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Original Research

Volatile organic compounds and mortality from ischemic heart disease: A case-cohort study

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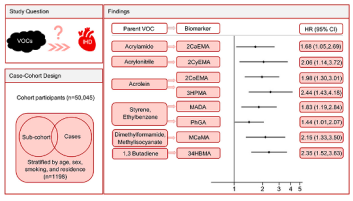
GRAPHICAL ABSTRACT

Associations of urinary volatile organic compound biomarkers (3rd vs. 1st tertiles) with ischemic heart disease mortality in the Golestan Cohort Study, a case-cohort analysis. Abbreviations: VOC, volatile organic compound; IHD, ischemic heart disease; HR, hazard ratio; CI, confidence interval; 2CaEMA, N-Acetyl-S-(2-carbamoylethyl)-L-cysteine; 2CyEMA, N-Acetyl-S-(2-cyanoethyl)-L-cysteine;2CoEMA, N-Acetyl-S- (2-carboxyethyl)-L-cysteine; 3HPMA, N-Acetyl-S- (3-hydroxypropyl)-L-cysteine; MADA, Mandelic acid; PhGA, Phenylglyoxylic acid; MCaMA, N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine; 34HBMA, N-Acetyl-S- (3,4-dihydroxybutyl)-L-cysteine.



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ABSTRACT

Background: Volatile organic compounds (VOCs) are major components of air pollution and tobacco smoke, two known risk factors for cardiovascular diseases. VOCs are ubiquitous in the environment and originate from a wide range of sources, including the burning of biomass, fossil fuels, and consumer products. Direct evidence for

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associations between specific VOCs and ischemic heart disease (IHD) mortality in the general population is scarce.

Methods: In a case-cohort study (stratified by age groups, sex, residence, and tobacco smoking), nested within the population-based Golestan cohort study (n = 50,045, 40–75 years, 58% women, enrollment: 2004–2008) in northeastern Iran, we measured urinary concentrations of 20 smoking-related VOC biomarkers using ultra high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. We calculated hazard ratio (HR) and 95% confidence interval (CI) for their associations with IHD mortality during follow-up to 2018, using Cox regression models adjusted for age, ethnicity, education, marital status, body mass index, physical activity, wealth, and urinary cotinine.

Results: There were 575 non-cases from random subcohort and 601 participants who died from IHD, mean (standard deviation) age, 58.2 (9.3) years, with a median of 8.4 years follow-up. Significant associations [3rd vs. 1st tertile, HR (95% CI), P for trend] were observed between biomarkers of acrylamide [1.68(1.05,2.69), 0.025], acrylonitrile [2.06(1.14,3.72), 0.058], acrolein [1.98(1.30,3.01), 0.003 and 2.44(1.43,4.18), 0.002], styrene/ethylbenzene [1.83(1.19,2.84), 0.007 and 1.44(1.01,2.07), 0.046], dimethylformamide/methylisocyanate [2.15 (1.33,3.50), 0.001], and 1,3butadiene [2.35(1.52,3.63),<0.001] and IHD mortality. These associations were independent of tobacco smoking, and they were only present in the non-smoking subgroup.

Conclusion: Our findings provide direct evidence for associations between exposure to several VOCs with widespread household and commercial use and IHD mortality many years after these exposures. These results highlight the importance of VOC exposure in the general population as a risk factor for cardiovascular diseases and underline the importance of bio-monitoring non-tobacco VOC exposure.

1. Introduction

Cardiovascular diseases remain the leading causes of premature mortality worldwide, with approximately 17.9 million deaths each year [1]. The Global Burden of Disease study estimated that ischemic heart disease (IHD), with 315.4 million prevalent cases and 9.2 million deaths, was the main cause of the cardiovascular disease burden in 2022 [2]. Pollution exposure and tobacco use (i.e., smoked, second-hand, and chewing) were responsible for approximately 5.5 and 3.2 million cardiovascular deaths worldwide in 2019—61.9% of all pollution-related deaths and 36.7% of all tobacco-related deaths, respectively [3-5]. As products of burning organic material, tobacco smoke and pollutants may share underlying toxic compounds and mechanisms leading to cardiovascular diseases. Identifying these mechanisms can potentially improve risk stratification, prevention, and early detection of cardiovascular diseases.

Volatile organic compounds (VOCs), a major component of air pollution and tobacco smoke, originate from a wide range of natural and anthropogenic sources, including the burning of biomass, fossil fuels, and consumer products [6]. VOCs are ubiquitous in the environment and may play crucial roles in human health [4,6]. Some VOCs, such as acrolein and benzene, are among cardiovascular toxicants listed by the U.S. Food and Drug Administration (FDA) as harmful and potentially harmful chemicals in tobacco smoke [7]. VOCs also contribute to fine particulate matter and tropospheric ozone formation [6].

Despite the well-documented cardiotoxic effects of air pollution, specifically fine particulate matter [4], the impact of specific gaseous pollutants, including VOCs, on cardiovascular risk remains unclear [6, 8]. This information gap is likely due to incomplete VOC emission inventories and problems with quantifying them [6]. Moreover, the health consequences of many VOCs have not been assessed in prospective epidemiologic studies [8]. Prospective studies can identify biologically relevant VOC exposure occurring years before health outcomes, helping establish causal relationships. Previous studies on the cardiovascular effects of specific VOCs have primarily focused on estimated environmental VOC levels in specific occupational settings with excessively high VOC levels and limited generalizability [9-11]. Compared with environmental VOC estimates, using specific and sensitive bio-monitoring methods for personal VOC exposure assessment is superior, as it takes all VOC sources and individual variations in absorption and metabolism into account [9]. Urinary VOC biomarkers, with longer physiological half-lives and easily collected samples, offer advantages over VOCs in blood and breath [8,9]. However, only a few large prospective cohort studies have collected the necessary urine samples for such analyses [12].

