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# Interval increase in the prevalence of symptomatic cholelithiasis-associated non-alcoholic fatty liver disease over a ten-year period in an Asian population

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**ABSTRACT**

**Introduction:** Non-alcoholic fatty liver disease (NAFLD) is frequently associated with cholelithiasis. The prevalence of NAFLD has been on the rise in Asia, but the magnitude of this increase has not been previously studied.

**Methods:** A retrospective cohort study was carried out on consecutive patients who underwent laparoscopic or open cholecystectomy from November 2001 to November 2004 (cohort 1) and from November 2011 to November 2014 (cohort 2) at Singapore General Hospital. Preoperative diagnostic scans (e.g. ultrasonography, computed tomography and magnetic resonance imaging) and clinical data were reviewed for the presence of fatty liver. Statistical analysis was performed.

**Results:** In cohorts 1 and 2, 127 patients and 99 patients were operated, respectively. Cohort 2 had significantly higher proportions of patients with NAFLD (56.6% vs. 40.2%; $p < 0.015$) and hyperlipidaemia (45.5% vs. 18.9%; $p < 0.001$) when compared to cohort 1. Binary logistic regression analysis showed that patients with hypertension (odds ratio [OR] 2.56; $p < 0.004$), and patients of Indian ethnicity (OR 5.45; $p < 0.043$) were significantly associated with NAFLD.

**Conclusion:** Similar to other international studies, we found a significant increase in the prevalence of patients with NAFLD presenting symptomatically for cholecystectomy over an interval of ten years in Singapore. Hypertension and Indian race were most significantly associated with NAFLD in both time periods. This trend supports the need for concerted public health intervention to stem the increasing incidence of NAFLD and prevent its progression to more advanced liver disease.

*Keywords: Asian, cholelithiasis, epidemiology, non-alcoholic fatty liver disease, Singapore*

## INTRODUCTION

It has been established that non-alcoholic fatty liver disease (NAFLD) and gallstone disease (GD) or cholelithiasis are both associated with obesity, insulin resistance, dyslipidaemia and high dietary cholesterol consumption.[1] In fact, both conditions are highly associated with each other and often occur together comorbidly.[2] NAFLD and metabolic syndrome are proposed to be independent risk factors for GD,[3] and patients with NAFLD have a high prevalence of GD.[4] However, it remains unclear if NAFLD is a precursor of GD or whether the presence of GD is merely co-associated with metabolic syndrome that accelerates the progression of NAFLD.[1]

In Western countries, cholesterol gallstones predominate, whereas pigment and mixed stones are more common in Asia,[5] which is interesting given that the pathogenesis of cholesterol gallstones and pigment stones are thought to share common factors that are related to bile metabolism.[6] Despite the possible differences in gallstone aetiology in the Asian population, NAFLD and markers of metabolic syndrome, such as low high-density lipoprotein cholesterol, high serum triglycerides, high waist circumference and body mass index (BMI), are similarly associated with GD.[7,8]

We were interested in studying the risk factors for NAFLD in our patient population that was comorbid with symptomatic GD because symptomatic patients can often be incidentally diagnosed for NAFLD during liver imaging, which needs to be performed preoperatively prior to cholecystectomy. Given that NAFLD is mainly asymptomatic[9,10] and often remains undiagnosed, as liver imaging is not routinely performed in the community setting, symptomatic patients with GD who may concurrently have NAFLD would be more readily targetable for disease management measures that are aimed at controlling the progression of NAFLD. This may be achieved by controlling the associated risk factors of NAFLD or by medical therapy.

The current management of NAFLD consists of diet control and lifestyle changes, and while there is some evidence that statins, bile acid conjugates, and cholesterol absorption inhibitors may be able to treat both GD and NAFLD, the efficacy of these treatments remain inconclusive.[11] A recent review on pharmacotherapies for NAFLD noted that vitamin E, pioglitazone and obeticholic acid have proven their benefits, although their effect is modest.[12] Nonetheless, vitamin E supplements are still prescribed in clinics for NAFLD patients. If left unreversed, NAFLD may progress to more advanced liver diseases, such as liver cirrhosis and hepatocellular carcinoma.[4]

The prevalence of NAFLD varies across different populations and is increasing globally.[13] The multiracial population of the city-state of Singapore is reflective of that of Southeast Asia. In Asian populations, non-obese patients reportedly have a greater tendency to develop NAFLD when compared to Western populations.[14] Reports have also suggested a rise in the prevalence of NAFLD in Asia, affecting up to 30% of the general population, although the associated obesity and diabetes pandemics are thought to have occurred later in Asia than in the West.[15,16] However, there is a dearth of literature that objectively compares multiple cohorts from the same patient population at different time points. Hence, we were also interested in characterising the magnitude of the increase in the prevalence of NAFLD among local symptomatic patients with GD from an epidemiological perspective.

