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Investigating tea temperature and content as risk factors for esophageal cancer in an endemic region of Western Kenya: Validation of a questionnaire and analysis of polycyclic aromatic hydrocarbon content

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Abstract

Background—Esophageal squamous cell carcinoma (ESCC) is common in certain areas worldwide. One area, western Kenya, has a high risk of ESCC, including many young cases (< 30 years old), but has limited prior study of potential risk factors. Thermal injury from hot food and beverages and exposure to polycyclic aromatic hydrocarbons (PAHs) have been proposed as important risk factors for ESCC in other settings. The beverage of choice in western Kenya is milky tea (chai).

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Authors' contributions

Study design: All authors. Data collection: RP, JOL, SR, RC, SLB. Data Analysis: MM, RP, GM, CCA, SMD, NP, MT. MM and RP drafted the initial manuscript. All authors read and approved the final manuscript

Competing interests

The authors declare that they have no competing interests

Conflict of Interest: None

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Declarations

Ethics approval and consent to participate

Ethical approval was granted for this study by the Institutional Research and Ethics Committee at Tenwek Hospital and the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee

Consent for publication

No Individual patient data has been shared in this paper.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Methods—Healthy individuals 18 years of age who were accompanying relatives to an endoscopy unit were recruited to participate. The preferred initial temperature of chai consumption in these adults was measured by questionnaire and digital thermometer. Comparisons of these results were assessed by kappa statistics. Concentrations of 26 selected PAHs were determined by gas chromatography/mass spectrometry in samples of 11 brands of commercial tea leaves commonly consumed in Kenya.

Results—Kappa values demonstrated moderate agreement between questionnaire responses and measured temperatures. The mean preferred chai temperatures were 72.1°C overall, 72.6°C in men (n=78), and 70.2°C in women (n=22; p<0.05). Chai temperature did not significantly differ by age or ethnic group. The PAH levels in the commercial Kenyan tea leaves were uniformly low (total PAH <300 ng/g of leaves).

Conclusions—Study participants drink chai at higher temperatures than previously reported in other high-risk ESCC regions. Chai is not, however, a source of significant PAH exposure. Very hot chai consumption should be further evaluated as a risk factor for ESCC in Kenya with the proposed questionnaire.

Keywords

Esophageal Neoplasms; Kenya; Tea; Hot Temperature; Polycyclic Aromatic Hydrocarbons

Introduction

Esophageal cancer is the eighth most common malignancy and the sixth most common cause of cancer death worldwide¹. Esophageal squamous cell carcinoma (ESCC), the most common histologic type of esophageal cancer, has a very unusual geographic distribution, with distinct areas of high risk across central Asia and from eastern to southern Africa². Western Kenya and the Eastern Cape Province of South Africa have long been reported as endemic areas of ESCC^{3–5}, but a larger corridor, from Ethiopia to South Africa, has recently been recognized as having a similar high risk^{6–8}. Esophageal cancer often presents late and carries a poor prognosis, but this is particularly true in low-resource settings, where 5-year survival is often < 5%⁹, and palliation is too often the only option¹⁰. Environmental causes of esophageal cancer are numerous, complex, and not fully understood¹¹. Research on the etiology and modifiable risk factors of ESCC in Africa is limited⁷.

ESCC is the most commonly diagnosed cancer at Tenwek Hospital, a 300-bed mission hospital in Bomet, western Kenya. In 2016, over 400 ESCC patients were diagnosed. A striking characteristic of Bomet, even among other endemic areas, is the high proportion of cases diagnosed at a young age. This finding is particularly striking in the majority Kalenjin population, where 9% of all cases are 30 years old and 20% are 40 years old⁴.

In rural western Kenya, the beverage of choice is *chai*, the Swahili word for tea, which is a mixture of black tea leaves and varying ratios of water and cow's milk (typically 1:1). Given the ubiquitous consumption of chai and the high rates of esophageal cancer, we set out to study two potential risk factors of tea consumption in rural Kenya. In other endemic areas, the presence of polycyclic aromatic hydrocarbons (PAH) have been associated in

beverages^{12,13} and are present in the epithelium of ESCC cases¹⁴. Therefore, we decided to examine the PAH content of tea leaves. Additionally, chai is consumed at hot temperatures. In this area, chai is made by first mixing the tea, water and milk together, and then bringing this mixture to a boil. This is different from other parts of the world where cold or warm milk is added to previously boiled water and tea. Sugar is also commonly added to chai. Tenwek Hospital has taken care of numerous children and adolescents with thermal esophageal injuries caused by ingestion of boiling hot chai. Anecdotally, the population seems to prefer hot drinking temperatures. But the actual preferred temperature of chai consumption has never been studied or reported in this population.

