Original Article

The Incidence and Outcome of Pregnancy-related Biliary Sludge/stones and Potential Risk Factors

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Abstract

Background: Diseases of the gall bladder have been reported to be the most common cause for non-obstetrical hospitalizations during the first year postpartum. Therefore we designed a prospective study to evaluate the incidence and natural outcome of pregnancy-related biliary sludge and stones, and to define the potential risk factors in a population of pregnant women in Sari, Iran.

Methods: A total of 380 pregnant women were scheduled for three ultrasound assessments: 1) during the first trimester of pregnancy (9 – 12 weeks), 2) during the early third trimester (26 – 32 weeks), and 3) during the fourth to sixth weeks postpartum. The incidence of biliary sludge, new stones and progression from sludge to stones in addition to the probable risk factors were analyzed with the t-test and chi-square test as appropriate (SPSS software, version 15).

Results: According to the first ultrasonography, 14 (3.7%) participants had evidence of biliary sludge and 3 (0.7%) had gallstones. Among those with normal baseline ultrasound findings, 28 (7.7%) developed biliary sludge and 7 (1.9%) formed stones by their second ultrasound examination ($P < 0.05$). Among the 14 participants with biliary sludge in their first ultrasound assessment, 1 (7.1%) developed gall stone and sludge remained in the other 13 as seen on the third trimester ultrasound results. According to the third ultrasound (performed postpartum), out of 41 participants with prior biliary sludge, 16 (39%) had no evidence of sludge, 22 (53.7%) had persistent sludge, and 3 (7.3%) developed stones. Out of 11 participants with prior gallstones, 1 had a normal ultrasound postpartum, but stones persisted in the other 10 ($P < 0.05$). In those with stones, 9.1% underwent cholecystectomies postpartum. The incidence of sludge and stones significantly correlated with the number of pregnancies and higher age at pregnancy.

Conclusion: Since the annual rate of pregnancy is high in Iran, pregnancy-related biliary disorders are important in this country. According to our results, the number of parities and age at pregnancy are potentially modifiable risk factors for lowering pregnancy-related biliary disorders.

Keywords: Biliary, gallstones, pregnancy, risk factors

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Introduction

Cholesterol gallstones are more prevalent in women than men and are primarily related to sex steroids, particularly progesterone.[1–8] Diseases of the gall bladder have been reported to be the most common cause of non-obstetrical hospitalizations during the first year postpartum.[9]

According to epidemiologic studies, pregnancy is associated with a significant risk for gallstones.[10] Both the frequency and number of pregnancies are strongly associated with higher risk for biliary sludge or gallstones.[6,7,11,12] During pregnancy, there are changes in bile composition and gall bladder motility that promote gallstone formation.[3–5] Progesterone, besides changing the lipid composition of bile, inhibits the mobilization of intracellular calcium within smooth muscle cells which leads to smooth muscle relaxation and therefore, lowers the motility of the gall bladder.[13–16]

In a literature review, the prevalence of biliary sludge was reported to be as high as 36%, whereas gallstones were reported as 11%.[17] A prospective study has also reported the development of new biliary sludge in 31% and new gallstones in 2% of the studied women during one year postpartum.[18] This risk remained high during five years after pregnancy.[10]

Most women with biliary sludge remain asymptomatic. However those with stones may experience pain or serious complications such as cholecystitis, choledocholithiasis or pancreatitis which may have a high morbidity or mortality for both mother and fetus.[19,20]

There is no study that has evaluated the incidence of biliary sludge or gallstone development during pregnancy in Iran. Therefore we have designed a prospective study to evaluate the incidence and natural out-come of pregnancy-related biliary sludge and stones, as well as to define the potential risk factors in a population of pregnant women who referred to Imam Hospital in Sari, Iran.

Patients and Methods

A total of 380 pregnant women entered the study during their first trimester of pregnancy. The exclusion criteria were age under 18 years or the presence of gallstones at entry. The study was

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approved by the Ethics Committee of Mazandaran University of Medical Sciences and written informed consents were obtained from all participants.

At entry, data that included the subject's age, body mass index (BMI), number of previous pregnancies that reached to at least 20 gestational weeks, previous use of oral contraceptive pills (OCP), history of diabetes mellitus, previous history of biliary sludge, and familial history of gallstones were recorded in a questionnaire. In addition, serum levels of triglycerides and cholesterol were tested; levels less than 200 mg/dl were considered normal.

