ORIGINAL RESEARCH PAPER

Volume-7 | Issue-11 | November-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CORRELATION BETWEEN SERUM IRON LEVEL AND CHOLESTEROL STONE IN CHOLELITHIASIS.

Clinical Research

Anshu Atreya* Junior Resident,Department of Surgery, Tribhuvan University Teaching Hospital, Kathmandu, Nepal *Corresponding Author

Bikal Ghimire Assistant Professor,Department of Surgery, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

Pradeep Vaidya Professor,Department of Surgery, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

ABSTRACT

Background: The old axiom that a typical gallstone carrier is a fat, fertile, flatulent, female in forties is only partially true as the disease has been found in women soon after their first delivery and also in underweight, thin people and in fetal life. Age, sex, obesity parity, socioeconomic status, alcohol consumption, cigarette smoking and built of individual are significant factors related with the disease. In recent times role of serum iron has also been considered to be one of the important factors in the formation of gallstones. The present work has been mainly taken up with a view to study the relationship between the cholesterol gallbladder stones with serum iron level.

Material and Method: In this prospective study, 80 patients with cholelithiasis were studied during a study period of one year (June 2009 to May 2010) in General Surgery unit, Department of Surgery, Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Patients were categorized based on serum iron level into low, normal and high serum iron group and bile/serum cholesterol ratio was compared for each category of the patient.

Result: Of the eighty patients studied, 14 patients were male and 66 females. Three patients with serum iron level <40 µg/dl were categorized into category 1 (low iron), 76 in category 2 (normal iron) and one in category 3 (high iron). The difference in mean age and mean serum cholesterol level in the three categories was not significant statistically (p value 0.501 and 0.065 respectively). However, the difference of bile/serum cholesterol ratio among the three categories was statistically significant (p value of 0.006).

Discussion: The present study shows that the gall bladder bile cholesterol level was significantly higher in the low serum iron category, as compared to that of the normal or high serum iron category patients. Further, no significant variation in the serum cholesterol values was detected between the three categories.

Conclusion: The study suggests that decreased serum iron level plays a significant role in the super saturation of gallbladder bile with cholesterol and may act as an independent factor in formation of cholesterol gallstones.

KEYWORDS

Bile/serum cholesterol ratio, Bile super saturation, Cholelithiasis

INTRODUCTION:

The concept of gall stone is very old and perhaps, as old as mankind. The earliest known gallstones dates back to 21st Egyptian Dynasty and was discovered in the mummy of a priestess of Amen (1085-945 BC).[1] Gallstone has been described by Greek physician Alexander of Tralles in 5th century AD and in 14th century, physician Gentile de Folingo, first postulated the relationship between cholecystitis and gallstones based on autopsy findings. Antonio Enivieni successfully diagnosed gallstone disease in a patient suffering from abdominal pain which was confirmed at autopsy. However, it was Jean Fernel in 1581, physician to the King of France, who provided the most accurate clinical description of symptoms associated with gallstones.[2]

In the modern world, cholelithiasis is one of the most common diseases often leading to surgical intervention. The prevalence of gallstone disease varies worldwide. Higher rates were reported in the United States, Chile, Germany, Sweden, and Austria. In the United States, an estimated ten to fifteen percent of adults have gallstone disease. The total cost incurred due to gallstones alone in the United States is estimated to be about $6.5 billion yearly.[3]

The importance of gall bladder calculi is due to the associated painful symptoms, dangerous pathological effects and troublesome complications. Studies have even reported association of biliary malignancy in patients with gallstone.[4]

The old axiom that a typical gallstone carrier is a fat, fertile, flatulent, female in forties, hold true partially since the disease has been found in females soon after their first delivery as well as in underweight, thin people and seldom even as early as in fetal life.[5,6] Several mechanisms play role in the formation of lithogenic bile, but the most important are due to disturbances of physio-chemical equilibrium of bile constituents, deranged cholesterol metabolism and biliary stasis.

