Annals of Nutrition and Metabolism

Ann Nutr Metab , DOI: 10.1159/000536196
Received: November 28, 2022
Accepted: January 8, 2024
Published online: January 11, 2024

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ISSN: 0250-6807 (Print), eISSN: 1421-9697 (Online)
https://www.karger.com/ANM
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Consumption Pattern of Tea is associated with Serum Ferritin Levels of Women of Childbearing Age in Nandi County, Kenya: A Cross-sectional Study

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Short Title: Tea consumption pattern and iron status among WCA

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Number of Tables: 5
Number of Figures: None
Word count: 2824

Keywords: serum ferritin, tea consumption patterns, polyphenols, iron status, and women of childbearing age

Abstract

Introduction: Tea consumption with meals affects iron absorption increasing the risk of iron deficiency. Our study investigated the association between tea consumption patterns and serum ferritin levels among women of childbearing age (WCA) in Nandi County, Kenya.

Methods: We conducted a cross-sectional analytical study among 160 WCA selected using a systematic random sampling technique from Kapsabet Ward. Information on tea consumption practices was gathered using a researcher-administered questionnaire, and serum ferritin and C-reactive protein were measured. We assessed associations between tea consumption and iron status of respondents by multivariable regression analysis, adjusting for potential confounders, including parasitic infections and recent severe blood losses.

Results: The prevalence of anaemia and iron deficiency among the study participants were 86.2% and 45%, respectively. Majority (90.6%) of the respondents consumed tea or coffee, with an infusion time of more than 5 minutes (60.0%) and a moderate tea strength (64.1%), within 1-hr before or after meals. Iron deficiency was associated the number of teacups consumed (adjusted odds ratio = 7.282, 95% CI = 3.580-14.812).

Conclusion: High tea consumption is positively associated with iron deficiency among WCA. Lower tea infusion strength, shorter tea infusion duration, and a lower number of teacups overall consumed, as well as consuming tea one hour before or after meals instead of with meals may be recommended for better outcomes in iron status among WCA.

Introduction

Kenya is the third leading producer of tea worldwide after China and India [1]. Tea is the second most popular beverage globally after packaged water [2]. Much lobbying has been undertaken to promote tea consumption among the local Kenyan people. As such, Kenya has seen a tremendous increase in the consumption of tea for over two decades [1,3]. A Kenyan adult is reported to take 2-3 cups of tea for breakfast daily [4]. Furthermore, high consumption of tea among pregnant women has been reported although the intake of tea is relatively low among youths aged between 15 and 25 years [5]. A growing body of evidence postulates that Women of Childbearing Age (WCA) consume tea with meals in Kenya [6–10], or close to mealtimes, within less than 20 minutes before or after meals [11].

The effect of tea consumption on iron absorption has been investigated extensively in various populations. A study conducted in the UK described that the simultaneous consumption of tea and an iron-containing porridge meal resulted in a notable decrease in nonheme iron absorption among women [12]. In Morocco, results of a randomized controlled trial indicated that tea consumption might reduce iron absorption from NaFeEDTA by more than 85% in both women with iron deficiency anemia and non-anaemic women [13]. Using rice meal, an Indian RCT found that the consumption of 1 or 2 cups of tea resulted in a reduction in iron absorption by 49% or 66%, respectively, and in the IDA group by 59% or 67%, respectively [14]. Contrasting findings emerged from a study conducted in South Africa, which found that tea consumption did not significantly increase the risk of iron deficiency in a mixed black adult population [15]. This assumption was confirmed in studies conducted in Western populations with generally adequate iron stores, where tea consumption did not influence iron status [16,17].

Plant-based food sources such as wine, tea, and coffee contain polyphenols [18]. The most abundant phenols in tea are catechins and tannins [16]. Phenols significantly reduce the dietary bioavailability of iron by inhibiting iron absorption from many meals in the gut. Tea consumption patterns, including the type of tea, frequency, steeping duration, and timing of consumption, may be determining factors in the potential effect of tea on iron status. Habitual tea consumption during meals might pose a significant risk of chronic depletion in iron stores if appropriate interventions are not implemented [19,20]. Therefore, investigating the association between tea consumption patterns, encompassing aspects such as the type of tea, frequency, steeping duration, and timing of consumption is key to understanding the link between tea consumption patterns and iron status. Iron deficiency is more pronounced among WCA [21]. However, most studies have primarily focused on assessing dietary iron intake, with less emphasis on investigating the consumption patterns of iron absorption inhibitors specifically among WCA. To address this gap, the present study aimed to examine the relationship between tea consumption patterns and iron status among WCA in Nandi County.