All participants were scheduled for three ultrasound assessments: 1) during the first trimester of pregnancy (9 – 12 weeks), 2) during the early third trimester (26 – 32 weeks), and 3) at the fourth to sixth weeks postpartum. If biliary sludge was found on the first ultrasound, the participant was followed for the probable progression to gallstones. The remaining participants were followed for probable development of new biliary sludge or gallstones.

All clinical data was obtained by the same examining physician and all ultrasounds were performed by the same sonographer who was blinded to the results of the patients' previous ultrasonographic findings. Ultrasounds were performed using a 3.5 MHz convex transducer (GE, Alpha 200, USA) and subjects were instructed to fast when the ultrasounds were performed. Sludge was defined as low-level echoes without post-acoustic shadowing which shifted by a change in position. Stones were defined as high-level echoes larger than 2 mm in diameter with post-acoustic shadowing.

According to serial ultrasounds, those with new biliary sludge or gallstones or those with progression of biliary sludge to gallstones were considered as having pregnancy-related gall bladder disease. Risk factors for gall bladder disease were analyzed and compared between different subgroups using the t-test, chi-square test and ANOVA as appropriate (SPSS software, version 15). Multivariate analysis was performed to identify independent risk factors. *P*-values less than 0.05 were considered statistically significant.

Results

A total of 380 pregnant women entered the study. The mean age was 26.26 ± 5.03 years. According to the initial ultrasonography performed during the first trimester, 14 (3.7%) had evidence of biliary sludge and 3 (0.7%) had gallstones. Prior to performing the second ultrasonography, three participants were excluded from the remainder of the study: two due to abortion before the 20th gestational week and one due to premature rupture of membranes (PROM) and labor. However, these three participants had normal sonographic findings.

Among those with normal baseline ultrasound findings, 28 (7.7%) developed biliary sludge and 7 (1.9%) formed stones by their second ultrasound examinations. There was a significant increase in the rate of developing biliary sludge or stones with increased gestational age ($P < 0.05$).

Among those 14 participants with biliary sludge noted on their first ultrasound assessments, 1 (7.1%) developed gallstones and sludge remained in 13. This showed a significant progression from sludge to stones as the gestational age increased ($P < 0.05$).

Therefore, according to the second ultrasound assessment (performed during the third trimester), 41 (10.8%) had biliary sludge and 8 (2.1%) formed new gallstones.

According to the third ultrasound (performed post-partum), of 41 patients with prior biliary sludge, 16 (39%) had no evidence of any remaining sludge; this occurred mainly in those whose first ultrasound assessments were normal ($P < 0.05$). There was persistent sludge in 22 (53.7%) patients and 3 (7.3%) developed gallstones. Gallstones resolved in 1 (12.5%) out of 8 patients with prior gallstones and persisted in the remaining 7 patients ($P < 0.05$; Table 1).

The cumulative incidence of new sludge was 7.7% and the cumulative incidence of new stones was 1.9%, whereas progression from sludge to stones was 7.1%.

Complications

Out of 11 participants with gallstones visualized during their third trimester, 3 (27.3%) experienced biliary pain. One (9.1%) experienced acute cholecystitis in the 34th gestational week, which led to premature labor in the 36th gestational week. This patient underwent a cholecystectomy postpartum. All participants had normal gall bladder wall thicknesses during their three ultrasound assessments, with the exception of the patient who experienced cholecystitis.

Risk factors

The mean age of those with normal ultrasound results during the third trimester was 25.5 ± 4.78 years and the mean age of those with biliary sludge/stones was 31.04 ± 3.8 years, which was significant ($P < 0.05$; Table 2). The mean duration of OCP use among all participants was 0.6 ± 1.1 years. The mean duration among those with biliary sludge/stones was 0.71 ± 1.3 years, whereas it was 0.59 ± 1.08 years in the normal population, which was not statistically significant ($P = 0.47$). Cholesterol ($P = 0.92$) and triglyceride ($P = 0.87$) levels did not show significant differences between those with sludge/stones and normal population. The mean parity number was significantly higher among those with biliary sludge/stones (1.73 ± 0.8) than normal participants (0.71 ± 0.9; $P < 0.05$).

Among 325 pregnant women with normal ultrasound findings during the third trimester, only 4 (1.2%) had previous histories of biliary sludge or gallstones, however 2 (4.9%) of those with biliary sludge and 1 (9.1%) of the 11 participants with gallstones had previous histories of gall bladder disease. These rates were higher than normal participants ($P < 0.05$).