Age, sex, obesity, parity, socioeconomic status, alcohol consumption, cigarette smoking and built of individual are significant predisposing factors of the disease. The role of trace elements (Ca, Zn and Fe) and defective pH in formation of gallstone has also been studied.[7,8] It is also noteworthy that iron deficiency has been in the recent times found to be an etiological factor in the formation of gallstone and the role of serum iron has also been considered to be one of the important factors in the formation of gallstones.[1,9] Iron deficiency has been shown to alter the activity of hepatic enzymes, leading to increased gallbladder bile cholesterol saturation and promotion of cholesterol crystal formation.[10,11] Iron acts as a coenzyme for nitric oxide synthetase (NOS), which synthesizes nitric oxide (NO) important for the maintenance of basal gall bladder tone and normal relaxation.[12,13] It is found that iron deficiency results in altered motility of gall bladder and sphincter of Oddi, leading to biliary stasis and thus, increased cholesterol crystal formation in the gall bladder bile.[14]

Based on extensive work that has been carried out in the past and in recent years for early recognition of cholelithiasis and to avoid its complications, this work was been taken up to study the relationship between the gallbladder stone diseases and serum iron level.

MATERIAL AND METHOD:

Tribhuvan University Teaching Hospital (TUTH), a tertiary care medical facility, situated in the centre of the capital city of Kathmandu, provides optimal health facility to the needy. The prospective study was undertaken in the general surgery unit of the Department of Surgery of this hospital from June 2009 to May 2010 after obtaining an approval from the Institutional Review Board, Institute of Medicine, Tribhuvan University. To maintain uniformity in the collection of the data, sampling of blood and bile, and analysis of the samples in the laboratory, a protocol was defined, and a pre-standardized laboratory test procedure was developed.

All patients in the general surgery units undergoing cholecystectomy were enrolled for the study.

Inclusion Criteria:

- All patients undergoing elective cholecystectomy.

Exclusion Criteria:

- Patients not giving consent for the study.

- Age less than 15 years.
- Patients with inadequate bile sample.
- Patients with empyema or mucocele of gall bladder.
- Patients with pure pigment bile stone.
- Patients who underwent cholecystectomy for reasons other than cholelithiasis.

Methodology:

The estimated sample size for the study was 80 patients, calculated in a population of 450 patients, which corresponds to the average number of patients undergoing cholecystectomy each year in the hospital (confidence level of 95%, margin of error 10% and response distribution of 50%). Informed written consent was obtained. On the day of surgery after an overnight *nil per os* for at least six hours, blood sample of the patient was drawn and sent to the biochemical laboratory of TUTH for analysis of serum iron and serum cholesterol level in a labeled vial. Intraoperatively, using a sterile syringe approximately 5 mL of bile was aspirated from the gall bladder and the stones were carefully observed by the operating surgeon. If the stones were that of cholesterol or mixed type, the bile sample was sent to the biochemistry laboratory for analysis of cholesterol level.

Laboratory standard operation procedures were maintained for all laboratory analysis. Internal quality control sera, both normal and pathological, were also run for each lot, for the validation of the results. In the post-operative period, the patients were managed as per the protocol of respective unit and were discharged accordingly.

Statistical analysis:

Patients were categorized according to the serum iron level into three categories of low, normal and high serum iron levels (Table 1):

TABLE 1: Categorization according to serum iron level.

Category	Category I	Category II	Category III
	Low serum iron	*Normal serum iron*	*High serum iron*
Serum iron	<40µg/dl	40-140 µg/dl	>140 µg/dl

Variables including age and sex, body mass index (BMI), previous history of biliary symptoms, previous hospital admission for acute biliary pathologies like acute calculous cholecystitis, acute biliary pancreatitis, biliary colic, obstructive jaundice due to choledocholithiasis or acute cholangitis, past history of anemia, parity and last menstrual period (LMP) in females, use of oral contraceptive pills (OCP), serum cholesterol level, serum iron level, bile/serum cholesterol ratio were analyzed, based on different iron categories.

The data were analyzed using the Statistical Program for Social Science (SPSS) for windows version 17.

RESULT:

A total of eighty patients were included in the study of which, 18% (14 patients) were male and the rest 82% (66 patients) were female. Classified according to category there were 3 patients in Category I, 76 and 1 in Category II and III respectively.

The mean age in years of the patients in the three categories were 32.7±7.0, 42.9±15.1 and 40.0 for Category I, II and III respectively (Figure 1).

