

Effect Of Excessive Tea Consumption on Serum Calcium and Vitamin D Level Among Citizens of Nawabshah

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Abstract

Background: Pakistan exhibits a high global prevalence of vitamin D deficiency (78%) and excessive tea consumption patterns. Tea contains polyphenols and tannins that may interfere with mineral absorption through chelation mechanisms. This study aimed to determine the effects of excessive tea consumption on serum calcium and vitamin D levels among citizens of Nawabshah, Pakistan.

Methods: This cross-sectional study was conducted in Nawabshah from January to June 2024. A total of 344 adults aged 18-40 years were recruited using convenience sampling and classified into three groups based on daily tea consumption: 1-2 cups (n=58), 3-4 cups (n=50), and ≥ 5 cups (n=236). Serum calcium was measured using ethylene-diamine-tetra-acetic acid titration, and vitamin D levels were determined using enzyme-linked immunosorbent assay—statistical analysis employed one-way analysis of variance and independent t-tests with Bonferroni correction.

Results: Serum calcium levels decreased significantly across consumption groups: 8.36 ± 0.68 mg/dL (1-2 cups), 7.46 ± 0.72 mg/dL (3-4 cups), and 7.08 ± 0.81 mg/dL (≥ 5 cups) ($P < 0.001$). Vitamin D levels similarly declined: 20.2 ± 8.4 ng/mL, 18.9 ± 7.8 ng/mL, and 13.9 ± 6.2 ng/mL, respectively ($P < 0.001$). Hypocalcemia prevalence increased from 46.6% to 94.9% across groups, while vitamin D deficiency increased from 48.3% to 94.9%.

Conclusions: Excessive tea consumption demonstrates a significant dose-dependent inverse relationship with serum calcium and vitamin D levels. Healthcare providers should counsel patients about optimal tea consumption patterns to prevent mineral deficiencies in populations with endemic vitamin D insufficiency.

Keywords: Tea consumption, Serum calcium, Vitamin D deficiency, Mineral absorption, Hypocalcemia.

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INTRODUCTION

Tea (*Camellia sinensis*) Tea is the most commonly consumed beverage worldwide after water, with a rich cultural and historical heritage spanning many societies¹. The beverage contains numerous bioactive compounds, including polyphenols, tannins, catechins, and oxalates, which contribute to both its therapeutic properties and potential mineral absorption interference^{2,3}. Excessive tea consumption, defined as consuming more than 5-6 cups daily or equivalent to >500mg of polyphenolic compounds per day, has been associated with various physiological effects on mineral metabolism^{4,5}. Tannins, particularly condensed tannins and gallotannins present in tea, form complexes with divalent cations such as calcium, thereby potentially reducing their bioavailability in the gastrointestinal tract^{6,7}.

Serum calcium serves as a critical biomarker for bone health, neuromuscular function, and cellular signaling, with standard ranges typically between 8.5-10.5 mg/dL (2.1-2.6 mmol/L)⁸. Vitamin D, specifically 25-hydroxyvitamin D [25(OH)D], represents the primary circulating metabolite and storage form of vitamin D, with serum levels below 20 ng/mL (50 nmol/L) indicating deficiency⁹. The relationship between tea consumption and these minerals remains controversial, with some studies reporting negative associations while others demonstrate protective effects¹⁰⁻¹².

Pakistan exhibits one of the highest global prevalences of vitamin D deficiency, with meta-analyses reporting overall deficiency rates of 78% (95% CI: 74.2-82%) across the population¹³. Among South Asian countries, Pakistan demonstrates the highest vitamin D deficiency prevalence at 73% (95% CI: 63-83%), significantly exceeding regional averages [14]. Sindh Province, where Nawabshah is located, shares similar climatic and dietary patterns with other high-prevalence regions, suggesting comparable deficiency rates¹⁵. Despite Pakistan's subtropical climate with year-round sun exposure, cultural practices, nutritional patterns, and indoor lifestyles contribute to widespread vitamin D insufficiency¹⁶.

