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The Lowest Prevalence of Cholelithiasis in the Americas - An Autopsy-based Study

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OBJECTIVES: This study used autopsy to evaluate the prevalence of cholelithiasis and its associated risk factors in a population of healthy, young subjects who suffered a violent or natural death.

METHODS: This study is a prospective evaluation of autopsies of 446 individuals from 2011 to 2013 in Brazil. Of that sample, 330 (74%) subjects died from violent deaths and 116 (26%) died naturally. The presence of biliary calculi, previous cholecystectomy, gender, age, ethnicity, body mass index (BMI) and alcohol use were evaluated.

RESULTS: In the natural death group, 6.9% (95% CI 3.39 to 13.28) (3.08% of the male subjects and 11.76% of the female subjects) exhibited evidence of gallbladder disease. In the violent death group, only 2.12% (95% CI 0.96 to 4.43) (2.17% of the male subjects and 1.85% of the female subjects) of the subjects exhibited evidence of gallbladder disease. Age was correlated with the prevalence of gallbladder disease, but BMI was correlated with only gallbladder disease in the natural death group.

CONCLUSIONS: This population has the lowest prevalence of cholelithiasis in the Americas. Dietary habits, physical activity, ethnicity, alcohol consumption and genetic factors may be responsible for this low prevalence.

KEYWORDS: Cholelithiasis; Gallstones; Prevalence; Autopsy.

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INTRODUCTION

Cholelithiasis is one of the most costly digestive diseases for the healthcare system and is the most common cause of death from nonmalignant disease of the gastrointestinal tract (1). The third National Health and Nutrition Examination Survey, or NHANES III, performed in the United States, included 14,000 subjects and reported that an estimated 6.3 million men and 14.2 million women have cholelithiasis. The highest prevalence in Hispanics (8.9% in men and 26.7% in women), followed by non-Hispanic whites (8.6% in men and 16.6% in women) and non-Hispanic blacks (5.3% in men and 13.9% in women) (1).

From 1890 to 1980, data regarding the prevalence of cholelithiasis were obtained through autopsy and oral cholecystography. A review paper analyzed the studies during this period and reported low rates in Africa (less than 1%, except in South Africa, where it reached 17% in whites) and Asia (maximum of 7% in Singapore) and an increased prevalence in Europe (10.5% before 1940 and 18.5% from 1940 to 1976). In the Americas, rates of cholelithiasis have been reported to be 9.1% (18.6% in whites and 6.6% in blacks) in Chicago, United States of America (USA) (2); 14.3% in Mexico (3); and 26.6% in Santiago, Chile (4). Among all of the studied populations throughout the world, the highest prevalence (48.6%) was found in Pima Indians in Phoenix, Arizona (USA) (5), which was potentially attributed to a super-saturation of biliary cholesterol in that population (6). In Brazil, the first surveys using autopsy results reported rates of cholelithiasis ranging from 9.07% in Triângulo Mineiro/MG (1) to 19.36% in São Paulo/SP (8).

Since the introduction of ultrasound in the early 1980s, studies of the prevalence of cholelithiasis and its associated risk have been conducted using this technology, which is more practical and less invasive than autopsy and oral cholecystography. Therefore, large population studies have been conducted in multiple countries. In Italy, the prevalence of cholelithiasis in a sample of 33,000 individuals between 30 and 69 years of age was 18.8% in women and 9.5% in men (9). In the USA, 20.5 million people were estimated to have

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gallstones based on a survey of 14,000 people between 20 and 74 years of age, with a prevalence ranging from 5.3% in non-Hispanic black men to 26.7% in Hispanic women (1). In Latin America, a prevalence of 20.5% and 28.5% was found in Argentina and in Chile (11), respectively. In Brazil, one study of 1000 people in the general population reported a prevalence of 12.9% in women and 5.4% in men, with an overall prevalence of 9.3% (12).