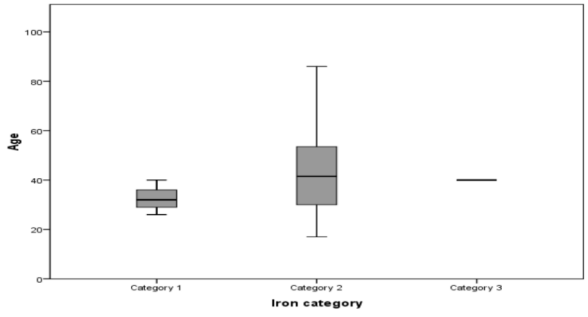


Figure 1: Age distribution of patients in different iron categories

Eight out of 80 patients had at some point of time been diagnosed with anemia, out of which seven were female and one was male. The relationship was statistically significant with *p* value equal to 0.004, suggesting females being more predisposed to develop anemia.

A total of 47 patients belonged to alcohol consumption group of which 39 were female and eight were male, six male patients and 27 female patients did not consumed alcohol. The mean serum cholesterol level in alcohol consumer group was found to be significantly higher (4.3±1.2 mg/dl) than that compared with the non-consumers (3.7±0.9 mg/dl), however bile/serum cholesterol ratio was comparable for the two groups (4.6 ± 2.4 in alcohol non-consumer versus 4.2 ± 1.6 in alcohol consumer group).

Of the total 66 female patients, most of them had parity 2 and 3 and the mean bile/ serum cholesterol ratio in these patients was 4.9±2.8 and 5.0±2.3 respectively, however for nulliparous females also the mean ratio was above 5. The difference in mean of bile/serum cholesterol for different parity was not significant statistically, *p* value = 0.639 (Table 2).

Table 2: Relation between parity and bile/serum cholesterol ratio

Parity	Mean	No. of patient	Minimum	Maximum	Range	p value
0	5.3±2.4	7	2.3	9.3	6.97	0.639
1	4.7±1.6	5	2.3	6.7	4.40	
2	4.9±2.8	17	2.1	11.8	9.63	
3	5.0±2.3	22	2.1	10.8	8.70	
4	3.1±1.1	9	2.0	4.9	2.93	
5	3.5±1.2	3	2.1	4.4	2.25	
6	4.3±0.1	3	4.1	4.4	0.24	

Twenty patients accounting for 30% of females included in the study were found to have used oral contraceptives whereas 46 female patients had never ever used oral contraceptives. The mean serum cholesterol level in OCP user group was 4.3±1.1 mg/dl and when compared to non users (3.7±0.8 mg/dl), the difference was statistically significant, p value = 0.013. The maximum bile/serum cholesterol ratio value was seen in OCP user group though the mean level of the ratio for the two group was not significant statistically, p value = 0.263

Table 3: Serum cholesterol, bile/serum cholesterol ratio in OCP users and non users

OCP	Frequency	Serum Cholesterol		Bile/serum cholesterol ratio	
		Mean ± SD (mg/dl)	*p* value	Mean ± SD	*p* value
Non user	46	3.7 ± 0.8	0.013	4.2 ± 2.2	0.263
User	20	4.3 ± 1.1		4.9 ± 2.3	

In the study the mean body mass index of patients in category 1 was found to be 22.5±3 Kg/m^2 with a range of 19.1 to 24.7 Kg/m^2, in category 2 and 3 it was found to be 23.3±2.7 and 19.1 Kg/m^2. The difference in the mean body mass index of patients in the three categories was not found to be significant with a *p* value of 0.298 (Figure 2).

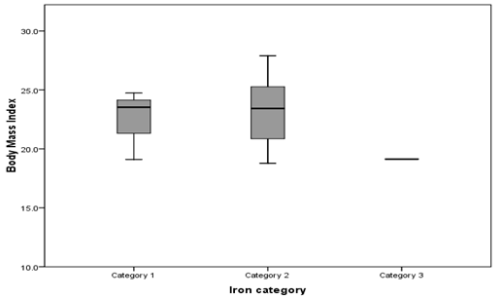


Figure 2: Distribution of BMI according to serum iron category

Also, no statistically significant difference in the mean serum cholesterol level (p value = 0.243) and mean bile/serum cholesterol ratio in the two categories of BMI to which the studied population belonged was observed (Table 4).