Familial history of gall bladder disease was not statistically different between those with or without biliary sludge/stones ($P = 0.229$).

We stratified BMI of participants into normal or higher than normal (BMI > 25),[21] but no significant difference was noted between those with normal ultrasound findings and those with new biliary sludge/stones ($P = 0.71$). The prevalence of diabetes mellitus was not statistically different between those with normal ultrasound findings and those with biliary sludge/stones ($P = 0.33$).

Since every pregnant woman has been routinely tested for hepatitis B infection, in this study there were 8 (2.1%) HBS-Ag positive pregnant women. However there was no significant correlation between HBS-Ag positivity and the incidence of biliary sludge/stones ($P = 0.24$)

Multivariate analysis of the risk factors showed a strong association between patient's age and the number of parities with the occurrence of new biliary sludge/stones (Table 3).

Table 1. Incidence and regression of biliary sludge and stones from first trimester until 4-6 weeks postpartum.

First trimester	Third trimester		Postpartum		
	Ultrasound findings	n	Normal	Sludge	Stones±sludge
Normal (n= 363)	Normal	325*	325	0	0
	Sludge	28	13	15	0
	Stones	7	1	0	6
Sludge (n= 14)	Normal	0	0	0	0
	Sludge	13	3	7	3
	Stones	1	0	0	1
Stones (n= 3)	Normal	0	0	0	0
	Sludge	0	0	0	0
	Stones	3	0	0	3

*Three patients with normal first ultrasound results were excluded from the remainder of study due to abortion and premature rupture of membranes (PROM).

Table 2. Potential risk factors for developing biliary sludge/stones.

Variables	No sludge/stones	New sludge/stones	*P**
Age (years)	31.4 ± 3.8	25.5 ± 4.7	<0.05
Duration of OCP** use (years)	1.3 ± 0.7	1.08 ± 0.5	0.47
Triglyceride level (mg/dl)	156 ± 28	163 ± 24	0.87
Cholesterol level (mg/dl)	204 ± 36	187 ± 31	0.92
Number of parity	1.73 ± 0.8	0.9 ± .07	<0.05
Previous history of biliary disease (%)	1.2	9.1	<0.05
Familial history of biliary disease (%)	4.3	2.4	0.22
BMI >25 (%)	24.9	25.3	0.71
Diabetes mellitus	2.5	2.4	0.33

**P*-values less than 0.05 were considered significant. **OCP: Oral contraceptive pills.

Table 3. Multivariate analysis of potential risk factors for the incidence of biliary sludge/stones.

Variable	Odds ratio	95% confidence interval (CI)	*P**
Age (years)			
<30	0.9	1.86–0.44	0.786
>30	3.96	5.68–2.24	<0.001
Duration of OCP** use	0.9	1.03–0.97	0.839
Hypertriglyceridemia	1.05	2.91–0.38	0.918
Hypercholesterolemia	2.44	7.14–0.83	0.105
Number of parities	4.42	6.24–2.60	<0.001
Previous history of biliary disease	1.07	1.17–0.98	0.115
Familial history of biliary disease	0.58	1.30–0.26	0.189
BMI	1.83	3.99–0.37	0.458
Diabetes mellitus	0.83	1.71–0.4	0.607

**P*-values less than 0.05 were considered significant. **OCP: Oral contraceptive pills.

Discussion

During pregnancy, changes in bile composition and gall bladder stasis can lead to nucleation of bile acids, sludge formation or the development of gallstones.[22–27] In the third trimester, the changes in bile composition are mainly due to the effects of estrogen.[28]

However, after delivery, bile composition and gall bladder movement return to normal and therefore, sludge or gallstones may resolve.[19,23,29–30] On the other hand, during the postpartum period, restoration of gall bladder motility may lead to biliary pain or other complications due to passage of sludge or stones from the gall bladder.

According to our results, the cumulative incidence of new sludge was 7.7% and the cumulative incidences of new stones or progression from sludge to stones were 1.9% and 7.1%, respectively. These results were similar to two studies performed on 3254 pregnant women in the United States and 69 pregnant women in Turkey, according to which the cumulative incidences of new sludge until 4 – 6 weeks postpartum were 5.1% in the United States and 10.9% in Turkey. The cumulative incidences of new stones were 2.8% in the United States and 6.3% in Turkey.[31,32] However a study performed on 272 pregnant women in Italy reported a higher (31%) incidence of new sludge until 2 – 4 weeks postpartum.[18]

During our study, one woman (0.26%) underwent a cholecystectomy due to gallstone and cholecystitis. Since more than 1,300,000 women give birth in Iran annually,[33] it has been estimated that about 3380 cholecystectomies are performed postpartum each year. Therefore, pregnancy-related biliary disorders are considerable in Iran and can lead to significant morbidity.

