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Cholelithiasis Following Bariatric Surgery: A New Approach to Deal with

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Abstract

Background Obesity and rapid weight loss induced by weight-reducing surgery are well recognized as a risk factor for the development of gallbladder stones. There is no standard policy whether to perform prophylactic cholecystectomy at the time of the bariatric operation or to give postoperative treatment to decrease the risk.

Objective To evaluate the incidence and risk factors of gallstone formation post-bariatric surgery. The results may help to decide how to deal with and follow up patients with post-bariatric surgery.

Methods A total of 120 patients who underwent weight-reducing operations were recruited for this study. Several factors expected to influence gallstone formation were recorded such as body mass index and excessive weight loss. Study population was followed up for 12 months postoperatively. Ultrasonography examination was performed for those who developed symptoms suggesting gallstone formation.

Results Twenty-six (21.7%) patients were found to develop gallstones. Of the studied risk factors, the percentage of excess weight loss, family history and carrying allele A of the variant rs670 were significant for predicting development of gallstone post-bariatric procedures.

Conclusion Based on the results of this study, it is reasonable to put an index for the risk of developing gallstone following bariatric surgery, and according to this index, the surgeon could decide whether to perform concomitant cholecystectomy along with the bariatric procedure or do not.

Keywords Bariatric surgery, cholelithiasis, single nucleotide polymorphism

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List of abbreviations: BMI = Body mass index, BS = Bariatric surgery, EWL = Excess weight loss, GS = Gall stone, LRYGB = Laparoscope Roux-en-y gastric bypass, LSG = Laparoscopic sleeve gastrectomy, MO = Morbid obesity, SNPs = Single nucleotide polymorphisms

Introduction

Morbid obesity (MO) is a leading preventable cause of death worldwide. In 2014, World Health Organization estimated that 600 million adults and 42 million children were obese [(1)]. This condition is associated with 2-fold increase in mortality compared with the general population, and with an increased number of serious pathological conditions such as hypertension, type 2 diabetes mellitus, depression and gallstone (GS) [(2,3)]. Three main options are considered in the treatment of MO. These are lifestyle change, pharmacotherapy, and surgery. In the first two options, the weight loss usually not maintained, and up to 66-90% of patients regain weight after cessation of the changed lifestyle or treatment

[4,5]. On the other hand, bariatric surgery (BS) offers the only means of delivering substantial weight loss.

However, this marked efficiency of BS in treatment of MO is not without penalties; may be the most important of which is the increased incidence of GS regardless of the operation type. As early as 1983, Wattchow et al. [6] reported an increased likelihood of GS after Laparoscopic Roux-en-Y Gastric Bypass (RYGB). Shiffman et al. (7) evaluated the incidence of GS formation in 105 morbidly obese patients undergoing gastric bypass surgery. After 6 months follow up, 36% of the patients became suffering from cholelithiasis, a percentage which remained stable until 18 months. These facts impose intensive investigation to reduce or even eliminate such incidence of GS following BS.

Three approaches have been suggested to address this problem. The first one pioneered by O'Brien et al. [8] called for cholecystectomy for all patients with BS. Additional operation and hospitalization time as well as possible complications of the procedure are among main disadvantages of this approach. The second approach [9] involves waiting and performing cholecystectomy for those who develop symptomatic GS after BS. Finally, the third approach depends on the using of preventive drugs. In the last two approaches, there is a relatively high cost of follow up and medications. An alternative approach is the calculation of expected risk of GS formation in patients undergoing BS and deciding whether to do simultaneous cholecystectomy or not. Some risk factors were already addressed like excess weight loss (EWL) and found to positively affect GS formation [10]. Other factors like the effect of genetic were less studied.