Table 4: Serum cholesterol and bile/serum cholesterol ratio as per BMI

BMI	Serum Cholesterol		Bile/serum cholesterol ratio	
	Mean ± SD (mg/dl)	*p* value	Mean ± SD	*p* value
18.5-24.9 Kg/m^2 (Normal weight)	4.0 ± 1.1	0.243	4.6 ± 2.2	0.525
25-29.9 Kg/m^2 (Over weight)	3.7 ± 0.7		4.2 ± 2.0	

The difference in mean value of serum iron in females (90.5±27.5 µg/dl) when compared to that of males (98.4±27.8 µg/dl) was not found to be statistically significant (p value = 0.349).

In the study it was observed that with decreased serum iron level there was no correlation of serum cholesterol as the difference was statistically not significant (p value = 0.065) but while comparing the mean bile/serum cholesterol ratio in the different iron categories, it was found that the difference in mean was statistically significant with a p value of <0.5 (Table 5) with higher bile/serum cholesterol ration observed in low serum iron level compared to lower ratio as observed in higher serum iron level.

Table 5: Relation between serum iron mean, serum cholesterol mean and bile/serum cholesterol ratio mean for different iron category

	Serum iron level (µg/dl)	Serum cholesterol level (mg/dl)	Bile/serum cholesterol ratio
Category 1	32.8±4.3	5.3 ± 2.1	8.1±3.2
Category 2	93.6 ± 24.3	3.9 ± 1.0	4.4 ± 2.0
Category 3	161.0 ± 0	4.2 ± 0	2.3 ± 0
p value	≤0.05	0.065	0.006

DISCUSSION

Of the eighty gallstone patients included in the present study, 66 (82%) were females, and 14 (18%) were males, a finding which supports the old axiom of gallstones being found more common in the female population. This male to female ratio of ~1:4 observed in the present study was different from that of seen in Pima Indians (1:17)[19] and Pakistanis (1:3).[15] The sex related difference of increased prevalence of cholelithiasis in females could be linked to female sex hormones and also to iron deficiency as mentioned by Channa NA *et al* in their study.[54] In the study, the mean serum iron level for the male patients was higher (98.4±27.8 µg/dl) than female (90.8±27.5 µg/dl), the difference was statistically not significant with a *p* value of 0.349.

Based on serum iron level, patients in the three categories had a comparable mean age with no statistical difference in the values, *p* value of 0.501 (Figure 1).

Further, 33 patients of the study population were found to be alcohol consumers and had a mean cholesterol level of 4.3±1.2 mg/dl compared to non consumers with a mean level of 3.7±0.9 mg/dl. The difference of mean cholesterol level for the two subset was statistically significant (*p* value = 0.026) and is at par with other studies that had concluded alcohol consumers to be having higher total serum cholesterol level.[16,17] However the mean bile/serum cholesterol ratio when compared for the alcohol consumer and non consumer group was not significant (*p* value=0.422), indicating that alcohol though alters the serum cholesterol level doesn't have influence on super saturation of cholesterol in bile.

In the study, the correlation between parity and bile/serum cholesterol ratio was not statistically significant, with *p* value of 0.639. Although studies have shown that with increasing parity, there is high risk of having cholelithiasis which has been attributed to decrease in serum iron levels after subsequent child birth. Such insignificance can be attributed to higher percentage of female population in the study using iron supplementation during and after pregnancy. The ratio of oral contraceptive users and non users in the study was found to be 30:70 and the mean cholesterol level in non users (3.7±0.8 mg/dl) when compared to users (4.3±1.1 mg/dl) was statistically significant (*p* value = 0.013) as in other studies [18,19] though the difference in mean bile/serum cholesterol level in both OCP user (4.2±2.2) and non user group (4.9±2.3) was not significant (*p* value = 0.263), suggesting OCP doesn't lead to cholesterol super saturation in bile (Table 3).

The patients were also categorized according to their respective body mass index and the mean serum cholesterol level and bile/serum cholesterol ratio were analyzed which were not significant statistically (*p* value = 0.243 and *p* value = 0.525 respectively) for the different BMI group (Table 4).

Iron deficiency alters the activity of several hepatic enzymes leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation.[10,11] Gallbladder filling and emptying are influenced by both inhibitory and excitatory stimuli, with nitric oxide (NO) playing a key role in normal relaxation. Iron acts as a coenzyme for nitric oxide synthetase (NOS), which synthesizes nitric oxide (NO).[12,13] Alteration of motility of the gallbladder and sphincter of Oddi leading to biliary stasis resulting in cholesterol crystal formation has been reported with iron deficiency.