Possibly, the real incidence of biliary sludge or gallstones could have been higher than reported by our study, as some cases of sludge or stones might have been transient and not detected at the ultrasound assessment. The sensitivity of ultrasound in detecting microsludge is about 50% – 60%, which therefore, lowers the

rate of sludge reports.[34,35] We have excluded pregnant women with previous cholecystectomies from our study, which also lowered the reported incidence of biliary sludge/stones.

According to our results, sludge resolved in 39% and stones in 9% of patients postpartum, which occurred mostly in those who had normal ultrasound findings during their first trimesters. One study has reported the spontaneous disappearance of biliary sludge and stones even in non-pregnant women.[36–40] This phenomenon was mainly due to passage of sludge or stones into the small bowel or a result of medical treatments.[36–40] However the results of our study showed a higher incidence of sludge disappearance compared with the abovementioned study.

In the current study, independent risk factors for the development of biliary sludge/stones were the mother's age and number of parities. A positive correlation between mother's age and the risk of sludge/stone formation was also shown in a study performed in Italy, but not in the United States.[18,31] Also, a correlation between number of parities and gallstones has been shown both in the general population[41–43] and in pregnant women.[32,44]

In contrast to the results of a previous study performed in the United States and reports from the general population, the results of our study did not show a significant correlation between higher BMI and sludge/stone formation.[12,31,45,46] Our results were similar to studies performed in Turkey and Italy.[18,32] This was possibly due to the lower BMI of our patients compared with studies performed in the United States and the general population and also, it might have been due to not considering the amount of weight loss or weight gain as a possible risk factor for sludge/stone formation during pregnancy. On the other hand, the low sensitivity of ultrasound could be another explanation for the low prevalence of stones in obese patients since obesity has been shown to interfere with ultrasound accuracy.[47]

Although the correlation between duration of OCP use and incidence of biliary sludge/stones has been shown in a number of previous studies,[5,13,22,23] the current study did not show a significant correlation.

In the general population, the correlation between hyperlipidemia and incidence of biliary sludge/stones has been reported and is mostly attributed to high triglyceride, but not cholesterol levels.[19,48–50] However the correlation between hyperlipidemia and pregnancy-related biliary sludge/stone is controversial. According to a study from the United States, pregnancy-related sludge/stones has been correlated with elevated triglyceride levels,[31] however this was not confirmed by our study or other studies.[32]

Previous studies have not shown any relation between the mother's blood glucose levels and incidence of biliary sludge/stones,[31] which was consistent with the results in our study.

A limitation of our study is the short duration of postpartum patient follow up. Longer durations of follow up might show a higher incidence of pregnancy-related biliary complications.

In conclusion, although most patients remained asymptomatic during pregnancy and two months postpartum, 9.1% needed surgical intervention. Since the annual rate of pregnancy is high in Iran, pregnancy-related biliary disorders are important in this country. According to our results, the number of parities and mother's age are potentially modifiable risk factors for lowering pregnancy-related biliary disorders. Further intervention to control the number of parities and educate mothers about the safe age for pregnancy may be beneficial.