The Golestan Cohort Study (GCS) collected baseline and repeat urine samples from a large sample of the general population of Golestan Province, Iran. Comparing two urine samples taken 5 years apart from the same individuals, we previously showed that many VOC biomarkers could be useful for exposure assessment in longitudinal studies [13]. In the present study, we evaluated the association between 20 smoking-related urinary VOC biomarkers (Table 1) in baseline urine with IHD mortality in the GCS.

2. Methods

2.1. Study design and setting

To evaluate the associations between individual VOC biomarkers and risk of different chronic diseases, including IHD, a collaboration was established between the FDA Center for Tobacco Products (CTP/FDA), the Division of Laboratory Sciences of the National Center for Environmental Health at the Centers for Disease Control and Prevention (CDC), the Division of Cancer Epidemiology and Genetics at the National Cancer Institute (NCI), and the Digestive Disease Research Institute of Tehran University of Medical Sciences (DDRI). In the current study, we conducted a stratified case-cohort analysis nested in the GCS. Details of the GCS have been described previously [14]. In brief, the study recruited a total of 50,045 people, 58% women, aged 40–75 years during 2004–2008 in northeastern Iran. At baseline, demographic, lifestyle, and medical information were collected along with a spot urine sample of all participants. Since then, the participants have been actively followed annually for cause-specific mortality and other outcomes. For this case-cohort study, the eligible cases were participants who died from IHD during follow-up until Jan 1, 2018 (n = 1226). Cause-specific IHD mortality rate in the GCS, during a median (25th-75th percentiles) follow-up of 11.0 (9.8–11.2) years, was 248.9 per 100,000 person-years. Among these cases, we included all individuals who smoked tobacco (n = 202); and a random sample of 407 cases from the 1024 who did not smoke tobacco at the time of enrollment. Cases were stratified into 16 strata defined by age (<55, ≥55 years), sex (male, female), place of residence (rural, urban), and current smoking status (yes, no). As the reference subcohort, we selected a random sample of 610 individuals, in the 16 strata described above, from all cohort participants with available urine samples, regardless of IHD death during follow-up (**Table S1**). We excluded participants with urine creatinine values outside the plausible range of 10–370 mg/dL. Finally, the data of 597 reference subcohort (including 575 non-cases)

Table 1
List of urinary biomarkers of volatile organic compounds.

Full compound name	Parent compounds	Analyte Code
2-Methylhippuric acid	Xylene	2MHA
3-Methylhippuric acid + 4-Methylhippuric acid	Xylene	3MHA+4MHA
N-Acetyl-S-(2-carbamoylethyl)-L-cysteine	Acrylamide	2CaEMA
N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	Acrylamide	2CaHEMA
N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	Acrylonitrile	1CyHEMA
N-Acetyl-S-(2-cyanoethyl)-L-cysteine	Acrylonitrile	2CyEMA
N-Acetyl-S-(2-hydroxyethyl)-L-cysteine	1,2-Dibromoethane, Vinyl chloride, Acrylonitrile, Ethylene oxide	2HEMA
N-Acetyl-S- (2-carboxyethyl)-L-cysteine	Acrolein	2CoEMA
N-Acetyl-S- (3-hydroxypropyl)-L-cysteine	Acrolein	3HPMA
N-Acetyl-S-(benzyl)-L-cysteine	Toluene, benzyl alcohol	BzMA
Mandelic acid	Styrene, ethylbenzene	MADA
Phenylglyoxylic acid	Styrene, ethylbenzene	PhGA
N-Acetyl-S-(phenyl)-L-cysteine	Benzene	PhMA
N-Acetyl-S- (2-hydroxypropyl)-L-cysteine	Propylene oxide	2HPMA
N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	N,N-Dimethylformamide, methylisocyanate	MCaMA
N-Acetyl-S- (3,4-dihydroxybutyl)-L-cysteine	1,3 Butadiene	34HBMA
N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	1,3 Butadiene	4HBeMA
N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	Crotonaldehyde	3HMPMA
N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	Isoprene	4HMBeMA
2-Thioxothiazolidine-4-carboxylic acid	Carbon-disulfide	TTCA

and 601 participants who died from IHD were included in the study.

All participants provided a written informed consent. The GCS protocol was approved by the ethical review committees of the DDRI, the NCI, and the International Agency for Research on Cancer (IARC).

2.2. Covariate assessment

Data on demographics and medical history, including age, sex, ethnicity, marital status, residential place, education, and lifestyle were collected by trained interviewers. The GCS questionnaire included detailed questions about potential sources of VOC biomarkers including tobacco and opiates [15]. Active use (smoked or oral) of tobacco, including chewing tobacco (nass), or opiates at least once a week for six months was considered as regular tobacco or opiate use, respectively. Former tobacco or opiate use included those who quit more than one year before cohort baseline. In two validation studies, there was excellent agreement between self-reported tobacco use and urinary cotinine [13], and between self-reported opiate use and urinary metabolites (i.e., codeine and morphine) [16]. A wealth score was calculated from appliance ownership, using multiple correspondence analysis [17]. Data on the intensity, duration, and frequency of past-year recreation and work physical activities were recorded and the metabolic equivalents (METs.min/week) were calculated. Body weight and height were measured using standard protocols. The body-mass index (BMI) was calculated as weight (in kg) divided by the squared height (in meters).