Methods

A cross-sectional analytical study was conducted in December 2019 among WCA drawn from Kapsabet Ward of Emgwen Sub-county in Nandi County, Kenya. The study area is located in the western region of Kenya, which has a subtropical highland climate. The area is characterized by hilly terrain with fertile soils, making it suitable for tea cultivation. Income is mainly agricultural, with tea farming being a major source of income for many households. Kapsabet ward covers Kapsabet town which serves its dwellers within a radius of 5 to 10 kilometres. The residents either purchase their tea from three tea factories in the ward or retail shops in Kapsabet town.

Non-expectant and non-lactating WCA aged between 15 and 49 years who consented to participate in the study were included in this study. Those respondents who had taken iron supplements in the past six months, donated blood or suffered severe blood loss in the previous six months, or had other chronic illnesses were excluded from the study. Our primary outcome was serum ferritin (SF) levels. Participants were classified as iron deficient when their SF was <15µg/l or SF 15-70 µg/l & c-reactive protein (CRP) > 5 mg/l [22,23], all other participants were considered to have normal iron status.

Sample Size Determination and Sampling Technique

G power software version 3.1.9.4 was used to determine the sample size. The level of significance (α) was 0.05, while the level of power (1-β) was 0.95, and the odds ratio of 3.57 with a proportion of successful outcome among the tea/coffee consumers ((Pr(y=1|x=1)= H1) of 0.65 and proportion of successful outcome among the non-tea/coffee consumers ((Pr(y=1|x=1)= Ho) at 0.35 [24]. A sample size of 160 was determined allowing 10% non-response [24].

Kapsabet ward has eight villages (Supplementary material) with a total target population of 4960. A proportionate sample was determined for each village and a systematic sampling technique was employed to select respondents at the village level. The households were mapped and numbered systematically. The selection interval ranged from 29th to 32nd household in the respective villages with the first household determined using a simple random sampling technique. For households that had more than one eligible respondent, an online random number generator was used to settle on one participant.

Data Collection Tools and Methods

A structured, researcher-administered questionnaire was used to collect information on the tea consumption patterns of the respondents. The tool was used to collect data on the common type of beverage consumed, reasons for taking or not taking tea/coffee, types of tea, preferred flavour, accompaniment, a household measure of a teacup, number of teacups consumed, tea infusion time, strength of tea in teacups and time WCA took tea (before, after or with meals). Three scales of tea strength based on infusion time used are low (less than 2 minutes), moderate (2-5 minutes), and high (more than 5 minutes), representing lighter, medium, and richer levels of colors, respectively (Supplementary material) [25].

Pre-testing of the questionnaire was carried out among 10% of the sample size [26], selected from Emgwen sub-county, before the actual data collection. We modified the content of the data collection tool based on the findings of the pre-test and expert consultations to ensure validity. The reliability of the tool was ensured through the test-retest method, the questionnaire was administered twice with a difference of two weeks, and the Cronbach coefficient was 0.8.

Seven undergraduate nutrition students and three phlebotomists were trained by PNN in data collection and blood collection procedures, respectively. Training included demonstrations and role-plays to improve data collectors interviewing skills. Members of the data collection team resided in Kapsabet Town, spoke fluent Nandi (local language) and were well versed in local Nandi culture and setting. During the data collection process, PNN provided supervision to each group of data collectors. These groups, comprising a phlebotomist and two interviewers, were assigned to specific villages. The paper questionnaire was filled by the trained research assistants after collecting information directly from participating WCA.