Although several studies throughout the twentieth century have elucidated much of the pathophysiology and risk factors associated with cholelithiasis, they are still incompletely understood. The impacts of some factors, such as diet, physical activity (13), ethanol consumption (14), ethnicity (15) and genetic factors (16), on the prevalence of cholelithiasis are still controversial. Furthermore, there are still populations, such as young people without comorbidities, for whom information that has been collected over the past few years regarding cholelithiasis is still highly restricted to the use of the single diagnostic modality of ultrasound. This diagnostic polarization between ultrasound and autopsy is problematic because autopsy provides a higher detection rate of cholelithiasis than ultrasonography (17). A study of prophylactic cholecystectomy in 62 patients undergoing bariatric surgery showed that 17% of the 47 individuals who had normal ultrasound images had cholelithiasis (<10 mm) based on the pathology analysis (18).

Thus, in the present study, autopsy results were used to evaluate the prevalence of cholelithiasis and its associated risk factors in a healthy, young population and in a population of people who died naturally.

METHODS

This was a prospective study conducted between January 2011 and December 2013 that included autopsy results of 446 individuals over 20 years of age who died in Palmas City, Tocantins State, Brazil. This study was approved by the Medical Ethics Committee of the Federal University of Tocantins, Brazil, and is linked to the School of Medicine of the University of São Paulo and the Federal University of Tocantins. All procedures performed were in accordance with the 1975 Helsinki Declaration and its later amendments or comparable ethical standards.

The cause of death (natural or violent), presence of cholelithiasis or previous cholecystectomy, gender, age, ethnicity (white and non-white), BMI (body mass index) and alcohol use were evaluated. BMI was calculated for each subject as follows: weight (kg)/height (m^2).

Statistical Analysis

Descriptive Analysis. For qualitative variables, such as gender, ethnicity, alcohol use and cholelithiasis, the data are presented as percentages. For quantitative variables, such as age and BMI, the data are presented as the mean with the corresponding standard deviation.

Inferential Analysis. The inferential analysis of qualitative variables (gender, ethnicity, alcohol use and cholelithiasis) was performed using Fisher's exact test.

For quantitative variables (age and BMI), we used the t test and the Mann–1Whitney test for variables with and without a normal distribution, respectively. Then, for each quantitative variable, we applied the ROC curve with the Youden maximizing index to determine the cutoff value.

Then, simple Poisson regression (19) was used for both qualitative and quantitative categorized variables, resulting in prevalence estimations with a robust variance estimator (19) and their respective 95% confidence intervals (CIs). Finally, variables with p values less than 0.20 were examined by multiple regression analysis.

In all analyses, p values <5% were considered significant. R software version 3.0.2 (20) was used.

RESULTS

Violent Death

Descriptive Analysis. Among the 446 individuals studied, 74% (330) died from violent deaths. In this group, 84% (276) were males, 91% (300) were nonwhite and 43% used alcohol. In addition, this population was young (276 men, average age ± standard deviation 41.33 ± 16.47 years, and 54 women, average age 41.19 ± 18.81 years) and thin (mean BMI 24.22 ± 4.56). Overall, 2.12% (95% CI 0.96 to 4.43) of all subjects who suffered a violent death (2.17% of the men and 1.85% of the women who suffered a violent death) exhibited evidence of current or past gallbladder disease (cholelithiasis or history of cholecystectomy) (Table 1).

Inferential Analysis. Age was the only variable associated with cholelithiasis. Individuals older than 48 years had a higher prevalence of cholelithiasis (PR or prevalence rate = 5.28, 95% CI 1.04 to 26.76) (Table 2). Multiple regression analysis revealed a p value for age of 0.044.

Natural Death

Descriptive Analysis. Among the 446 individuals studied, 26% (116) died naturally. In this group, 56% (65) were male, 71% (82) were non-white, and 20% (23) used alcohol. This population was thin (mean BMI 24.29 ± 5.42) and older than the people in the violent death group (65 men, average age ± standard deviation 53.86 ± 17.52 years, and 51 women, average age 54.27 ± 18.6 years). Overall, 6.9% (95% CI 3.39 to 13.28) of the subjects who died naturally (3.08% of the men and 11.76% of the women) exhibited evidence of current or past gallbladder disease (cholelithiasis or history of cholecystectomy) (Table 3).

