Biomarker-based assessment of tobacco exposure and oral cancer risk: Serum cotinine, dose-response patterns, and population disparities - A cross - sectional study

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Abstract

Background: Tobacco exposure is a leading cause of oral cancer, but self-reported tobacco use is often biased. Serum cotinine, a validated biomarker of nicotine intake, provides a more reliable measure of exposure.

Objectives: To evaluate serum cotinine as a predictor of oral cancer risk, analyse dose- response patterns, and examine demographic disparities in exposure among United States adults using National Health and Nutrition Examination Survey (NHANES) 2013-2014 data.

Methods: A cross-sectional analysis of NHANES participants (≥30 years) was performed. Serum cotinine levels were categorized into low, moderate, and heavy tobacco exposure groups. Oral cancer risk was assessed using a composite score. Logistic regression models adjusted for age, sex, race/ethnicity, and socioeconomic status were used to evaluate associations with oral cancer prevalence.

Results: Higher serum cotinine levels were significantly associated with increased oral cancer prevalence (p < 0.001). A clear dose-response gradient was observed. Demographic disparities were evident: The association was strongest among younger adults (18-49 years), females (OR: 2.90, 95% CI: 1.99-4.21), and Non-Hispanic Whites and Blacks.

Conclusion: Serum cotinine is a robust biomarker for assessing tobacco-related oral cancer risk, revealing dose-response effects and disparities masked by self-report. Biomarker-based surveillance can strengthen prevention and targeted interventions.

Keywords: Serum cotinine; Oral cancer; Biomarker; Tobacco exposure; Dose-response; NHANES; Demographic disparities

Introduction

Oral cancer represents a significant global health burden, with 377,713 new cases and 177,757 deaths reported worldwide in 2020[1]. In the United States, approximately 54,000 new cases were diagnosed in 2021, with an estimated 10,850 deaths[1]. The 5-year survival rate is 64.3%, dropping to 38.5% when cancer has metastasized at diagnosis[1], emphasizing the importance of identifying risk factors and prevention strategies. Tobacco use is a leading cause of oral cancer[2,3]. Tobacco products contain carcinogens that induce DNA damage and mutations in oral epithelial cells, potentially causing malignant transformation[3].

Other risk factors include alcohol consumption, poor oral hygiene, HPV infection, and dietary deficiencies[4,5]. Serum cotinine's half-life of 16-20 hours compared to nicotine's 2 hours, cotinine provides a more stable measure than self-reported tobacco uses [6,7]. Serum cotinine levels reflect recent tobacco use, including active smoking and secondhand smoke exposure[8]. High-risk regions include Palau and certain Asian areas, with middle SDI regions showing the greatest burden growth[9]. This study examines the association between serum cotinine levels and oral cancer risk using NHANES 2013-2014 data and explores effect modifications by age, gender, and race/ethnicity,

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thereby contributing to the understanding of oral cancer risk factors across diverse populations.

Oral cancer risk involves multifactorial interactions between behavioral, inflammatory, and nutritional pathways. Prior studies have highlighted limitations of relying solely on diagnosed cancer cases in population surveys[10,11]. We developed a composite risk score integrating five established indicators: self-reported oral pain, poor oral health, frequent alcohol consumption, extensive tooth loss, and nutritional deficiencies. This approach aligns with Oral Cancer Foundation recommendations and Carolina Head and Neck Cancer Study methodologies[11,12,13], which demonstrated improved risk stratification through cumulative markers.

Objectives

Primary Objective: To evaluate serum cotinine as an objective predictor of oral cancer risk in U.S. adults.

Secondary Objectives: To examine dose-response patterns between serum cotinine levels and oral cancer prevalence and also to explore demographic disparities (age, sex, race/ethnicity, SES) in cotinine levels and risk associations.
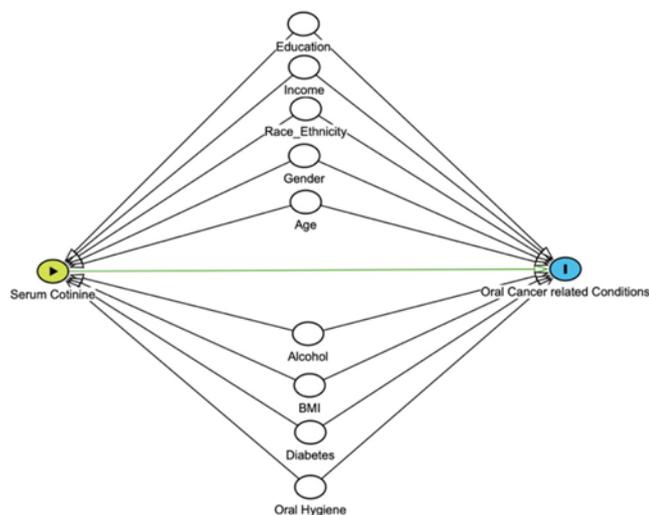
Directed acyclic graph (DAG)