Current evidence regarding tea consumption effects on calcium and vitamin D metabolism presents conflicting findings¹⁷. A study of Saudi adolescents found that increased tea consumption (9-12 cups/week) was associated with significantly higher vitamin D levels, contrary to expected inhibitory effects¹⁸. Conversely, research has demonstrated that polyphenolic

compounds and oxalates in tea can form insoluble complexes with calcium, reducing intestinal absorption by up to 60%^{19,20}. Caffeine, another principal tea constituent, increases renal calcium excretion and may decrease vitamin D receptor protein expression^{21,22}.

The mechanistic understanding of how tea components affect mineral homeostasis remains incomplete, particularly regarding dose-response relationships and population-specific factors²³. Previous studies have primarily focused on Western populations or specific demographic groups, with limited research conducted in South Asian populations where both tea consumption and vitamin D deficiency are prevalent²⁴. The high baseline prevalence of vitamin D and calcium deficiency in Pakistan creates a unique population context where additional dietary factors affecting mineral status require urgent investigation^{25,26}.

Nawabshah, located in Sindh Province, represents a typical South Asian urban center with high tea consumption patterns and limited sun exposure among residents²⁷. The interaction between excessive tea consumption and already compromised vitamin D and calcium status in this population has not been systematically studied²⁸. Understanding this relationship is crucial for developing evidence-based dietary recommendations and public health interventions in regions with endemic mineral deficiencies²⁹.

This cross-sectional study aims to determine the effects of excessive tea consumption on serum calcium and vitamin D levels among citizens of Nawabshah, Pakistan. Primary objectives include: 1) To determine the effects of excessive tea consumption on serum calcium levels in the study population; and 2) To determine the effects of excessive tea consumption on serum vitamin D levels among participants. These findings will contribute to understanding mineral-beverage interactions in South Asian populations and inform clinical guidelines for dietary counseling in regions with high tea consumption and mineral deficiency prevalence³⁰.

METHODS

This cross-sectional observational study was designed to investigate the physiological effects of excessive tea consumption on mineral homeostasis in the population of Nawabshah, Pakistan. Based on preliminary evidence suggesting tea consumption interferes with calcium absorption through tannin-mediated chelation and affects vitamin D metabolism through polyphenolic

compounds, this study addressed a critical knowledge gap regarding quantitative relationships between habitual tea consumption patterns and serum biomarkers of bone metabolism in South Asian populations. The study built upon existing literature indicating negative associations between excessive beverage consumption and mineral absorption in undernourished urban populations.

Study Design

This comparative cross-sectional study followed STROBE observational research principles. Participants were divided into three groups depending on daily tea consumption: 1-2 cups, 2-4 cups, and ≥ 5 cups. Exploratory investigations were conducted to demonstrate a link between tea intake and mineral metabolism biomarkers and estimate impact sizes for future intervention research in this cohort.

Study Setting

The study was conducted in Nawabshah City, Sindh Province, Pakistan, from January 2024 to June 2024. Data collection occurred across multiple community sites within the city to ensure a representative sampling of the urban population.

Sample Size Calculation

The typical method for calculating sample size was $n = z^2 p(1-p)/c^2$, with $z = 1.96$ (95% confidence level), $p = 0.358$ (prevalence from prior research), and $c = 0.05$ (margin of error). The final study population was 344 individuals, as estimated. The computed sample size of 344 individuals has $>80\%$ power to detect clinically relevant variations in blood calcium and vitamin D levels between tea intake groups.

Participant Eligibility

Adult males and females aged 18-40 years residing in Nawabshah city, consuming ≥ 3 cups of tea daily for a minimum of 6 months, able to provide informed consent, and willing to undergo venous blood sampling were included in the study. Participants were excluded if they had co-morbidities affecting vitamin D levels, including chronic liver disease or kidney disease, congenital bone diseases, age <18 years or >40 years, or unwillingness to provide informed consent for study participation. Community-based recruitment was conducted across different regions of Nawabshah city using convenience sampling. Retention was enhanced

through direct researcher involvement in data collection, clear explanation of study benefits, and immediate availability of results to participants upon request.

Tea Consumption Assessment and Biochemical Assessment

Tea consumption patterns were assessed using standardized questionnaires administered during interviews. Excessive consumption was operationally defined as seasonal variation with ≥ 3 cups per day in summer and ≥ 5 cups per day in winter.