Inferential Analysis. Age and BMI were the only variables associated with cholelithiasis. Individuals older than 70 years had a higher prevalence of cholelithiasis (PR=9.88, 95% CI 2.12 to 46.13). Subjects with a BMI greater than 30 also had a higher prevalence of the disease (PR=4.37, 95% CI 1.17 to 16.32) (Table 4). Multiple regression analysis revealed p values for age and BMI of 0.001 and 0.022, respectively.

DISCUSSION

In this cross-sectional analysis, we found a prevalence of cholelithiasis of 6.9% (95% CI 3.39 to 13.28) (3.08% in men and 11.76% in women) in individuals who died naturally and 2.12% (95% CI 0.96 to 4.43) (2.17% in men and 1.85% in

Table 1 - Prevalence of Cholelithiasis (and 95% Confidence Interval) by Sex and Age – Violent Death.

Age (yrs)	Male			Female			Total		
	N	Prevalence	95% CI	N	Prevalence	95% CI	N	Prevalence	95% CI
<48	185	0.54	0 – 3.37	39	2.56	0 – 14.59	224	0.89	0.05 – 3.46
⩾48	91	5.49	2.11 – 12.62	15	0	0 – 24.33	106	4.72	1.8 – 10.93
Total	276	2.17	0.91 – 4.81	54	1.85	0 – 10.87	330	2.12	0.96 – 4.43

Table 2 - Prevalence Rates of Qualitative and Categorized Quantitative Variables – Violent Death.

Factor	Group	PR	95% CI	*p* value
Gender	Male	1.17	0.14 – 9.56	0.881
Ethnicity	Non-white	*	*	0.994
BMI	⩾24 kg/m^2	7.2	0.88 – 59.09	0.066
Age	⩾48 yrs	5.28	1.04 – 26.76	0.044
Alcohol use	Yes	0.98	0.22 – 4.32	0.979

Note: * Could not be calculated due to computing reasons.

women) in individuals who suffered violent deaths. Both values are lower than those previously reported in Brazil and other countries in the Americas. In Brazil, studies of autopsy results in people over 20 years of age have reported a prevalence of cholelithiasis of 9.07% in Triângulo Mineiro/MG (7), 11.7% in Campinas/SP (21) and 19.3% in São Paulo/SP (8), whereas studies using ultrasound found values of 9.3% in Curitiba/PR (12) and 18.4% in the state of Maranhão (22). The most recent data from Brazil reported a prevalence of cholelithiasis of 11.43% based on ultrasound evaluations, although the study included patients with GERD (gastroesophageal reflux disease) and esophageal achalasia (23). Regarding other countries in the Americas, studies of autopsy results found a prevalence of cholelithiasis of 9.1% in Chicago, USA (2); 14.3% in Mexico (3); and 26.6% in Santiago, Chile (4), whereas studies using ultrasonography found values of 28.5% in Chile (11) and 20.5% (10) and 21.9% (24) in Argentina and, in the USA, the prevalence was 5.3% and 13.9% in non-Hispanic black men and women, respectively, the ethnic group with the lowest prevalence (1).

The great difference in the prevalence of cholelithiasis between the violent death group in this study and the populations studied in Brazil and other countries in the Americas may be due to differences in ethnic distribution, age, gender, alcohol consumption and BMI.