Figure 1. Directed acyclic graph (DAG) illustrating the hypothesized relationships between serum cotinine, confounders, and oral cancer risk. This DAG was constructed using DAGitty (www.dagitty.net)[14]

Data source

Data collection in NHANES 2013-2014 involved both interview and examination components. The interview component included demographic, socioeconomic, dietary, and health-related questions. The examination included medical, dental, and physiological measurements, as well as laboratory tests conducted in mobile examination centres (MECs) [15]. Serum cotinine levels were measured using an automated isotope- dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS) method. This method demonstrates high accuracy (coefficient of variation: 1.5-3.8%) and sensitivity (limit of detection: 0.015 ng/mL), validated through rigorous interlaboratory comparisons[15]. The NHANES protocol aligns with CDC-established quality assurance standards for cotinine quantification in population studies.

Study design: Cross-sectional analysis of NHANES 2013-2014.

Population: Adults ≥18 years with serum cotinine measurements and oral health outcomes.

Exposure: Serum cotinine categorized into:

1. **Low (<0.05 ng/mL)**: Minimal or no exposure to tobacco smoke.
2. **Medium (0.05-3 ng/mL)**: Indicative of secondhand smoke exposure.
3. **High (≥3 ng/mL)**: Indicative of active smoking.

Outcome:

Participants were assigned a composite oral cancer risk score (0-5 points) using five criteria: oral pain, poor oral health, heavy alcohol use, extensive tooth loss, and nutritional deficiencies, with high risk defined as meeting three or more of these criteria. This threshold was chosen to balance sensitivity and specificity, reflecting the additive nature of risk factors in oral carcinogenesis. Prior studies have demonstrated improved risk stratification using cumulative markers[10,11]. This approach also aligns with evidence linking behavioral, structural, and nutritional factors to synergistic increases in oral cancer risk[12,13].

Covariates: Age, Sex, Race/Ethnicity, Income, Education, Alcohol Consumption Covariates were selected based on their established role as confounders in oral cancer risk from prior literature and their availability in NHANES 2013-2014.

Variables were prioritized if they were:

1. Biologically plausible risk factors for oral cancer.
2. Associated with both serum cotinine (exposure) and oral cancer-related conditions (outcome).
3. Not mediators of the exposure-outcome relationship.

Inclusion criteria: Adults aged 30 years and above, participants with available serum cotinine measurements and participants with completed oral cancer-related conditions status data were included in the study.

Exclusion criteria: Participants with missing serum cotinine data, participants with missing or incomplete oral cancer status and pregnant women (due to altered cotinine metabolism) were excluded from the study

Methodology

Statistical analysis

Descriptive analysis

Descriptive statistics were calculated to characterize the study population and examine patterns across exposure levels. For categorical variables, frequencies and percentages were computed. For continuous variables, means, standard deviations, and ranges were calculated. Chi-square tests were used to examine differences in categorical variables across cotinine categories, while analysis of variance (ANOVA) was used for continuous variables.

Regression analyses

Binary logistic regression models were used to examine the association between serum cotinine levels and oral cancer risk, with the low cotinine group serving as the reference category. Four sequential models were constructed:

1. Model 1: Unadjusted association between serum cotinine levels and oral cancer risk

2. Model 2: Adjusted for demographic factors (age, gender, race/ethnicity)

3. Model 3: Further adjusted for socioeconomic factors (income, education)

4. Model 4: Additionally adjusted for health-related factors (BMI, diabetes status, alcohol consumption, oral hygiene practices)

Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs).