Consumption of hot food and beverages has been proposed as a risk factor for esophageal cancer in a number of different geographical locations¹⁵, including drinking hot maté in South America¹⁶; drinking hot tea in Iran^{17,18}; and consumption of hot food and beverages in China^{19,20}. Recently, the International Agency for Research on Cancer (IARC) defined drinking temperatures above 65°C as “very hot” and considered this exposure as probably carcinogenic (Group 2A)^{21,22}. However, very limited research on this topic has been conducted in East Africa, which is known as a high-risk area for ESCC⁴. One recent study from Tanzania has measured beverage temperature, and it found that the participants drank their beverages (mainly tea) at an average temperature of 70.6 °C, higher than that reported in previous studies from other populations²³. A recent case-control study by Middleton et al. in Kenya, showed an association between self-reported ingestion of hot beverages with ESCC²⁴. Historically, studies have utilized participant self-assessed questionnaires to report on their preferences for the temperature. The actual temperature of consumption is rarely reported. So, questionnaires could naturally reflect relative preferences compared to the region and not the actual temperature. Therefore, it is necessary to determine how questionnaires might reflect temperatures in the regional context.

We conducted an observational study to determine the preferred initial temperature of chai consumption among healthy asymptomatic subjects at Tenwek Hospital and to evaluate the presence of PAHs in Kenyan tea leaves that are used to make chai.

Methods

Consecutive healthy individuals 18 years of age who were accompanying relatives to the Tenwek Hospital endoscopy unit were recruited to participate in the study. After signing an informed consent, all subjects were given a brief questionnaire by one of two trained interviewers. Questions included demographic information (age, sex, and ethnic group), the kind of beverage most commonly consumed, how many cups of this beverage were consumed daily (a typical cup is equivalent to 300mL), the temperature of the drink (self-reported: warm, hot, or very hot), and how many minutes the subject typically waited for the drink to cool after pouring, before drinking. Then the subject was offered a cup of chai, using methods similar to Islami *et al.*¹⁸. Briefly, when the chai was at 80°C, two cups of tea were poured. One cup was offered to the subject, and a digital thermometer was placed in the second. The subject was then asked if he or she preferred the chai at that temperature. If not, when the chai reached 75°C, the subject was asked again if they preferred drinking at

that temperature. This was repeated at 70°C, 65°C, 60°C, etc. until the preferred initial temperature at first drink was reached.

T-tests were used to evaluate the association of measured chai temperature categories and demographic factors. We tested the agreement between chai temperature-related questionnaire responses and between these responses and actual chai drinking temperature categories by weighted kappa statistics, weighted for ordinal data, and Spearman's rank correlation coefficients. Comparisons of our results and the preferred chai temperatures reported in prior studies in other geographic locations were analyzed using unpaired, two-sample t-tests. We considered two-sided P values <0.05 to be statistically significant. All statistical analyses were done using Stata version 10.0 software (STATAcorp, College Station, TX).

Eleven brands of commercial black Kenyan tea leaves were purchased and analyzed by gas chromatography/mass spectrometry to quantify the concentrations of 26 selected individual PAHs, using methods previously described¹³. The measurements were conducted at the National Institute of Standards and Technology (NIST) in the Analytical Chemistry Division. Briefly, the tea samples were stored in their packages at room temperature until sub-sampled for analysis. SRM 2260a Aromatic Hydrocarbons in Toluene, SRM 1649a Urban Dust, SRM 2269 Perdeuterated PAH-I, and SRM 2270 Perdeuterated PAH-II were utilized from the Standard Reference Materials Group, NIST. One subsample from each brand of tea and one subsample from one bottle of SRM 1649a were placed into pressurized fluid extraction (PFE) cells containing hydromatrix (Isco, Lincoln, NE) and mixed with the hydromatrix. The void space of the cells was filled with hydromatrix. For the internal standards, a diluted solution prepared from SRM 2269 and SRM 2270 was added to each extraction vessel. The extraction used dichloromethane as a solvent heated for 5 minutes at 100°C after preheating the cell for one minute. The PAHs of interest were quantified using gas chromatography/mass spectrometry.