Collection of Blood Sample

A phlebotomist drew 2 ml venous blood from participants aseptically, half ml of the collection was aliquoted into BD vacutainer tubes (purple tubes) with K2E anti-coagulant for haemoglobin determination within 24 hours of collection and the rest into serum vacutainers. Samples were coded for identification, using a fine-point permanent marker pen. The tubes were packed in a cooler box at 15^{0}C and transported within one hour of collection to Chepsoo Medical Centre, they were centrifuged, serum separated (maximum time: 60 minutes), aliquoted into vials and refrigerated at 4^{0}C for five days. A five-day collection of serum was packed in a cooler box at 15^{0}C and transported (maximum time: 7 hours) to the University of Nairobi/Kenyatta National Hospital Paediatric laboratory where they were stored at a temperature below -20^{0}C (the biomarkers are stable for 12 months in this temperature). Repeated cycles

of freezing and thawing were avoided to reduce interference with the integrity of the samples. Analyses for serum ferritin and CRP were conducted after one month of sample storage in the frozen state.

Biomedical Methods for Determining Biomarkers

Blood in vacutainers with K2E anti-coagulant was used to form a drop that was placed on a strip inserted in the Mission® Plus machine (San Diego, USA) for haemoglobin determination. Serum ferritin was quantitatively determined using an automated chemilumiscence LIASON® Analyser (DiaSorin S.p.A. - Saluggia- Italy). C-reactive protein (CRP) was analyzed using an automated HumaStar 600 quantitatively by the immunoassay method. The standards were stored for two days at a temperature of 2-8 °C. During biochemical analysis, the serum ferritin samples were first retrieved from storage, defrost and thoroughly mixed using a vortex mixer.

Data Analysis

Data was cleaned and coded. Data on tea consumption patterns, biomarkers and health-related data was entered into Statistical Package for Social Sciences software version 22 (Illinois, Chicago). Descriptive statistics, such as percentage, means and standard deviation, were performed on tea consumption patterns and iron status. Multivariable regression was conducted to determine the association between tea consumption patterns and iron status (as a dichotomous variable) of WCA [15]. Seven respondents were excluded from the final multivariable analysis to control for malaria and severe blood losses (two had recent episodes of malaria, three had recent severe blood loss and one had both) (Supplementary material). The consumption of milk and milk products with meals were adjusted for during the final (regression) analysis.

Results

Characteristics of the Study Respondents

A total of 160 WCA participated in the study. Data and blood samples were obtained from all of them. Table 1 presents the sociodemographic characteristics of the study respondents. Majority of the respondents were single (57.5%) with a mean age of 24.7 years (SD: 7.31). A total of 145 WCA reported to be tea/coffee consumers. Finally, 139 WCA were included in the analyses. Reasons for exclusions are shown in Supplementary Figure 1.

Tea Consumption Patterns among the Study Respondents

The tea consumption patterns of the study respondents are presented in Tables 2 and 3. Tea or coffee was the most consumed beverage (90.6%) among the study participants. Nearly, two-thirds (64.1%) of the respondents reported drinking tea with moderate infusion strength and the majority (75.2%) consumed tea with milk. Majority (71.7%) of the respondents consumed tea with meals during breakfast and slightly more than half (53.4%) at lunch meal and over one-third (37.7%) during supper. Those who did not take tea with meals mainly consumed the tea one hour before or less than one hour after the meals (Table 3).

Iron Deficiency among WCA in Nandi County, Kenya

The prevalence of crude iron deficiency was 21.3% but increased to 45.0% after adjusting for inflammation among the respondents (Table 4).

Association between Consumption of Tea and Iron Deficiency

An increase in the number of teacups consumed was associated with an increased likelihood of iron-deficiency among the respondents (adjusted odds ratio (AOR) = 7.282, 95% confidence interval (CI) = 3.580-14.812, $p < 0.001$). Low compared to high tea-infusion strength in teacups (AOR = 0.060, 95% CI =

0.060 – 0.270, $p < 0.001$) or short (3-5 minutes) in comparison to long (more than 5 minutes) duration of tea infusion (AOR = 0.318, 95% CI = 0.318 - 0.144, $p < 0.001$) were less associated with iron deficiency (Table 5).

Discussion

This cross-sectional analytical study conducted among 160 WCA from Nandi Country, Kenya investigated the relationship between consumption patterns of tea and iron status. The prevalence of iron deficiency among the study participants was 45%. Majority of the respondents consumed tea or coffee, with an infusion time of more than 5 minutes (60.0%) and a moderate tea strength (64.1%), within 1-hr before or after meals. The majority of participants consumed tea 2-3 times/day and 2-4 cups (250 ml) daily. The chances of iron deficiency increased with an increasing number of teacups consumed. Lower tea-infusion strength in teacups and a duration of tea infusion shorter than 5 minutes both decreased the chance of iron deficiency.