2.3. Urinary biomarker assays

Spot urine samples were collected at the time of interview, and stored at −20 °C before long-term storage at −80 °C. In a pilot study, 320 urine samples collected from 225 participants (95 participants had two samples from baseline cohort and 5 years later—the repeated measurement phase of the GCS) were evaluated by the Division of Laboratory Sciences of the National Center for Environmental Health (NCEH) at the CDC, Atlanta, GA. This pilot showed excellent validity of self-reported tobacco use and showed that urinary biomarkers were reliable for exposure assessment in longitudinal studies [13]. Biomarkers used in the current study consisted of the 20 VOC biomarkers tested in the pilot study and they were measured in the same CDC laboratory (Table 1). Urinary VOC biomarkers were evaluated using ultra high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry [9]. The limit of detection (LOD) for these VOC biomarkers ranged from 0.5 to 13 ng/mL, in our study (**Table S2).** We included masked vials containing pooled quality control samples randomly placed in the batches sent to the laboratory, and we used these samples to calculate assay coefficients of variation (CV). As shown in Table S2, all the assays had CVs below 15%, except for the 4HMBeMA biomarker which had a CV of 17%. We also measured urinary cotinine concentrations using a modified version of isotope dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry [18]. The LOD for cotinine was 0.03 ng/mL. Urine creatinine was measured using a commercial automated, colorimetric enzymatic (creatinase) method implemented on a Roche/Hitachi cobas 6000 Analyzer.

2.4. Follow-up and outcome ascertainment

In the GCS, trained interviewers actively follow all participants annually by phone calls or home visits and record their vital status. In the case of death, the team gathers copies of all available and relevant medical documents and, if needed, completes a verbal autopsy. Using all gathered information, two internists determine the cause of death independently, based on the International Classification of Diseases 10th version (ICD-10) codes. In case of disagreement, a third senior internist reviews all documents and the two initial diagnoses to make the final diagnosis. For the present study, follow-up continued until death, loss to follow-up, or January 1, 2018, whichever came first, and we considered ICD-10 codes of I20-I25 as IHD death.

2.5. Statistical methods

Biomarker concentrations below the LOD were replaced by the LOD divided by the square root of 2. Then all biomarker concentrations were divided by urinary creatinine to adjust for urinary dilution. After calculating sampling fractions in all 32 strata of case and subcohort groups, the participants in each stratum were weighted by the inverse of the sampling fractions (inverse probability weighting). We estimated weighted geometric means (GM) and 95% confidence intervals (95% CI) of VOC biomarkers. Creatinine-corrected biomarker concentrations were categorized into tertiles, with low levels of biomarker concentrations (including those below LOD) classified as the first (reference) tertile. We calculated hazard ratio (HR) and 95% CI for the associations between biomarker tertiles and IHD mortality using weighted Cox proportional hazard models. P for trend tests were calculated across biomarker tertiles using the median of tertile categories. We used stratified design to account for the potential roles of stratifying variables (age groups, sex, residential place, and self-reported tobacco smoking) in exposure-outcome associations. The models were also adjusted for age (continuous in years), ethnicity (Turkmen/non-Turkmen), years of schooling (0, ≤5, >5 years), marital status (married/non-married), BMI (continuous), physical activity (tertiles), wealth score (tertiles), nass use (never/ past/current), enrolment year, and urinary cotinine (μg/g

creatinine; continuous). Cotinine is a specific nicotine metabolite and widely used as a biomarker of recent exposure to tobacco (smoked, secondhand, and chewing) [19]. The proportional hazard assumption was evaluated by testing variable interactions with time. In cases of the assumption violation, the stratified Cox regression (stratified on the violating covariate) was used.

We also constructed sensitivity and subgroup analyses. In a sensitivity analysis, we used non-creatinine-corrected biomarker concentrations and included creatinine as a covariate in the models to re-evaluate our findings with another common method of urinary biomarker studies as opposed to direct creatinine correction. We also excluded nass users to eliminate the potential effect of oral tobacco use in the results. We analyzed the associations in subgroups of people who reported current tobacco smoking (smoking subgroup) and those who did not report current tobacco smoking (non-smoking subgroup). In another sensitivity analysis, we defined the non-smoking subgroup based on the self-report combined with a urinary cotinine cutoff to reduce the potential misclassification bias. Therefore, we excluded individuals whose urinary cotinine was above 100 μg/g creatinine [20], except for current nass users because chewing tobacco increases urinary cotinine [13].

There were no missing values for VOC biomarkers in our study, except for 2CaHEMA and MADA in 4 and 1 urine samples, respectively. Physical activity levels were missing in 47 cases and 25 non-cases, so we used separate missing indicators to keep them in the models. A two-sided p-value of less than 0.05 or 95% CI not including one were considered to indicate statistical significance. All statistical analyses were conducted with the Stata statistical software, version 12 (StataCorp, College Station, TX).

3. Results

The 601 case and 575 non-case participants had a total of 8707 person-years of follow-up, with a median (25th-75th percentiles) of 8.4 (4–11) years. The mean (standard deviation) age of these participants was 58.24 (9.3) years and 443 (37.7%) were women. Most participants (75.4%) were rural residents, 69.9% were Turkmen, 84.5% were married, and 71.8% had no formal education. Current smoking was reported by 33.2%, current opiate use by 28.1%, and current nass use by 10.4% of participants. The cases were slightly older than non-cases, and were more likely to have high BMI, no formal schooling, low physical activity, and current opiate use. Individuals in smoking subgroup were more likely to be younger, male, married, more educated, current users of opiates, and have lower BMI than participants of non-smoking subgroup (Table 2).

Table 3 shows the weighted GM and 95% CI of VOC biomarkers, based on smoking status, in both case and non-case groups. Concentrations of almost all measured biomarkers tended to be higher in smoking subgroup than non-smoking subgroup, except for BzMA and TTCA. In **Table S3,** these two groups are further subdivided by whether or not they used opiates. Dual users of opiates and tobacco smoking was associated with the highest concentrations of almost all biomarkers except for BzMA, PhGA, and TTCA. In addition, individuals who exclusively used opiates had higher concentrations of 2CaEMA and 2CaHEMA (acrylamide biomarkers) than those who exclusively smoked tobacco. As **Table S4** shows, nass use did not impact VOC biomarker concentrations, although it was strongly associated with urinary cotinine (**Table S5**).