Accordingly, the aim of this study was to establish the relative prevalence of cholelithiasis-associated NAFLD in patients who presented symptomatically for cholecystectomy over two cohorts spaced ten years apart in a Southeast Asian population with BMI stratification, and to establish the risk factors associated with NAFLD for this group. Changes in the patient pool were likely to be representative of changes in the general population. We hypothesised that there would be an increase in the prevalence of NAFLD in accordance with international trends, with a null hypothesis that there would be no significant increase over ten years.

## METHODS

A retrospective cohort study was performed on consecutive patients who underwent laparoscopic or open cholecystectomy at Singapore General Hospital, Singapore, over two time periods separated by ten years, namely between November 2001 and November 2004 (cohort 1) and between November 2011 and November 2014 (cohort 2), who fulfilled the inclusion and exclusion criteria (Box 1). Surgery was carried out by a single surgeon whose surgical practice has remained consistent over the study period. Complete diagnostic scans were available for all patients. In addition, information on relevant demographic factors and medical comorbidities was retrieved from patients' medical records (Box 2). Patient history of alcohol consumption was derived from the preoperative anaesthesia notes.

**Box 1. Inclusion and exclusion criteria for patient enrolment:**

Inclusion criteria:

- Aged 21–90 years
- Previously underwent laparoscopic or open cholecystectomy
- Calculous gallbladder
- Underwent preoperative imaging (e.g. ultrasonography, computed tomography or magnetic resonance imaging)

Exclusion criteria:

- History of chronic alcohol consumption
- Medical history of viral hepatitis
- Acalculous gallbladder
- No imaging data
- Carcinoma of the gallbladder

**Box 2. Demographic, medical and lifestyle characteristics retrieved:**

Demographic

- Age
- Gender
- Ethnicity
- Body mass index

| |
|---|
| Comorbidity<br>• Type 2 diabetes mellitus<br>• Hypertension<br>• Hyperlipidaemia<br>• Ischaemic heart disease<br>Lifestyle<br>• Chronic alcohol consumption |

Reports of diagnostic imaging, such as ultrasonography, computed tomography or magnetic resonance imaging, were reviewed for the presence of fatty liver disease. If multiple modalities were used, a positive reporting was taken to be true. If there was no report of fatty changes to the liver, the result was taken to be negative. This study was approved by the SingHealth Centralised Institutional Review Board (CIRB Ref. No.: 2015/2035).

Chi-square test was used to analyse if any change in the proportion of patients with NAFLD, other medical comorbidities and demographic characteristics between the two cohorts occurred by chance, while a two-tailed Student's *t*-test ($\alpha = 0.05$) was used to compare for changes between mean age and BMI. Univariate binary logistic regression was performed on all factors to be analysed, and factors with $p < 0.2$ were included in the multivariate logistic regression model. The Hosmer-Lemeshow goodness-of-fit test was used to test for adequacy of fit during multivariate logistic regression analysis. Statistical analysis was performed using IBM SPSS Statistics version 20.0 (IBM Corp, Armonk, NY, USA). A p-value of $< 0.05$ was considered to be statistically significant.

## RESULTS

A total of 226 patients fulfilled the study criteria – 127 patients were from cohort 1, for whom surgery was performed between November 2001 and November 2004, and 99 patients were from cohort 2, who were operated from November 2011 to November 2014. The demographic profiles of both cohorts were largely similar, except that there was a decrease in the proportion of Chinese patients over time ($p < 0.006$). Accordingly, multivariate adjustment was performed to account for ethnicity in the subsequent logistic regression analysis. Table I presents the differences found between the two study cohorts with respect to demographic variables following the two-tailed *t*-test and chi-square test.

**Table I. Demographic characteristics of patients.**

| Variable | No. (%) | | $\chi^2$ | p-value |
|---|---|---|---|---|
| | **Cohort 1 (n = 127)** | **Cohort 2 (n = 99)** | | |
| Age (yr)* | 54.4 ± 13.4 | 53.9 ± 13.4 | 0.226† | 0.822 |
| BMI (kg/m$^2$)* | 24.8 ± 4.00 | 24.1 ± 3.30 | 0.527† | 0.599 |
| Gender | | | | |
| Women | 80 (63.0) | 58 (58.6) | 0.451 | 0.502 |
| Men | 47 (37.0) | 41 (41.4) | 0.451 | 0.502 |
| Ethnicity | | | | |
| Chinese | 110 (86.6) | 71 (71.7) | 7.702 | 0.006‡ |
| Malay | 7 (5.51) | 9 (9.09) | 1.081 | 0.299 |
| Indian | 6 (4.72) | 4 (4.04) | 0.061 | 0.806 |
| Other | 4 (3.15) | 15 (15.2) | 10.407 | 0.001‡ |

**Data presented as mean ± standard deviation. †T-statistic values. ‡p < 0.05 was considered statistically significant. BMI: body mass index*

Cohort 2 had a significantly higher proportion of patients with NAFLD (56.6% vs. 40.2%, $p < 0.015$) and hyperlipidaemia (45.5% vs. 18.9%, $p < 0.001$) when compared to cohort 1 (Table II). There was no significant increase in the proportion of patients with other comorbidities over the ten-year period.

