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The Association Between Cotinine-Verified Smoking Status and Risk of Gallstones: A Cohort Study

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ABSTRACT

Background & Aims: Previous epidemiological data on the association between cigarette smoking and risk of gallstone development remain controversial, and most relevant studies have relied on self-reported questionnaires. We aimed to elucidate this association using both an objective biomarker of tobacco exposure (urinary cotinine) and a self-reported questionnaire.

Methods: We analyzed 221,721 asymptomatic adults who underwent abdominal ultrasonography and urinary cotinine measurement between January 2011 and December 2016. Cotinine-verified current smokers were defined as participants with urinary cotinine levels ≥50 ng/mL.

Results: The mean age of the study population was 35.9 years, and the proportion of men was 55.8%. The proportions of self-reported and cotinine-verified current smokers were 21.3% and 21.2%, respectively. After adjusting for confounding factors, self-reported current smoking was associated with an increased risk of gallstone development [adjusted odds ratio (aOR) 1.14; 95% confidence interval (95%CI), 1.04–1.25]. Moreover, among the current smokers, the risk of gallstone development increased with an increase in the amount of cigarette smoking (<20 and ≥20 pack-years vs. never smoked; aOR=1.11 and 1.25; 95%CI: 1.01–1.22 and 1.07–1.45, respectively). Cotinine-verified current smoking was also associated with an increased risk of gallstone development (aOR=1.16; 95%CI: 1.07–1.25). Among the self-reported never or former smokers, the cotinine-verified current smokers (aOR=1.20; 95%CI: 1.01–1.44) showed a significantly higher risk of gallstones than cotinine-verified never smokers.

Conclusions: Cotinine-verified and self-reported current smoking were independent risk factors for gallstones, suggesting a distinct role of tobacco smoking in gallstone development.

Key words: smoking – cotinine – gallstone.

Abbreviations: aOR: adjusted odd ratio; BMI: body mass index; BP: blood pressure; CI: confidence interval; FBG: fasting blood glucose; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; HEPA: health-enhancing physically active; MET: metabolic equivalent; OR: odd ratio; US: ultrasonography.

INTRODUCTION

Gallstone disease is one of the most common biliary tract diseases and a major public health concern in many countries [1]. Previous ultrasonography (US)-based epidemiologic studies have shown prevalence rates of 9.7–19.5% in European countries, 10–12% in the United States, and 2–5% in Asian countries [2-4]. The prevalence of gallstones has increased with the increased prevalence of obesity and the westernization of the socioeconomic environment in Asian countries, including Korea [3]. Although gallstones are discovered incidentally in many patients during abdominal US without symptoms and are classified as benign, their potential to cause significant morbidity through critical complications such as cholecystitis, cholangitis, and pancreatitis highlights their clinical importance [5]. In addition, twin studies indicate that environmental factors account for the majority of symptomatic gallstone disease, while genetic susceptibility is responsible for approximately 25%, a significant minority, of symptomatic gallstone disease [6, 7]. Hence, identifying and addressing modifiable risk factors for the development of gallstones is crucial to mitigate the increase in gallstone formation and its related complications.

The results of previous epidemiological studies on the association between cigarette smoking and the risk of developing gallstones have not been entirely consistent. Some studies have shown a significantly increased risk of gallstones in smokers [8, 9], whereas other studies have suggested null [10] or even inverse [11] associations between smoking and gallstones. The modest sample sizes of prior studies may have contributed to these inconsistent findings. Furthermore, these discrepancies may be partly attributed to the predominant use of self-reported questionnaires, which may not accurately capture the smoking status. The main concern regarding the reliability of self-reported smoking status is potential underreporting. In circumstances where a substantial social stigma exists against smoking, smokers may inaccurately identify themselves as nonsmokers [12].

Cotinine, the main nicotine metabolite, is widely used to indicate cigarette smoking status. Establishing a connection between cotinine, an objective marker of tobacco exposure, and development of gallstones could provide an accurate insight into the relationship between smoking and gallstone formation. However, most previous studies have assessed the association between self-reported smoking status and gallstone formation risk, and no data on the association between cotinine and gallstone formation has been reported. Therefore, this study aimed to clarify the association between cigarette smoking and the risk of gallstones using urinary cotinine measurements and self-reported questionnaires in a large sample of Korean adults.