References

1. Bockus HL, Willard WB, HN M. Role of infection and of disturbed cholesterol metabolism in gallstones genesis. *Pa Med J*. 1935; **39:** 482 – 493.
2. Bennion LJ, Grundy SM. Risk factors for the development of cholelithiasis in man (second of two parts). *N Engl J Med*. 1978; **299(22):** 1221 – 1227.
3. Kern F Jr, Everson GT. Contraceptive steroids increase cholesterol in bile: mechanisms of action. *J Lipid Res*. 1987; **28(7):** 828 – 839.
4. Everson GT, Fennessey P, Kern F Jr. Contraceptive steroids alter the steady-state kinetics of bile acids. *J Lipid Res*. 1988; **29(1):** 68 – 76.
5. Kern F Jr, Everson GT, DeMark B, McKinley C, Showalter R, Erfling W, et al. Biliary lipids, bile acids, and gallbladder function in the human female. Effects of pregnancy and the ovulatory cycle. *J Clin Invest*. 1981; **68(5):** 1229 – 1242.
6. Friedman GD, Kannel WB, Dawber TR. The epidemiology of gallbladder disease: observations in the Framingham Study. *J Chronic Dis*. 1966; **19(3):** 273 – 292.
7. Barbara L, Sama C, Morselli Labate AM, Taroni F, Rusticali AG, Festi D, et al. A population study on the prevalence of gallstone disease: the Sirmione Study. *Hepatology*. 1987; **7(5):** 913 – 917.
8. Mauer KR, Everhart JE, Ezzati TM, Johannes RS, Knowler WC, Larson DL, et al. Prevalence of gallstone disease in Hispanic population in the United States. *Gastroenterology*. 1989; **96:** 487 – 492.
9. Lydon-Rochelle M, Holt VL, Martin DP, Easterling TR.. Association between method of delivery and maternal rehospitalization. *JAMA*. 2000; **283:** 2411 – 2416.
10. Thijs C, Knipschild P, Leffers P. Pregnancy and gallstone disease: an empiric demonstration of the importance of specification of risk periods. *Am J Epidemiol*. 1991; **134(2):** 186 – 195.
11. The epidemiology of gallstone disease in Rome, Italy. Part II. Factors associated with the disease. The Rome Group for Epidemiology and Prevention of Cholelithiasis (GREPCO). *Hepatology*. 1988; **8(4):** 907 – 913.
12. Stampfer MJ, Maclure KM, Colditz GA, Manson JE, Willett WC. Risk of symptomatic gallstones in women with severe obesity. *Am J Clin Nutr*. 1992; **55(3):** 652 – 658.
13. Ryan JP. Effect of pregnancy on gallbladder contractility in the guinea pig. *Gastroenterology*. 1984; **87(3):** 674 – 678.
14. Everson GT. Gastrointestinal motility in pregnancy. *Gastroenterol Clin North Am*. 1992; **21(4):** 751 – 776.
15. Ryan JP, Pellecchia D. Effect of progesterone pretreatment on guinea pig gallbladder motility in vitro. *Gastroenterology*. 1982; **83:** 81 – 83.
16. Xiao ZL, Chen Q, Biancani P, Behar J. Mechanisms of gallbladder hypomotility in pregnant guinea pigs. *Gastroenterology*. 1999; **116(2):** 411 – 419.
17. Mendez-Sanchez N, Chavez-Tapia NC, Uribe M. Pregnancy and gallbladder disease. Ann Hepatol. 2006; **5(3):** 227 – 230.
18. Maringhini A, Ciambra M, Baccelliere P, Raimondo M, Orlando A, Tine F, et al. Biliary sludge and gallstones in pregnancy: incidence, risk factors, and natural history. *Ann Intern Med*. 1993; **119(2):** 116 – 120.
19. Valdivieso V, Covarrubias C, Siegel F, Cruz F. Pregnancy and cholelithiasis: pathogenesis and natural course of gallstones diagnosed in early puerperium. *Hepatology*. 1993; **17(1):** 1 – 4.
20. Ramin KD, Ramsey PS. Disease of the gallbladder and pancreas in pregnancy. *Obstet Gynecol Clin North Am*. 2001; **28(3):** 571 – 580.
21. Davidson S, Passmore R, Eastwood MA. , editors. Davidson and Passmore Human nutrition and dietetics. 8th ed: Edinburgh ; New York : Churchill Livingstone; 1986.
22. Braverman DZ, Johnson ML, Kern F Jr. Effects of pregnancy and contraceptive steroids on gallbladder function. *N Engl J Med*. 1980; **302(7):** 362 – 364.
23. Everson GT, McKinley C, Lawson M, Johnson M, Kern F Jr. Gallbladder function in the human female: effect of the ovulatory cycle, pregnancy, and contraceptive steroids. *Gastroenterology*. 1982; **82(4):** 711 – 719.
24. Cohen S. The sluggish gallbladder of pregnancy. *N Engl J Med*. 1980; **302(7):** 397 – 399.
25. Small DM. Cholesterol nucleation and growth in gallstone formation. *N Engl J Med*. 1980; **302(23):** 1305 – 1307.
26. Holan KR, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology*. 1979; **77:** 611 – 617.