These samples were analyzed at the same time as eight samples of yerba maté leaves from Brazil, the results of which have been previously published¹³. For each PAH and the sum of all PAHs we calculated the mean and standard deviation of the 11 Kenyan brands and compared them to the means and standard deviations of the same PAHs in the 8 yerba maté brands.

Ethical approval was granted for this study by the Institutional Research and Ethics Committee at Tenwek Hospital and the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee.

Results

One hundred subjects, including 78 men and 22 women, were interviewed in Bomet, Kenya. The median age of participants was 30.5 years, with a range of 18 to 78 years. All participants chose chai as their preferred beverage, and on average they reported drinking 4.2 (± 1.9) cups of chai daily. The self-reported temperature for desired tea temperature was "warm", "hot", and "very hot", 25%, 64% and 11% of participants, respectively. The

reported length of time between pouring and drinking tea was > 4 minutes, 2–4 minutes, and <2 minutes for 29%, 49% and 22% (including 8 who said they drank their tea “immediately” after pouring) of participants, respectively (Table 1).

The mean measured preferred chai temperature was 72.1°C (+/- 4.6) overall. The majority (88%) of the participants were from the local Kipsigis tribe and their mean preferred chai temperature was 72.2°C (+/-4.6), which was non-significantly greater than the 70.8°C (+/- 4.2) mean preferred temperature of the 12 non-Kipsigis subjects ($p=0.33$). There was no association between the mean preferred chai temperature and participant age; however, there was a statistically significant difference between men (72.6°C +/- 4.7) and women (70.2°C +/- 3.6) ($p=0.033$) (Table 1).

Kappa values showed moderate agreement between the two chai temperature-related questionnaire responses (weighted kappa = 0.541) and between each of these responses and the actual measured temperature categories (weighted kappas = 0.464 and 0.505) (Table 2).

Table 3 shows the concentrations (ng/g of leaves) of benzo[a]pyrene (B[a]P), and the sum of all 26 selected PAHs in the 11 tested commercial brands of Kenyan black tea, and compares the mean (+/- SD) of these measures with the mean (+/- SD) of B[a]P and total PAHs in 8 commercial brands of Brazilian maté which were measured at the same time as the Kenyan tea. The data for all 26 PAHs in all 11 Kenyan brands are shown in the Supplementary Table.

Discussion

We performed a study to assess a questionnaire on the preferred initial temperature for drinking milky tea (chai) among healthy adults in Bomet, Kenya, a region of high ESCC incidence. There was moderate agreement between the interview questions related to chai temperature preference and measured chai temperatures (weighted kappa values of 0.464 and 0.505). We found that study participants in Bomet drank chai at a mean temperature of 72.1°C. The preferred chai temperature was significantly higher for men than for women, but not significantly different by participant age or ethnic group. We also measured the PAH content of the tea leaves used to make chai. All of the PAH concentrations were low in all of the tea samples tested.

Prior studies have demonstrated an association between ingesting hot temperature food and beverages and ESCC. This theory was first proposed by Watson in 1939²⁵, but it was initially thought that the temperature of hot foods and beverages would not affect the esophagus after cooling rapidly in the upper digestive tract²⁶. However, De Jong and colleagues showed that drinking hot coffee (65°C) could increase intra-esophageal temperature by 6 – 12°C²⁷, and an endoscopic survey in southern Brazil demonstrated that drinking very hot maté was associated with esophageal injury (esophagitis)²⁸. Recent systematic reviews and meta-analyses have demonstrated an association between ingesting hot temperature food and beverages and ESCC^{15,29,30}. A working group from the IARC has now classified very hot beverages as possibly carcinogenic^{21,22}.