METHODS

Study Population

The Kangbuk Samsung Health Study included a cohort of adult Korean men and women aged ≥18 years who underwent a comprehensive annual or biannual health examination at the Kangbuk Samsung Hospital Total Healthcare Center in Seoul or Suwon, South Korea [13, 14]. The present study used a subset of participants from the Kangbuk Samsung Health Study who underwent abdominal US screening between January 2011 and December 2016 (n=419,058). Korea's Industrial Safety and Health Law stipulates that employees must undergo health examinations once or twice a year. Over 80% of the participants were employees of diverse companies or local government organizations (or their spouses), whereas the remaining participants voluntarily enrolled in the screening program.

The exclusion criteria were as follows (Fig. 1): 1) history of cholecystectomy; 2) history of malignancy, including gall bladder cancer and cholangiocarcinoma; and 3) incomplete data on urinary cotinine levels, self-reported smoking status, or the presence of gallstones on US. After excluding 197,337 participants who met one or more exclusion criteria, the final sample comprised 221,721 participants.

This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (no. KBSMC 2023-02-023). As this study used anonymized retrospective data collected from regular health screening examinations, the requirement for informed consent was waived.

Measurements and Definitions of Variables

During the health examinations, trained staff measured anthropometric dimensions, blood pressure, and serum biochemical parameters, while standardized self-administered questionnaires were used to gather information on demographic characteristics, health-related behaviors, and medical histories [13, 14].

Smoking status was categorized as never, former, or current smokers. Participants who had smoked 100 or more cigarettes in their lives and who smoked cigarettes at the time of the study were defined as "current smokers." Participants who had smoked 100 or more cigarettes in their lives but reported that they did not currently smoke were defined as "former smokers". Participants who had never smoked a cigarette or who had smoked fewer than 100 cigarettes in their lives were defined as "never smokers" [15]. The average daily alcohol consumption was calculated based on the weekly frequency of alcohol consumption and the amount of alcohol consumed per drinking day. Alcohol intake was defined as >10 g/d in men and >5 g/d in women. Physical activity was estimated using the Korean version of the International Physical Activity

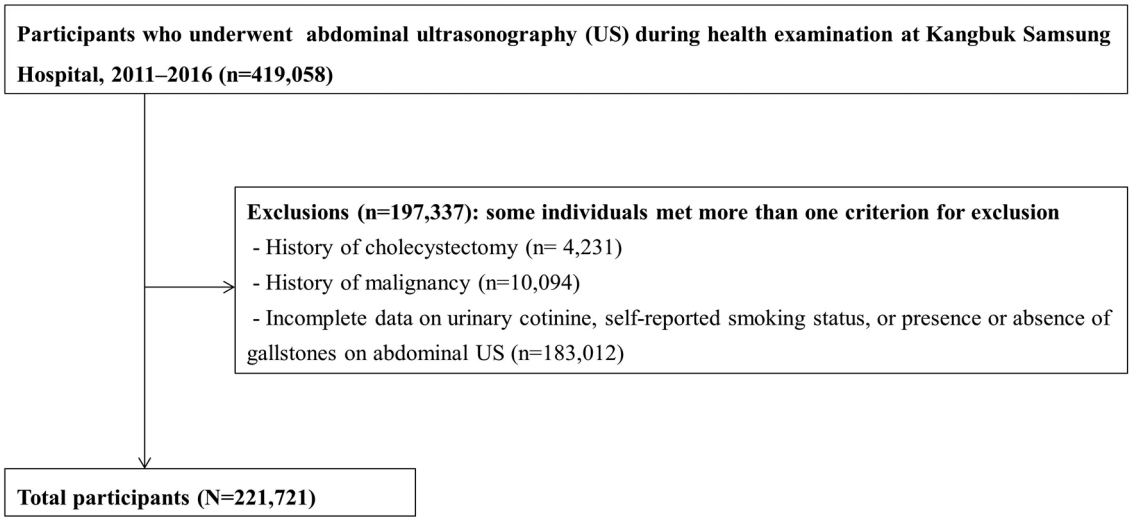

Fig. 1. Flowchart of the study population.