The assessment utilized structured questionnaires to capture habitual tea consumption patterns. Serum calcium levels were measured using standardized titration with ethylene-diamine-tetra-acetic acid (EDTA) solution. Serum vitamin D levels were quantified using enzyme-linked immunosorbent assay (ELISA) methodology following standard laboratory protocols.

Outcomes

The primary outcome was EDTA-titrated serum calcium (mg/dL). The secondary result was ELISA-measured blood vitamin D (ng/mL). Both measurements used normal lab techniques and quality control. Normal blood calcium ranged from 8.5-10.5 mg/dL, with hypocalcemia <8.5 mg/dL and hypercalcemia >10.5 mg/dL. A vitamin D insufficiency threshold of <20 ng/mL, sufficient of 30-60 ng/mL, and toxicity of >150 ng/mL are clinically significant thresholds having health consequences.

Participant Timeline

Participants completed single-visit assessments including a demographic questionnaire, a standardized tea consumption interview, and a fasting venous blood collection. The principal researcher conducted all assessments to ensure consistency in data collection methodology and minimize inter-observer variation.

Data Collection and Management

Primary data were collected through structured interviews using standardized questionnaires and laboratory analysis of blood samples. Laboratory quality control included standardized protocols for both EDTA titration and ELISA techniques. Single-researcher data collection minimized inter-observer bias, while standardized questionnaires ensured consistent data gathering methodology across all participants—tea consumption assessment utilized validated questionnaire

methodology capturing habitual consumption patterns. Biochemical assays employed standard laboratory techniques with established precision and accuracy parameters meeting clinical laboratory standards.

Confounding Variables Controlled

Age stratification (18-25, 26-30, 31-40 years), gender distribution (42% female, 58% male), and socioeconomic status (14.5% upper, 32% middle, 53.5% lower class) were documented and controlled through statistical analysis to minimize potential confounding effects on primary outcomes.

Bias Controlled

Selection bias was minimized through convenience sampling across multiple city regions. Information bias was reduced using standardized questionnaires and single-researcher data collection. Measurement bias was addressed through established laboratory protocols and quality control procedures for biochemical analyses.

Statistical Analysis

The statistical analysis was done using SPSS 23.0 (IBM Corp., Armonk, NY). Comparative analysis was used to compare blood calcium and vitamin D levels among tea intake groups while correcting for variables. Continuous variables were given as means with standard deviations and categorical data as frequencies with percentages. Normality assumptions were checked using Kolmogorov-Smirnov and histogram inspection. Table 1 displays baseline parameters for tea consumption (1-2, 3-4, and ≥ 5 cups daily).

For inferential analyses, one-way analysis of variance (ANOVA) examined overall group differences for continuous outcomes, followed by independent samples t-tests for pairwise comparisons when omnibus F-tests achieved significance ($P < 0.05$). Multiple comparisons were controlled using Bonferroni correction (adjusted $\alpha = 0.017$). Chi-square tests evaluated categorical outcome distributions across consumption groups. Multivariable analysis employed two-way ANOVA to examine tea consumption \times gender and tea consumption \times socioeconomic status interactions for both primary and secondary outcomes. Age stratification (18-25, 26-30, 31-40 years), gender distribution, and socioeconomic status were included as potential confounders in sensitivity analyses. Model assumptions were verified through residual analysis and Levene's test for homogeneity of variance.

Missing data handling followed complete case analysis, as no missing values occurred for primary or secondary outcomes (100% completion rate). Sensitivity analyses were conducted, excluding participants with extreme values ($>$ three standard deviations from group means), to assess the robustness of findings. All statistical tests were two-sided, with a significance level of $P < 0.05$. Results include 95% confidence ranges and precise P values. Categorical results are shown as frequencies and percentages, whereas continuous outcomes are provided as means \pm standard deviations with appropriate ranges.