There was no significant difference (*p* value = 0.065) in the mean serum cholesterol level of the three iron categories, whereas bile/serum cholesterol ratio was significantly increased (*p* value < 0.006) in the low iron group, thus suggesting that lower iron levels may be contributing to the super saturation of gall bladder bile with respect to cholesterol independent of serum cholesterol levels (Table 5). Two separate studies done in India, had similar findings and both had concluded that low serum iron levels lead to bile super saturation with respect to cholesterol, leading to gallstone formation.[1,9]

Probably low serum iron is an independent risk factors operating for the causation of gallstones and if present together with other known risk factors, it might produce synergistic effects.

CONCLUSION

In patients with cholesterol gallstones with low serum iron level, cholesterol supersaturated bile is observed.

REFERENCES

1. Sahu SK, Jain R, Prakash A, et al. Correlation of gallstone disease with iron deficiency anaemia: A Prospective Study. Internet J Surg. 2008 Volume 14 Number 2
2. Odze RD, Goldblum JR. Surgical pathology of GI tract, Liver, Biliary Tract and Pancreas. 2nd ed. Philadelphia, Saunders Elseiver: 2004
3. Gallstones, www.health.usnews.com 2009; [Cited: Nov 2, 2010]. Available from:http://health.usnews.com/health-conditions/digestive-disorders/gallstones
4. Hsing AW, Gao YT, Han TQ, et al. Gallstones and the risk of biliary tract cancer: A population-based study in China. Br J Cancer. 2007 December 3; 97(11): 1577–1582.
5. Maringhini A, Ciambra M, Baccelliere P, et al. Biliary Sludge and Gallstones in Pregnancy: Incidence, Risk Factors, and Natural History. Ann Intern Med, July 15, 1993vol. 119 no. 2 116-120
6. Devonald KJ, Ellwood DA, Colditz PB. The variable appearances of fetal gallstones. J Ultrasound Med. 1992 Nov;11(11):579-85
7. Strasberg SM, Clavien PA, Harvey PR. Pathogenesis of cholesterol gallstones. HPB Surg 1991; 3(2): 79-102
8. Verma GR, Pandey AK, Bose SM, et al. Study of serum calcium and trace elements in chronic cholelithiasis. Aust NZ J Surg 2002; 72: 596-9.
9. Kumar M, Goyal BB, Mahajan M, et al. Role of iron deficiency in the formation of gall stones. Indian J Surgery, Vol. 68, No. 2, March-April, 2006, pp. 80-83
10. Roslyn JJ, Conter RL, Julian E, et al. The role of dietary iron in pigment gallstone formation. Surgery 1987; 102: 327-33.
11. Johnston SM, Murray KP, Martin SA, et al. Iron deficiency enhances cholesterol gallstone formation. Surgery 1997;122:354-61
12. Swartz-Basile DA, Goldblatt MI, Blaser C, et al. Iron deficiency diminishes gallbladder neuronal nitric oxide synthase. J Surg Res 2000;90:26-31
13. Salomons H, Keaveny AP, Henihan R, et al. Nitric oxide and gallbladder motility in prairie dogs. Am J Physiol 1997; 272: G770-8.
14. Goldblatt MI, Swartz-Basile DA, Choi SH, et al. Iron deficiency transiently suppresses biliary neuronal nitric oxide synthase. J Surg Res 2001;98:123-8
15. Channa NA, Khand FD, Bhangar MI, et al. Surgical incidence of cholelithiasis in Hyderabad and adjoining areas (Pakistan). Pak J Med Sci 2004;20:13-7
16. Gaziano JM, Buring JE, Breslow JL, et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N Engl J Med.1993;329:1829–1834
17. De Oliveira E Silva ER, Foster D, McGee HM, et al. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of Apolipoproteins A-I and A-II. Circulation 2000; 102: 2347-2352
18. Banaszewska B, Pawelczyk L, Spaczynski RZ, et al. Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: Prospective, randomized, crossover trial. J Clin Endocrinol Metab. 2007 Feb; 92(2):456-61
19. Frempong BA, Ricks M, Sen S, et al. Effect of low-dose oral contraceptives on metabolic risk factors in African-American women. J Clin Endocrinol Metab. 2008 Jun; 93(6):2097-103