In the violent death group, 83% of subjects were male, 91% were non-white, the average age was 41.31 years (±16.84 years), the average BMI was 24.22 kg/m^2 (±4.56 kg/m^2), and 43% consumed alcohol. In comparison, the average age and average BMI in other studies performed in Brazil and in other countries in the Americas were higher and male and non-white individuals with moderate alcohol consumption were not predominant. This difference likely exists because most autopsy studies are performed on individuals who die of natural causes and because studies that use diagnostic ultrasound exams include representative samples from the general population. Therefore, the population who suffered violent deaths had a lower prevalence of some of the major risk factors for the development of cholelithiasis, such as female gender (25,26), advanced age (27), high BMI (28) and white ethnicity (15,21). Furthermore, the violent death population had a high rate of moderate alcohol consumption, which is theoretically a protective factor for cholelithiasis (14,29). These differences provide, at least in part, an explanation for the lower prevalence of cholelithiasis in subjects who suffered violent deaths in this study than the rates found in other studies.

No other study has prospectively evaluated the presence of gallstones in a population with the same characteristics as the violent death group (young individuals without comorbidities) based on autopsy results. One study, which examined a similar population, relied on ultrasound (30). That study was conducted in former West Germany and included 1116 blood donors with a mean age of 36.4 years (*versus* 41.31 years in the violent death group in our study), 34.3% of whom had a BMI above 25 kg/m^2 (*versus* 33.6% in the violent death group from our study). A higher prevalence of cholelithiasis was reported in the German study than in the group that suffered violent deaths in our study: 6% *versus* 2.12%. Although the German study did not show such a marked prevalence of males (58.7% *versus* 83.64% in the violent death group in our study), the prevalence of cholelithiasis among men (5.8%) in the German study was higher than the prevalence of disease in both men (2.17%) and women (1.85%) who suffered violent deaths in our study. Another study that also used ultrasound and examined a similar population (96.9% males with a mean age of 42.2 years) found a prevalence of cholelithiasis of 12.5% (31). However, that study was conducted in HIV-positive patients and exposure to ritonavir-boosted atazanavir in and of itself was associated with cholelithiasis (31).

Although the German study (30) did not provide data about the ethnic distribution of its study population, it is unlikely that the percentage of non-white individuals was as high as that in the violent death in our study. Because white skin is a risk factor for cholelithiasis (15,21), this difference in the ethnic distribution between the German study and the

Table 3 - Prevalence of Cholelithiasis (and 95% Confidence Interval) by Sex and Age – Natural Death.

Age (yrs)	Male			Female			Total		
	N	Prevalence	95% CI	N	Prevalence	95% CI	N	Prevalence	95% CI
<70	50	0	0 – 8.74	39	5.13	0.62 – 17.98	89	2.25	0.18 – 8.42
⩾70	15	13.33	2.72 – 39.38	12	33.33	13.78 – 61.22	27	22.22	10.4 – 41.21
Total	65	3.08	0.28 – 11.31	51	11.76	5.23 – 23.86	116	6.9	3.39 – 13.28

Table 4 - Prevalence Rates of Qualitative and Categorized Quantitative Variables – Natural Death.

Factor	Group	PR	95% CI	*p* value
Gender	Male	0.26	0.06 – 1.24	0.092
Ethnicity	Non-white	0.66	0.17 – 2.62	0.557
BMI	$\geqslant$30 kg/m^2	4.37	1.17 – 16.32	0.028
Age	$\geqslant$70 yrs	9.88	2.12 – 46.13	0.004
Alcohol use	Yes	0.58	0.07 – 4.46	0.599

violent death group in our study might explain the lower prevalence of cholelithiasis in the latter group. However, we cannot exclude other important risk factors related to cholelithiasis, such as diet and physical activity level (13), ethanol consumption (14), ethnicity (15), and genetic factors (16), as potential causes for the lower prevalence of the disease in the violent death group in our study than in the German study population.

Although the literature indicates that age (27), female gender (26,25), white skin (15,21) and BMI (28) are risk factors for cholelithiasis and that alcohol is a potentially protective factor (14), we did not find a positive correlation between cholelithiasis and gender, ethnicity, BMI, or alcohol consumption in the violent death group. There was a positive correlation only between cholelithiasis and age. Individuals aged $\geqslant$48 years had a higher prevalence of cholelithiasis (PR=5.28, 95% CI 1.04 to 26.76), which was confirmed by the multiple regression analysis (p=0.044). The German study also found a positive correlation between age and cholelithiasis. However, while a positive correlation between BMI and cholelithiasis was not found in the violent death group, such a correlation was reported in the German study population.