Table 4 shows the associations between VOC biomarker tertiles and IHD mortality. We observed the associations [3rd vs. 1st tertile, HR (95% CI), P for trend] for biomarkers of acrylamide [2CaEMA, 1.68 (1.05,2.69), 0.025], acrylonitrile [2CyEMA, 2.06 (1.14,3.72), 0.058], acrolein [2CoEMA, 1.98 (1.30,3.01), 0.003 and 3HPMA, 2.44 (1.43,4.18), 0.002], styrene/ethylbenzene [MADA, 1.83 (1.19,2.84), 0.007 and PhGA, 1.44 (1.01,2.07), 0.046], dimethylformamide (DMF)/methylisocyanate [MCaMA, 2.15 (1.33,3.50), 0.001], and 1,3butadiene [34HBMA, 2.35 (1.52,3.63), <0.001]. These associations persisted in the sensitivity analyses (**Table S6**).

We also examined our findings in the non-smoking and smoking subgroups, separately (Table 5). In the non-smoking subgroup, increased risk of IHD mortality [3rd vs. 1st tertile, HR (95% CI)] was associated with the biomarkers of acrylamide [2CaEMA, 2.02 (1.19,3.44)], acrylonitrile [1CyHEMA, 7.87 (1.93,32.18) and 2CyEMA,

Table 2
Baseline characteristics of the cases and non-cases by tobacco smoking status.

	Cases			Non-cases		
	All (n = 601)	Smoking (n = 201)	Non-smoking (n = 400)	All (n = 575)	Smoking (n = 190)	Non-smoking (n = 385)
Age (years)	59.03 (9.1)	57.51 (9.4)	59.80 (8.9)	57.41 (9.4)	56.03 (9.7)	58.09 (9.2)
Sex, women	224 (37.3)	18 (9.0)	206 (51.5)	219 (38.1)	21 (11.1)	198 (51.4)
Ethnicity, Turkmen	430 (71.6)	145 (72.1)	285 (71.3)	392 (68.2)	133 (70.0)	259 (67.3)
Residence, rural	455 (75.7)	150 (74.6)	305 (76.3)	432 (75.1)	140 (73.4)	292 (75.8)
Marital status, married	504 (83.9)	181 (90.1)	323 (80.8)	490 (85.2)	175 (92.1)	315 (81.8)
Body-mass index (kg/m^2)	26.17 (5.4)	24.26 (4.5)	27.13 (5.5)	25.15 (5.3)	23.51 (5.1)	25.95 (5.2)
Education (years)						
No	446 (74.2)	129 (64.2)	317 (79.3)	398 (69.2)	109 (57.4)	289 (75.1)
≤5 years	93 (15.5)	40 (19.9)	53 (13.3)	96 (16.7)	44 (23.2)	52 (13.5)
>5 years	62 (10.3)	32 (15.9)	30 (7.5)	81 (14.1)	37 (19.5)	44 (11.4)
Wealth score						
1st tertile	249 (41.4)	84 (41.8)	165 (41.3)	236 (41.0)	74 (39.0)	162 (42.1)
2nd tertile	176 (29.3)	56 (27.9)	120 (30.0)	157 (27.3)	54 (28.4)	103 (26.8)
3rd tertile	176 (29.3)	61 (30.4)	115 (28.8)	182 (31.7)	62 (32.6)	120 (31.2)
Physical activity						
1st tertile	284 (47.3)	82 (40.8)	202 (50.5)	199 (34.6)	51 (26.8)	148 (38.4)
2nd tertile	125 (20.8)	37 (18.4)	88 (22.0)	163 (28.4)	50 (26.3)	113 (29.4)
3rd tertile	145 (24.1)	72 (35.8)	73 (18.3)	188 (32.7)	82 (43.2)	106 (27.5)
unknown	47 (7.8)	10 (5.0)	37 (9.3)	25(4.4)	7 (3.7)	18 (4.7)
Opiate use						
Never users	383 (63.7)	92 (45.8)	291 (72.8)	414 (72.0)	85 (44.7)	329 (85.5)
Past users	24 (4.0)	13 (6.5)	11 (2.8)	24 (4.2)	10 (5.3)	14 (3.6)
Current users	194 (32.3)	96 (47.8)	98 (24.5)	137 (23.8)	95 (50.0)	42 (10.9)
Nass use						
Never users	516 (85.9)	171 (85.1)	345 (86.3)	512 (89.0)	167 (87.9)	345 (89.6)
Past users	10 (1.7)	5 (2.5)	5 (1.3)	16 (2.8)	5 (2.6)	11 (2.9)
Current users	75 (12.5)	25 (12.4)	50 (12.5)	47 (8.2)	18 (9.5)	29 (7.5)

Data are mean (standard deviation) or n (%).

Table 3
Volatile organic compound biomarkers (μg/g creatinine) in cases and non-cases stratified by tobacco smoking.