Individual's genetic background is undoubtedly involved in GS formation either in obese or non-obese persons [11,12]. Certain single nucleotide polymorphisms (SNPs) in some genes were found to be associated with different diseases [13]. Of these, the SNPs rs670 in ApoA1 gene and rs351855 in FGFR4 gene were found to be associated with cholilithiasis. Dixit et al. [14] reported high risk for G allele of the SNP rs670 on GS disease in India, while Chen et al. [15] linked rs351855 with the aggravation of GS disease among Chinese population.

The current study aimed to evaluate the risk factors predisposing for gallstone formation in Iraqi patients undergoing BS in order to establish an index that help surgeon to decide how to deal with the problem of GS following BS.

Methods

Study Population

This is a prospective study of 120 MO-patients who underwent Laparoscopic Roux-en-Y Gastric Bypass (LRYGB) and Laparoscopic Sleeve Gastrectomy (LSG) at Al-Imamein Al-Kadhimein Medical City, Baghdad during the period from August 2013 to January 2015. Consent form explaining the objective and the scope of the study was obtained from each participant. Exclusion criteria were prior cholecystectomy, presence of GS by abdominal ultrasonography and refusal of participation in the study. During 12 months' post-operative follow up, the patients who were symptomatic for acute cholecystitis, acute cholangitis, abnormal and/or biliary pancreatitis as first presentation, were examined by abdominal ultrasonography along with liver function tests. Cholecystectomy was performed to those who developed gallstone during the follow-up period.

Data were collected by direct interview with each patient. These data included age, gender, preoperative body mass index (BMI), family history of GS, diabetes mellitus, and percentage of excess weight loss (%EWL), which was calculated according to Broca formula [16].

Blood samples were collected from each patient before the operation and kept in EDTA tubes. DNA was extracted from these samples using ready kit (gSYNCTM DNA Mini Kit Whole Blood Protocol/ Geneaid/ Korea) according to the manufacturer's instructions. The target sequence containing the SNPs rs670 in ApoA1 gene and rs351855 in FGFR4 gene were

amplified with specific primers using polymerase chain reaction technique (PCR).

The ApoA1 gene was amplified using the primers: forward 5'-AGGGAC AGA GCT GATCCT TGA ACT CTTAAG-3' and reverse 5'-TTAGGG GAC ACC TACCCGTCAGGA AGA GCA-3' [17]. Primers for FGFR4 gene were 5'-GACCGCAGCAGCGCCCGAGGCCAG-3' as forward primer and 5'-AGAGGGAAGAGGGAGAGCTTCTG-3' as reverse primer [18]. Each amplification was performed using 100 ng to 300 ng of genomic DNA in a volume of 25 µL using 12.5 pmol of each primer, 200 µM dinucleotide triphosphate, 15 mM magnesium chloride, 100 mM Tris (pH 8.0) and two units of Taq polymerase (Bioneer/ Korea). PCR conditions for ApoA1 involved an initial denaturation at 95°C for 5 min followed by 30 and with denaturation at 95 °C for 30s, annealing at 60 °C respectively for 45 s, extension at 72 °C for 60 s and final extension at 72 °C for 5 min. Almost similar conditions were applied for FGFR4 gene, but involved 35 cycles and the annealing temperature was 60 °C.

The PCR products were detected by 1% agarose electrophoresis and visualized under U. V light after staining with ethidium bromide. The amplified products were determined by comparison with a commercial 1000 bp ladder (Kappa Biosystem/USA). PCR products (435-bp and 168-bp for ApoA1 and FGFR4 respectively) were directly sequenced with ABI system (Macrogen/Korea).

Statistical analysis

Quantitative variables were expressed as means ± standard deviation (SD), while qualitative data were presented as absolute frequencies and proportions. Binary regression analysis was used to find out odds ratios (OR) with 95% confidence interval (CI) for categorical variables with respect to GS formation. Chi square test with 2x2 tables was employed to analyze the differences in proportions. Student t-test was used to compare means between two groups for quantitative variables. All analysis was performed with SPSS for windows software, version 16.0. A P-value of less than 0.5 was considered statistically significant.