We reviewed the literature for other studies of preferred beverage temperature^{16–18,23,31–40} (Table 4), and found that the mean preferred tea temperature in our study was 1.5°C higher

than any other studied population. It should be noted that the various studies presented in Table 4 use various methodologies to assess temperature and this limits the ability to compare them, and numerous studies concerning beverage temperature do not report descriptive statistics for temperature. Previously, the hottest beverage temperature reported in any geographic location was from Tanzania, with a mean of 70.6°C²³. Such hot temperatures could have an important effect on the risk of esophageal cancer in our population. A previous study from Golestan, Iran reported an 8.2-fold increase in ESCC risk for individuals who consume hot tea (>70°C) versus those who drink warm tea (<65°C)^{18,41}. Interestingly, those who consumed hot tea at >70°C in Iran included only 5% of the population. In contrast, 85% of the participants in our study preferred their chai at or above 70°C (Table 4). Only one participant in our study preferred their initial chai drinking temperature less than the “very hot” potentially carcinogenic threshold of 65°C^{21,22}. All participants preferred their initial drinking temperature > 60°C, which has been shown to be a risk factor for ESCC in a different population as evidenced by a prospective cohort study in Iran⁴¹. This Kenyan experience also contrasts with that reported from non-endemic areas, where mean beverage temperatures are nearly always <65°C and are often <60°C (Table 4). The importance of validating a questionnaire in this region rests on the impressive differences between populations.

Of note, 12 (12%) of the participants in this study started drinking the chai immediately, at the poured temperature of 80°C. Thus, the true mean preferred drinking temperature was underestimated in this series. All twelve of these drinkers of extremely hot chai were Kipsigis men. In addition to the starting temperature, there may be additional factors that potentiate the thermal injury potential of Kenyan chai. In our population, all participants preferred chai and drank multiple cups a day with an average of 4.2 (±1.9) cups per day, roughly equivalent to 1260mL (±570mL). In Iran, the association with tea and ESCC is dose-dependent with an increase in risk for those who drink more than 3 cups (270mL) per day⁴¹. It is not clear what impact the volume of beverage consumed, the quantity that is sipped or swallowed, has on ESCC risk, but higher volumes increase intra-esophageal temperatures in experimental studies²⁷ and should increase the time that the esophageal mucosa is exposed to potential thermal injury.

Studies in experimental animals have demonstrated the potentiating effects of thermal injury by hot liquids on carcinogenic agents in the esophagus. After instilling very hot water (>65°C) into the esophagus of rats, Li et al. observed an increase in nitrosamine-induced esophageal tumors, and the effect increased with increasing water temperatures⁴². In addition, Tobey et al. demonstrated that exposure of rabbit esophageal epithelium to hot beverages negatively impacted epithelial structure and function⁴³.

The questionnaire agreement was less strong than the agreement of similar questions and measured tea temperatures in the study of Islami *et al.* in Iran (weighted kappa values of 0.49 and 0.39)¹⁸. This difference in the agreement between interview questions and measured temperatures reminds us that questionnaires designed to evaluate associations between hot beverage consumption and clinical outcomes should be validated against measured temperatures for each population. Asking Europeans if they consume “hot beverages” and drawing conclusions about cancer risk is very different than asking East

Africans (since the meaning of “hot beverages” probably differs by about 15°C as we have shown). In addition, systematic reviews of hot beverages and esophageal cancer risk should analyze studies that include measured temperatures of consumption separately from those that report only subjective estimates of relative beverage temperature. As someone drinking “warm” chai in our region would still be consuming very hot (>65°C) beverages in other regions. The mean serving temperature in many other populations^{44–46} is similar to the mean initial temperature of drinking in our population. Our institution is undertaking a case-control trial on the risk factors for ESCC, the validation and information of the questionnaire will help in the understanding of hot chai as a risk factor. A case-control study from Eldoret in Kenya reported an association between hot beverages and ESCC²⁴. Although no temperature measurements were included, the controls reported consuming “very hot”, “hot”, and “warm” tea in 7%, 68%, and 25%, respectively, which is quite similar to our distribution of 11%, 64%, and 25%.