The lack of positive correlations between cholelithiasis and gender and ethnicity may be due to the small number of female and white subjects in the violent death group. Furthermore, it is difficult to establish an ethnic classification in mixed populations, such as those in Brazil, especially in Palmas/TO, a city composed of individuals from all over Brazil. Other studies of autopsy results conducted in Brazil, in São Paulo/SP (8) and in Triângulo Mineiro/MG (7), also did not find an association between the disease and ethnicity. Of note, in the German study (30), a positive correlation between female gender and cholelithiasis was not found. Because a higher female-to-male ratio of cholelithiasis has been observed only for subjects under 60 years of age (2.2:1) (32), this finding is in contrast to what was expected.

For the group that died naturally, the prevalence of cholelithiasis – 6.9% (95% CI 3.39 to 13.28) of all subjects (3.08% of the male subjects and 11.76% of the female subjects) – was lower than those reported by other autopsy studies conducted in the Americas, such as in São Paulo/SP, Brazil (19.3%) (8); Campinas/SP, Brazil (11.7%) (21); Chicago, USA (9.1%) (2); Mexico (14.3%) (3); and Santiago, Chile (26.6%) (4). These differences cannot be attributed to demographic differences among the study populations, such as age, gender or BMI, because all of those studies were conducted in subjects who died naturally. Compared to studies using ultrasound, the prevalence of cholelithiasis in the natural death group was also lower than in other studies, such as those conducted in Curitiba/PR, Brazil (9.3%) (12); Maranhão State, Brazil (18.4%) (22); Argentina (20.5%) (10); Chile (28.5%) (11); and in non-Hispanic black people in the USA, the ethnic group with the lowest prevalence (5.3% in men and 13.9% in women) (1). This finding reinforces the hypothesis that was previously raised for the violent death group: environmental factors, such as diet and physical activity level (13), ethanol consumption (14) and ethnicity (15), and genetic factors (13,16) may influence the prevalence of cholelithiasis.

Similar to the violent death group, no positive correlation was found between cholelithiasis and gender, ethnicity or alcohol consumption in the natural death group, but a positive correlation between cholelithiasis and age was found. The cutoff value at which a higher prevalence of the disease was observed was 70 years of age (PR=9.88, 95% CI 2.12 – 46.13). However, in contrast to the violent death group, a positive correlation between cholelithiasis and BMI was found in the natural death group. The cutoff value at which a higher prevalence of disease was observed was BMI$\geqslant$30 kg/m^2 (PR=4.37, CI 95% 1.17 – 16.32). Both age and BMI remained significantly correlated with cholelithiasis in the multiple regression analysis, with p values of 0.001 and 0.022, respectively.

Unfortunately, after conducting this study, we were not able to fully clarify the impact of some controversial factors on the incidence of cholelithiasis, such as diet, physical activity level, ethanol consumption, ethnicity and genetic factors. However, our results indicate that these factors and others still unknown may be responsible for the low prevalence of cholelithiasis found in both the natural death and violent death groups from the city of Palmas/TO, Brazil.

We conclude that the city of Palmas/TO, Brazil, has the lowest prevalence of cholelithiasis in the Americas and that dietary habits, physical activity, ethnicity and alcohol consumption, as well as genetic factors, may be responsible for that low prevalence.

AUTHOR CONTRIBUTIONS

Asperti AM and D'Albuquerque LA contributed to the results and discussion. Reis P, Pinto MD, and Silva Júnior EC conducted the data collection and contributed to the discussion. Diniz MA contributed to the methods and results sections. Silva DF conducted data collection and the literature review and contributed to the results.

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