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RESEARCH ARTICLE

ROLE OF IRON DEFICIENCY IN THE FORMATION OF GALL STONE

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ABSTRACT

Background: The old axiom that a typical gall stone sufferer is a fat, fertile, female of fifty, is only partially true, as the disease is found in women soon after their first delivery and also in underweight and thin people. So while searching for other parameters, iron deficiency was found to be a new parameter of interest in the etiology of gall stones.

Aim and Objectives: To study the prevalence (numbers of patients) of iron deficiency in patient with gall stone disease. To study Serum Iron, Serum Cholesterol and Bile Cholesterol in patient with Gallstone Disease. To evaluate correlation between Serum Iron and Bile Cholesterol in patient with Gallstone Disease.

Methods: The study was carried out at Sir Sayajirao Gaekwad Hospital from February 2016 to November 2016. Patient with gall stone who meet the inclusion criteria was selected. This was Cross sectional study. Minimum 60 patients were required for the present study based on the results of the review article for this study.

Results: Total 67 patients were studied. Out of 67 patients 32 patients were included in Iron Deficiency Group A and 35 patients were included in Non Iron Deficiency Group B. On comparing mean serum cholesterol levels of both groups with independent t-test, p-value was 0.0797 which was >0.05. So there was no significant difference in serum cholesterol of the two groups. On comparing mean bile cholesterol levels of both groups with independent t-test, p-value was <0.0001 which was <0.05. So there was significant difference in bile cholesterol of the two groups.

Conclusion: There was significant difference ($p<0.0001$) in groups A and B. This suggesting that bile cholesterol is one of a significant criterion in the supersaturation of bile with respect to cholesterol. Low serum iron levels lead to bile supersaturation with respect to cholesterol, which leads to gallstone formation.

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INTRODUCTION

Gallstone disease is common clinical entity affecting the adult population of both sexes. Gallstone disease is more common in females than males. Gallstone disease is common in fatty, fertile, flatulent female of forty. These were old concepts. Recent studies have defined the role of trace elements (Fe, Ca, Zn and Cu) and defective pH in the formation of gallstone (Strasberg, 1991 and Verma, 2002). Gallstones are classified into either pure cholesterol stones, black or brown pigmented stones or mixed stones. The conditions that favor the formation of cholesterol gallstones are as follow (Verma, 2002). Bile must be supersaturated with cholesterol. Nucleation must be kinetically favorable. Cholesterol crystals must remain in the gall bladder long enough to agglomerate into stones. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation (Roslyn, 1987). Iron acts as a coenzyme for nitric oxide synthetase (NOS), and that is important for the maintenance of basal gall bladder tone and normal relaxation (Swartz-Basile, 2002; Salomons, 1997). It was found that iron deficiency resulted in altered motility of gall bladder and sphincter of oddi (SO), leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile (Goldblatt, 2001). This study was carried out to evaluate the role of Serum Iron, Serum Cholesterol and Bile Cholesterol in patients with Gallstones and to find(if any) correlation between low Serum Iron and Bile Cholesterol.

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MATERIALS AND METHODS

The study was carried out at Sir Sayajirao Gaekwad Hospital from February 2016 to November 2016. Patient with gall stone

who meet the inclusion criteria was selected. This was Cross sectional study.

Inclusion criteria

- Patient with ultrasound confirmation of their gall stones, irrespective of their age, sex, physique and parity.
- Only those patients were included, whose serum as well as bile could be procured for analysis.
- Patients who underwent open as well as laparoscopic cholecystectomy both were included.

Exclusion criteria

- Those patients whose serum as well as bile cannot be procured for analysis were excluded.
- Those with empyaema and mucocele of gall bladder were excluded.
- Those patient has taken iron supplements within 15 day were excluded.

Sample size and Study population

Minimum 60 patients were required for the present study based on the results of the review article for this study, considering the objective of the study, the variables included and the results expected, to get mean difference of Bile Cholesterol of 0.5 with Standard deviation 0.5 at 1% alpha error at 90% power. The sample size was calculated using software "n-Master 2.0".

Method of study

Serum Iron level and Thin Peripheral Smear Examination of all patients who satisfy inclusion criteria were estimated. Then Patients were divided in to 2 groups according to serum iron level and thin peripheral smear examination. Patients whose serum iron levels equal to or less than normal reference limit and thin peripheral smear suggestive of microcytic hypochromic RBCs were included in 'Iron Deficiency' group 'A'. Patients whose serum iron levels within normal reference range and normocytic normochromic RBCs were included in 'Non Iron Deficiency' group 'B'. The normal reference values for serum iron level, for males (60-160 ug/dl) and for females (35-145 ug/dl) are used to label the patients as iron deficiency and non iron deficiency i.e. males with serum iron < 60 ug/dl and females with serum iron <35 ug/dl was labeled as Iron deficiency. During the operation for open cholecystectomy, bile was aspirated with a needle mounted on a sterilized syringe.