Effect modification analysis

To test for effect modification, interaction terms between serum cotinine levels and each potential effect modifier (age, gender, and race/ethnicity) were added to the fully adjusted model (Model 4). The factors were categorized as follows:

1. Age: Used as a continuous variable in the interaction model and later categorized for stratified analysis.

2. Gender: Categorical variable with levels as defined in the NHANES dataset(male and female).

3. Race/ethnicity: Categorical variable with levels as defined in the NHANES dataset.

Interaction models were created using the *svyglm* function to account for the complex survey design of NHANES:

1. Age interaction: oral_cancer_risk - serum_cotinine_category*continuous age variable + covariates

2. Gender interaction: oral_cancer_risk - serum_cotinine_category*gender + covariates

3. Race/ethnicity interaction: oral _ cancer _ risk - serum _ cotinine _ category* race _ ethnicity + covariates

Likelihood ratio tests, implemented using the anova function with a Chi-square test, were used to assess the statistical significance of these interactions. Stratified analyses were subsequently performed for all interactions to examine the association between cotinine levels and oral cancer risk within each subgroup. Age was categorized into groups for the stratified analysis. (Table 3) showing odds ratios with 95% confidence intervals and p-values for each stratum. Interaction plots were generated to visually represent the effect modification, with predicted probabilities of oral cancer risk plotted against serum cotinine categories for different levels of each effect modifier.

Statistical software and survey weights

All analyses were conducted using R Studio. NHANES survey weights were applied to account for the complex sampling design and ensure nationally representative estimates. The survey package in R Studio was used to incorporate sampling weights, stratification, and clustering in all analyses.

Results

Descriptive results

The study analysed 4,464 participants with a mean age of 54 years, categorizing them into low, medium, and high serum cotinine groups, which comprised 57.3%, 17.1%, and 25.6% of the sample, respectively. High cotinine participants were younger (mean age 50), predominantly male (57%), and disproportionately Non-Hispanic Black or White compared to the overall group. Socioeconomic disparities were evident: only 10% of the high cotinine group were college graduates, and 35% reported low income, while those with low cotinine had much higher educational attainment (36% college graduates) and lower rates of low income (12%). Health measures varied, with more underweight individuals in the high cotinine group and more obesity in the medium cotinine group, and diabetes was less prevalent among those with high cotinine. Alcohol use was more common in those with high cotinine, while the low cotinine group had more non-drinkers; notably, the proportion classified as higher risk for oral cancer

was twice as high (34%) in the high cotinine group compared to the low cotinine group (15%).

Regression results

The association between serum cotinine levels and oral cancer risk was analyzed using a series of logistic regression models with progressive adjustment for confounders. Results demonstrated a dose-response relationship between cotinine levels and oral cancer risk, though this association varied across adjustment stages (Table 2A-D).

In the **unadjusted model** (Table 2A), participants with medium cotinine levels (0.05- 3 ng/mL) had 1.93 times higher odds of oral cancer risk compared to the low cotinine group (<0.05 ng/mL) (95% CI: 1.33-2.80, *p*<0.001). The high cotinine group (≥3 ng/mL) showed even stronger effects, with 3.00 times higher odds (95% CI: 2.53-3.55, *p*<0.001). After adjusting for **demographic factors** (age, gender, race/ethnicity), the effect estimates attenuated slightly but remained

significant: medium cotinine (OR=1.82, 95% CI: 1.21-2.72, *p*<0.001) and high cotinine (OR=2.55, 95% CI: 2.01-3.22, *p*<0.001). (Table 2B)