Questionnaire scores, which were converted to metabolic equivalent (MET) units and classified into three categories: inactive, minimally active, and health-enhancing physically active (HEPA) [16]. Participants who met at least one of the following criteria were categorized as having a HEPA level of physical activity: engaging in >1,500 MET min/week of physical activity that includes at least 3 days of vigorous activity or engaging in >3,000 MET min/week of physical activity [16]. Body mass index (BMI) was calculated by dividing measured weight (kg) by height squared (m^2). Obesity was defined as BMI ≥25 kg/m^2, which is the proposed cutoff value for diagnosing obesity in Asians [17].

Blood samples were collected from the participants after a minimum fasting period of 10 hours. Blood tests included viral hepatitis indices [hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV)], lipid profiles, levels of liver enzymes, fasting blood glucose (FBG), and glycated hemoglobin.

Metabolic syndrome was defined as having three or more of the following five metabolic abnormalities used in the National Cholesterol Education Program and Adult Treatment Panel-III criteria: abdominal obesity (waist circumference ≥90 cm in men and ≥85 cm in women, the proposed cutoff values for Asians) [18], elevated FBG levels (≥100 mg/dL) or use of glucose-lowering medications, elevated blood pressure (BP) (≥130 mmHg systolic, ≥85 mmHg diastolic), use of antihypertensive drugs, elevated triglyceride levels (≥150 mg/dL) or use of lipid-lowering agents, and reduced high-density lipoprotein cholesterol levels (<40 mg/dL in men and <50 mg/dL in women) [19].

Urinary cotinine levels were determined using the DRI Cotinine Assay (Microgenics Corp., Fremont, CA, USA) with a modular P800 analyzer (Roche Diagnostics, Tokyo, Japan). Cotinine-verified current smokers were defined as participants with urinary cotinine levels of ≥50 ng/mL [20].

Diagnosis of Gallstones

The presence or absence of gallstones was examined on abdominal US, which was performed using a 3.5MHz transducer (LOGIQ 9; General Electric, Madison, WI, USA) after the participants had fasted for a minimum of 10 h. Abdominal US examinations were performed by 11 experienced radiologists who were unaware of the study aims and were blinded to the clinical information. Gallstones were defined as US-documented gallstones by the presence of strong intraluminal echoes that were gravity-dependent or that attenuated US transmission (acoustic shadowing) [2, 21]. The inter- and intra-observer reliability for the diagnosis of gallstones were excellent (kappa statistics: 0.90 and 0.94, respectively) [2].

Statistical Analysis

The baseline characteristics of the participants and the prevalence of gallstones according to self-reported or cotinine-verified smoking status were compared using the chi-square test for categorical variables and Student's t test or one-way analysis of variance for continuous variables. Data were expressed as mean (standard deviation), median (interquartile range), or frequency (%).

To assess the association between development of gallstones and self-reported or cotinine-verified smoking status, we used a logistic regression model to estimate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) for gallstones. Multivariate logistic regression analysis was subsequently performed, adjusting for confounders that showed statistical significance in the univariate analysis. Confounders included age, sex, obesity, hypertension, diabetes, dyslipidemia, fatty liver, metabolic syndrome, alcohol intake, and the presence or absence of HBsAg and anti-HCV antibodies. All reported p values were two-tailed, and a p value <0.05 was considered statistically significant. SPSS version 21 (IBM Corp., Armonk, NY, USA) was used for all the statistical analyses.

RESULTS

The mean age of the 221,721 participants at baseline was 35.9 years (standard deviation, 7.4 years), and the proportion of male participants was 55.8%. The proportions of never, former, and current smokers based on the self-reported questionnaire were 62.3, 16.5, and 21.3%, respectively, whereas the proportions of cotinine-verified never and current smokers were 78.8 and 21.2%, respectively. Among participants who self-reported as current smokers, cotinine verification reclassified 12.6% as never smokers. In contrast, among those who self-reported as never smokers, 21.2% were reclassified as current smokers after cotinine verification (Table I).

Comparisons of the baseline characteristics and prevalence of gallstones according to self-reported and cotinine-verified smoking status are shown in Table II. Both self-reported and cotinine-verified current smokers tended to be older and had a higher prevalence of male gender, obesity, hypertension, diabetes, and metabolic syndrome than self-reported and cotinine-verified never smokers. The proportion of participants with alcohol intake, HEPA level of physical activity, and presence of HBsAg were also higher in self-reported and cotinine-verified current smokers than in self-reported and cotinine-verified never-smokers. The prevalence rates of gallstones in never, former, and current smokers were 2.4, 2.5, and 2.5%, respectively, whereas those in cotinine-verified never and current smokers were 2.4 and 2.6%, respectively.

