

Patterns of thyroid gland function and iodine level in *Moringa stenopetala* consuming and non-consuming areas in Southern Ethiopia: A Case-Control study

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Abstract

Background: *Moringa stenopetala* is widely used as food, fodder and medicine in the South and South-western areas of Ethiopia. Association of *M. stenopetala* consumption with occurrence of goiter is still controversial. This study was aimed to investigate the association of consumption of *M. stenopetala* with thyroid function as well as urine iodine level, and to study iodized salt availability and utilization.

Methods: A community based unmatched case-control study was conducted from August to September, 2016. Data collection was done using WHO NCD Steps and locally constructed structured questionnaires. Physical measurements and clinical parameters were determined. Data were analyzed using SPSS version 22. Multivariate data analyses were performed to determine the associated factors based on p-value less than 0.05 and odd ratio with 95% confidence level.

Result: There was no significant difference in the mean levels of T3, T4 and TSH among case and control study participants in moringa consuming and non-consuming areas. The mean total T4 and T3 values were within the normal range of interval of the test kits. Iodized salt test indicated the presence of sufficient iodine (> 15 ppm) in more than 40% of households of the study area. Thyroid gland enlargement was not observed in the majority (> 90%) of the study participants. Urine iodine level was also normal in the majority of female participants.

Conclusion: *M. stenopetala* consumption did not cause significant effect on thyroid gland function and urine iodine. There was no significant association between consumption of *M. stenopetala* and occurrence of goiter.

Key words: *Moringa stenopetala*, case-control, thyroid gland function, iodine level

Introduction

Moringa stenopetala (Baker f.) Cufodontis is a multi-purpose tree widely used as food, fodder and medicine in the South and South-western Ethiopia (Anjelo, 2009). *M. stenopetala* leaf is nutritionally rich and an excellent source of concentrated proteins, vitamins and minerals. The cooked leaf is one of the best vegetable foods. It is also used to make the famous local meals “*Kurkufa*, *Fosose* and *Dama*” (Armelle de and Melanie, 2010; Engels. and Götsch, 1991; Yisehak et al. 2011; Edwards et al. 2002).

The extract of the leaves and solvent partitions from these extracts showed hypoglycemic and anti-hyperglycemic activity as well as anti-hypertriglyceridemia and cholesterol lowering effects (Mussa 2008; Nardos 2011; Toma 2016; Seleshi 2014). The extract also showed vasodilator and diuretic (saluretic) activities (Mengistu et al. 2012; Geleta et al. 2015; 2016A and 2016B). The extracts of different parts of *M. stenopetala* were reported to contain benzyl glucosinolates and benzylisothiocyanates glycosides that may be

responsible for goiter, if consumed for a long period of time (Dietz et al. 1987; Abuye et al. 1999; Mekonen, and Gebreyesus 2000; Bennett et al. 2003; Mekonnen and Dräger 2003).

There are controversial reports pertaining to the association of long-time consumption of *M. stenopetala* with the occurrence of goiter. This community-based unmatched case control study, therefore, assessed the effect of *M. stenopetala* consumption on thyroid function tests in the study participants.

Materials and Methods

Study design and study population: The study design was cross sectional community based unmatched case control cross. The study was conducted in selected rural and urban villages of the moringa consuming and non-consuming woredas of South Ethiopia from August to September, 2016. The study areas have similar culture, agroecological and disease burden.

Sample size determination and sampling technique: The sample size was calculated using double

population proportion formula by considering 95% confidence level, 5% margin of error and 50% prevalence to get sufficient sample size. Multistage sampling techniques were employed to recruit 109 cases (hypertension and diabetes) and 227 controls study participants in *M. stenopetala* consuming group for non-consuming group. Rural and urban study sites were selected randomly from each district, then sampling frame was prepared from the list of households and WHO, KISH methods.

Data collection techniques: WHO NCD-STEPS questionnaire and a locally constructed structured questionnaire were modified following the pretest was employed for data collection from 336 study participants. The data collectors were well oriented health care professionals on the tools. Data were collected digitally using personal digital assistants (PDAs) transferred to central server using internet file streaming system (IFSS). Data were collected on socio-demographic characteristics, nutrition and diet factors, health status, physical and biochemical parameters measurements, *M. stenopetala* use as food and/or medicine from the study participants.