Associations between hot food or beverages and cancer may reflect a combination of temperature effects (thermal injury) and effects of constituents of the foods or beverages themselves. For example, recent studies have shown that maté, a staple hot temperature beverage in southern South America which has been associated with high ESCC risk^{47,48}, contains PAHs, one of the important classes of carcinogens in tobacco smoke, suggesting that drinking hot maté exposes the esophagus to two potentially carcinogenic influences, thermal injury and PAHs¹². All of the PAH levels were uniformly low in the Kenyan tea samples: the total PAH levels ranged from 92 ng/g to 287 ng/g of leaves, compared to a range of 621 ng/g to 3360 ng/g of leaves in the maté samples from Brazil¹³. Thus, an additional important finding in this study was the PAH content of commercially available Kenyan tea leaves was low in comparison, making it very unlikely that chai consumption is a significant source of PAH exposure in this population. Our results from an endemic area for esophageal cancer in Kenya are consistent with a meta-analysis on beverage temperature and content which demonstrated that temperature, but not PAH content, should be examined as potential a risk factor⁴⁹.

This study has several limitations. One was the convenience sampling of hospital visitors, which led to a relatively young age distribution in the study population. In our analysis, however, preferred tea temperature was not related to age ($p=0.73$). The sample size was also relatively small compared to the study by Munishi et al, which had twice as many subjects²³. There were also limitations to our method of temperature assessment. We measured the desired temperature at first drink and not the temperature throughout the consumption of the beverage, which can be influenced by the initial tasting⁴⁰. Although most participants drank their beverage at what seemed to be a fast pace, neither the rate nor the volume were measured. Also, the sip size has been shown to be predictive of intra-esophageal temperature²⁷ and future studies would benefit from this measurement to improve inter-population comparisons. Another limitation, mentioned above, was starting the temperature measurements at 80°C, which may have been below the actual preferred drinking temperature of some of the participants. The restriction to 5-degree intervals may also resulted in some loss of information.

The IARC has classified the consumption of “very hot” beverages above 65°C as “probably carcinogenic” to the esophagus (Group 2A)^{21,22}. The question of hot beverages as a risk factor for ESCC in Kenya should be further explored, and self-reported preferences seem reasonable to do so. There is growing epidemiological evidence that in areas with endemic ESCC, such as China, Iran, southern South America and East Africa, the occurrence of ESCC is impacted to some degree by the effects of hot beverages. With the ubiquitous consumption of chai, and the high temperature at which it is ingested, this increasingly recognized risk factor should be considered in the investigation of ESCC etiology in the population of western Kenya.

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Abbreviations

ESCC	Esophageal squamous cell carcinoma
PAH	polycyclic aromatic hydrocarbons
IARC	International Agency for Research on Cancer
NIST	National Institute for Standards and Technology

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Table 1:

Temperatures of chai consumption at Tenwek Hospital, by demographic characteristic

Participant Characteristics	N	Preferred Chai Temperature			Interval (minutes) between pouring and drinking Chai			Mean Measured Chai Temperature (°C) (SD)	
			N (%)	p-value		N (%)	p-value		p-value
Overall	100	Warm	25 (25.0)		>4	29 (29.0)		72.1 (4.6)	
		Hot	64 (64.0)		2–4	49 (49.0)			
		Very hot *	11 (11.0)		<2	22 (22.0)			
Age				0.73			0.89	72.3 (4.1)	0.59
< 30 years	50	Warm	10 (20.0)		>4	12 (24.0)			
		Hot	36 (72.0)		2–4	29 (58.0)			
		Very hot	4 (8.0)		<2	9 (10.0)			
> 30 years	50	Warm	15 (30.0)		>4	17 (34.0)		71.8 (5.0)	
		Hot	28 (56.0)		2–4	20 (40.0)			
		Very hot	7 (14.0)		<2	13 (26.0)			
Sex				0.43			0.13	72.6 (4.7)	0.033
Men	78	Warm	19 (24.4)		>4	19 (24.4)			
		Hot	49 (62.8)		2–4	41 (52.6)			
		Very hot	10 (12.8)		<2	18 (23.1)			
Women	22	Warm	6 (27.3)		>4	10 (45.5)		70.2 (3.6)	
		Hot	15 (68.2)		2–4	8 (36.4)			
		Very hot	1 (4.6)		<2	4 (18.2)			
Ethnicity				0.08			0.95	72.2 (4.6)	0.33
Kipsigis	88	Warm	20 (22.7)		>4	26 (29.6)			
		Hot	57 (64.8)		2–4	42 (47.7)			
		Very hot	11 (12.5)		<2	20 (22.7)			
Non-Kipsigis	12	Warm	5 (41.7)		>4	3 (25.0)		70.8 (4.2)	
		Hot	7 (58.3)		2–4	7 (58.3)			
		Very hot	0 (0.0)		<2	2 (16.7)			