Table I. Misclassification of cotinine-verified and self-reported smoking status

	Total (n = 221,721)	Never smokers (n = 138,037)	Former smokers (n = 36,541)	Current smokers (n = 47,143)
Cotinine-verified smoking status				
Cotinine-verified never smoker	174,788 (78.8)	135,275 (98.0)	33,578 (91.9)	5,935 (12.6)
Cotinine-verified current smoker	46,933 (21.2)	2,762 (2.0)	2,963 (8.1)	41,208 (87.4)

Data are expressed as numbers (percentages).

Table II. Baseline characteristics and prevalence of gallstones according to self-reported and cotinine-verified smoking statuses

	Total (n = 221,721)	Never smokers (n = 138,037)	Former smokers (n = 36,541)	Current smokers (n = 47,143)	p	Cotinine-verified never smokers (n = 174,788)	Cotinine-verified current smokers (n = 46,933)	p
Age (years)	35.9 ± 7.4	35.1 ± 7.2	37.7 ± 8.0	37.2 ± 7.4	<0.001	35.6 ± 7.5	37.1 ± 6.7	<0.001
Male	123,766 (55.8)	45,705 (33.1)	33,058 (90.5)	45,003 (95.5)	<0.001	80,433 (46.0)	43,333 (92.3)	<0.001
BMI (kg/m^2)	23.2 ± 3.4	22.4 ± 3.3	24.4 ± 3.1	24.6 ± 3.2	<0.001	22.9 ± 3.4	24.5 ± 3.3	<0.001
Obesity (≥25 kg/m^2)	61,455 (27.7)	27,429 (19.9)	14,283 (39.1)	19,743 (41.9)	<0.001	42,160 (24.1)	19,295 (41.1)	<0.001
Hypertension	19,725 (8.9)	8,263 (6.0)	5,352 (14.7)	6,110 (13.0)	<0.001	13,903 (8.0)	5,822 (12.4)	<0.001
Diabetes mellitus	6,078 (2.7)	2,321 (1.7)	1,575 (4.3)	2,182 (4.6)	<0.001	3,926 (2.2)	2,152 (4.6)	<0.001
Dyslipidemia	78,696 (35.5)	38,636 (28.0)	16,365 (44.8)	23,695 (50.3)	<0.001	55,326 (31.7)	23,370 (49.8)	<0.001
Fatty liver	58,101 (26.2)	25,231 (18.3)	13,873 (38.0)	18,997 (40.3)	<0.001	39,582 (22.6)	18,519 (39.5)	<0.001
Metabolic syndrome	21,465 (9.7)	8,008 (5.8)	5,222 (14.3)	8,235 (17.5)	<0.001	13,365 (7.6)	8,100 (17.3)	<0.001
Alcohol intake[a]	69,367 (37.7)	27,594 (25.1)	16,028 (50.6)	25,745 (60.5)	<0.001	44,242 (31.2)	25,125 (59.5)	<0.001
Physical activity[b]	35,102 (16.0)	20,115 (14.7)	7,092 (19.6)	7,895 (16.9)	<0.001	27,231 (15.7)	7,871 (16.9)	<0.001
High education level[c]	165,688 (82.7)	102,651 (81.6)	28,312 (85.3)	34,725 (83.8)	<0.001	131,580 (82.7)	34,108 (82.7)	0.720
HBsAg	6,804 (3.1)	4,058 (2.9)	1,163 (3.2)	1.583 (3.4)	<0.001	5,194 (3.0)	1,610 (3.4)	<0.001
Anti-HCV	180 (0.07)	94 (0.10)	37 (0.10)	49 (0.08)	0.021	123 (0.07)	57 (0.12)	0.001
Gallstones	5,376 (2.4)	3,291 (2.4)	907 (2.5)	1178 (2.5)	0.278	4,173 (2.4)	1,203 (2.6)	0.028

Values are presented as mean ± standard deviation or number (percentage). [a] >10 g/d for men and >5 g/d for women. [b] Health-enhancing physical activity level: physical activity consuming over 1,500 MET-minutes per week, including more than 3 days of vigorous activity, or physical activity consuming over 3,000 MET-minutes per week. [c] College graduate or higher. Anti-HCV: hepatitis C antibody; BMI: body mass index; HBsAg: hepatitis B surface antigen.