Biological specimen collection: Blood samples (5-8 ml) were collected from ante cubital vein of the arm of overnight fasted participants, using evacuated yellow tip tubes (gel and clot activated) designated for clinical test parameters. The serum was then separated from the blood after centrifugation, collected in a Nancy serum collection tube and stored in a mobile deep freezer at -20°C and transported to EPHI. The serum specimen was then transferred and stored at -80°C until analysis. Early morning urine samples were also collected in 20 ml polypropylene tubes from the study participants of child bearing mothers and transferred to 5 ml cryotubes and stored in mobile deep freezer at -20°C and transported to EPHI.

The urine specimen was then transferred and stored at -80°C until analysis to determine the content of iodine, sodium, potassium, chlorine and total protein

Blood chemistry and Urine iodine analysis: The serum was used to analyze T3, T4 and TSH using COBAS 6000 (e601) and COBAS e41 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) at clinical chemistry laboratory, EPHI. The urine was used to test iodine level using spectrophotometer at the food science laboratory, EPHI.

Data management and analysis: The data were collected using tablets, Cispro-software was used to design and program the data collection tools and analyzed using IBM SPSS Statistics version 22 (Armonk- NY: IBM-Corp. 2013). Descriptive analysis was used to describe the mean, median, frequency and percentages for relevant variables in the study. Logistic regression was used to fit the data to identify

factors associated with outcome variables. Chi-square test was used to determine association of independent with dependent variables. All factors with a p-value ≤ 0.05 in the bivariate analysis were entered into the multivariate logistic regression model to control confounding effects. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. Statistical significance was accepted at the 5% level ($p \leq 0.05$).

Operational definitions

- **Cases:** Volunteered study participants whose systolic blood pressure (SBG) ≥ 140 mmHg and diastolic blood pressure (DBG) ≥ 90 mmHg and/or fasting blood sugars ≥ 120 mg/dl.
- **Controls:** Volunteered study participants whose systolic blood pressure (SBG) < 140 mmHg and diastolic blood pressure (DBG) < 90 mmHg and/or fasting blood sugars < 120 mg/dl.
- **Moringa stenopetala consuming:** Study participants consuming *M. stenopetala* as cooked vegetable, soup, tea infusion or the staple local meals *kurkufa*, *fosese* and *dama* that are prepared by cooking *M. stenopetala* leaves and rolled maize or sorghum flour together.
- **Moringa stenopetala non-consuming:** Study participants who do not consume *M. stenopetala* as cooked vegetable, soup, tea infusion or the staple local meals made from it.

Ethical Consideration: Scientific and ethical approval was obtained from Institutional Review Board of Ethiopian Public Health Institute, approval letter EPHI with reference number 6.13/796. Data were collected anonymously and confidentiality was maintained. Letter of support was obtained from Health Bureau of Southern Nations, Nationalities and Peoples Regional State to facilitate the study. Adequate explanation was given to the study participants on the survey procedure, risk, benefit, confidentiality, and the right to withdraw from the study. Informed consent was then obtained from each participant before administering the questionnaire and collecting blood samples. Assent was obtained from the children between 16 and 18 years to collect information about the children and blood samples besides the proxy consent obtained from guardians.

Results

Socio-demographic characteristics: A total of 336 participants were included of whom 32.4% were patients with hypertension and diabetes and 67.6% non-hypertension and non-diabetics (controls). The majority of study participants were the residents of

study areas. Indigenous ethnic groups mainly Gamo, Derashe and Konso were the majority of the study subjects. The age group 31-45 years accounted for more than 35% of the study participants in both moringa consuming and non-consuming area. The mean age of the study participants was 30.7 ± 3.9 years. Female were the major participants compared to males accounting for 66.1% vs 67.8% for the case and control, respectively. The majority of survey participants (23.3%) fell under middle quintile. Fourth quintiles belong to small proportion (18%) of the study participant. The majority (78.6%) of the participants were married. More than half, i.e., 57.4% of the study participants did not read and write and more than 40% of the participants were making their livelihood using farming (Table 1).

Household iodized salt and *M. stenopetala* consumption pattern: There was no significant difference (χ^2 Test, $p=0.07$ and 0.06) pertaining the presence of iodine in salt used for making food in households between case and controls in both moringa consuming and non-consuming area, respectively.