	Cases		Non-cases	
	Smoking	Non-smoking	Smoking	Non-smoking
2MHA	178.07 (155.96,203.32)	83.13 (74.34,92.96)	166.56 (141.70,195.78)	87.81 (75.43,102.22)
3MHA+4MHA	797.54 (696.46,913.30)	319.86 (288.83,354.22)	755.59 (640.65,891.15)	318.90 (275.86,368.64)
2CaEMA	152.17 (133.97,172.84)	65.02 (59.54,71.00)	155.17 (130.61,184.35)	53.99 (48.90,59.61)
2CaHEMA	18.83 (16.90,20.97)	12.22 (11.33,13.17)	20.91 (18.11,24.13)	11.03 (10.06,12.09)
1CyHEMA	15.06 (12.00,18.91)	1.34 (1.18,1.52)	16.64 (12.90,21.47)	1.00 (0.89,1.13)
2CyEMA	74.23 (59.77,92.18)	2.76 (2.35,3.24)	76.68 (59.92,98.13)	1.81 (1.53,2.13)
2HEMA	3.49 (3.02,4.03)	1.50 (1.38,1.63)	3.67 (3.04,4.43)	1.73 (1.57,1.90)
2CoEMA	215.51 (194.51,238.77)	105.79 (99.12,112.91)	186.12 (164.36,210.76)	80.27 (73.69,87.43)
3HPMA	980.47 (849.14,1132.10)	240.45 (221.30,261.25)	922.76 (782.98,1087.50)	184.67 (166.79,204.46)
BzMA	5.98 (5.32,6.72)	6.29 (5.69,6.95)	5.51 (4.83,6.27)	5.65 (5.06,6.31)
MADA	399.17 (369.32,431.43)	236.04 (223.29,249.52)	388.26 (349.36,431.50)	207.63 (193.37,222.95)
PhGA	124.98 (108.23,144.31)	113.04 (102.91,124.17)	103.65 (85.28,125.98)	91.18 (80.91,102.75)
PhMA	1.66 (1.51,1.83)	1.14 (1.06,1.23)	1.53 (1.37,1.71)	1.11 (1.01,1.22)
2HPMA	52.74 (47.14,59.00)	26.82 (25.18,28.57)	50.91 (44.94,57.69)	26.87 (24.51,29.46)
MCaMA	398.52 (348.95,455.14)	147.04 (133.74,161.66)	397.01 (331.50,475.48)	107.72 (96.56,120.18)
34HBMA	423.38 (392.35,456.86)	337.21 (320.13,355.21)	407.56 (381.22,435.71)	300.53 (279.14,323.56)
4HBeMA	25.87 (22.09,30.31)	5.56 (5.15,5.99)	24.31 (20.68,28.58)	4.82 (4.37,5.31)
3HMPMA	881.27 (759.24,1022.91)	248.25 (229.62,268.39)	886.06 (767.47,1022.96)	209.67 (190.07,231.30)
4HMBeMA	24.27 (19.81,29.74)	3.09 (2.84,3.37)	22.49 (17.56,28.81)	2.48 (2.28,2.69)
TTCA	11.87 (10.17,13.86)	12.34 (11.07,13.76)	11.31 (9.43,13.57)	11.70 (10.32,13.26)

Data are weighted geometric means (95% confidence intervals). Abbreviations: 2MHA, 2-Methylhippuric acid; 3MHA+4MHA, 3-Methylhippuric acid and 4-Methylhippuric acid; 2CaEMA, N-Acetyl-S-(2-carbamoylethyl)-L-cysteine; 2CaHEMA, N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; 1CyHEMA, N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine; 2CyEMA, N-Acetyl-S-(2-cyanoethyl)-L-cysteine; 2HEMA, N-Acetyl-S-(2-hydroxyethyl)-L-cysteine; 2CoEMA, N-Acetyl-S- (2-carboxyethyl)-L-cysteine; 3HPMA, N-Acetyl-S- (3-hydroxypropyl)-L-cysteine; BzMA, N-Acetyl-S-(benzyl)-L-cysteine; MADA, Mandelic acid; PhGA, Phenylglyoxylic acid; PhMA, N-Acetyl-S-(phenyl)-L-cysteine; 2HPMA, N-Acetyl-S- (2-hydroxypropyl)-L-cysteine; MCaMA, N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine; 34HBMA, N-Acetyl-S- (3,4-dihydroxybutyl)-L-cysteine; 4HBeMA, N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine; 3HMPMA, N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine; 4HMBeMA, N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine; TTCA, 2-Thioxothiazolidine-4-carboxylic acid.

2.54 (1.29,5.02)], acrolein [2CoEMA, 2.11 (1.31,3.40) and 3HPMA, 2.99 (1.60,5.59)], styrene/ethylbenzene [MADA, 1.94 (1.18,3.20) and PhGA, 1.54 (1.00,2.37)], DMF/methylisocyanate [MCaMA, 2.56 (1.49,4.40)], and 1,3butadiene [34HBMA, 2.49(1.50,4.15)]. In the sensitivity analysis, when we restricted non-smoking subgroup based on the urinary cotinine level, these associations remained unchanged (**Table S6**). In the smoking subgroup, none of the associations between biomarkers and the outcome were statistically significant (Table 5).

4. Discussion

In this population-based case-cohort study, urinary biomarkers of acrylamide (2CaEMA), acrylonitrile (2CyEMA), acrolein (2CoEMA and 3HPMA), styrene/ethylbenzene (MADA and PhGA), DMF/methylisocyanate (MCaMA), and 1,3butadiene (34HBMA) were associated with IHD mortality. These associations were independent of tobacco smoking. In the tobacco smoking subgroup, such associations were not detected.

All the significant associations we observed were among participants who did not smoke tobacco. Although we showed that people who use combustible tobacco are exposed to higher concentration of VOCs, the quantitative evaluation of an individual VOC effect among people who smoke tobacco is complicated due to co-exposure to a complex mixture of thousands constituents with known and unknown cardiovascular consequences [21]. In fact, other smoke-related toxins, such as hydrogen cyanide, arsenic, and carbon monoxide, are important contributors to the cardiovascular risk [21], and these powerful predictors may mask other associations in the smoking subgroup. Our findings suggest that specific VOCs may contribute to the risk of IHD mortality, independent of tobacco smoke.