Table III shows the association between self-reported and cotinine-verified smoking statuses and the risk of developing gallstones. In the multivariate analysis adjusted for age, gender, obesity, hypertension, diabetes, dyslipidemia, fatty liver, metabolic syndrome, alcohol intake, and the presence or absence of HBsAg and anti-HCV, which showed statistical significance in the univariate analysis, self-reported current smoking emerged as a significant risk factor for gallstones [adjusted OR (aOR) =1.14; 95%CI: 1.04–1.25]. Furthermore, among the self-reported current smokers, the risk of gallstones was found to increase with an increasing daily amount of cigarettes smoked (10–19, and ≥20 cigarettes/day vs. never smokers; aOR were 1.18 and 1.19; 95%CI: 1.05–1.31 and 1.04–1.37, respectively), duration of smoking (≥20 years vs. never smokers; aOR=1.18; 95%CI: 1.05–1.33), and total amount of cigarette smoked (<20 and ≥20 pack-years vs. never smokers; aOR were 1.11 and 1.25; 95%CI: 1.01–1.22 and 1.07–1.45, respectively), even after adjusting for confounding factors. In addition, cotinine-verified current smokers had a significantly increased risk of gallstones (aOR=1.16; 95%CI: 1.07–1.25) compared with cotinine-verified never smokers.

Table IV shows the effect of participant misclassification between self-reported and cotinine-verified smoking statuses on the risk of developing gallstones. Among the self-reported never or former smokers, the cotinine-verified current smokers (adjusted OR=1.20; 95%CI: 1.01–1.44) exhibited a significantly higher risk of gallstones than cotinine-verified never smokers.

DISCUSSION

This study investigated the association between smoking and the risk of developing gallstones using cotinine and a self-reported questionnaire in a large cohort of young and middle-aged Korean adults. Self-reported current smoking was independently associated with an increased risk of gallstone development. The risk of developing gallstones increased with the increasing amount and duration of cigarette smoking in a dose-dependent manner. Cotinine-verified current smoking status is also an independent risk factor for gallstones. Moreover, among self-reported never or former smokers, cotinine-verified current smokers had a significantly higher risk of developing gallstones than cotinine-verified never smokers. Our results confirmed a positive association between tobacco smoking and developing gallstones. Thus, tobacco smoking, whether direct or secondary, may play an important role in gallstone pathogenesis.

Epidemiological studies of the association between cigarette smoking and developing gallstones have shown inconsistent results. One prospective study demonstrated that smokers were more likely to develop symptomatic gallstones than non-smokers in women (relative risk = 1.19; 95%CI: 1.06–1.34) [22]. In another prospective study from southern Italy, cigarette smoking was related to an approximately twofold increase in the development of gallstones in men and women combined [23]. A population-based prospective study from Japan also found that smokers were associated with an increased risk of gallstones in women (aOR, 1.66; 95%CI: 1.14–2.40), but not in men [7]. A recent meta-analysis including 10 prospective studies showed an estimated 11% increased risk of developing gallstone disease per 10 cigarettes per day compared to non-smokers [9]. In agreement with these studies, our study identified that self-reported current smoking was independently associated with an increased risk of developing gallstones. Additionally, among self-reported current smokers, the risk of gallstones increased with an increase in the daily

Table III. Multivariate analysis and relationship between self-reported and cotinine-verified smoking status and risk of gallstones