The aspiration needle was passed obliquely into the fundus of gall bladder and as much of bile as possible, was withdrawn from the gall bladder. During laparoscopic Cholecystectomy, bile was aspirated from any one of the 5mm port with the help of Veress Needle/Gall Bladder Aspirator. Minimum 2cc bile was needed for estimation of bile cholesterol. Bile was kept in a sterile labeled container. Serum cholesterol and bile cholesterol of all patients were estimated. Hemoglobin level of all patients were estimated. Comparison of serum cholesterol levels of iron deficiency group A and non iron deficiency group B were done. Comparison of bile cholesterol levels of iron deficiency group A and non iron deficiency group B were also done.

Statistical Methods

Data amongst the two groups were subjected to statistical analysis using Independent t-test. The P-value <0.05 was considered significant. M.S Excel was used for data entry and S.T.A.T.A – 13 softwere was used for analysis.

RESULTS

A cross sectional study was carried out in the Department of General Surgery, during the period from February 2016 to December 2016 in 67 patients having Gallstone Disease. The patients belonged to various surgical units in Sir Sayajirao Gaekwad General Hospital and full details of the patients were recorded in the proforma. Out of 67 patients 32 patients were included in Iron Deficiency Group A and 35 patients were included in Non Iron Deficiency Group B. Serum cholesterol and Bile Cholesterol levels between the two groups were recorded as mean ± standard deviation and compared by the Independent t-test. A p-value <0.05 was considered statistically significant. All stastical analyses were performed in M.S. Excel and S.T.A.TA.-13 software. Observations and analysis of all the parameters studies are as follows:

Sex Distribution

Table 1. Sex distribution in both groups

Sex	Group a	Group b	Total
Male	8	8	16
Female	24	27	51
Total	32	35	67

Out of 67 patients 51(76%) patients were females and 16(24%) patients were males. So Male:Female ratio in this study was 1:3. The above sex distribution shows that the gall bladder diseases have a higher frequency in female than in males.

In Group A, there were 24(75%) females and 8(25%) were males. In Group B, there were 27(77%) females and 8(23%) were males.

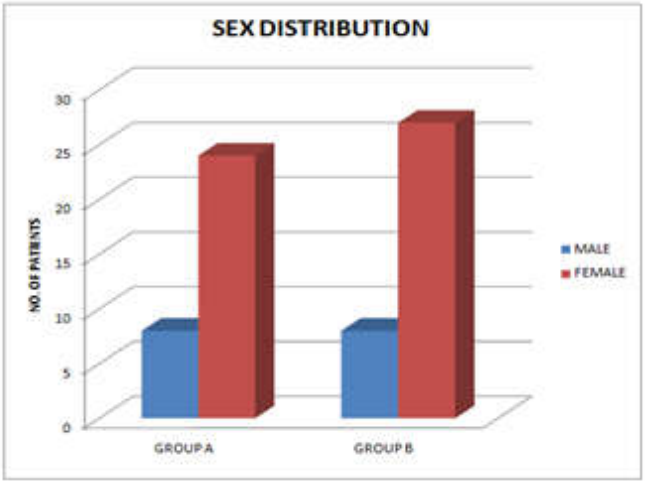

Figure 1. Sex distribution in both groups

The mean age of all 67 patients analysed was 40.8 years ± 13.6 SD. The age group of the patients ranged from 17 years to 72 years. The maximum incidence is seen in the age group of 31-40 years followed by 41-50 years of age.

Age Distribution

Table 2. Age Distribution in Both Groups

Age group(in years)	Group a	Group b	Total	Percentage
17-20	3	0	3	4%
21-30	8	7	15	22%
31-40	9	8	17	25%
41-50	4	12	16	24%
51-60	5	6	11	16%
61-70	3	1	4	6%
71-72	0	1	1	1%

The mean age of patients in Group A was 38.6 years ± 14.8 SD. The mean age of patients in Group B was 42.6 years ± 12.8 SD.

