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Original Article

Surgery Section

Correlation between Serum Iron, Serum Ferritin and Bile Cholesterol Level in Gallstone Disease

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ABSTRACT

Introduction: The cholesterol stones are formed by super-saturation of the bile with cholesterol. Iron deficiency alters activity of several hepatic enzymes, leading to increased cholesterol saturation of bile in gall bladder thus promoting cholesterol crystallisation. Iron has a role in gallstone pathogenesis and ferritin is the most specific marker for iron levels in the body, but there are less studies which depict correlation between serum iron, serum ferritin and bile cholesterol.

Aim: To study the correlation between serum iron, serum ferritin and bile cholesterol level in gallstone patients.

Materials and Methods: The observational study was conducted in the Department of Surgery. The study population was 45 patients with gallstone disease. Serum iron, Serum ferritin and Bile cholesterol contents were analysed. Serum cholesterol levels were estimated using Folch method and estimation of bile cholesterol was done by Enzopak kit.

Statistical analysis was done by using descriptive and inferential statistics using chi square test and software used in the analysis were SPSS 24.0 version and $p<0.05$ was considered as level of significance.

Results: A positive but negligible correlation between serum iron and serum ferritin was observed (Pearson's correlation coefficient of 0.247). Negative correlation between serum iron vs serum cholesterol and serum ferritin vs serum cholesterol was observed.

Conclusion: It can be concluded that a low value of serum iron and serum ferritin is a risk factor for cholelithiasis, whereas, if the value of bile cholesterol increases then there is a high probability of cholelithiasis.

Keywords: Bile cholesterol, Cholelithiasis, Iron deficiency anaemia, Risk factor

INTRODUCTION

Cholelithiasis is a common prevailing disorder of abdomen. The percentage of adults suffering from gallstones in western countries is 10-12% [1,2]. In India, the prevalence of gall stone ranges from 6% to 9% in the adult population [3]. The common bile duct stones with gallstones have prevalence varying from 8 to 16% [4].

Cholesterol stones are more common (80%) in Western countries and pigment stones are more common (80%) in Asian countries [5]. The pathogenesis in the development of cholesterol stone is by super-saturation of the bile with cholesterol. Super saturation usually caused by cholesterol hyper secretion rather than reduced secretion of phospholipids or bile salts [5].

Iron deficient diet alters hepatic enzyme metabolism, increases gallbladder bile cholesterol and promotes cholesterol crystal formation and activity alteration of many hepatic enzymes also occurs [6].

Iron is essential because it maintains basal tone of gall bladder and its normal relaxation [7,8]. Iron deficiency alters the activity of many hepatic enzymes that elevates bile cholesterol saturation in gall bladder and promotes formation of cholesterol crystals. It was found that deficiency of iron caused altered motility of sphincter of oddi and gall bladder, thus leading to stasis of bile and increased formation of cholesterol crystal in the gall bladder bile [7]. Hence, iron plays a significant role in gallstone pathogenesis and ferritin is the most specific marker for iron levels in the body [9]. There are studies which show correlation between serum iron and serum ferritin and between serum iron and bile cholesterol in gall stone patients. But there are paucity of studies which depict correlation between serum iron, serum ferritin and bile cholesterol, so this study aimed to understand the correlation between serum iron, serum ferritin and bile cholesterol level in gallstone patients.

MATERIALS AND METHODS

This Observational study was conducted after approval of Ethical Committee (letter number: IEC/2017-18/6642) from September 2017 to August 2019. The study population included all cases of cholelithiasis. Sample size was calculated as per the following formula and study by Babu RG et al., which was a similar study thus was taken as reference [10].

Sample size calculating formula and Sample size formula for difference between two proportions:

This calculator uses the following formula for the sample size n:

$$n=(Z_{\alpha/2}+Z_{\beta})^{2*}\ (p_1(1-p_1)+p_2(1-p_2))/(p_1-p_2)^2,$$

where, $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g., for a confidence level of 95%, α is 0.05 and the critical value is 1.96),

Z_{β} is the critical value of the Normal distribution at β (e.g., for a power of 80%, β is 0.2 and the critical value is 0.84) and

P1 and P2 are the expected sample proportions of the two groups.