Further adjustment for **socioeconomic factors** (income, education) maintained the significant association for high cotinine (OR=2.46, 95% CI: 1.73-3.49, *p*<0.001), while the medium cotinine group's effect estimate widened and became non-significant (OR=1.79, 95% CI: 0.96-3.33, *p*=0.067) (Table 2C). In the **fully adjusted model** (demographic, socioeconomic, and health factors), only the high cotinine group retained statistical significance (OR=2.74, 95% CI: 1.82-4.11, *p*<0.001). The medium cotinine group showed no significant association (OR=1.91, 95% CI: 0.88-4.14, *p*=0.102) (Table 2D). This relationship is visually represented in Figure 2.
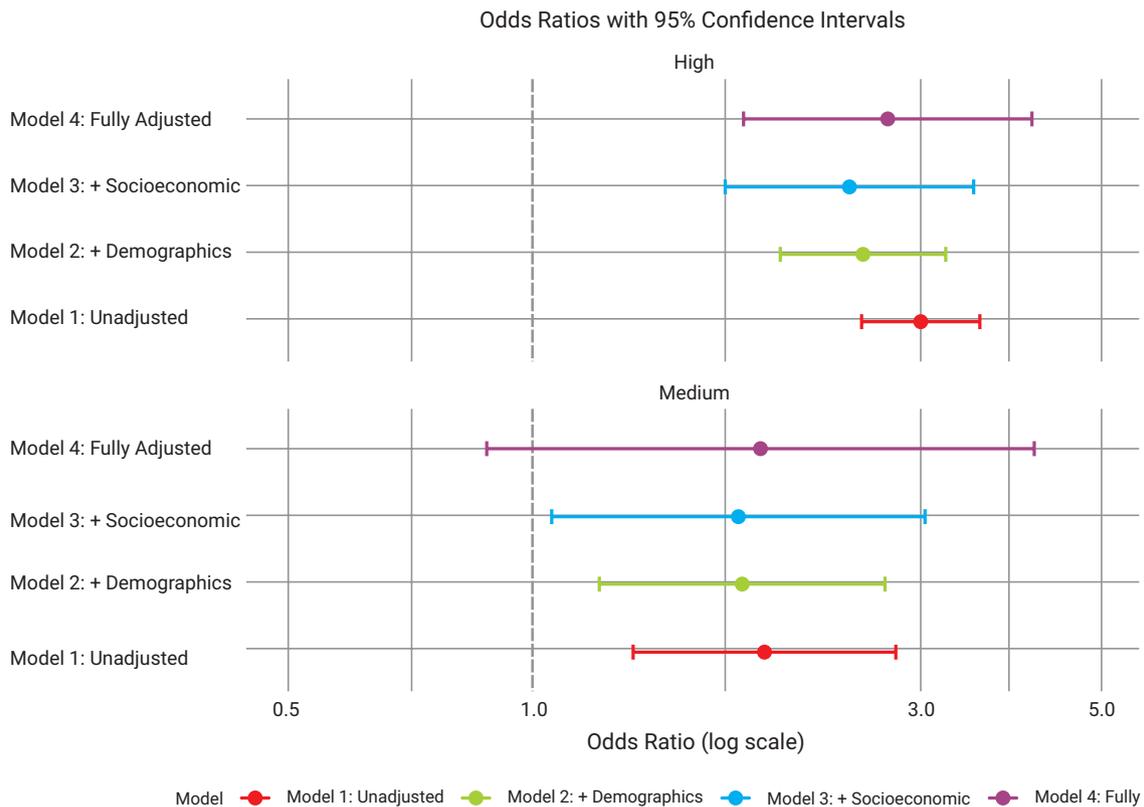

Figure 2: Association between serum cotinine category and oral cancer risk

The high cotinine group's effect size remained robust across all models, suggesting tobacco exposure (active smoking) is independently associated with oral cancer risk even after accounting for sociodemographic and clinical factors. The attenuation of medium cotinine effects in adjusted models implies that second-hand smoke exposure's association may be mediated by

socioeconomic disparities, as supported by prior studies[4].

Effect modification

Analyses revealed significant effect modification by age, gender, and race/ethnicity (Table 3). Among ages 18-49, both medium and high cotinine levels

were linked to increased oral cancer risk, while in ages 50-64, only high levels were significant. Age-related modification is illustrated in Figure 3a. High cotinine showed a stronger association in females (OR: 2.90), though medium levels were significant only in males. Gender modification of the association is presented in Figure 3b. By race/ethnicity, high cotinine was significant among Hispanics, Non-Hispanic Blacks, and Non-Hispanic Whites, while medium levels were significant only in Non- Hispanic Whites. The modifying effect of race/ethnicity on the association is shown in Figure 3c. Overall, younger participants, females, and certain racial/ethnic groups demonstrated stronger associations.
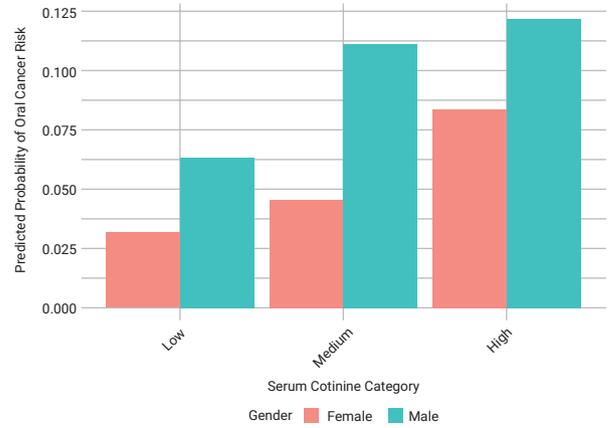