One of the most consistent associations in our study was between acrolein metabolites and IHD mortality. These findings are consistent with other studies that demonstrated acrolein exposure was associated with increased cardiovascular risk [8,22]. A cross-sectional study among people who did not smoke tobacco found a significant association between 3HPMA and high systolic blood pressure and endothelial dysfunction [8]. Another cross-sectional study demonstrated that acrolein exposure from tobacco smoke as well as non-tobacco sources was associated with increased cardiovascular risk among U.S. adults [22]. In line with our results, these researchers concluded that acrolein exposure was associated with increased cardiovascular risk regardless of its sources. Results in our study, set in a very different population, support this conclusion. Acrolein may be involved in atherosclerotic lesion initiation and progression via mechanisms including oxidative stress, inflammation, and matrix metalloproteinase activation. Acrolein could also affect plaque instability, platelet activation, and thrombus formation often resulting in myocardial infarction and stroke [23]. Acrolein is formed mainly from incomplete combustion of biomass and cooking of foods and is classified by IARC as probable human carcinogen (IARC group 2A) [24].

Styrene and ethylbenzene are the monomers of polystyrene and polyethylene, respectively, and are used in the manufacturing of plastics and synthetic rubbers. Occupational exposures and cigarette smoking are the main sources of human exposure. Although they are known hazardous air pollutants, their adverse cardiovascular effects have not been well established [10]. An occupational case-cohort study indicated that environmental styrene in rubber-manufacturing plants was associated with an elevated risk of IHD mortality, but little is known about the cardiovascular toxicity of exposure to low level of styrene in the general population [25]. A recent prospective study indicated that blood levels of ethylbenzene was associated with total and heart disease mortality among U.S. adults [26]. In line with these findings, we showed that both urinary biomarkers of ethylbenzene and styrene were associated with IHD mortality in the general population. Another study among U.S. participants indicated the association of blood level of ethylbenzene with dyslipidemia and heart attack, but not coronary artery disease. The cross-sectional design and self-reported outcome report were some limitations of this study [27]. Heart rate variability alteration has been suggested as a mechanism of their cardiotoxicity [10].

1,3Butadiene mainly originates from combustion products (e.g. motor vehicle emissions, burning of rubber and plastic, and tobacco smoke). The highest exposure to 1,3butadiene occurs in occupational

Table 4
Association of urinary volatile organic compound biomarkers with ischemic heart disease mortality.

	2nd vs. 1st tertile	3rd vs 1st tertile	P trend
2MHA	1.20 (0.83,1.73)	0.95 (0.65,1.38)	0.594
3MHA+4MHA	1.01 (0.71,1.44)	0.99 (0.67,1.47)	0.958
2CaEMA	1.13 (0.79,1.62)	1.68 (1.05,2.69)	0.025
2CaHEMA	1.00 (0.69,1.46)	1.15 (0.75,1.78)	0.522
1CyHEMA	1.38 (0.88,2.16)	1.99 (0.94,4.23)	0.072
2CyEMA	1.42 (0.99,2.02)	2.06 (1.14,3.72)	0.058
2HEMA	0.52 (0.36,0.74)	0.64 (0.43,0.96)	0.125
2CoEMA	1.70 (1.19,2.43)	1.98 (1.30,3.01)	0.003
3HPMA	1.34 (0.93,1.93)	2.44 (1.43,4.18)	0.002
BzMA	0.94 (0.65,1.37)	0.94 (0.66,1.36)	0.800
MADA	1.49 (1.03,2.16)	1.83 (1.19,2.84)	0.007
PhGA	1.14 (0.78,1.65)	1.44 (1.01,2.07)	0.046
PhMA	0.91 (0.65,1.28)	1.39 (0.94,2.04)	0.092
2HPMA	0.91 (0.64,1.30)	1.13 (0.74,1.71)	0.468
MCaMA	0.99 (0.69,1.43)	2.15 (1.33,3.50)	0.001
34HBMA	1.19 (0.82,1.72)	2.35 (1.52,3.63)	<0.001
4HBeMA	1.22 (0.85,1.75)	1.13 (0.70,1.84)	0.790
3HMPMA	1.17 (0.83,1.65)	1.41 (0.88,2.26)	0.178
4HMBeMA	1.37 (0.97,1.95)	1.32 (0.78,2.22)	0.575
TTCA	0.59 (0.35,1.00)	1.14 (0.68,1.89)	0.527

Data are weighted hazard ratios with 95% confidence intervals stratified by age groups, sex, residence, and smoking and adjusted for age (years), ethnicity, education, marital status, body-mass index, physical activity, wealth score, nass use, enrolment year, and cotinine. Abbreviations: 2MHA, 2-Methylhippuric acid; 3MHA+4MHA, 3-Methylhippuric acid and 4-Methylhippuric acid; 2CaEMA, N-Acetyl-S-(2-carbamoylethyl)-L-cysteine; 2CaHEMA, N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; 1CyHEMA, N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine; 2CyEMA, N-Acetyl-S-(2-cyanoethyl)-L-cysteine; 2HEMA, N-Acetyl-S-(2-hydroxyethyl)-L-cysteine; 2CoEMA, N-Acetyl-S- (2-carboxyethyl)-L-cysteine; 3HPMA, N-Acetyl-S- (3-hydroxypropyl)-L-cysteine; BzMA, N-Acetyl-S-(benzyl)-L-cysteine; MADA, Mandelic acid; PhGA, Phenylglyoxylic acid; PhMA, N-Acetyl-S-(phenyl)-L-cysteine; 2HPMA, N-Acetyl-S- (2-hydroxypropyl)-L-cysteine; MCaMA, N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine; 34HBMA, N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine; 4HBeMA, N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine; 3HMPMA, N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine; 4HMBeMA, N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine; TTCA, 2-Thioxothiazolidine-4-carboxylic acid.