**Table II. Comorbidities of patients from both study cohorts.**

| Variable | No. (%) | | $\chi^2$ | p-value |
|---|---|---|---|---|
| | **Cohort 1 (n = 127)** | **Cohort 2 (n = 99)** | | |
| Hyperlipidaemia | 24 (18.9) | 45 (45.5) | 18.520 | 0.001† |
| NAFLD | 51 (40.2) | 56 (56.6) | 5.904 | 0.015† |
| Diabetes mellitus | 20 (15.7) | 22 (22.2) | 1.552 | 0.213 |
| Hypertension | 57 (44.9) | 44 (44.4) | 0.006 | 0.940 |
| Ischaemic heart disease | 11 (8.66) | 8 (8.08) | 0.043 | 0.835 |

*Chi-squared test (α = 0.05) for change in proportion of NAFLD and medical comorbidities. †p < 0.05 was considered statistically significant. NAFLD: non-alcoholic fatty liver disease*

As the Asian population presents with obesity at lower BMI ranges,[17,18] the definitions of overweight, obesity and severe obesity were stratified in our study according to World Health Organization guidelines for the Asia-Pacific region as: normal (BMI 18.5–22.9 kg/m$^2$); overweight (BMI 23.0–24.9 kg/m$^2$); obese (BMI 25.0–29.9 kg/m$^2$); and severely obese (BMI ≥ 30.0 kg/m$^2$).[19] Logistic regression analysis of possible risk factors revealed that hypertension (odds ratio [OR] 2.56; p < 0.004) and Indian ethnicity (OR 5.45; p < 0.043) were factors that were positively associated with NAFLD in our study after multivariate adjustment for ethnicity, BMI and medical comorbidities (Table III).

**Table III. Logistic regression analyses of medical and demographic variables, with NAFLD as the dependent variable.**

| **Variable** | **Univariate analysis** | | **Multivariate analysis** | |
|---|---|---|---|---|
| | **OR (95% CI)** | **p-value** | **OR (95% CI)** | **p-value** |
| BMI (kg/m$^2$) | | | | |
| < 23.0 | 0.709 (0.334–1.507) | 0.372 | 0.675 (0.302–1.509 | 0.339 |
| 23.0–24.9 | 2.172 (0.629–7.499) | 0.220 | 2.075 (0.548–7.859) | 0.283 |
| 25.0–29.9 | 2.457 (1.039–5.808) | 0.041* | 1.996 (0.795–5.009) | 0.141 |
| ≥ 30.0 | 2.539 (1.253–5.147) | 0.010* | 2.010 (0.950–4.255) | 0.068 |
| Ethnicity (Chinese ethnicity as reference) | | | | |
| Indian | 5.282 (1.091–25.572) | 0.039* | 5.448 (1.054–28.170) | 0.043* |
| Malay | 2.201 (0.767–6.315) | 0.142 | 1.828 (0.571–5.853) | 0.310 |
| Other | 1.816 (0.697–4.728) | 0.222 | 1.549 (0.561–4.280) | 0.398 |
| Gender (men vs. women) | 1.316 (0.768–2.253) | 0.317 | | |
| Risk factor | | | | |
| Hypertension | 3.052 (1.770–5.262) | 0.000* | 2.558 (1.361–4.808) | 0.004* |
| Diabetes mellitus | 1.829 (0.926–3.615) | 0.082 | 1.125 (0.517–2.445) | 0.767 |
| Hyperlipidaemia | 1.701 (0.961–3.012) | 0.068 | 1.029 (0.516–2.052) | 0.936 |
| Ischaemic heart disease | 1.260 (0.492–3.229) | 0.630 | | |

*Hosmer-Lemeshow test: $\chi^2$ = 6.66, significant p = 0.574 (adequate fit). *p < 0.05 was considered statistically significant. BMI: body mass index; CI: confidence interval; NAFLD: non-alcoholic fatty liver disease; OR: odds ratio*