P1=Proportion of patients with iron deficiency in anaemia=81.8% =0.818

P2=Proportion of patients with iron deficiency in non anaemia =44.4%=0.444

$$N=\frac{(1.96+0.84)^2\ *\{0.818*(1-0.818)+0.444*(1-0.444)\}}{(0.818-0.444)^2}$$

=22.18=25 patients needed

Inclusion Criteria

All patients with cholelithiasis confirmed by ultrasonography with age group of 15-70 years.

Exclusion Criteria

1. Patients taking iron supplements in any form
2. Previous case of biliary tract surgery.
3. The patients having the following disorders/ disease were not considered: Patient of empyema, mucocele of gall bladder, hematological disorders, cirrhosis of liver, cystic fibrosis, blood dyscrasias, Crohn's disease, familial hyperlipoproteinemia, hyperlipoprotenimia type-3, pyruvate kinase deficiency, drugs causing gallstones (oestrone, clofibrate, cholesterol lowering agents) and pregnant females will also be excluded.

Method

In this study, a detailed history of all the patients suffering from gall stone disease was taken. Routine investigations such as Liver Function Test (LFT), ultrasound of abdomen was done in all the patients. Venous blood sample of 4 mL was taken in 2 red vacutainers for evaluation of serum iron, serum ferritin levels. Serum ferritin level was assessed by chemiluminescence. Serum iron was estimated by the ferrozine kit method. Serum cholesterol levels were estimated using Folch method. Bile cholesterol sample was taken post cholecystectomy from gall bladder and was sent to the biochemistry lab for estimation of bile cholesterol by Enzopak kit.

The normal reference values as per the kit manual for bile cholesterol were 60-160 µg/dL for males and 35-145 µg/dL for females. The reference value of serum ferritin in males was 23-336 ng/mL and in females was 11-306 ng/mL [11].

STATISTICAL ANALYSIS

Statistical analysis was done by using descriptive and inferential statistics using chi-square test and software used in the analysis were SPSS 24.0 version and $p<0.05$ was considered as level of significance.

RESULTS

Distribution of Serum Iron Level in Patients

Out of 18 male patients,7 (38.9%) had low serum iron levels. Out of 27 female patients, 13 (48.2%) had low serum iron levels [Table/Fig-1].

Serum iron level	Male (60-160 µg/dL)	Female (35-145 µg/dL)
Low	7 (38.9%)	13 (48.2)
Normal	11 (61.1%)	14 (51.8)
Total	18 (100%)	27 (100%)
Mean±SD	70.08±29.97	56.10±32.69

[Table/Fig-1]: Serum Iron levels in patients.

Distribution of Patients According to Serum Ferritin Level

Out of 18 male patients, 6 (33.3%) had low levels of serum ferritin while 10 (37%) of the female patients had low levels of serum ferritin [Table/Fig-2].

Serum ferritin level	Male (23-336 ng/mL)	Female (11-306 ng/mL)
Low	6 (33.3%)	10 (37%)
Normal	12 (66.7%)	17 (63%)
Total	18 (100%)	27 (100%)
Mean±SD	41.69±24.26	23.57±16.95

[Table/Fig-2]: Distribution of patients according to serum ferritin level.

Distribution of Serum Iron and Serum Ferritin among Cases

Overall, 20 patients(44.4%) had low serum iron level and 16 patients (35.5%) had low serum ferritin levels. The cross tabulation is shown in [Table/Fig-3].

Parameter	Serum iron low	Serum iron normal	Total
Serum ferritin low	9 (20%)	7 (15.5%)	16 (35.5%)
Serum ferritin normal	11 (24.5%)	18 (40%)	29 (64.5%)
Total	20 (44.5%)	25 (55.5%)	45 (100%)

[Table/Fig-3]: Distribution of serum iron and serum ferritin among cases.

Bile Cholesterol Levels in Patient

In this study, bile cholesterol level varied between 351 mg/dL-950 mg/dL. Mean bile cholesterol level was 542 mg/dL±116 [Table/Fig-4].

Bile cholestrol level (in mg/dL)	No. of Patient	% of patient
351-450	12	26.6%
451-550	8	17.78%
551-650	19	42.22%
651-750	4	8.89
751-850	1	2.22
851-950	1	2.22
Total	45	100%
Mean±SD	542±116 mg/dL	

[Table/Fig-4]: Bile cholesterol levels in patients in this study.

The mean serum iron level was 66.22 µg/dL, mean serum ferritin level was 30.82 ng/dL and mean bile cholesterol level was 544 mg/dL [Table/Fig-5].