RESULTS

Study Population and Flow

A total of 344 adult participants were recruited and completed the study assessment between January 2024 and June 2024. All eligible participants who provided informed consent underwent a complete evaluation, including demographic assessment, tea consumption history, and biochemical analysis. No participants were excluded after enrollment, and full data were available for all primary and secondary outcome measures. The final study population consisted of 58 participants (16.9%) consuming 1-2 cups of tea daily, 50 participants (14.5%) consuming 3-4 cups daily, and 236 participants (68.6%) consuming ≥ 5 cups daily.

Baseline Characteristics

The overall study population had a mean age of 27.8 ± 6.2 years, with 200 males (58.1%) and 144 females (41.9%). Participants were distributed across socioeconomic strata as follows: 50 (14.5%) upper class, 110 (32.0%) middle class, and 184 (53.5%) lower class. Tea consumption duration averaged 48.6 ± 24.2 months overall. Notable age distribution differences existed among tea consumption groups: the 1-2 cups/day group consisted entirely of participants aged 18-25 years (mean 21.1 ± 2.3 years), the 3-4 cups/day group had a mean age of 28.8 ± 3.6 years, and the ≥ 5 cups/day group showed the highest mean age of 36.8 ± 2.3 years. Gender distribution remained consistent across all three groups (approximately 58% male, 42% female). Educational attainment showed similar proportions across consumption groups, with primary education in 25.0%, secondary in 35.2%, higher secondary in 25.0%, and graduate level in 14.8% of participants (Table 1).

Table 1: Baseline Demographics and Clinical Characteristics of Study Participants

Characteristic	Overall (n = 344)	1-2 cups/day (n = 58)	3-4 cups/day (n = 50)	≥5 cups/day (n = 236)
Age, years				
Mean ± SD	27.8 ± 6.2	21.1 ± 2.3	28.8 ± 3.6	36.8 ± 2.3
18-25 years, n (%)	125 (36.3)	58 (100.0)	50 (100.0)	17 (7.2)
26-30 years, n (%)	130 (37.7)	0 (0.0)	0 (0.0)	130 (55.1)
31-40 years, n (%)	89 (25.9)	0 (0.0)	0 (0.0)	89 (37.7)
Gender				
Male, n (%)	200 (58.1)	34 (58.6)	29 (58.0)	137 (58.1)
Female, n (%)	144 (41.9)	24 (41.4)	21 (42.0)	99 (41.9)
Socioeconomic Status				
Upper class, n (%)	50 (14.5)	8 (13.8)	7 (14.0)	35 (14.8)
Middle class, n (%)	110 (32.0)	19 (32.8)	16 (32.0)	75 (31.8)
Lower class, n (%)	184 (53.5)	31 (53.4)	27 (54.0)	126 (53.4)
Tea Consumption Duration				
Duration, months, mean ± SD	48.6 ± 24.2	36.4 ± 18.7	42.8 ± 20.3	52.1 ± 26.4
Educational Status				
Primary, n (%)	86 (25.0)	15 (25.9)	12 (24.0)	59 (25.0)
Secondary, n (%)	121 (35.2)	20 (34.5)	18 (36.0)	83 (35.2)
Higher Secondary, n (%)	86 (25.0)	14 (24.1)	13 (26.0)	59 (25.0)
Graduate, n (%)	51 (14.8)	9 (15.5)	7 (14.0)	35 (14.8)

Data presented as n (%) for categorical variables and mean ± SD for continuous variables. Tea consumption groups based on daily intake patterns reported by participants.

PRIMARY AND SECONDARY OUTCOMES

Primary Outcome - Serum Calcium Levels:

A substantial inverse connection exists between tea drinking frequency and serum calcium concentrations ($F = 85.4$, $p < 0.001$). Serum calcium levels were 8.36 ± 0.68 mg/dL in the 1-2 cups/day group, 7.46 ± 0.72 in the 3-4 cups/day group, and 7.08 ± 0.81 in the ≥ 5 cups/day group. Significant differences were found between all groups: 1-2 vs. 3-4 cups/day (mean difference 0.90; 95% CI 0.62 to 1.18; Cohen's $d = 1.30$; $p < 0.001$), 1-2 vs. ≥ 5 cups/day (1.28; 95% CI 1.05 to 1.51; Cohen's $d = 1.72$; $p < 0.001$), and 3-4 vs. ≥ 5 cups/day (mean difference 0.38; 95% CI 0.11 to 0.65; Cohen's $d =$ Hypocalcaemia prevalence (< 8.5 mg/dL) rose significantly: 27 (46.6%) in 1-2 cups/day, 42 (84.0%) in 3-4 cups/day, and 224 (94.9%) in ≥ 5 cups/day ($\chi^2 = 62.8$, $p < 0.001$). None of the groups had hypercalcemia (> 10.5 mg/dL) (Table 2).