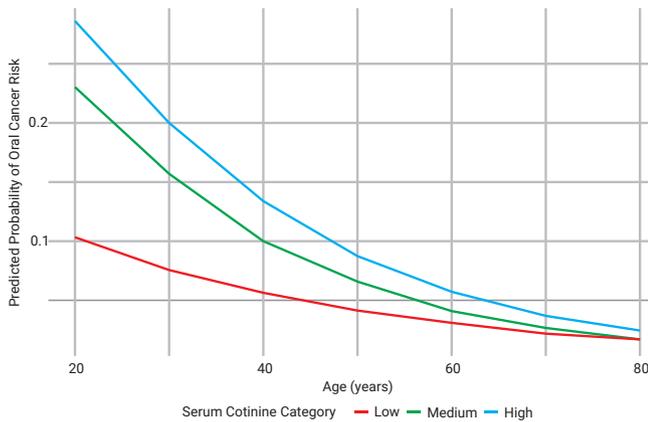Figure 3b: Interaction between gender and oral

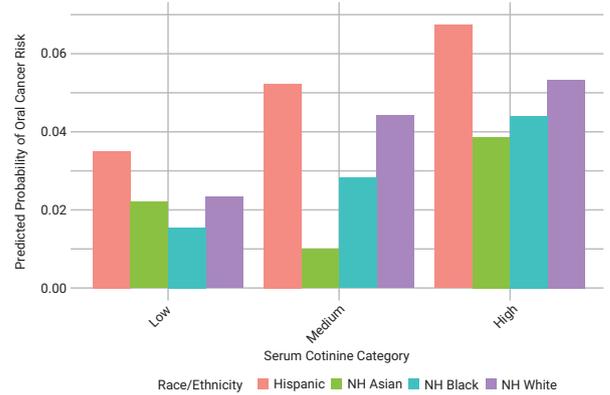Figure 3a: Interaction between age and oral cancer risk

Figure 3c: Interaction between race and ethnicity and oral cancer risk

Table 1: Characteristics of study population by serum cotinine category

Characteristic	N	Serum Cotinine Category			
		Overall, N = 4,464	Low N = 2557	Medium N = 762	High N = 1145
Age (years)	4,464	54 (15)	56 (15)	54 (15)	50 (13)
Gender	4,464				
Female		2,355 (53%)	1,466 (57%)	398 (52%)	491 (43%)
Male		2,109 (47%)	1,091 (43%)	364 (48%)	654 (57%)
Race/Ethnicity	4,464				
Hispanic		976 (22%)	670 (26%)	151 (20%)	155 (14%)
NH Asian		629 (14%)	387 (15%)	126 (17%)	116 (10%)
NH Black		875 (20%)	350 (14%)	220 (29%)	305 (27%)
NH White		1,984 (44%)	1,150 (45%)	265 (35%)	569 (50%)
Income	4,118				
High		1,645 (40%)	1,201 (51%)	208 (30%)	236 (22%)
Low		847 (21%)	280 (12%)	192 (27%)	375 (35%)
Medium		1,626 (39%)	874 (37%)	302 (43%)	450 (42%)
Missing		346	202	60	84
Education Level	4,460				
College grad+		1,173 (26%)	912 (36%)	145 (19%)	116 (10%)
High school graduate		1,003 (22%)	467 (18%)	192 (25%)	344 (30%)
Less than high school		991 (22%)	454 (18%)	210 (28%)	327 (29%)
Some college		1,293 (29%)	723 (28%)	213 (28%)	357 (31%)