Results

Demographic Characteristics of the Study Population

The demographic characteristics of the study population are shown in table 1.

Table 1. Demographic Characteristics of the study population

Parameter	Characteristics	Value
Age (years)	Mean	40.2
	Range	20-57
	SD	7.18
Gender	Male	44 (36.67%)
	Female	74 (63.33%)
BMI	Mean	41.19
	Range	37.1-43.9
	SD	6.62
Type 2 diabetes mellitus	Yes	12 (10%)
	No	108 (90%)
Family history of gallstone	Yes	7 (5.83%)
	No	113 (94.17%)
Bariatric Surgery	Roux-en-Y gastric bypass	14 (11.67%)
	Sleeve gastrectomy	106 (88.33%)

Incidence and risk factors of gallstones among MO-patients undergone BS

Out of 120 MO-patients underwent BS, 26 (21.6%) developed symptomatic GS diagnosed by transabdominal ultrasonography 2 to 12 months (mean 10 months) post BS. Elective cholecystectomy was performed by laparoscopic operation. Table 2 shows the association of different risk factors with the development of gallstone after BS. Only three of these factors (family history, %EWL and the SNP rs670) were significantly associated.

Five (19.23%) GS patients had family history of first or second relative with GS compared to 2 (2.13%) among patients with no GS with significant difference (OR=10.952, 95%CI = (1.987-60.371, P=0.005). Similarly, mean %EWL among GS and no GS patients were 24.6 and 21.65 with significant difference (P=0.028). The SNP rs670 had three genotypes; CC, CT, and TT (figure 1). In patients who developed GS, these genotypes account for 11 (42.31%), 9 (34.61%), and 6 (23.08%) respectively, compared to 61(64.89%), 29(30.86%), and 4(4.25%) respectively, in patients with no GS with significant differences for both heterozygous genotype (OR=8.318, 95%CI=2.013-34.37, P=0.003) and for homozygous mutant genotype (OR=4.833, 95%CI=1.112-21.014, P=0.036). Analysis of allele's frequencies of this SNP confirmed the significant association of T allele with the incidence of GS. The frequency of T allele between patients with and without GS was 40.38% and 19.68% respectively (OR=2.765, 95%CI=1.428-5.351, P=0.002).

On the other hand, each of the age, sex, BMI, operation type, DM and different genotypes of the SNP rs351855 did not show significant influence on the incidence of GS after BS. For age the two groups (GS and non GS patients) had very closed means of age (40.2 and 40.35 years respectively, P=0.488). Although female represented 73.08% among GS patients compared to 60.64 in non-GS patients, the difference was insignificant (OR=1.762, 95%CI=0.674-4.603, P=0.175).

Similar to rs670, the SNP rs351855 appeared with three genotypes which were CC, CT and TT (Table 2, figure 2). These genotypes represented 65.38%%, 26.93% and 7.69% respectively in patients with GS and 73.4%, 25.53% and 1.07% respectively in non GS patients. However, the differences were not significant.

At allelic level, the frequency of T allele in patients with GS was 21.15% compared with 13.83% in those with no GS with insignificant difference (OR=1.672, 95%CI=0.763-3.661, P=0.199).

Discussion

Bariatric surgery is known worldwide as a cost-effective treatment for MO patients. Cholecystitis and GS formation are the main unfavorable sequelae of this maneuver. Three approaches have been adopted to prevent or treat GS after BS. However, each of these approaches implies some disadvantages. In fact, the aim of this study is beyond determination of the incidence and risk factors associated with GS formation post-BS. That is because both the incidence and risk factors were well addressed, and there is almost a general agreement that only the %EWL is significant risk factor, and this what the current result revealed. However, for the best of our knowledge, it is the first time to investigate genetic risk factors in this regard. Thus, the study was intended to formulate an alternative approach to deal with GS formation in those patients. Although needs for maturation, this approach bases upon calculation of significant risk factors to get a value according which surgeon can take the decision whether to perform simultaneous cholecystectomy with BS or not. That means we have to establish a standardized language to evaluate these risks.