	Mean	Std. deviation
Serum iron level	66.22 µg/dL	32.04
Serum ferritin level	30.82 ng/dL	21.85
Bile cholesterol level	544 mg/dL	116
Serum cholestrol	155.92 mg/dL	28.60

[Table/Fig-5]: Mean serum iron, serum ferritin and bile cholesterol level and serum cholesterol.

The Pearson coefficient correlation between serum iron, serum ferritin and serum cholesterols shown in [Table/Fig-6].

Parameters	Statistical significance	Serum iron level	Serum ferritin level	Bile cholesterol level
Serum iron level	r-value	1	0.247	-0.047
	p-value		0.102, NS	0.757, NS
Serum ferritin level	r-value	0.247	1	-0.082
	p-value	0.102, NS		0.590, NS
Bile cholesterol level	r-value	-0.047	-0.082	1
	p-value	0.757, NS	0.590, NS	

[Table/Fig-6]: Pearson coefficient correlation between serum iron, ferritin and bile cholesterol.

DISCUSSION

In this study, prevalence of gallstones was higher in females compared to males and was consistent with findings of other studies [12-14]. It was also observed that gallstones were more common in between 31-40 years of age group which is consistent when compared to other studies [13]. Pain in abdomen was found to be the most consistent symptom which is similar to other studies [11,12] mentioned in the [Table/Fig-7] [11-16]. In this study, more percentage of females had low serum iron, low serum ferritin and high serum cholesterol as compared to males. However when compared to other studies [11,12,17], the results varied. [Table/Fig-8] shows a compilation of few studies based on the mean Serum iron, serum, ferritin and bile cholesterol.

Limitation(s)

It was a single centre study. This could have an impact on the reproducibility of the reported results. Sample size of patient was 45 which further might have an impacted on the reproducibility of

	Male and female prevalence	Peak incidence of gallstone	Pain in abdomen	Vomiting	Fatigue	Diarrhoea	Dyspepsia	Serum iron* (Normal:Low) in males and females	Serum ferritin* (Normal:Low) in males and females	Serum cholesterol* (Normal:High) in males and females
Index study	Males: 40% Females: 60%	31-40 years	100%	53.3%	20%	8.8%	17.7%	M-61.1: 38.9 F-51.8: 48.2	M-66.7: 33.3 F-63: 37	M-94.40: 5.60 F-88.8: 11.20
Arora BK et al., [11]	-	-	98%	37.5%	21%	9.5%	9.5%	M-58.06: 41.93 F-76.92: 23.07	M-35.5: 64.5 F-64.5: 35.5	-
Sarhan HH et al., [12]	Males: 20% Females: 80 %	-	98%	-	-	-	-	-	-	
Halgaonkar Pet al., [13]	Males: 15% Females: 85%	31-40 years	-	-	-	-	-	-	-	-
Chandran P et al., [14]	Males: 31% Females: 69%	-		-	-	-	-	-	-	-
Prasad PC et al., [15]	-	-	-	-	-	-	-	M-39: 61 F-16.3: 83.7	M-69.3: 30.7 F-65: 35	-
Akhtar N et al., [16]	-	-	-	-	-	-	-	-	-	M-61.5: 7.69 F-62.16: 2.7

[Table/Fig-7]: Comparison of different variables of index study with literature [11-16].
M: Male; F: Female; *Data is in percentage

	Mean serum iron (µg/dL)	Mean serum ferritin (ng/dL)	Mean bile cholesterol (mg/dL)
Index study	56.10	41.69	542 mg/dL
Arora BK et al., [11]	65.45	29.68	-
Sarhan HH et al., [12]	-	-	700 mg/dL
Misra PK et al., [17]	-	-	Group A-212.8 mg/dL Group B-747.1 mg/dL

[Table/Fig-8]: Mean Serum iron, ferritin and bile cholesterol values in various studies [11,12,17].
Group A: Nonanaemic patients with gallstones; Group B: Anaemic patients with gallstones

the results. Only few literatures are available on correlation between serum iron, serum ferritin and bile cholesterol in patients with cholelithiasis, so further multicentric studies need to be done for validation of our hypothesis. Authors recommend that every patient above 30 years with gallstones should be screened for serum iron, serum ferritin and bile cholesterol. It may be used as marker of iron store and progression to severe iron deficiency can be prevented. Further studies are needed to establish the role of oral iron in high risk patients in the treatment and prevention of cholelithiasis.