Risk factors	Multivariate-adjusted OR	95% CI	p
Age (year)	1.05	1.04–1.05	<0.001
Male gender	0.72	0.67–0.78	<0.001
Obesity (≥25 kg/m^2)	1.42	1.32–1.53	<0.001
Hypertension	1.07	0.97–1.19	0.170
Diabetes mellitus	1.06	0.91–1.23	0.462
Dyslipidemia	0.99	0.92–1.42	<0.001
Fatty liver	1.26	1.17–1.36	<0.001
Metabolic syndrome	1.08	0.97–1.20	0.175
Alcohol intake[a]	0.82	0.77–0.88	<0.001
HBsAg	1.50	1.30–1.73	<0.001
Anti-HCV	0.55	0.27–1.13	0.103
Self-reported smoking status			
Never smokers	1	Reference	
Former smokers	1.02	0.92–1.12	0.769
Current smokers	1.14	1.04–1.25	0.005
Among self-reported current smokers			
Daily amount of cigarette smoked			
<10 cigarettes/day*	0.98	0.83–1.14	0.765
10–19 cigarettes/day*	1.18	1.05–1.31	0.004
≥20 cigarettes/day*	1.19	1.04–1.37	0.012
Duration of cigarette smoke			
<20 years*	1.10	0.98–1.22	0.101
≥20 years*	1.18	1.05–1.33	0.006
Total amount of cigarette smoke			
<20 pack-years*	1.11	1.01–1.22	0.040
≥20 pack-years*	1.25	1.07–1.45	0.005
Cotinine-verified smoking status**			
Cotinine-verified never smoker	1	Reference	
Cotinine-verified current smoker	1.16	1.07–1.25	<0.001

The multivariate-adjusted model was adjusted for confounders, including age, sex, obesity, hypertension, diabetes, dyslipidemia, fatty liver, metabolic syndrome, alcohol intake, presence or absence of HBsAg and anti-HCV, and self-reported or cotinine-verified smoking status. a >10 g/d for men and >5 g/d for women. *The reference group consisted of never smokers. **Self-reported smoking status was excluded, and cotinine-verified smoking status was added for the analysis. CI: confidence interval; OR: odds ratio. For the rest of abbreviations see Table II.

amount and duration of cigarette smoking in a dose-dependent manner. Indeed, a recent Mendelian randomization study revealed an independent causal role of smoking in developing gallstone disease (for one standard deviation increase in smoking prevalence: OR=1.25; 95%CI: 1.16–1.34) [24]. Another Mendelian randomization study also supported a causality of gallstone disease incidence with lifetime smoking (OR=1.008; 95%CI: 1.003–1.013) and current smoking (OR=1.007; 95%CI: 1.002–1.011) [25]. In contrast, some other studies have failed to establish an association between

Table IV. Effects of participant misclassification based on self-reported and cotinine-verified smoking status on the risk of gallstones

	Multivariate-adjusted OR (95% CI)	P value
Among self-reported never or former smokers (N = 174,578)		
Cotinine-verified never smokers (n = 168,853)	1 (Reference)	0.042
Cotinine-verified current smokers (n = 5,725)	1.20 (1.01–1.44)	0.042

For abbreviations see Table III.

smoking and developing gallstones. In a large multicenter study including 14 cohorts from Italy, cigarette smoking and gallstone disease were not significantly associated [26]. A case-control study in Japanese men also showed that cigarette smoking was not significantly associated with prevalent gallstones, cholecystectomy, newly diagnosed gallstones, or known gallstone disease [27]. Recently, a prospective cohort study involving 2,848 participants without gallstones at baseline, along with an additional systematic review and meta-analysis, showed that smoking did not significantly increase the risk of incident gallstones [28].

The discrepancies in the outcomes of previous studies could be partially due to the reliance on self-reported smoking questionnaires. Self-reported questionnaires are prone to recall biases and reporting errors [12]. Smokers may downplay their smoking habits or falsely identify themselves as non-smokers because of social pressures advocating smoking cessation [12]. These self-reported surveys may also fail to accurately reflect exposure to secondhand smoking and individual variations in nicotine metabolism [29, 30]. Using objective biomarkers indicative of tobacco exposure may help more accurately assess an individual's smoking status.

Cotinine, the main metabolite of nicotine, is widely used to assess the accuracy of self-reported smoking status. This is because it is not affected by diet or exposure to pollution, and it has a relatively longer half-life and less dependence on temporal factors than nicotine [30-34]. Furthermore, it encompasses all forms of tobacco exposure and its metabolic process [29, 30]. Establishing a link between cotinine levels and gallstones could further clarify the relationship between smoking and gallstone formation. However, to date, no studies have examined the association between cotinine-verified smoking status and the risk of developing gallstones. To the best of our knowledge, this is the first study to examine the association between smoking status and risk of gallstones using both cotinine levels and self-reported questionnaires. We found that cotinine-verified smoking status objectively indicated a positive association between smoking and gallstone risk, and higher cotinine levels, even among those who self-reported as never or former smokers, which also significantly increased the risk of developing gallstones. This suggests that cotinine-verified smoking status reflects direct and passive smoking as well as individual metabolic processes, which might contribute to the risk of gallstones. Cotinine could play a supplementary role in self-reported smoking status for assessing gallstone risk.