Characteristic	N	Serum Cotinine Category			
		Overall, N = 4,464	Low N = 2557	Medium N = 762	High N = 1145
Missing		4	1	2	1
BMI Category	4,408				
Normal weight		1,138 (26%)	665 (26%)	159 (21%)	314 (28%)
Obese		1,737 (39%)	979 (39%)	346 (46%)	412 (37%)
Overweight		1,477 (34%)	866 (34%)	239 (32%)	372 (33%)
Underweight		56 (1.3%)	21 (0.8%)	5 (0.7%)	30 (2.7%)
Missing		56	26	13	17
Diabetes Status	4,309				
Absent		3,641 (84%)	2,065 (84%)	608 (82%)	968 (87%)
Present		668 (16%)	389 (16%)	131 (18%)	148 (13%)
Missing		155	103	23	29
Alcohol Intake	4,464				
Never		1,170 (26%)	793 (31%)	232 (30%)	145 (13%)
Former		499 (11%)	271 (11%)	77 (10%)	151 (13%)
Light		1,273 (29%)	713 (28%)	194 (25%)	366 (32%)
Moderate		532 (12%)	274 (11%)	87 (11%)	171 (15%)
Heavy		623 (14%)	324 (13%)	102 (13%)	197 (17%)
Missing		367 (8.2%)	182 (7.1%)	70 (9.2%)	115 (10%)
Oral Cancer Risk	4,464				
Lower Risk		3,534 (79%)	2,176 (85%)	604 (79%)	754 (66%)
Higher Risk		930 (21%)	381 (15%)	158 (21%)	391 (34%)
Mean (SD); n (%)					

Table 2A: Association between exposure and oral cancer risk (unadjusted)

Characteristic	Unadjusted OR	95% CI	p-value
Serum Cotinine Levels			<0.001
Low	-	-	
Medium	1.93	1.33, 2.80	
High	3.00	2.53, 3.55	
1 OR (95% CI)			
2 OR = Odds Ratio, CI = Confidence Interval			

Table 2B: Association between exposure and oral cancer risk (adjusted for demographic characteristics)

Characteristic	Demographics OR	95% CI	p-value
Serum Cotinine Levels			<0.001
Low	-	-	
Medium	1.82	1.21, 2.72	
High	2.55	2.01, 3.22	
Age in years at screening	0.96	0.95, 0.97	<0.001
Gender			<0.001

Characteristic	Demographics OR	95% CI	p-value
Female	-	-	
Male	2.08	1.46, 2.96	
Race and ethnicity			<0.001
Hispanic	-	-	
NH Asian	0.33	0.22, 0.50	
NH Black	0.55	0.45, 0.68	
NH White	0.75	0.67, 0.84	
1 OR (95% CI)			
2 OR = Odds Ratio, CI = Confidence Interval			

Table 2C: Association between exposure and oral cancer risk (adjusted for socioeconomic characteristics)

Characteristic	Socioeconomic OR	95% CI	p-value
Serum Cotinine Levels			<0.001
Low	-	-	
Medium	1.79	1.06, 3.04	
High	2.46	1.73, 3.49	
Age in years at screening	0.95	0.94, 0.97	<0.001

Characteristic	Demographics OR	95% CI	p-value
Gender			<0.001
Female	-	-	
Male	2.11	1.28, 3.48	
Race & Ethnicity			<0.001
Hispanic	-	-	
NH Asian	0.34	0.18, 0.66	
NH Black	0.53	0.37, 0.76	
NH White	0.73	0.59, 0.90	
Income Category			0.14
High	-	-	
Low	0.82	0.57, 1.16	
Medium	0.92	0.61, 1.38	
Education			0.004
College grad+	-	-	
High school graduate	1.52	0.86, 2.70	
Less than high school	1.01	0.55, 1.86	
Some college	1.67	0.99, 2.81	
1 OR (95% CI)			
2 OR = Odds Ratio, CI = Confidence Interval			

Disparities: Non-Hispanic Blacks showed disproportionately higher cotinine levels than Whites at comparable smoking levels.

Table 2D: Association between exposure and oral cancer risk (fully adjusted)

Characteristic	Adjusted OR	95% CI	p-value
Serum Cotinine Levels			<0.001
Low	-	-	
Medium	1.91	0.88, 4.14	
High	2.74	1.82, 4.11	
Age in years at screening	0.96	0.94, 0.98	<0.001
Gender			<0.001
Female	-	-	
Male	2.12	1.05, 4.29	
Race & Ethnicity			<0.001
Hispanic	-	-	
NH Asian	0.37	0.15, 0.91	
NH Black	0.53	0.34, 0.82	
NH White	0.75	0.58, 0.95	
Income			0.12
High	-	-	
Low	0.85	0.55, 1.31	
Medium	0.98	0.58, 1.68	
BMI			0.013
Normal weight	-	-	
Underweight	0.46	0.07, 3.06	
Obese	1.29	0.58, 2.90	
Overweight	1.04	0.39, 2.75	
Diabetes status			0.004
Absent	-	-	
Present	0.62	0.30, 1.28	
1 OR (95% CI)			
2 OR = Odds Ratio, CI = Confidence Interval			