Our study is the first of its kind to assess tea consumption patterns and its potential impact on iron-related outcomes specifically among WCA in Kenya. Our results highlight that intensive tea consumption may pose significant health risks for WCA. Strength of our study include a sample size determined based on a sample-size calculation; the random sampling method used, a data collection team with extensive knowledge of the local culture and setting; and the large study participation rate. Our study has some weaknesses. As it is a cross-sectional study, no clear causal relationship can be established based on our results. Further, we noticed that students were overrepresented among the participants of our study, therefore despite our best efforts results might not be representative to the broader local society of women aged 15-49, as students might represent WCA of different age, education level and socioeconomic status.

In agreement with our study's findings, three studies described that tea or coffee is the most preferred beverage in Kenya [4,11,27]. The study area (Nandi County) is a tea-growing region harbouring 19 tea factories such as Chebut Tea, Kibwari Tea and Kipchabo Tea, which could explain the high availability and utilization of loose tea leaves. The county has hills that normally have very cold weather which could contribute to the high consumption of tea by the locals to keep themselves warm [28]. Similarly, other studies have found that WCA consume tea with meals or within one hour before or after meals in Kenya [7–9,11]. When tea is taken less than an hour before or after a meal, a situation is created where phenolic compounds complex with iron in food subsequently reducing dialyzable iron [12].

Our findings demonstrate that the respondents were less associated with iron deficiency when they consumed tea with milk alone as compared to taking milk tea with an accompanying meal. When tea is consumed with milk alone without an accompanying meal, the phenolic compounds in the tea will not have iron to complex with. However, this advantage can be reduced if there is an overlap between the food- and tea-gastric emptying phases [12]. The milk in tea introduces calcium, which is an important inhibitor of iron bioavailability further compounding the iron absorption inhibition. Earlier studies suggested that the dialyzability of iron increased when both milk and tea are consumed together [29,30]. It may imply that calcium availed by milk complex with tannic acid sparing more dialyzable iron. On the contrary, more recent studies established that the addition of milk to tea reduced significantly the dialyzable iron as compared to when tea or milk is taken alone with meals [31,32].

A review of determinants of iron bioavailability suggested that an overall increase in the intake of polyphenols attracted an equivalent greater reduction in the dialyzable iron [31]. This might be an explanation for the results of this study, as increased tea intake might result in a higher provision of tannic acid in the gut, and as a consequence, reduced bioavailability of iron. While similar studies have reported contrasting results on relationship between tea consumption and iron status [15–17], certain investigations have exclusively focused on iron absorption [12–14]. The results described in our study might be applicable to WCA living in a country or region with high tea/coffee consumption pattern.

Conclusion

Lower tea infusion strength, shorter tea infusion duration and a lower number of teacups overall consumed decreased the chance of having iron deficiency among WCA in Nandi County, Kenya. A more favourable iron status may be achieved by recommending healthier tea consumption habits to WCA and other risk groups. Further, recommending the consumption of tea one hour before or after meals instead of consuming it with meals might have further beneficial effects on iron status of WCA.

Statements

Acknowledgement

The authors are grateful to the respondents who accepted to participate in the research and to the research assistant team who collected data and blood samples.

Conflict of Interest

The researchers express no conflict of interest.

Statement of Ethics

This study protocol was reviewed and approved by Kenyatta University Ethics Review Committee (PKU/2029/11176). Written informed consent was solicited from the respondents before data and blood sample collection. PNN obtained written informed consent from parents/legal guardians for ALL participants aged under 18.

Financial Support

The study received no funding.

Author Contributions

PNN designed the study, collected data, analysed, interpreted the work, drafted the manuscript and approved the final version. JK and AWM supervised the design of the work, data collection, interpretation of data, and drafting of the manuscript and approved the final version of the paper. DAG and SL interpreted the data, revised the manuscript critically for important intellectual content and approved the final version of the work for publication.

Data Availability

Data used to reach conclusion in this study is not publicly available due to ethical reasons. Further enquiries can be directed to the corresponding author.