Table 5
Association of urinary volatile organic compound biomarkers with ischemic heart disease mortality based on the tobacco smoking status.

	Non-smoking subgroup (n = 785)		Smoking subgroup (n = 391)	
	2nd vs. 1st tertile	3rd vs 1st tertile	2nd vs. 1st tertile	3rd vs 1st tertile
2MHA	1.25 (0.83,1.89)	0.88 (0.57,1.35)	0.97 (0.41,2.32)	1.16 (0.49,2.73)
3MHA+4MHA	1.10 (0.75,1.62)	0.97 (0.61,1.52)	0.35 (0.14,0.92)	0.54 (0.21,1.37)
2CaEMA	1.16 (0.79,1.70)	2.02 (1.19,3.44)	0.42 (0.15,1.13)	0.41 (0.16,1.06)
2CaHEMA	0.99 (0.65,1.52)	1.18 (0.66,2.12)	0.95 (0.46,1.95)	0.95 (0.51,1.76)
1CyHEMA	1.33 (0.80,2.21)	7.87 (1.93,32.18)	0.52 (0.22,1.27)	0.39 (0.16,0.95)
2CyEMA	1.44 (0.99,2.08)	2.54 (1.29,5.02)	0.18 (0.03,1.22)	0.13 (0.02,0.83)
2HEMA	0.53 (0.36,0.78)	0.60 (0.37,0.98)	0.42 (0.18,1.02)	0.66 (0.30,1.46)
2CoEMA	1.80 (1.22,2.64)	2.11 (1.31,3.40)	0.60 (0.22,1.60)	0.82 (0.36,1.87)
3HPMA	1.33 (0.91,1.96)	2.99 (1.60,5.59)	0.87 (0.31,2.47)	0.71 (0.27,1.86)
BzMA	0.94 (0.60,1.45)	0.97 (0.63,1.49)	0.99 (0.52,1.86)	0.84 (0.45,1.55)
MADA	1.51 (1.01,2.26)	1.94 (1.18,3.20)	0.99 (0.38,2.55)	1.08 (0.42,2.80)
PhGA	1.11 (0.73,1.71)	1.54 (1.00,2.37)	1.24 (0.64,2.41)	1.10 (0.61,1.99)
PhMA	0.87 (0.60,1.28)	1.28 (0.81,2.03)	1.22 (0.63,2.37)	1.85 (0.97,3.53)
2HPMA	0.90 (0.61,1.32)	1.18 (0.72,1.93)	0.86 (0.37,1.99)	0.74 (0.31,1.74)
MCaMA	0.97 (0.66,1.43)	2.56 (1.49,4.40)	0.76 (0.26,2.24)	0.73 (0.26,2.06)
34HBMA	1.25 (0.84,1.88)	2.49 (1.50,4.15)	0.59 (0.26,1.36)	1.23 (0.60,2.53)
4HBeMA	1.33 (0.91,1.94)	1.12 (0.62,2.03)	0.17 (0.05,0.57)	0.29 (0.11,0.79)
3HMPMA	1.19 (0.83,1.71)	1.63 (0.95,2.79)	0.46 (0.15,1.35)	0.38 (0.14,1.02)
4HMBeMA	1.34 (0.93,1.93)	1.56 (0.85,2.84)	2.51 (0.77,8.20)	0.72 (0.28,1.83)
TTCA	0.49 (0.26,0.92)	1.16 (0.63,2.14)	1.27 (0.53,3.02)	1.00 (0.43,2.34)

Data are weighted hazard ratios with 95% confidence intervals stratified by age groups, sex, residence, and smoking and adjusted for age (years), ethnicity, education, marital status, body-mass index, physical activity, wealth score, nass use, enrolment year, and cotinine. Abbreviations: 2MHA, 2-Methylhippuric acid; 3MHA+4MHA, 3-Methylhippuric acid and 4-Methylhippuric acid; 2CaEMA, N-Acetyl-S-(2-carbamoylethyl)-L-cysteine; 2CaHEMA, N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; 1CyHEMA, N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine; 2CyEMA, N-Acetyl-S-(2-cyanoethyl)-L-cysteine; 2HEMA, N-Acetyl-S-(2-hydroxyethyl)-L-cysteine; 2CoEMA, N-Acetyl-S- (2-carboxyethyl)-L-cysteine; 3HPMA, N-Acetyl-S- (3-hydroxypropyl)-L-cysteine; BzMA, N-Acetyl-S-(benzyl)-L-cysteine; MADA, Mandelic acid; PhGA, Phenylglyoxylic acid; PhMA, N-Acetyl-S-(phenyl)-L-cysteine; 2HPMA, N-Acetyl-S- (2-hydroxypropyl)-L-cysteine; MCaMA, N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine; 34HBMA, N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine; 4HBeMA, N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine; 3HMPMA, N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine; 4HMBeMA, N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine; TTCA, 2-Thioxothiazolidine-4-carboxylic acid.

settings involved in the production of synthetic rubbers and polymers [11]. Although 1,3butadiene is a known human carcinogen (IARC group 1), its cardiovascular complications are less clear [8,11]. A few studies among workers at butadiene facilities showed statistically significant elevated mortality rates for cardiovascular diseases [28]. We showed that in the general population, the 1,3butadiene biomarker was associated with IHD mortality. In line with our results, some nonoccupational studies reported the association of 1,3butadiene with cardiovascular risk due to oxidative stress, lipid dysfunction, and hypertension [8,11]. For example, a cross-sectional study showed that urinary metabolites of 1, 3butadiene among participants who did not smoke tobacco were associated with endothelial dysfunction and elevated risk of hypertension. The researchers indicated that low levels of 1,3butadiene may contribute to cardiovascular disease effects, independent of smoking [8].

