less than 15 µg/l and zinc level less than 66 µg/l

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Table 3: Prevalence of micronutrient deficiency and malnutrition among lactating mothers in two agro-ecological zones of rural Ethiopia (N = 202)

Prevalence of iodized salt utilization and Total Goitre Rate

Adequate iodized salt (with >15ppm) was found in 36.6% of the households (Table 3), about half (50.5%) of households have salt with less than 15 ppm and the remaining households (12.9%) use non-iodized salt. The median Urinary Iodine Concentration (UIC) of lactating mothers was 120 µg/litre, and 42.6% of the mothers had UIC less than 100 µg/litre (Table 3). The weighted total goitre (TGR) rate among lactating mothers in this study group was 35.2% (95%, CI = 28.6 - 42.1), 32.7% palpable and 2.5% visible goitre (Table 3).

Prevalence of low serum zinc, iron and urinary iodine by agro-ecological zone

The proportion of lactating mothers with zinc, iron deficiency and iron deficiency anaemia was considerably higher among lowland residents compared to midland (Table 3). Greater than 96% of mothers in the lowland region had serum zinc levels less than 66 µg/litre. On the other hand, about 46% of mothers had zinc level below this cut-off in the midland zone. Higher prevalence of ID (serum ferritin levels <15 µg/litre) was observed among lowland agro-ecological zones (37.5% versus 3.1% in midland) (Table 3). The levels of anaemia in the two zones paralleled the ID rates. Overall levels of ID in this study population, as assessed by serum ferritin concentration, were very high. Nearly 21% of surveyed mothers had serum ferritin concentrations less than 15 µg/litre which is an indicator of ID (Table 3).

The prevalence of Total Goitre Rate (TGR) was very similar in both zones with slightly higher incidence in the lowland (36.5%) agro-ecological zone compared to the midland (33.7%). The number of mothers with visible goitre was 4 in the lowland and 1 individual in midland region (Table 3).

About 20% of mothers surveyed were deficient in both biomarkers (zinc and iron) with higher rate (37.5%) in the lowland zone while only 2.0% of mothers in the midland zone were deficient in both biomarkers measured in this study (Table 3).

Table 4 shows the correlation between micronutrient status, malnutrition and dietary diversity scores among lactating mothers in rural Ethiopia. Maternal serum ferritin concentration levels were significantly associated with serum zinc ($r = 0.374$, $p = 0.000$) and haemoglobin level ($r = 0.21$, $p = 0.003$). Haemoglobin levels were associated with maternal BMI ($r = 0.165$, $p=0.019$), MUAC

($r = 0.175$, $p = 0.013$) and Women Dietary Diversity Score ($r = 0.192$, $p = 0.006$) in addition to serum ferritin. Maternal MUAC was positively associated with maternal BMI ($r = 0.619$, $p = 0.000$) in addition to haemoglobin levels. Maternal age was negatively but not significantly associated with concentrations of zinc, ferritin and haemoglobin. As the age of the mother increased, maternal BMI tended to increase also.

Variables		Ferritin	Zinc	Haemo-globin	BMI	MUAC	WDDS
Ferritin	P. Correlation Sig. (2-tailed)	1.00					
Zinc	P. Correlation Sig. (2-tailed)	0.374** <0.001	1.00				
Haemoglobin	P. Correlation Sig. (2-tailed)	0.211** 0.003	0.096 0.173	1.00			
BMI	P. Correlation Sig. (2-tailed)	-0.075 0.291	-0.146* 0.039	0.165* 0.019	1.00		
MUAC	P. Correlation Sig. (2-tailed)	-0.078 0.270	-0.074 0.295	0.175* 0.013	.619** <0.001	1.00	
WDDS	P. Correlation Sig. (2-tailed)	0.093 0.189	0.104 0.139	0.192** 0.006	-0.034 0.627	-0.030 0.668	1.0
Age of the mothers	P. Correlation Sig. (2-tailed)	-0.052 0.46	-0.025 0.72	-0.061 0.39	0.25 <0.001	0.10 0.145	-0.01 0.87

P: Pearson correlation; **BMI:** Body Mass Index calculated as weight in kg/height in meter (kg/m^2); **MUAC:** Mid Upper Arm Circumference; **WDDS:** Women Dietary Diversity Score

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Table 4: Correlation between micronutrients, malnutrition and Women Dietary Diversity Score among lactating mothers in rural Ethiopia (N = 202)

Discussion

This study conducted in lactating mothers confirmed that 71.8% had low serum zinc, 60.4% had low iron status and 42.6% of the mothers had UIC less than 100 $\mu\text{g}/\text{L}$. Of the mothers with low iron status, 10.9% had IDA. The deficiencies were more pronounced in lowland (96.2% for zinc and 89.4% for iron) compared to midland (45.9% for zinc and 29.6% for iron) agro-ecological zones.