Test for the presence of iodine in the household salt used for food preparation indicated presence of sufficient iodine (> 15 ppm) in 44% and 60% for case study participants in moringa consuming and non-consuming area, respectively (Table 5). Similarly, it

was 49% and 62% for control study participants in moringa consuming and non-consuming area, respectively. Salts that were lacking iodine were not used in most households in both study areas. The majority of women participants in child bearing age had normal urine iodine level for case and control study participants in moringa consuming and non-consuming areas, respectively (Table 4). No anterior neck mass or enlarged thyroid gland was also observed in the household members of the study participants. There are no reports of goiter in the last five years in the majority of households for case and control study participants in studied sites. There were also no reports of thyroidectomy by the household heads (Table 5).

Thyroid gland function Tests: The majority of the case and control study participants in the study area had normal T3, T4 and TSH values. The mean T3, T4 and TSH value were lower in moringa consuming compared to non-consuming areas for the cases and controls study participants, respectively, for both groups. There was no significant variation among the mean values of T3, T4 and TSH (Table 2-4). Moreover, the mean value of thyroid hormones was within the normal clinical reference interval value (Wu 2006; Lohr et al. 2009; Katayey et al. 2010).

Table 1: Socio-demographic and economic characteristics of the case-control study population in *M. stenopetala* consuming and non-consuming areas (N=336)

Variable characteristics		Case-control study participants	
		Case n (%)	Control n (%)
Age			
	16-30	30 (27.5%)	86 (37.9%)
	31-45	39 (35.8%)	79 (34.8%)
	46 and above	40 (36.7%)	62 (27.3%)
Sex			
	Male	37 (33.9%)	73 (32.2%)
	Female	72 (66.1%)	154 (67.8%)
Area of residence			
	Urban	115 (58.1%)	83 (41.9%)
	Rural	44 (32.6%)	91 (67.4%)
Religion			
	Orthodox	54 (49.5%)	97 (42.7%)
	Protestant	49 (45%)	115 (52.4%)
	Others	6 (5.5%)	11 (4.9%)
Ethnicity			
	Derashe	27 (24.8%)	52 (22.9%)
	Konso	14 (12.8%)	41 (16.1%)
	Gamo	56 (51.4%)	112 (48.3%)
	Others	13 (11.1%)	22 (9.8%)
Marital status			
	Married	89 (81.7%)	174 (77.5%)
	Others	20 (18.4%)	52 (22.9%)
Level of Education			
	Do not read and write	63 (57.8%)	129 (55.9%)
	Can read or write	20 (18.4%)	38 (16.1%)
	Complete elementary school	9 (8.3%)	21 (9%)
	Secondary and above	17 (15.6%)	39 (17%)
Work status			
	Farming (crop or livestock)	40 (35.7%)	100 (44.1%)
	Housewife	20 (35.7%)	43 (35.7%)
	Trader	17 (15.6%)	22 (9.7%)
	others	32 (35.7%)	62 (35.7%)
Family size			
	Less than 5	48 (44%)	102 (44.9%)
	5 to 14	61 (56%)	123 (54.2%)
	15 and above	0 (15.6%)	2 (0.9%)
	Government employee	9 (7.3%)	17 (7.5%)
	Others	79 (11.8%)	43 (39.5%)
Wealth Index			
	Lowest quintiles	17 (15.3%)	49 (21%)
	Second quintiles	18 (16.2%)	49 (21%)
	Middle quintiles	22 (19.8%)	58 (24.9%)
	Fourth quintiles	24 (21.6%)	38 (16.3%)
	Highest quintiles	30 (27%)	39 (16%)

Table 2: Independent sample test for thyroid function tests among the case-control study population in selected study sites (N=336)

Characteristics	T-test for equality of means for Case-Control study			
	t	2-tailed Sig (P value)	Mean diff	95% CI
T3	t (267, 197) = -1.66	0.10	-0.156	-0.34 – 0.030
T4	t (267, 198) = 0.917	0.36	0.366	-0.42 – 1.15
TSH	t (267, 192) = 1.95	0.32	-0.133	-0.40 – 0.13
Urine iodine	t (51, 28) = 1.95	0.95	-0.0517	-1.64 – 1.54

T3: Triiodothyronine, T4: Tetraiodothyronine (thyroxine), TSH: Thyroid stimulating hormone

Table 3: Thyroid function test result among the case-control study population in selected study sites (N=336)