We found associations between urinary metabolites of acrylamide and acrylonitrile with IHD mortality, especially among participants who did not smoke tobacco. A few studies have shown associations between acrylamide and cardiovascular diseases [29,30]. Oxidative stress and chronic inflammation have been proposed as IHD-related consequences of long-term acrylamide exposure [29,30]. Tobacco smoke and rich carbohydrate-content foods cooked at high temperatures are known sources of acrylamide [31]. Acrylonitrile is used as a raw material for the manufacture of fibers, resins, plastics, elastomers, and synthetic rubbers. Cardiovascular consequences of acrylonitrile exposure remain unclear. A cross-sectional study showed that higher levels of 2CyEMA were correlated with increased levels of an oxidative stress product (8-hydroxydeoxyguanosine), but it was not related to other cardiovascular risk factors including blood lipids, BMI, and blood glucose [32]. In the GCS, among non-smoking subgroup individuals, who generally have lower levels of VOCs, opiate use is the main source of several VOCs including acrylonitrile and acrylamide, particularly the latter, which is almost exclusively produced by heating opium, in the area [15]. Long-term opiate use (ingestion and/or smoking) has been associated with increased cardiovascular mortality, independent of traditional risk factors such as hypertension, tobacco use, physical

inactivity, dyslipidemia, diabetes, low fruit/vegetable intake, and obesity [33]. Opiates are mixtures of two broad groups of compounds: alkaloids (e.g. morphine and codeine) and non-alkaloids (e.g. water, sugar, and several organic acids) [34]. One of the mechanisms linking opiate use and cardiovascular mortality may involve VOCs produced by heating these mixtures, either during processing or use.

The DMF/methylisocyanate urinary biomarker, MCaMA, was associated with IHD mortality in our study. DMF is a powerful solvent extensively used in a wide variety of industrial applications such as acrylic-fiber, synthetic-leather, electronics, pesticides, paint-stripping, and pharmaceutical industries [35]. DMF is probably carcinogenic to humans (IARC group 2A) [35]. Its hepatotoxicity has been demonstrated in both animal studies and occupational poisoning cases [36], but only a few studies have shown cardiac damage. A study suggested that the oral administration of DMF to mice for 90 days was associated with increased levels of cardiac troponin and creatine kinase and oxidative stress was involved in this injury [37]. A cross-sectional study examined the effects of DMF exposure in the air on elderly residents living near synthetic leather factories in China. The results showed an increased risk of heart, liver, and kidney injuries associated with DMF exposure [38]. Methylisocyanate is used in the production of pesticides, polyurethane foam, and plastics and has been detected in cigarette smoke [39]. Acute exposure to high concentrations could result in severe lung damage and death [39], while no information is available on its long-term cardiovascular effects.

A major advantage of this study is using data from a large population-based prospective study. In addition to excessively high exposure levels in occupational studies, the healthy worker effect and survivor effect are among limitations to generalize their findings [40]. To assess the effects of pollutants on IHD, biomonitoring, compared with environmental monitoring, is more useful because it directly measures internal dose from all different sources, regardless of metabolism variations among individuals. We measured urinary mercapturic acid metabolites as appropriate biomarkers compared with blood and breath to assess individual-level VOC exposure [9]. Temporal variability in the urinary levels of VOC biomarkers, due to their relatively short half–lives and temporal changes in sources, is an important limitation. However, using urine data of a repeated measurement phase, 5 years apart, we previously showed that most VOC biomarkers could be appropriate for exposure assessment in longitudinal studies, especially in the presence of a strong source of exposure such as tobacco smoke [13]. Some VOC sources among other populations likely differs from those observed in Golestan cohort (e.g., opiates); nevertheless, our biomonitoring-based findings for specific VOC biomarkers suggest that these compounds may increase risk of IHD mortality regardless of their sources. We did not have data on second-hand tobacco smoke exposure and nicotine replacement therapy. While nicotine replacement therapy is very rare in this population, we adjusted the models for urinary cotinine levels that could overcome these limitations. Although we adjusted for multiple important confounders, residual confounding and the influence of other unmeasured factors cannot be completely ruled out. Although there were multiple exposure biomarkers in the study, we did not correct for multiple testing because the panel of biomarkers in this study included smoke-related biomarkers, with *a priori* data indicating their potential roles in the link between tobacco exposure and cardiovascular diseases. Additionally, for acrolein and styrene/ethylbenzene, both biomarkers exhibited statistically significant associations with IHD mortality, which reduces the chances of a random finding. Nevertheless, these associations should be verified in future studies.

Although the carcinogenicity of several VOCs has received considerable attention by health organizations, their cardiovascular effects are relatively under-studied. Cancer and cardiovascular diseases share several common risk factors (e.g., tobacco smoking and air pollution) and pathophysiological processes for disease development and progression (i.e., inflammation and oxidative stress) [41]. Our findings provide strong evidence for the association between exposure to several VOCs at baseline and IHD mortality many years later in the general population. These results have three major implications: first, these associations may help us understand the underlying mechanisms for cardiovascular toxicity of complex mixtures, second, our findings highlight the importance of investigating VOC exposure in the general population as a risk factor for the development of cardiovascular diseases, and finally, they underline the importance of non-tobacco VOC exposure, providing important clues for bio-monitoring specific compounds with widespread household and commercial use by regulatory bodies and policymakers.

Data sharing

Data are available from the corresponding authors on reasonable request.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Department of Health and Human Services or any of its affiliated institutions or agencies.

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Author agreement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajpc.2024.100700.

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