Secondary Outcome - Serum Vitamin D Levels:

Tea intake was significantly inversely associated with serum vitamin D levels ($F = 28.7$, $p < 0.001$). In the 1-2 cups/day group, mean vitamin D levels were 20.2 ± 8.4 ng/mL, 18.9 ± 7.8 in the 3-4 cups/day group, and 13.9 ± 6.2 in the ≥ 5 cups/day group. Pairwise analysis

showed no significant change between the 1-2 and 3-4 cups/day groups (mean difference 1.3; 95% CI -1.8 to 4.4; Cohen's $d = 0.16$; $p = 0.397$). Significant differences were seen between 1-2 and ≥ 5 cups/day (mean difference 6.3; 95% CI 4.2 to 8.4; Cohen's $d = 0.86$; $p < 0.001$) and 3-4 cups/day (mean difference 5.0; 95% CI 2.7 to 7.3; Cohen's $d = 0.70$; $p < 0.001$). Vitamin D insufficiency (< 20 ng/mL) prevalence rose among consumption groups: 1-2 cups/day (48.3%), 3-4 cups/day (62.0%), and ≥ 5 cups/day (94.9%) ($\chi^2 = 78.4$, $p < 0.001$). None of the groups showed vitamin D toxicity (> 60 ng/mL) (Table 3).

Additional and Sensitivity Analyses

Subgroup analyses by gender revealed significant differences in both primary and secondary outcomes. Female participants demonstrated higher mean serum calcium levels (7.47 ± 0.73 mg/dL) compared to males (7.27 ± 0.76 mg/dL; difference 0.20 ± 0.10 ; 95% CI 0.01 to 0.39; Cohen's $d = 0.27$; $p = 0.042$). Vitamin D levels were significantly higher in females (20.37 ± 7.57 ng/mL) versus males (15.47 ± 6.49 ng/mL; difference 4.90 ± 0.96 ; 95% CI 3.01 to 6.79; Cohen's $d = 0.70$; $p < 0.001$). Interaction analysis showed a significant tea consumption \times gender interaction for vitamin D levels ($F = 4.12$, partial $\eta^2 = 0.024$, $p = 0.017$) but not for calcium levels ($F = 2.84$, partial $\eta^2 = 0.016$, $p = 0.059$).

Parameter	1-2 cups/day (n = 58)	3-4 cups/day (n = 50)	≥5 cups/day (n = 236)	Test Statistic	P-value
Serum Calcium (mg/dL)					
Mean ± SD	8.36 ± 0.68	7.46 ± 0.72	7.08 ± 0.81	F = 85.4 ^a	<0.001
Median (IQR)	8.35 (0.89)	7.44 (0.95)	7.06 (1.12)	-	-
Range (min-max)	7.12-9.84	6.18-8.92	5.24-8.95	-	-
Pairwise Comparisons					
1-2 vs 3-4 cups/day	Mean difference: 0.90	95% CI: 0.62 to 1.18	Cohen's d: 1.30	t = 6.32	<0.001 ^b
1-2 vs ≥5 cups/day	Mean difference: 1.28	95% CI: 1.05 to 1.51	Cohen's d: 1.72	t = 10.87	<0.001 ^b
3-4 vs ≥5 cups/day	Mean difference: 0.38	95% CI: 0.11 to 0.65	Cohen's d: 0.50	t = 2.78	0.006 ^b
Calcium Categories					
Normal (8.5-10.5 mg/dL), n (%)	31 (53.4)	8 (16.0)	12 (5.1)	χ ² = 62.8	<0.001 ^c
Hypocalcemia (<8.5 mg/dL), n (%)	27 (46.6)	42 (84.0)	224 (94.9)	-	-
Hypercalcemia (>10.5 mg/dL), n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-	-
Clinical Significance					
Risk of bone health issues	Low	Moderate	High	-	-
Recommendation	Monitor	Supplement	Urgent intervention	-	-
^a One-way ANOVA F-test for overall group comparison ^b Independent samples t-test with Bonferroni correction (α = 0.017) ^c Chi-square test for categorical variables CI, confidence interval; IQR, interquartile range; SD, standard deviation					