Table 3: Combined effect modification analysis for cotinine-associated oral cancer risk

Demographic Stratum	Category	Medium Cotinine OR (95% CI)	p-value	High Cotinine OR (95% CI)	p-value
Age Group	18-34	2.07 (1.24-3.45)	0.0053	2.58 (1.24-5.39)	0.0115
	35-49	2.15 (1.12-4.12)	0.0212	2.85 (1.75-4.62)	<0.0001
	50-64	1.07 (0.62-1.85)	0.8083	1.74 (1.13-2.69)	0.0123
	65+	2.16 (0.75-6.25)	0.1545	1.12 (0.51-2.46)	0.7777
Gender	Female	1.43 (0.94-2.18)	0.0956	2.90 (1.99-4.21)	<0.000001
	Male	1.84 (1.14-2.97)	0.0126	1.99 (1.42-2.81)	0.000077
Race/Ethnicity	NH White	2.05 (1.19-3.52)	0.0095	2.62 (2.01-3.42)	<0.0001
	NH Black	1.78 (0.81-3.91)	0.1511	2.66 (1.09-6.50)	0.0317
	Hispanic	1.28 (0.85-1.92)	0.2350	1.85 (1.19-2.88)	0.0064
	NH Asian	0.48 (0.19-1.22)	0.1218	1.92 (0.83-4.41)	0.1258

Gender and socioeconomic gradients persisted, with lower-income groups exhibiting higher cotinine exposure.

Discussion

This study examined the association between serum cotinine levels and oral cancer-related conditions using nationally representative data from NHANES 2013-2014. The findings confirm serum cotinine as a robust biomarker for tobacco exposure and oral cancer risk, outperforming self-reports in sensitivity, specificity, and dose-response detection [16]. Specifically, individuals with high cotinine levels (≥3 ng/mL), indicative of active smoking, had 2.74 times higher odds of oral cancer-related conditions compared to those with low cotinine levels (<0.05 ng/mL), even after adjusting for various confounding factors. Medium cotinine levels (0.05-3 ng/mL), which may reflect secondhand smoke exposure, showed a positive but statistically non-significant association with oral cancer risk. These findings support our hypothesis that high serum cotinine levels are associated with an increased risk of oral cancer-related conditions in the US population.

The study revealed significant effect modification by age, gender, and race/ethnicity, suggesting that the association between cotinine levels and oral cancer risk varies across different demographic subgroups. Our findings highlight demographic disparities, particularly elevated cotinine levels among non-Hispanic Black participants despite similar or lower reported smoking rates. This aligns with prior evidence of racial/ethnic variation in nicotine metabolism (e.g., CYP2A6 polymorphisms) and underscores the influence of both biology and social determinants on exposure [17,18].

Age-specific patterns showed strongest associations among younger cohorts, with 18-34 year-olds experiencing 2.58 times higher odds (95% CI: 1.24 - 5.39) and 35-49 year-olds showing 2.85 times higher odds (95% CI: 1.75 - 4.62), while no significant associations were observed in those ≥65 years. Gender analysis revealed females exhibited stronger associations (OR=2.90) compared to males (OR=1.99). Racial/ethnic differences were pronounced, with Non-Hispanic Blacks demonstrating the highest risk (OR=2.66, 95% CI: 1.09 - 6.50), followed by significant associations among Hispanics (OR=1.85, 95% CI: 1.19 - 2.88) and Non-Hispanic Whites (OR=2.62, 95% CI: 2.01 - 3.42). These findings mirror international patterns, including gender-specific oral cancer development in India where women develop oral cancer earlier despite lower tobacco use [19], and align with disproportionately high oral cancer mortality rates among Non-Hispanic Blacks in the U.S., even

after adjusting for socioeconomic factors [20]. This finding highlights the importance of considering demographic factors when assessing tobacco-related oral cancer risk and developing targeted interventions.