Out of nine studied risk factors, only 3 had significant association with the incidence of GS.

Table 2. Risk factors of gallstone formation following bariatric surgery

Risk factor		Gallstones 26 cases	No gallstones 94 cases	*P*-value	OR (95%CI)
Age (yr)	mean±SD	40.2±6.03	40.35±5.53	0.488	—
Sex	Male Female	7 (26.92%) 19 (73.08%)	37 (39.36%) 57 (60.64%)	0.175	1.762 (0.674-4.603)
BMI (Kg/m^2)	mean±SD	42.8±4.17	41.75±6.22	0.104	—
Operation	LRYGB LSG	4 (15.38%) 22 (84.15%)	10 (10.64%) 84 (89.36%)	0.357	0.655 (0.187-2.287)
Family History	No Yes	21 (80.77%) 5 (19.23%)	92 (97.87%) 2 (2.13%)	0.005	10.952 (1.987-60.371)
DM	No Yes	22 (84.62%) 4 (15.38%)	11 (11.7%) 83 (88.3%)	0.415	1.372 (0.398-4.727)
%EWL	Mean	24.6	21.65	0.028	—
rs670 Genotypes	CC CT TT	11 (42.31%) 9 (34.61%) 6 (23.08%)	61 (64.89%) 29 (30.86%) 4 (4.25%)	0.013 0.003 0.036	8.318 (2.013-34.370) 4.833 (1.112-21.014)
rs670 Alleles	C T	31 (59.62%) 21 (40.38%)	151 (80.32%) 37 (19.68%)	0.002	2.765 (1.428-5.351)
rs351855 Genotypes	CC CT TT	17 (65.38%) 7 (26.93%) 2 (7.69%)	69 (73.4%) 24 (25.53%) 1 (1.07%)	0.246 0.095 0.138	8.118 (0.695-94.866) 6.857 (0.539-87.279)
rs351855 Alleles	C T	41 (78.85%) 11 (21.15%)	162 (86.17%) 26 (13.83%)	0.199	1.672 (0.763-3.661)

SD: standard deviation; LRYGB: laparoscopic Roux-en-Y gastric bypass; LSG: laparoscopic sleeve gastrectomy; DM: diabetes mellitus; EWL: excessive weight loss

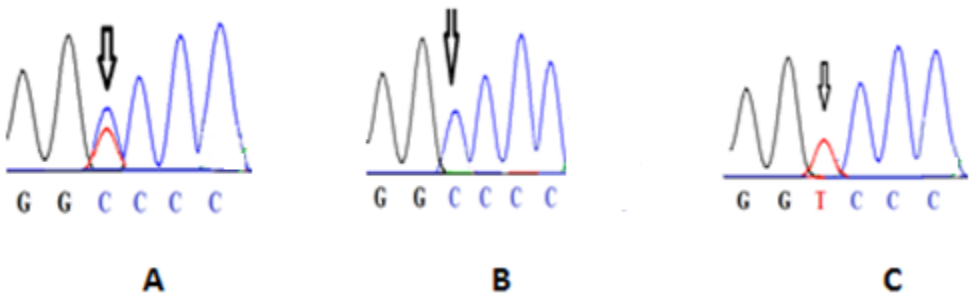

Figure 1. Different genotype patterns of the SNP rs670 (reverse strand). Heterozygous (CT) genotype (A), homozygous wild type (CC) genotype (B) and homozygous mutant (TT) genotype (C)