Variable	Case study participants								Control study participants							
	<i>M. stenopetala</i> consuming participants n (%)				<i>M. stenopetala</i> non-consuming participants n (%)				<i>M. stenopetala</i> consuming participants n (%)				<i>M. stenopetala</i> non-using participants n (%)			
	N	Mean	Std dev	95% CI	N	Mean	SD	95% CI	N	Mean	S D	95% CI	N	Mean	Std dev	95% CI
T3 (nmol/L)	48	1.490	0.411	1.370 -1.609	33	1.555	0.384	1.419 – 1.691	104	1.576	0.851	1.419- 1.691	76	1.820	0.774	1.643 -1.997
T4 (mg/dl)	48	8.926	2.610	8.168 -9.683	33	10.012	2.303	9.202 -10.835	104	8.487	2.91	7.923 -9.05	76	9.701	3.510	8.899 – 10.503
TSH (mIU/ml)	48	1.543	0.730	1.331-1.755	33	1.7948	1.021	1.433- 2.157	104	1.528	0.818	1.319 – 1.513	76	2.07	1.319	1.768 – 2.371

Normal or reference interval of value the test kit **T3** value: 1.3 to 3.1nmol/L; (nmol/L $\times$ 0.651 = ng/mL 0.85 to 2.02 ng/ml); **Normal or** reference interval of value the test kit **T4** value: 5.1 to 14.1 mg/dl [nmol/L $\times$ 0.077688 mg/dl $\times$ 1000 = 77.688 ng/dL is equivalent to 396.22 to 1095.43 ng/dl]; Normal **TSH** value: 0.27 to 4.20 mIU/ml

Table 4: Effects of *M. stenopetala* consumption on thyroid function test for the case-control study population in selected sites (N=336)

Variable characteristics	<i>M. stenopetala</i> using (as food source or medicine) study participant					
	Case			Control		
	<i>M. stenopetala</i> consuming participants n (%)	<i>M. stenopetala</i> non-consuming participants n (%)	χ^2 Test, p-values	<i>M. stenopetala</i> consuming participants n (%)	<i>M. stenopetala</i> non consuming participants n (%)	χ^2 Test, p-values
T3 level			0.181, 0.671			1.273, 0.259
Normal	30 (91%)	43(93.5%)		60 (83.3%)	91(81.3%)	
Raised	3 (9%)	3 (6.5%)		12 (16.7%)	11 (9.8%)	
T4 level			0.315, 0.574			7.257, 0.007
Normal	29 (87.9%)	44 (91.7%)		63 (82.9%)	98 (95.1%)	
Raised	4 (12.1%)	4 (8.3 %)		13 (17.1%)	5 (4.9 %)	
TSH level			8.527, 0.01			5.647, 0.02
Normal	33 (100%)	48 (100%)		70 (92.1%)	103 (99%)	
Raised	0 (0%)	0 (0 %)		6 (7.9%)	1 (1 %)	
Urine iodine level (for women participants in child bearing age)			1.847, 0.34			7.847, 0.04
Normal	32 (100%)	48 (100%)		73 (96%)	102 (98%)	
Raised	0 (0%)	0 (0 %)		3 (4%)	2 (2%)	
Moderate						

Table 5: Iodized salt kit test result in households among the Case-Control study population in selected study sites (N= 336).

Variable characteristics	Case		χ^2 Test, p-values	Control		χ^2 Test, P-values
	<i>M. stenopetala</i> consuming n (%)	<i>M. stenopetala</i> non-consuming n (%)		<i>M. stenopetala</i> consuming n (%)	<i>M. stenopetala</i> non-consuming n (%)	
Test for the presence of iodine in the salt used by households			6.181, 0.071			6.671, 0.059
Iodine present sufficiently (> 15 ppm is present in the tested salt from HH)	27 (44%)	34 (60%)		57 (49%)	69 (62%)	
Iodine present in small amount (< 15 ppm is present in the tested salt from HH)	34 (55%)	23 (40%)		51 (44%)	41 (37%)	
Iodine presence is not detected (0 ppm in the tested salt from HH)	1 (1%)	0 (0%)		8 (7%)	1 (1%)	
Urine laboratory test iodine level			11.181, 0.061			17.181, 0.071
Normal	6 (67%)	7 (64%)		14 (82%)	15 (80%)	
Mild	2 (22%)	2 (22%)		2 (12%)	3 (16%)	
Moderate	1 (11%)	1 (18%)		1 (6%)	1 (4%)	
Severe	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Examination of anterior neck mass for enlarged thyroid gland			9.181, 0.091			6.671, 0.159
No neck mass was detected during palpitation	47 (90%)	55 (96%)		103(89%)	109 (98%)	
Presence of neck mass detected during palpitation	5 (10%)	2 (496%)		13(11%)	2 (2%)	
Presence of goiter in the past 5 years			12.181, 0.141			13.181, 0.431
Yes	2 (4%)	1 (2%)		6 (5%)	3 (3%)	
No	50 (96%)	56 (98%)		110 (95%)	108 (97%)	
Surgery of thyroid gland in the past 5 years			9.181, 0.051			12.181, 0.051
Yes	2 (4%)	0 (0%)		0 (0%)	0 (0%)	
No	50 (96%)	116 (100%)		57 (100%)	111 (100%)	