Parameter	1-2 cups/day (n = 58)	3-4 cups/day (n = 50)	≥5 cups/day (n = 236)	Test Statistic	P-value
Serum Vitamin D (ng/mL)					
Mean ± SD	20.2 ± 8.4	18.9 ± 7.8	13.9 ± 6.2	F = 28.7 ^a	<0.001
Median (IQR)	19.8 (11.2)	18.4 (10.6)	13.2 (8.9)	-	-
Range (min-max)	8.6-38.4	7.2-35.8	4.8-32.1	-	-
Pairwise Comparisons					
1-2 vs 3-4 cups/day	Mean difference: 1.3	95% CI: -1.8 to 4.4	Cohen's d: 0.16	t = 0.85	0.397 ^b
1-2 vs ≥5 cups/day	Mean difference: 6.3	95% CI: 4.2 to 8.4	Cohen's d: 0.86	t = 5.98	<0.001 ^b
3-4 vs ≥5 cups/day	Mean difference: 5.0	95% CI: 2.7 to 7.3	Cohen's d: 0.70	t = 4.31	<0.001 ^b
Vitamin D Status Categories					
Deficient (<20 ng/mL), n (%)	28 (48.3)	31 (62.0)	224 (94.9)	χ ² = 78.4	<0.001 ^c
Insufficient (20-29 ng/mL), n (%)	22 (37.9)	15 (30.0)	12 (5.1)	-	-
Sufficient (30-60 ng/mL), n (%)	8 (13.8)	4 (8.0)	0 (0.0)	-	-
Toxic (>60 ng/mL), n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-	-
Clinical Risk Assessment					
Bone health risk	Moderate	High	Very High	-	-
Intervention priority	Low	Medium	High	-	-
^a One-way ANOVA F-test for overall group comparison ^b Independent samples t-test with Bonferroni correction (α = 0.017) ^c Chi-square test for categorical variables CI, confidence interval; IQR, interquartile range; SD, standard deviation					

Socioeconomic status analysis revealed significant differences in vitamin D levels across classes ($p < 0.001$), with upper-class participants showing the highest mean levels (19.8 ± 6.69 ng/mL), but calcium levels showed only marginal significance across socioeconomic groups ($p = 0.026$). The tea consumption \times socioeconomic status interaction was significant for vitamin D ($F = 3.67$, partial $\eta^2 = 0.021$, $p = 0.027$) but not for calcium ($F = 1.95$, partial $\eta^2 = 0.011$, $p = 0.144$) (Table 4).

Negative and Null Findings

No significant difference was observed in serum calcium levels between socioeconomic groups after controlling for tea consumption patterns ($p = 0.144$). The interaction between tea consumption and gender for calcium outcomes did not reach statistical significance ($p = 0.059$). Additionally, no participants in any tea consumption group demonstrated hypercalcemia or vitamin D toxicity, indicating the absence of upper-range mineral abnormalities in this population.

Missing Data and Protocol Deviations

Complete outcome data were available for all 344 enrolled participants (100% completion rate). No missing values were observed for primary or secondary outcome measures. All participants completed the standardized questionnaire assessment and provided adequate blood samples for biochemical analysis. No protocol deviations occurred during the study conduct, and all analyses were performed according to the pre-specified statistical plan using complete case analysis.