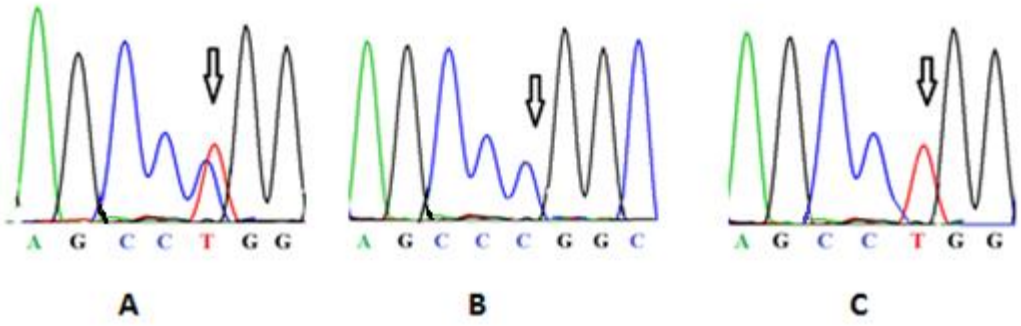

Figure 2: Different genotype patterns of the SNP rs351855; heterozygous (CT) genotype (A), homozygous wild type (CC) genotype (B) and homozygous mutant (TT) genotype (C)

For EWL percentage, there is no odds ratio because there is no reference EWL and it cannot be calculated at time of BS. However, the intensive investigations in this respect have determined the %EWL for every operation type. For example, after two years of LSG or LRYGB, the %EWL is 65-70% [16]. Body weight at the operation time does not seem to affect the GS stone formation as evidenced by the insignificant association of BMI with this disease, but this weight certainly influence %EWL. However, the range of this loss is very narrow and can be considered for every patient. It is the presence of other risk factors (genetics) that contribute the variation of GS incidence among different patients.

Family history can be easily obtained, and the risk of which can, also, be easily calculated through obtaining odds ratio. In normal population, studies revealed an increased frequency of cholelithiasis; nearly 3 times elevated risk in the relatives of GS patients [19]. The result of the current study showed that patient who has first or second-degree relative with GS exposes 10.95-fold risk of developing GS compared with patient without such relative. This implies predisposing factors among certain family mainly related to genetic components, similar dietary and other common lifestyles.

On the other hand, identification of different variants associate with the risk of GS is rather a hard task. With the rapid development of molecular technique, this task becomes less difficult. Fortunately, limited number of SNPs were reported to influence GS formation [14,20-22]. Of course, These SNPs, could be enrolled under genetic factors influencing family history, but not all family history is genetic, nor all family members carry the same variant. Therefore, study of these variants is important in putting an expectation for GS formation. Among the most studied gene in this respect is ApoA1 gene which encodes a major protein component of the HDL and a co-factor for lecithin cholestrolacyltransferase (LCAT). The later catalyzes the formation of plasma cholesterol ester [23]. Thus, the product of this gene is directly involved in GS formation because accumulation of the cholesterol ester in the gallbladder predispose for cholelithiasis [24]. The minor allele of the SNP rs670 (allele T) in ApoA1 gene was found to be associated with abnormal levels in serum lipids in several populations [25-27]. Furthermore, carriers of this allele were 1.7-times more chance to increase LDL-C than those who carry C allele.

In line with these results, the current study revealed that carrier of T allele has 2.76-fold risk of developing GS post BS compared to C allele carriers. Thus, patients who has a family history of GS and carries allele T of the SNP rs670 is highly predisposed to GS formation after BS, and therefore it is recommended in such patient to perform concurrent cholecystectomy with BS. Of course, the list of risk fact, especially SNPs, does not end at this point, and many SNPs could be added [27], but,

the present data strongly suggest a new approach for dealing with GS formation after BS through calculation of absolute risk. More studies with larger patients and other SNPs are needed to formulate such number. Then each significant risk factor will be given a value according to the obtained odds ratio. Summation of these values will result a number used by surgeon to decide how to deal with the problem of GS formation in patients with BS.

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Author contribution
Dr. Alhefy: work design and sample collection. Dr. Al-Mayah: Statistical analysis, DNA sequence analysis and writing. Dr. Allami: PCR, reviewing and writing of references.

Conflict of interest
The authors declare that they have no conflict of interest.

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