The biochemical mechanisms that connect smoking to the formation of gallstones are not entirely understood yet, but potential mechanisms exist. Bile acids play an important role in eliminating excess cholesterol from the body and processing dietary fat by promoting micelles formation [35]. When bile acid metabolism is disrupted, cholesterol is unable to maintain its micellar state, gradually forming crystals and precipitates, which subsequently result in cholesterol stones [35, 36]. Indeed, almost all patients with gallstone are reported to have abnormal bile acid metabolism [37]. Smoking may induce dysregulation in bile cholesterol metabolism by altering the levels of gallstone-related proteins in the gallbladder, as well as serum concentrations of free fatty acid, lipid profile and lipoproteins [35, 38]. This alteration can stimulate hepatic synthesis and cholesterol secretion, thereby promoting the formation of gallstones [38].

This study has several limitations that should be considered when interpreting our findings. Firstly, we lacked information on the reasons for cholecystectomy, which prevented us from differentiating cholecystectomy cases that were unrelated to gallstones such as gallbladder polyps or cancer. Therefore, all participants who underwent cholecystectomy were excluded from the analysis. Although incorporating cholecystectomy cases into the analysis did not change the outcomes, if any of these omitted cases were associated with gallstones, the actual association between cigarette smoking and developing gallstones could be more pronounced than that revealed in our study. Secondly, information regarding the size or volume of the stones on abdominal US was not available. In addition, we did not evaluate interobserver variations in the sonographic diagnosis of gallstones. However, in the current study, only cases with definitive abdominal US-documented gallstones were considered to have gallstones and not those with sludge alone. All sonographic examinations were performed by experienced board-certified radiologists using the same classification system with a clear definition of gallstones. Therefore, we believe that the risk of significant variations in the ultrasonographic diagnosis of gallstones is relatively low. Third, this was a hospital-based rather than a population-based study, and our results were based on a sample of young, middle-aged, and relatively healthy Koreans. Therefore, our findings may not be generalizable to older populations or other ethnic groups. Nonetheless, our study population, predominantly composed of healthy young and middle-aged individuals, may be better suited to estimate the impact of cigarette smoking on the risk of gallstones because most of those who undergo cholecystectomy are in their 50s, 60s, or older [39]. In fact, the number of cholecystectomy cases that were excluded from the analysis was minimal (1%, n=4,231/419,058). Finally, the cross-sectional design precluded the determination of causality and did not allow the establishment of temporal relationships. While both self-reported and cotinine-verified smoking statuses, which reflect actual cigarette use, may change over time, we could not evaluate the influence of these dynamic changes on the risk of gallstones because most participants were only assessed during their initial visit. Nonetheless, the substantial sample size of this study may have mitigated any potential bias arising from disease variability.

Despite these limitations, our study offers valuable insights into the distinct role of smoking in the risk of gallstones. The major strengths of our study include the use of abdominal US for gallstone detection, which also captures asymptomatic cases, and the substantial sample size that offers robust statistical power to evaluate the association between smoking and gallstone development risk. Furthermore, our findings were strengthened by the comprehensive inclusion of potential confounding variables, the availability of high-quality laboratory procedures with thorough quality controls, and the relative homogeneity of the participants, which reduced the likelihood of confounding factors such as socioeconomic status and access to healthcare [2].

CONCLUSIONS

Cotinine-verified current smoking and self-reported current smoking status were significant independent risk factors for gallstones. In addition, the risk of developing gallstones increases with a rise in the number and duration of cigarettes smoked. Considering the increasing prevalence of gallstones, our observations may have important public health implications for identifying potentially modifiable factors associated with gallstones, suggesting that direct or secondhand smoking should be avoided. Further large-scale prospective studies are required to clarify the impact of smoking on the development of gallstones and whether smoking cessation leads to a decrease in the incidence and complications of gallstone disease.

Conflicts of interest: None to declare.

Authors' contribution: N.H.K was involved in conceptualization, data curation, formal analysis, investigation, methodology, original drafting and revising processes. J.H.K.: contributed to conceptualization, investigation, supervision, and revision. H.J.K. conceived the study, collected data, and supervised the study.

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