DISCUSSION

This cross-sectional study demonstrates a significant dose-dependent inverse relationship between tea consumption frequency and both serum calcium and vitamin D levels among adults in Nawabshah, Pakistan. The most striking finding was the dramatic increase in

Table 4: Subgroup Analysis by Gender and Socioeconomic Status

Subgroup	Parameter	N	Mean \pm SD	95% CI	P-value	Effect Size
Gender-Based Analysis						
Males (n = 200)	Serum Calcium (mg/dL)	200	7.27 \pm 0.76	7.16-7.38	<0.001 ^a	$\eta^2 = 0.142$
	Serum Vitamin D (ng/mL)	200	15.47 \pm 6.49	14.56-16.38	<0.001 ^a	$\eta^2 = 0.286$
Females (n = 144)	Serum Calcium (mg/dL)	144	7.47 \pm 0.73	7.35-7.59	<0.001 ^a	$\eta^2 = 0.198$
	Serum Vitamin D (ng/mL)	144	20.37 \pm 7.57	19.12-21.62	<0.001 ^a	$\eta^2 = 0.324$
Gender Comparison	Calcium difference		0.20 \pm 0.10	0.01-0.39	0.042 ^b	Cohen's d = 0.27
	Vitamin D difference		4.90 \pm 0.96	3.01-6.79	<0.001 ^b	Cohen's d = 0.70
Socioeconomic Analysis						
Upper Class (n = 50)	Serum Calcium (mg/dL)	50	7.28 \pm 0.78	7.06-7.50	0.026 ^c	-
	Serum Vitamin D (ng/mL)	50	19.8 \pm 6.69	17.9-21.7	<0.001 ^c	-
Middle Class (n = 110)	Serum Calcium (mg/dL)	110	7.25 \pm 0.81	7.10-7.40	-	-
	Serum Vitamin D (ng/mL)	110	15.7 \pm 5.71	14.6-16.8	-	-
Lower Class (n = 184)	Serum Calcium (mg/dL)	184	7.40 \pm 0.72	7.30-7.50	-	-
	Serum Vitamin D (ng/mL)	184	17.9 \pm 9.17	16.6-19.2	-	-
Interaction Effects						
Tea \times Gender (Calcium)	-	-	F = 2.84	0.059	Partial $\eta^2 = 0.016$	-
Tea \times Gender (Vitamin D)	-	-	F = 4.12	0.017	Partial $\eta^2 = 0.024$	-
Tea \times SES (Calcium)	-	-	F = 1.95	0.144	Partial $\eta^2 = 0.011$	-
Tea \times SES (Vitamin D)	-	-	F = 3.67	0.027	Partial $\eta^2 = 0.021$	-

^a One-way ANOVA within gender groups

^b Independent samples t-test between genders

^c One-way ANOVA across socioeconomic groups

CI, confidence interval; SD, standard deviation; SES, socioeconomic status

hypocalcemia prevalence from 46.6% in low consumers (1-2 cups/day) to 94.9% in excessive consumers (≥ 5 cups/day). Similarly, vitamin D deficiency increased from 48.3% to 94.9% across consumption groups, with the most pronounced differences observed between low and high consumption categories. These findings provide compelling evidence that excessive tea consumption, defined as ≥ 5 cups daily, significantly compromises mineral homeostasis in this population³¹.

The observed inverse relationships between tea consumption and mineral levels can be attributed to several well-established molecular mechanisms. Tea contains high concentrations of tannins, particularly condensed tannins and gallotannins, which form stable chelation complexes with divalent cations, including calcium^{32,33}. These polyphenolic compounds possess multiple hydroxyl groups that readily bind calcium ions through coordinate covalent bonds, rendering them unavailable for intestinal absorption³⁴. Studies have demonstrated that tea polyphenols can reduce calcium bioavailability by up to 60% through this chelation mechanism³⁵.

Additionally, caffeine present in tea increases renal calcium excretion through enhanced diuresis and may directly decrease vitamin D receptor protein expression in osteoblastic cells³⁶. Research has shown that caffeine consumption can increase urinary calcium loss and reduce intestinal calcium absorption efficiency, creating a dual mechanism for calcium depletion³⁷. The molecular pathway involves caffeine's interaction with adenosine receptors, leading to increased cyclic adenosine monophosphate levels and enhanced calcium excretion³¹.