**DISCUSSION**

Over the ten-year interval, there was a significant increase in both NAFLD and hyperlipidaemia in patients who underwent cholecystectomy at our centre. NAFLD was also significantly associated with hypertension, BMI and Indian ethnicity. Although a significant increase in the proportion of patients with hyperlipidaemia was found, and there was some degree of association between hyperlipidaemia and NAFLD in the univariate analysis (OR 1.701; $p < 0.068$), we found no significant association after multivariate adjustment. This contrasts against another multiethnic Asian study performed on a general population pool where hypertriglyceridaemia and low high-density lipoprotein were found to be independent predictors in a multivariate model,[(20)] as well as the wider international literature, where hyperlipidaemia is strongly associated with NAFLD.[(21)] This discrepancy could be due to the smaller sample size in our study and the fact that our study only included patients with GD. As GD is already known to be associated with hyperlipidaemia and other manifestations of metabolic syndrome,[(22)] the significance of hyperlipidaemia may be negated as an independent risk factor in our patient pool.

However, our study concurs with established literature that arterial hypertension, a manifestation of metabolic syndrome, is independently associated with NAFLD. It was previously found that the prevalence of NAFLD in non-obese and non-diabetic hypertensive patients was at least twice that in the general population.[(23)] Therefore, our results indicate that hypertension, in addition to BMI, needs to be better controlled in our local population.

BMI stratification showed that overweight and non-severely obese patients may be at increased risk of NAFLD, although this association did not reach statistical significance in our study. However, it is worth noting that the risk for developing NAFLD increased as we progressed up the BMI strata. Overweight patients with BMI in the range 23.0–24.9 $kg/m^2$ (OR 2.08; $p < 0.283$) and obese patients with BMI in the range 25.0–29.9 $kg/m^2$ (OR 1.99; $p <$

0.141) demonstrated increasing ORs and statistical significance. These findings were similar to those of another multiethnic Asian study, where all patients with BMI > 23.0 kg/$m^2$ were grouped together and found to be significantly associated with NAFLD.[20] As such, by using a greater degree of stratification, our study does not directly concur with the notion of non-obese NAFLD being prevalent in the Asian population.[14] Instead, our results indicate that clinicians should target patients in the higher BMI groups and graduate management accordingly. Our findings assist in establishing BMI targets for patients in the local population for the purposes of weight management.

Although our study showed that Indian patients were at a greater risk for NAFLD when compared to Chinese patients, we noted that the confidence interval was very wide due to the small sample number of Indian (n = 10) patients in our study. However, this result is in line with regional studies that have shown that patients of Indian ethnicity are more predisposed to NAFLD than Chinese patients, ostensibly due to the higher prevalence of obesity in the former.[20,24] Placing Indian ethnicity as an independent risk factor during multivariate logistic regression analysis in our study supported the notion that there are likely other genetic predispositions in this patient population. Interestingly, it has been previously shown that Asian Indians have a higher body fat percentage and abdominal adiposity even when the BMI is within normal limits.[25]

It is possible that changes in the prevalence of NAFLD may be due to lifestyle or diet choices. For example, it has been shown that the dietary consumption of fructose is linked to NAFLD, given that fructose is metabolised by the liver.[26] As fructose is commonly added to fruit juice and sweet drinks, it is a candidate apt for dietary control measures. One could also postulate that an increasingly westernised diet, sedentary lifestyles, readily accessible fast foods and increased calorie intake could be contributing factors for this change in prevalence.[15] However, there is a dearth of detailed primary studies in the recent literature to identify the specific lifestyle factors accounting for this shift in Singapore.

We acknowledge that there are several limitations to this study. The radiological reporting may not have been consistent, as this was a retrospective study, and the sensitivity of the diagnostic imaging may have changed over time. As there was no specific request to report for fatty liver disease, our results were likely to be conservative, even though all abnormalities, including incidental findings, were to be reported as part of routine protocol. Patient history on chronic alcohol consumption was taken from preoperative anaesthesia notes, and may not have specified the exact consumption amount, which is ideally defined by alcohol consumption of more than 20 g daily (or 140 g weekly) for men and more than 10 g daily (or 70 g weekly) for women.[16] Finally, we were limited to NAFLD data on a subset of patients with symptomatic GD, which we assumed to have followed trends in the general population from an epidemiological standpoint. Nonetheless, such data was still useful to our objective of proactively identifying patients for further management.

In conclusion, there was a significant increase in the proportion of patients with symptomatic cholelithiasis comorbid with NAFLD and hyperlipidaemia over the span of ten years at our centre, which is alarming from an epidemiological perspective. Hypertension and BMI > 30.0 kg/m$^2$ are the main factors that need to be more aggressively controlled in our patient population, so as to stem the increasing incidence of NAFLD before it progresses to more advanced liver disease. These results allow us to take steps at an advisory level by establishing the characteristics of individuals who are likely to be at risk and also help to identify at-risk patients for future prospective trials.

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