* The term “very hot” in the questionnaire should not be confused with the IARC classification of “very hot” beverages which refers to temperatures greater than 65°C

Table 2:

Comparisons of questionnaire data and measured chai temperatures

A. Comparison of self-reported preferred chai temperature and the interval between pouring and drinking tea							
Descriptive variable Interval * (minutes)	Self-Reported Preferred Chai Temperature				Weighted kappa	Correlation coefficient $\hat{r}$	
	All	Warm	Hot	Very Hot			
	>4	29	17	11			1
	2–4	49	7	41			1
	<2	22	1	12			9
All	100	25	64	11			
B. Comparison of questionnaire data and measured chai temperatures							
Descriptive variable	Measured Chai Temperature Categories				Weighted kappa	Correlation coefficient $\hat{r}$	
	All	<70°C	70°C	75°C			
Self-assessment of Chai Temperature							
Warm	25	11	13	1	0.464	0.586	
Hot	64	4	26	34			
Very Hot	11	0	1	10			
Interval * (minutes)							
>4	29	9	18	2	0.505	0.594	
2–4	49	6	20	23			
<2	22	0	2	20			

* Interval between chai being poured and drunk.

[†] Spearman's rank correlation coefficient.

Table 3.

Concentration (ng/g) of the 26 PAHs measured in commercial Kenyan tea brands

	Kenya											Brazil	
	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7	Brand 8	Brand 9	Brand 10	Brand 11	Mean (SD) *	Mean (SD)
naphthalene	23.7	<5	40.7	54.9	11.7	72.9	60.5	36.7	21.9	32.5	10.0	43 (32)	223 (76)
biphenyl	13.7	<5	9.0	5.2	30.3	12.7	6.5	7.9	3.1	14.4	8.59	11 (8)	163 (255)
acenaphthene	44.0	97.2	24.9	21.2	68.4	35.7	22.0	29.4	23.7	32.8	25.8	39 (24)	90 (132)
acenaphthylene	<5	<5	<5	<5	<5	<5	<5	5.0	8.1	<5	<5	5 (1)	167 (83)
fluorene	<5	<5	<5	<5	<5	8.0	<5	9.2	3.4	11.8	<5	6 (2)	56 (35)
phenanthrene	24.0	26.4	16.0	13.4	25.5	22.3	12.7	30.9	41.1	55.1	14.0	26 (13)	498 (344)
anthracene	<2	<2	<2	<2	2.4	<2	<2	2.0	5.1	2.6	<2	2 (1)	26 (22)
4H-cyclopenta[de]phenanthrene	6.5	6.7	<5	<5	<5	<5	<5	<5	<5	<5	<5	5 (1)	28 (23)
fluoranthene	21.4	24.1	11.3	10.0	14.9	11.2	8.8	20.6	31.0	16.9	13.8	17 (7)	283 (216)
pyrene	16.0	18.5	8.8	8.3	11.8	8.9	6.4	15.3	31.6	12.5	10.2	13 (7)	247 (173)
benzo[ghi] fluoranthene	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	49 (36)
benzo[c] phenanthrene	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	25 (20)
benzo[a] anthracene	<5	<5	<5	<5	<5	<5	<5	6.4	<5	<5	<5	<5	99 (67)
triphenylene & chrysene	12.2	12	7.5	7.5	9.5	<5	5.3	13.4	9.1	7.6	9.5	9 (3)	114 (94)
benzo[b] fluoranthene	2.1	2.5	<1	1.6	2.0	1.4	<1	5.8	2.3	1.2	<1	2 (1)	44 (37)
benzo[k] fluoranthene	1.4	<1	<1	1.1	1.0	<1	<1	2.5	1.2	<1	<1	1 (0)	10 (8)
benzo[j] fluoranthene	1.4	1.9	<1	<1	<1	9.2	<1	9.1	<1	<1	<1	3 (3)	21 (15)
benzo[a] fluoranthene	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	12 (9)
benzo[e] pyrene	<1	<1	<1	<1	1.3	<1	<1	3.0	1.2	<1	<1	1 (1)	29 (22)
benzo[a] pyrene	1.2	1.4	<1	1.2	2.6	1.7	1.4	4.3	2.2	1.7	<1	2 (1)	41 (30)
perylene	<1	<1	<1	<1	<1	<1	<1	2.4	<1	<1	<1	1 (0)	8 (5)
indeno[1,2,3-cd] pyrene	7.4	5.6	2.0	<1	<1	<1	1.2	6.0	7.7	<1	<1	3 (3)	31 (21)
benzo[ghi] perylene	2.7	<1	<1	<1	<1	1.2	<1	4.0	2.5	<1	<1	2 (1)	41 (27)
dibenzo[a,h] anthracene	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	17 (6)
picene	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	9 (3)
Sum of the PAHs quantified	177.7	196.3	120.21	124.4	286.7	185.2	124.8	213.9	195.2	189.1	91.89	173 (55)	2256 (1435)