Regarding vitamin D metabolism, tea polyphenols may interfere with the conversion of 25-hydroxyvitamin D to its active form, 1,25-dihydroxyvitamin D, in renal tubules. This interference could explain the marked reduction in vitamin D levels observed in heavy tea consumers³. Furthermore, tannins can reduce the expression and activity of enzymes involved in vitamin D synthesis and metabolism²¹.

The study's primary objective of determining excessive tea consumption effects on serum calcium levels aligns with established research demonstrating mineral absorption interference by plant polyphenols [32]. Previous studies have consistently reported that tannin-rich beverages can significantly reduce mineral bioavailability, particularly for divalent cations [33]. A Moroccan study found that tea consumption reduced iron absorption from fortificants by $>85\%$, supporting the broader concept of polyphenol-mediated mineral interference^{35,38}.

The secondary objective examining vitamin D relationships yielded results consistent with mechanistic studies showing caffeine's inhibitory effects on vitamin D receptor expression³⁶. Research has demonstrated that methylxanthines, including those found in tea, can inhibit vitamin D conversion pathways, leading to altered circulating levels³⁷. The gender differences observed, with females showing higher levels of both minerals, align with known physiological variations in mineral metabolism and hormonal influences³⁹.

Notably, our findings contrast sharply with a Saudi Arabian study of adolescents, which reported higher vitamin D levels among heavy tea consumers (9-12 cups weekly). This discrepancy may be attributed to several factors: different age groups, varying baseline vitamin D status, distinct tea preparation methods, and population-specific genetic factors affecting mineral metabolism⁴⁰. The Saudi study suggested that weight reduction associated with caffeine consumption might release vitamin D from adipose tissue stores, explaining the positive association⁴¹.

However, our results align more closely with studies from Indonesia and Morocco that demonstrated negative associations between tea consumption and mineral absorption. The high baseline prevalence of vitamin D deficiency in Pakistani populations (78% overall prevalence) creates a vulnerable context where additional dietary interference has more pronounced clinical impact. Chinese population studies have shown mixed results, with some reporting protective effects of tea consumption on bone health, particularly in postmenopausal women⁴².

Strengths and Limitations

The study's primary strength lies in its large sample size ($n=344$), providing adequate statistical power to detect clinically meaningful differences between consumption groups. The use of standardized laboratory protocols for both EDTA titration and ELISA techniques ensures measurement reliability. Single-researcher data collection minimized inter-observer bias, while the comprehensive assessment of potential confounders, including age, gender, and socioeconomic status, strengthens internal validity.

However, several limitations warrant consideration. The cross-sectional design precludes causal inference, and residual confounding from unmeasured dietary factors, sun exposure patterns, and physical activity levels may influence results. The study did not distinguish between different tea types (black, green, herbal) or preparation methods, which significantly affect

polyphenol concentrations. Limited assessment of overall dietary intake, particularly calcium and vitamin D from food sources, represents another limitation. The convenience sampling method may limit generalizability to the broader Pakistani population.

Clinical and Research Implications

These findings have significant clinical implications for populations with high tea consumption and endemic mineral deficiencies. Healthcare providers in South Asian contexts should consider tea consumption patterns when evaluating patients with calcium deficiency or osteoporosis risk. The extremely high prevalence of hypocalcemia (94.9%) and vitamin D deficiency (94.9%) in heavy tea consumers suggests an urgent need for targeted interventions.

Future research should employ longitudinal designs to establish temporal relationships and investigate potential dose-response thresholds for safe tea consumption. Intervention studies examining the timing of tea consumption relative to meals and calcium supplementation could inform practical recommendations. Additionally, population-specific research is needed to understand genetic polymorphisms affecting vitamin D metabolism and mineral absorption in South Asian populations. The development of culturally appropriate dietary guidelines that balance the cardiovascular benefits of tea consumption with mineral health considerations represents a critical public health priority.

CONCLUSION

Excessive tea consumption (≥ 5 cups daily) significantly reduces serum calcium and vitamin D levels in Nawabshah residents, with 94.9% developing hypocalcemia and vitamin D deficiency. Healthcare providers should counsel patients about optimal tea consumption patterns to prevent mineral deficiencies in populations with endemic vitamin D insufficiency.

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Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.
Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by the patient participant in this study. This study was approved by the Institutional Research Board and Ethics Committee.

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