27. Sedaghat A, Grundy SM. Cholesterol crystals and the formation of cholesterol gallstones. *N Engl J Med*. 1980; **302(23):** 1274 – 1277.
28. Lynn J, Williams L, O'Brien J, Wittenberg J, Egdahl RH. Effects of estrogen upon bile: implications with respect to gallstone formation. *Ann Surg*. 1973; **178(4):** 514 – 524.
29. Maringhini A, Ciambra M, Baccelliere P, Raimondo M, Pagliaro L. Sludge, stones, and pregnancy. *Gastroenterology*. 1988; **95(4):** 1160 – 1161.
30. Braverman DZ, Herbet D, Goldstein R, Persitz E, Eylath U, Jacobsohn WZ. Postpartum restoration of pregnancy-induced cholecystoparesis and prolonged intestinal transit time. *J Clin Gastroenterol*. 1988; **10(6):** 642 – 646.
31. Ko CW, Beresford SA, Schulte SJ, Matsumoto AM, Lee SP. Incidence, natural history, and risk factors for biliary sludge and stones during pregnancy. *Hepatology*. 2005; **41(2):** 359 – 365.
32. Bolukbas FF, Bolukbas C, Horoz M, Ince AT, Uzunkoy A, Ozturk A, et al. Risk factors associated with gallstone and biliary sludge formation during pregnancy. *J Gastroenterol Hepatol*. 2006; **21(7):** 1150 – 1153.
33. Registration NOfC. A collection of population statistics. 2010.
34. lee SP, Nicholls JF, Park HZ. Biliary sludge as a cause of acute pancreatitis. *N Engl J Med*. 1992; **326:** 589 – 593.
35. Ros E, Navarro S, Bru C, Garcia-Puges A, Valderrama R. Occult microlithiasis in 'idiopathic' acute pancreatitis: prevention of relapses by cholecystectomy or ursodeoxycholic acid therapy. *Gastroenterology*. 1991; **101(6):** 1701 – 1709.
36. Lee SP, Maher K, Nicholls JF. Origin and fate of biliary sludge. *Gastroenterology*. 1988; **94(1):** 170 – 176.
37. Hoppe-Seyler. G. Northnagel's Encyclopedia of Practical Medicine: Philadelphia: W.B. Saunders; 1905.
38. Kennedy R. On the use of olive oil in large doses for softening the easy expulsion of biliary calculi. *Lancet*. 1880; **2:** 456.
39. Dworken. H. Recent experiences with spontaneous disappearing gallstones. *Gallstones*. 1960; **38:** 76 – 86.
40. Schoenfield LJ, Lachin JM. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: the National Cooperative Gallstone Study. A controlled trial of efficacy and safety. *Ann Intern Med*. 1981; **95(3):** 257 – 282.
41. Hossain GA, Islam SM, Mahmood S, Chakrabarty RK, Akhter N. Gall stone in pregnancy. *Mymensingh Med J*. 2003; **12(2):** 112 – 116.
42. Jorgensen T. Gall stones in a Danish population: fertility period, pregnancies, and exogenous female sex hormones. *Gut*. 1988; **29(4):** 433 – 439.
43. Scragg RK, McMichael AJ, Seamark RF. Oral contraceptives, pregnancy, and endogenous oestrogen in gall stone disease--a case-control study. *Br Med J (Clin Res Ed)*. 1984; **288:** 1795 – 1799.
44. Tsimoyiannis EC, Antoniou NC, Tsaboulas C, Papanikolaou N. Cholelithiasis during pregnancy and lactation. Prospective study. *Eur J Surg*. 1994; **160:** 627 – 631.
45. Maclure KM, Hayes KC, Colditz GA, Stampfer MJ, Speizer FE, Willett WC. Weight, diet, and the risk of symptomatic gallstones in middle-aged women. *N Engl J Med*. 1989; **321(9):** 563 – 569.
46. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors. *JAMA*. 2003; **289(1):** 76 – 79.
47. Amaral JF, Thompson WR. Gallbladder disease in the morbidly obese. *Am J Surg*. 1985; **149(4):** 551 – 557.
48. The epidemiology of gallstone disease in Rome, Italy. Part I. Prevalence data in men. The Rome Group for Epidemiology and Prevention of Cholelithiasis (GREPCO). *Hepatology*. 1988; **8(4):** 904 – 906.
49. Thijs C, Knipschild P, Brombacher P. Serum lipids and gallstones: a case-control study. *Gastroenterology*. 1990; **99(3):** 843 – 849.
50. Jonkers IJ, Smelt AH, Ledeboer M, Hollum ME, Biemond I, Kuipers F, et al. Gall bladder dysmotility: a risk factor for gall stone formation in hypertriglyceridaemia and reversal on triglyceride lowering therapy by bezafibrate and fish oil. *Gut*. 2003; **52(1):** 109 – 115