CONCLUSION(S)

It can be concluded that, females are more prone to developing gallstone disease and there were no specific symptoms found during this period specific to the gallstone disease. Also, pain in abdomen was the most consistent symptom found during the study. There was negative correlation between serum iron and bile cholesterol whereas there is positive correlation seen between serum iron and serum ferritin.

REFERENCES

[1] Diehl AK. Epidemiology and natural history of gallstone disease. Gastroenterol Clin Nor Am. 1991;20:01-19.
[2] Heaton KW, Braddon FE, Mountford RA, Hughes AO, Emmett PM. Symptomatic and silent gallstones in the community. Gut. 1991;32:316-20. Doi: 10.1136/gut.32.3.316. PMID: 2013429.
[3] Dhamnetiya D, Goel MK, Himan B, Pathania OP. Gallstone disease andquantitative analysis of independent biochemicalparameters: Study in a tertiary care hospital of India. J Lab Physicians. 2018;10:448-52. Doi: 10.4103/JLP.JLP_75_18. PMID: 30498320.
[4] Kratzer W, Mason RA, Kachele V. Prevalence of gallstones in sonographic surveys worldwide. J Clin Ultrasound. 1999;27:01-07. Doi: 10.1002/(SICI)1097-0096(199901)27:1<1::AID-JCU1>3.0.CO;2-H.
[5] Conlon K. The gall bladder and bile ducts. In: Williams NS, Bulstrode C, O'Connell PR, editors. Bailey & Love's Short Practice of Surgery, 26th edn. CRC Press 2013;67:1097-117.
[6] Johnston SM, Murray KP, Martin SA, Fox-Talbot K, Lipsett PA, Lillemoe KD, et al. Iron deficiency enhances cholesterol gallstone formation. Surgery. 1997;122(2):354-61. Doi: 10.1016/S0039-6060(97)90027-1.
[7] Swartz-Basile DA, Goldblatt MI, Blaser C, Decker PA, Ahrendt SA, Sarna SK. Iron deficiency diminishes gallbladder neuronal nitric oxide synthase. J Surg Res. 2000;90:26-31. Doi: 10.1006/jsre.2000.5827. PMID: 10781371.
[8] Salomons H, Keaveny AP, Henihan R, Offner G, Sengupta A, Lamorte WW, et al. Nitric oxide and gallbladder motility in prairie dogs. Am J Physiol. 1997;272:G770-78. Doi: 10.1152/ajpgi.1997.272.4.G770. PMID: 9142907.
[9] Bhadre RB, Verma R, Halgoankar P. Correlation of gallstone formation with serum iron levels. J Evid Based Med Healthc. 2016;3(60):3243-47. Doi: 10.18410/jebmh/2016/702.
[10] Babu RG,Bille S. Correlation of gallstone disease with iron deficiency anaemia. Arch Int Surg. 2017;7:121-25. Doi: 10.4103/ais.ais_3_18.
[11] Arora BK, Yadav AK. Serum iron and serum ferritin levels in cholelithiasis: A randomized study. Int Surg J. 2018;5:1411-16. Doi: 10.18203/2349-2902.isj20181121.
[12] Sarhan HH, Hamed MS, Khalaf SJ. Relationship between iron deficiency and gall stones formation. Tikrit Medical Journal. 2009;15(2):119-23.
[13] Halgaonkar P, Verma R, Bhadre R, Unadkat P, Vaja C, Unadkat P. Study to establish the clinical correlation between chemical constituents of gallstones and serum biochemical parameters. Int J Sci Stud. 2016;4(3):97-102.
[14] Chandran P, Garg P, Pundir CS. Correlation between chemical components of billary calculi and bile & sera and bile of gallstone patients. Indian J Clin Biochem.2005;20(2):81-85. Doi: 10.1007/BF02867405. PMID: 23105538.
[15] Prasad PC, Gupta S, Kaushik N. To study serum iron levels in patients of gall bladder stone disease and to compare with healthy individuals. Indian J Surg. 2015;77(1):19-22. Doi: 10.1007/s12262-012-0739-6. PMID: 25829706.
[16] Akhtar N, Nomani A, Salim B, Baig SJ. To study serum iron levels in patients of gall bladder stone disease. Int J Med Health Res. 2017;3(3)126-28.
[17] Misra PK, Dalal S, Kumawat M, Kharb S. Role of trace elements in the formation of gall stones. Ashian J Biochem. 2014;9:213-20. Doi: 10.3923/ajb.2014.213.220.

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