Cotinine's strength lies in reducing misclassification, which often obscures associations in self-reported data, especially across racial, ethnic, and lower socioeconomic groups [21]. The use of serum cotinine as an objective measure of tobacco exposure provides a more accurate assessment of exposure compared to self-reported smoking status, which may be subject to recall bias, social desirability bias, or misclassification. Incorporating biomarkers into surveillance improves accuracy for oral cancer and other tobacco-related diseases.

The study's findings are consistent with previous research on the association between tobacco exposure and oral cancer risk. Wu et al. (2025) conducted a systematic analysis showing that smokeless tobacco is strongly linked to oral cancer. Our study supports this association by demonstrating that serum cotinine levels, as an objective measure of tobacco exposure, are significantly associated with oral cancer-related conditions. Similarly, Gupta & Johnson (2014)[22] performed a systematic review and meta-analysis confirming a significant association between smokeless tobacco use and oral cancer, particularly in South Asia. Our study extends these findings to the US population using a continuous measure of tobacco exposure, providing further evidence of this association in a different geographical and cultural context.

Pontes et al. (2020)[23] found an association between serum cotinine levels and oral mucosal lesions in a South African population, which aligns with our findings of increased oral cancer risk with higher cotinine levels. Our study builds on this by examining a more comprehensive set of oral cancer-related conditions and exploring effect modification by demographic factors in a nationally representative US sample. The observed variations in the association across demographic subgroups are also consistent with previous research suggesting differential susceptibility to tobacco-related cancers.

We also confirm that tobacco synergizes with alcohol and poor oral health to substantially elevate oral cancer risk, consistent with mechanistic and epidemiologic studies [24]. These findings stress the need for integrated prevention strategies targeting high-risk groups, particularly those with multiple co-occurring risk factors.

Given the rising global burden of oral cancer, cotinine-based surveillance offers a valuable tool for identifying

disparities and guiding precision prevention and public health policy. The demographic variations observed in this study underscore the need for tailored interventions that address both biological differences in nicotine metabolism and social determinants of health that contribute to differential tobacco exposure patterns. Future research should examine the mechanisms underlying these demographic disparities and evaluate the effectiveness of targeted prevention strategies in reducing oral cancer burden among high-risk populations.

Strengths & limitations

Strengths: Use of NHANES, a nationally representative dataset. Objective biomarker measurement of tobacco exposure. Dose-response and subgroup analysis.

Limitations: Cross-sectional design precludes causal inference. Oral cancer outcomes were partly self-reported. Residual confounding (e.g., diet, genetics) may remain.

Conclusion

Serum cotinine is a reliable predictor of oral cancer risk, with clear dose response associations and disparities across racial/ethnic and socioeconomic groups. Biomarker-based assessments should complement self-report for accurate exposure monitoring.

Implications for public health and future research

Integration of cotinine testing into population surveys can improve risk prediction models. Interventions must consider racial/ethnic metabolic differences and socioeconomic disparities. Longitudinal studies are needed to confirm causal pathways and refine biomarker thresholds for oral cancer prevention.

Ethical clearance statement

The NHANES protocol was approved by the NCHS Research Ethics Review Board, and all participants provided informed consent. As the present study used publicly available, de-identified data, it was exempt from further IRB review.

Acknowledgements

Data source

This study utilized data from the National Health and Nutrition Examination Survey (NHANES) 2013-2014 cycle [25]. NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States, combining interviews and physical examinations. It is conducted by the National Center for Health Statistics (NCHS), part of the Centers for Disease Control and Prevention (CDC). The survey examines a nationally representative sample of approximately 5,000 individuals each year, with data released in two-year cycles.
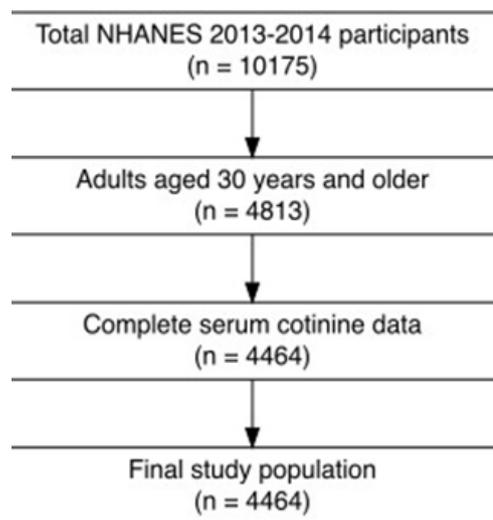
Supplementary Figures

Fig S1: Strobe Diagram

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