Our observation of zinc deficiency of 71.8% is similar to that reported in pregnant women in Ethiopia at 74% [31]. The higher prevalence of low serum zinc found in the present study could be due to an inadequate zinc intake and/or poor bioavailability. It is worthy to note that several dietary factors are known to affect zinc absorption as a result of physico-chemical interactions in the intestine. Phytate, a component in plants with the highest concentration found in seeds (cereal grains/legumes/nuts), inhibits zinc absorption [32]. This might hold true in the subjects of the present study, as cereal is a staple food in both agro-ecological zones. Additionally, good sources of bioavailable zinc, like meat and egg, were poorly consumed especially in the lowland agro-ecological zone.

On the basis of the WHO cut-off points for serum ferritin [23], the prevalence of iron deficiency in the present study population was 20.8%. When we compare prevalence of iron deficiency among agro-ecological zones, an exceptionally higher prevalence was recorded among lowland community (37.5% versus 3.1%). This prevalence is larger than reported in other studies conducted in Ethiopia [16,33].

The WDDS was similar but low in both agro-ecological zones (Table 2). Evidence for a close association between dietary diversity and micronutrient deficiencies in developing countries is well documented [34,35]. Some cereal crops, notably teff the main cereal consumed in the midland agro-ecological zone, are high in iron and fermented enset may increase non-heme iron absorption. Additionally, a marked observation in the present study is the lower intakes of meat (source of heme iron) and eggs among mothers from the lowland zone (Table 1). Heme iron from animal foods is much better absorbed than non-heme iron from plant source food where iron absorption may range from as low as 1 to 10%. A striking difference was noted for legume consumption among the study participants between the two agro-ecological zones. Ninety nine percent of mothers in the midland zone reported consuming legumes the day prior to the study while only 43.3% of mothers in the lowland zone consumed these foods. Legumes are a rich source of vitamin C and this could be another factor related to the differences in iron deficiency anaemia between the two zones. Frequent consumption of coffee and tea was observed. Consumption of these beverages was much higher in the lowland zone. Coffee and tea contain phenolic compounds that inhibit the absorption of iron. In addition to their appetite-suppressing effects, these beverages are of low nutrient density, thus when consumed especially with sugar, they may displace more nutritious foods.

Women frequently enter pregnancy with insufficient nutrient stores, and the increased demands associated with pregnancy and lactation are reported to increase risk of developing anaemia [36]. Our data show that antenatal care and birth interval were greater in the midland agro-ecological zone compared to lowland. These factors could also be contributing to the differences in anaemia rates between the two zones. On the other hand, no differences in parity and family size were seen between the zones.

Although the presence of parasitic infections, particularly hookworm, is associated with bleeding and hence higher levels of iron deficiency, this did not appear to contribute significantly to the differences in iron deficiency between the lowland and midland zones in the present study. There was no significant difference between the lowland and midland zones with respect to the practice of taking deworming tablets, households with toilet, hand washing practices and mother being sick in past 15 days. Thus, our findings suggest that difference in iron deficiency between the two agro-ecological zones is mainly caused by dietary factors. Additionally, we have previously reported variations in dietary diversity and traditional feeding habits between these two agro-ecological zones in rural Ethiopia [37].

The 10.9% prevalence of IDA in the present study is lower than reported for lactating mothers in an urban slum of Addis Ababa (22%) [17], non-pregnant Ethiopian women [16], and similar to the figure (13%) reported in pregnant mothers in southern Ethiopia [31]. Prevalence of IDA was higher in the lowland agro-ecological zone (37.5%) compared to lactating mothers from Addis Ababa (22%) [17]. These differences are again most likely to be related to variations in dietary pattern in the different zones of rural Ethiopia [16].