Discussion

Thyroid hormones play important role in growth and development of the body and regulate metabolism. They affect almost every cell in the body with the exception of the brain, spleen, testes and uterus (Huang *et al.* 2008). In the current case-control study of moringa consuming and non-consuming participants, the majority of the study participants in the study area have normal T3, T4 and TSH values. In addition, no anterior neck mass or thyroid gland enlargement was detected in the study participants during palpitation.

Determination of T3, T4 and TSH concentrations in the blood plasma is considered a substantive evaluation of thyroid function. Alterations in the normal levels of these hormones usually lead to clinical abnormalities, such as hypothyroidism and hyperthyroidism (Becker 1993; Mariotti 2011). The mean T3, T4 and TSH values were lower in moringa consuming compared to non-consuming areas in all study participants, respectively. There was no significant variation (2 tailed test, $p=0.10$, 0.36 and 0.32 , respectively) among the mean values of T3, T4 and TSH (Table 2-4). Moreover, the mean value of thyroid hormones was found within the normal clinical reference interval value (Wu 2006; Lohr *et al.* 2009; Katayey *et al.* 2010). This finding is in agreement with the reports of the previous community-based study (Zekewos 2013) and animal study (Debela 2015).

Thyroid hormones are unique in a way that they require trace element iodine for biological activity, and the daily iodide requirement is between 150 and 200 μg . About 50 μg of iodide is secreted each day, with an average uptake of 25-30% of the iodide ingested in the form of iodized salt (Robert *et al.* 1993). In the present study, test for the presence of iodine in the household salt used for food preparation in the studied area indicated the presence of sufficient iodine ($> 15 \text{ ppm}$) for case study participants in moringa consuming and non-consuming area, respectively (Table 5). There was no significant difference (χ^2 Test, $p=0.07$ and 0.06) pertaining the presence of iodine in salt used for making food in households between case and controls in moringa consuming and non-consuming area, respectively.

Iodine deficiency that may affect thyroid function may partly be attributed to the consumption of plant foods containing goitrogens (Van Etten., 1969; Steinmaus *et al.*, 2007). Isothiocyanates have goitrogenic effect which could affect thyroid function through inhibition of iodine uptake by thyroid gland thereby aggravating iodine deficiency. Goitrogenic substances are present in certain widely used food plants. The local meals “Kurkufa, Fosose and Dama” are made from the

cooked fresh leaves of *M. stenopetala* mixed with either rolled maize or sorghum flour consumed 1-3 times per day. The majority consumes daily a handful or more parcel of the leaves branches which is about 1Kg or above. The dried leaves of *M. stenopetala* can be also used for making soup or sauce or tea infusion.

M. stenopetala is reported to contain benzylglucosinolates and benzylisothiocyanates glycosides claimed to have an exacerbating effect on goiter, if consumed for a long period of time (Dietz, *et al.* 1987; Abuye, *et al.* 1999; Mekonen, and Gebreyesus, 2000; Bennett *et al.* 2003; Mekonnen, and Dräger, 2003). Moreover, the mean value of thyroid hormones in the moringa consuming Vs non-consuming area for the case and control study participants in the current study was found to be within the clinical reference interval value (Table 2-4).

This may be attributable to the processing of the traditional meal containing *M. stenopetala* through cooking before consumption that may alter the chemical structure of goitrogenic substances thereby preventing the occurrence of goiter. The findings from this case-control study demonstrate that *M. stenopetala* consuming or non-consuming may not infer on thyroid gland function. From the present study, It is not possible to associate *M. stenopetala* use with induction of goiter.

Strength and Limitation of the study

The strength of the current case-control community-based study is being the first in its kinds for assessment of the effect of moringa consumption on thyroid function test, it can serve as a baseline for other studies. The limitations of this study is its cross-sectional nature that may not be used to establish temporal relationship between the exposures and outcome variables.

Conflict of interests

The authors declare that they have no conflicts of interest related to this study.

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