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Table 1: Socio-demographic characteristics of the Respondents

Characteristics	n=160 n (%)
Sex of household head	
Male	77 (48.1%)
Female	83 (51.9%)
Marital status	
Single	92 (57.5%)
Married	65 (40.6%)
Separated	2 (1.3%)
Widowed	1 (0.6%)
Age (years)	
Mean age (SD)	24.7 (7.31)
15-24	86 (53.8%)
25-34	56 (35.0%)
35-44	16 (10.0%)
45-49	2 (1.3%)
Highest education level attained	
No formal education	2 (1.3%)
Primary	48 (30.0%)
Secondary	69 (43.1%)
Tertiary	41 (25.6%)
Occupation	
Casual worker	27 (16.9%)
Formal employment	14 (8.8%)
Businessman/woman	44 (27.5%)
Student	67 (41.9%)
Others	8 (5.0%)

Table 2: Consumption Patterns of Tea among the Study Participants

Characteristics	n=145[a] n (%)
Beverage consumed (n = 160)	
Tea and/or coffee[b]	145 (90.6%)
Other drinks [c]	15 (9.4%)
Reasons for taking tea	
Health/dietary reasons benefits	39 (26.9%)
To keep warm	12 (8.3%)
Prefer tea taste	18 (12.4%)
Drinking tea is relaxing	11 (7.6%)
Habitual	65 (44.8%)
Type of tea taken	
Black	138 (95.2%)
Green	5 (3.4%)
Blend	2 (1.4%)
Preferred flavour in tea	
Ginger/masala	36 (24.8%)
Lemon	10 (6.9%)
Plain	98 (67.6%)
Others	1 (0.7%)
Accompaniment	
Without milk	36 (24.8%)
Tea with milk	109 (75.2%)
Duration of tea infusion	
0-2 minutes	6 (4.1%)
3-5 minutes	52 (35.9%)
> 5 minutes	87 (60.0%)
Frequency of tea consumption	
1-2 times a week	5 (3.4%)
Breakfast only	34 (23.4%)
2-3 times a day	79 (54.5%)
> 3 times a day	27 (18.6%)
Volume of tea taken	
200-500	53 (36.6%)
501-1000	61 (42.1%)
1001-1500	25 (17.2%)
1501-2000	6 (4.1%)
Strength of black tea Concentration	
Low	30 (20.7%)
Moderate	93 (64.1%)
High	22 (15.2%)

[a] 15 respondents did not consume tea or coffee [b] tea was the predominant beverage among the majority of respondents, however, since coffee is equally a potential inhibitor of iron absorption and demonstrates similar inhibitory effects as tea, both beverages (tea and coffee) were included [c] i.e. cocoa/drinking cholate and juices

Table 3: Consumption Patterns of Tea among Women of Childbearing Age in Nandi County, Kenya

		n=145[a]			
Breakfast (n=145)	**Tea with breakfast** 104 (71.7%)	**tea before breakfast (n=18)**		**Tea after breakfast (n=23)**	
		< 1-hr[b]	>1-hr	< 1-hr	>1-hr
		13 (72.2%)	5 (27.8%)	15 (65.2%)	8 (34.8%)
Lunch (n=88)	**Tea with lunch** 47 (53.4%)	**tea before lunch (n=7)**		**Tea after lunch (n=34)**	
		< 1-hr	>1-hr	< 1-hr	>1-hr
		4 (57.1%)	3 (42.9%)	29 (85.3%)	5 (14.7%)
Supper (n=77)	**Tea with supper** 29 (37.7%)	**tea before supper (n=7)**		**Tea after supper (n=41)**	
		< 1-hr	>1-hr	< 1-hr	>1-hr
		6 (85.7%)	1 (14.3%)	36 (87.8%)	5 (12.2%)

[a] 15 respondents did not use tea or coffee [b] As assessed using a 1-hr reference scale [12]