This study also demonstrated that deficiencies of zinc and iron occur concurrently. Once again, prevalences were higher in the lowland agro-ecological zone. The coexistence of zinc and iron deficiencies is probably related to high intakes of cereal which is rich in phytate and very low intakes of animal proteins [32,37]. In addition to dietary factors, mothers living in less developed countries, like Ethiopia, are exposed to frequent diarrhoea and respiratory infections, recurrent pregnancy and prolonged lactation all of which can influence micronutrient status [38].

Among the participants, 20% mothers were deficient in both zinc and iron. This prevalence is greater than reported in studies in Gondar [14]. Our data indicate that the majority of the mothers in the study area are affected by at least a single micronutrient deficiency, with the coexistence of micronutrient deficiencies most likely due to common aetiology and underlying mechanisms. For instance, a diet rich in phytate and low in animal proteins is common in most developing countries including Ethiopia; such a dietary pattern predisposes individuals to insufficient intake and absorption of zinc and iron [39,40].

This study showed that ferritin level is positively and significantly correlated with haemoglobin and zinc. As the level of ferritin increases, the serum levels of haemoglobin and zinc will also increase. The associations between zinc, iron and anaemia were reported in others studies [41,42]. Likewise haemoglobin levels were significantly correlated with maternal BMI, MUAC and WDDS which suggests that intervention directed to alleviate micronutrient deficiencies should be multidimensional.

The prevalence of goitre observed among lactating mothers in this study (35.2%) is similar to that reported in other studies among reproductive age Ethiopian women [19,43]. Our data are in accord with previous work in Ethiopia [19] which reported >30% TGR in Tigray, Oromia, the Southern Nations, and Beshangul-Gumuz. According to WHO/UNICEF/ICCIDD [29], a total goitre rate of 5% is a cut-off point indicating a public health risk of adverse functional consequences and when TGR is higher than 30% the problem is severe. The percentage of lactating mothers with UIC less than 100 µg/L was 42.6%. However, even higher prevalences have been reported in Papua New Guinea, 60% [44] and Niger, 69.7% [45]. WHO/UNICEF/ICCIDD recommended cut-off point of <100 µg/L for urinary iodine concentration (UIC) is for population groups [29]. This cut-off point is a good indicator of iodine status in populations but there is no good established urinary biomarker for iodine status of individuals. UIC is associated with high intra- and inter-individual variation and hence the determination of UIC in a single spot sample should not ideally be used to categorise an individual. Konig, *et al.* (2011) presented data indicating that 10 spot urine samples were needed to assess individual iodine status with 20% precision [46]. These authors noted the major limitation of this large number of repeated urine samples needed to estimate individual iodine status. This is particularly the case with respect to our study conducted in rural zones in Ethiopia. Thus, our findings and inferences with respect to UIC need to be evaluated in light of this limitation. High prevalence of suboptimal status of iodine nutrition among lactating mothers is of concern because of the association between subclinical iodine deficiency in lactating mothers and the potential risk of abnormal brain development in breastfed infants. The breast feeding mother provides the nursing infant with its sole source of iodine while the infant is exclusively breastfed [47,48].

Iodized salt was found in 36.6% of the households in our study which is greater than that reported in other studies conducted elsewhere in Ethiopia [43,48,49]. However, the Ethiopian government has set up a Universal Salt Iodization program even though there is a lot more to do to achieve WHO/UNICEF guidelines [47].

A limitation of this study is that although bloods were collected from apparently healthy individuals, acute phase proteins, including C-reactive protein (CRP), tests were not conducted due to financial reasons and absence of reagents at national level during this study period. Due to shortage of budget, some more micronutrients, like serum Vitamin A and copper were not examined. As data were collected by interview, social desirability bias could not be totally excluded.

Conclusions

In summary, the results of the present study reveal a high magnitude of micronutrient deficiencies, independently as well as concurrently, among lactating mothers in both agro-ecological zones. However, the deficiencies were more pronounced in lowland compared to midland agro-ecological zone of rural Ethiopia. These data suggest the need to undertake agro-ecological based studies in various parts of Ethiopia to determine appropriate interventions in all reproductive age mothers. Increased animal food consumption and increased consumption of vegetables and fruits is recommended in this population group.

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