* Mean and SD calculated using absolute values for all measurements listed

Table 4:**Measured Temperatures of Hot Beverages in Various Locations**

Author	Location	Beverage	No. of subjects	Mean temperature at which the subject started drinking
Mwachiro 2018	Bomet, Kenya	Tea	100	Overall 72.1°C (+/- 4.6) 12% Greater than 80°C 73% 70–79°C 14% 65–69°C 1% 60–64°C
Munishi 2015 ²³	Kilimanjaro, Tanzania	Tea	188	70.6°C (+/-3.9)
Victora CG 1990 ¹⁶	Southern Brazil	Maté	1400	69.5°C
Borchgrevink 1999 ⁴⁶	United States	Coffee	250	65.6°C
Chen 2011 ³²	Guangdong Province, China Cases of Esophageal Cancer Control Group (Healthy Volunteers)	Green Tea	150 300	64.9°C* 8% Greater than 80°C 12% 70–79°C 20% 60–69°C 10% 50–59°C 8% <50°C 42% Never Drink Tea 60.5°C* 3% Greater than 80°C 8% 70–79°C 19% 60–69°C 15% 50–59°C 15% <50°C 40% Never Drink Tea
Ghisolfi 2000 1999 ³⁴	Southern Brazil	Maté	107	63.4°C
Graham 1996 ³⁵	United States Group A: <i>H. pylori</i> (+) Group B: <i>H. pylori</i> (–)	Water	43 12	63.4°C 61.3°C
Dirler 2018	Germany	Coffee	87	63°C
Islami F 2009 ¹⁸	Golestan, Iran	Tea	48592	62.4°C 5.4% Greater than 70°C 16.6% at 65–70°C 38.9% at 60–64°C 39% Less than 60°C
Pearson 1989 ³⁸	United Kingdom Group A: Disease – patients with known disease of esophagus, stomach, and duodenum Group B: Control asymptomatic volunteers	Tea & Coffee	59 65	Median 62°C Median 56°C
Ghadirian 1987 ¹⁷	Iran High-Risk – Shahsavari Low-Risk – Gorgan	Tea	100 100	61.3°C 50.1°C
Hunt 1947 ³⁶	United Kingdom Medical student volunteers	‘Oxo’	236	60°C
Lee 2002 ³⁷	United States Healthy volunteers	Coffee	300	59.8°C
Brown 2008 ³¹	United States	Tea, Coffee, Hot Chocolate	300	Defined optimal drinking temperature 57.8°C**
Edwards 1956 ³³	United Kingdom Patients with “Indigestion” Normal Miscellaneous findings Superficial gastritis Atrophic gastritis	Tea	78 32 9 36	53.6°C 55.1°C 54.9°C 57.3°C

* Calculated average temperature (estimated from percentages) of those consuming green tea

** Brown 2008 calculated the optimal temperature by utilizing the data from Lee 2002 and a mathematical model for scald burn injuries.

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