Table 4: Iron Status of the Respondents

Parameters	n= 160 **n (%)**
Haemoglobin levels (g/dl)	
Mean [SD]	9.6 [1.9]
Normal ≥ 12.0 g/dl	22 (13.8%)
Mild anaemia (11.0-11.9 g/dl)	24 (15.0%)
Moderate anaemia (8.0- 10.9 g/dl)	86 (53.8%)
Severe anaemia (< 8 g/dl)	28 (17.5%)
Anaemia (<12.0 g/dl)	138 (86.2%)
C-reactive protein (CRP) concentrations (mg/l)	
Mean [SD][a]	4.826 [2.017]
Elevated levels (CRP > 5 mg/l)	49 (30.6%)
Serum Ferritin (SF) concentrations (µg/l)	
Mean (SD)	35.3 [42.2]
Crude Iron deficient stores[b]	34 (21.3%)
SF 15 – 70 µg/l & CRP> 5mg/l	38 (23.8%)
Adjusted[c] iron stores status	
Iron deficient (ID)[d]	72 (45.0%)
Frequency of deworming (n=94)	
Regularly (every 3 months)	48 (51.1%)
Irregularly	46 (48.9%)
Had malaria episode within 2 weeks preceding data collection	3 (1.9%)
Had recent (<3 months) severe blood loss	5 (3.1%)

[a] Statistical measure is mean and standard deviation [b]Crude Iron deficient stores is SF < 15 µg/l [c] adjusted iron stores for inflammation

[d] ID is (SF <15µg/l or SF 15 - 70 µg/l & CRP> 5mg/l)

Table 5: Association between Consumption Patterns of Tea and Serum Iron Status[a] among Women of Childbearing Age in Nandi County, Kenya

	n=145		n=139		Serum ferritin in µg/l	
Determinants	**COR[b] [95% CI]**	***p****	**AOR[c] [95% CI][d]**	***p****	**Mean (SD)**	**95% CI**
Accompaniment						
Tea (without milk)	0.055 [0.006-0.505]	0.010	0.044 [0.005- 0.413]	0.006	37.453 (35.696)	27.252- 50.553
Tea (with milk)	0.080 [0.009-0.687]	0.021	0.070 [0.008-0.605]	0.016	40.423 (56.161)	29.763- 56.198
Tea (without milk) with food	0.143 [0.016-1.290]	0.083	0.134 [0.015-1.218]	0.074	29.318 (27.152)	20.515- 40.134
Tea (plus milk) with food **(ref)**		0.031		0.017	15.962 (8.260)	10.120- 22.298
Duration of tea infusion						
0-2 minutes	0.512 [0.089-2.940]	0.453	0.098 [0.98-3.250]	0.502	36.167 (22.914)	19.925- 56.729
3-5 minutes	0.341 [0.160-0.726]	0.005	0.318 [0.318-0.144]	0.005	44.578 (63.416)	30.068- 62.885
> 5 minutes (ref)		0.019		0.018	30.239 (28.090)	24.981- 36.789
Tea-infusion strength in teacups[e]						
Low	0.052 [0.012-0.231]	<0.001	0.060 [0.013- 0.270]	< 0.001	38.420 (24.900)	30.398- 48.102
Moderate	0.352 [0.131-0.945]	0.038	0.366 [0.133-1.006]	0.051	37.040 (51.531)	27.806- 49.902
High **(ref)**		0.001		0.001	25.325 (29.581)	15.495- 39.902
Frequency of tea intake						
1-2 times a week		0.999		0.999	159.840 (155.410)	51.576- 330.400
Breakfast only		0.997		0.997	52.062 (38.536)	39.773- 66.430
2-3 times a day	0.026 [0.003-0.203]	<0.001	0.023 [0.003-0.179]	<0.001	27.636 (16.570)	24.032- 31.685
> 3 times a day **(ref)**		0.005		0.005	13.127 (6.156)	10.966- 15.671
# of cups (250 ml) per day [f]	7.300 [3.643-14.626]	<0.001	7.282 [3.580-14.812]	<0.001		

[a] the iron status indicator was serum ferritin levels [b] COR stands for crude odds ratio, [c] AOR stands for adjusted odds ratio, [d]adjusted for parasitic infections, severe blood losses, milk and milk products [e] low, moderate and high tea infusion strength is define by short (< 2 minutes) and lighter color, moderate (2-5 minutes steeping) timing and moderate co intensity, long (> 5 minutes) and richer color, respectively [f]The consumption of tea also caters for the infrequent consumption of coffee by the respondents [*]Significance level < 0.05 ** a multivariable logistic regression model was used