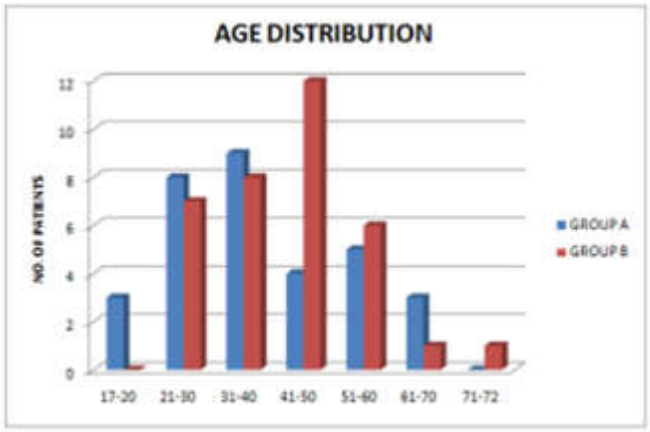

Figure 2. Age distribution in both groups

Table 3. Comparison of serum cholesterol

No	Group	No of patients	Serum cholesterol(mg/dl)		P-value
			Mean±sd	Range	
1	A	32	179.3±15.8	163.5-195.1	0.0797
2	B	35	187.5±21.3	166.2-208.8	(>0.05)

The mean serum cholesterol of the patients in Group A was 179.3mg/dl with SD of 15.8 which ranging from 163.5mg/dl to 195.1mg/dl. The mean serum cholesterol of the patients in Group B was 187.5mg/dl with SD of 21.3 which ranging from 166.2mg/dl to 208.8mg/dl. On comparing mean serum cholesterol levels of both groups with independent t-test, p-value was 0.0797 which was >0.05. So there was no significant difference in serum cholesterol of the two groups.

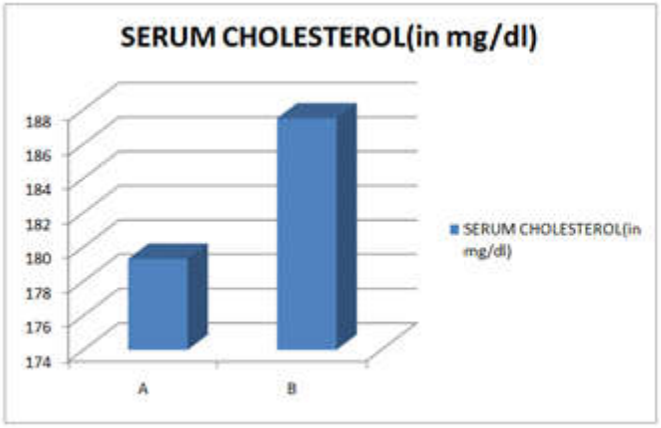

Figure 3. Comparison of mean serum cholesterol of both groups

Comparison of bile cholesterol in both groups

Table 4. Comparison of bile cholesterol

No	Group	No of patients	Bile cholesterol(gm/dl)		P-value
			MEAN±SD	RANGE	
1	A	32	1.397±0.129	1.268-1.526	<0.0001
2	B	35	0.767±0.231	0.536-0.998	(<0.05)

The mean bile cholesterol of the patients in Group A was 1.397gm/dl with SD of 0.129 which ranging from 1.268gm/dl to 1.526gm/dl. The mean bile cholesterol of the patients in Group B was 0.767gm/dl with SD of 0.231 which ranging from 0.536gm/dl to 0.998gm/dl. On comparing mean bile cholesterol levels of both groups with independent t-test, p-value was <0.0001 which was <0.05. So there was significant difference in bile cholesterol of the two groups.

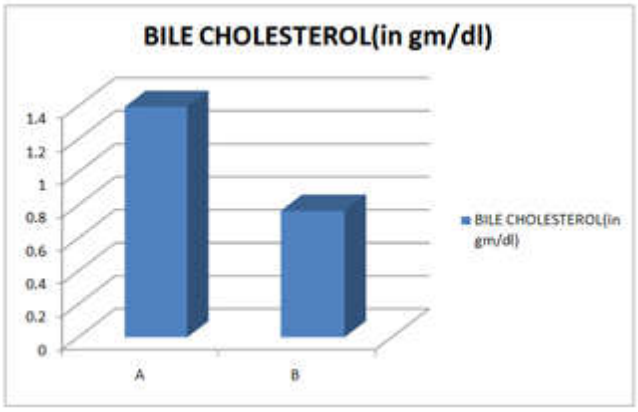

Figure 4. Comparison of mean bile cholesterol of both groups

DISCUSSION

Gall stone disease is a very common gastrointestinal problem in day to day practice. In present study, 67 patients with gallstone disease were satisfied inclusion criteria. Out of 67 patients 32 patients had serum iron level equal to or less than normal reference limit and thin peripheral smear suggestive of microcytic hypochromic RBCs. So these were included in Iron Deficiency Group A. While rest 35 patients had serum iron within normal reference range and normocytic normochromic RBCs on thin peripheral smear. So these patients were included in Non Iron Deficiency Group B.

No age is said to be immune to gallbladder disease, however they were more common in the third, fourth and fifth decades of life as 71% of the cases belonged to these decades. Maximum incidence was seen in the age group of 31-40 years i.e. 17 patients (25%) followed by that in 41-50 years i.e, 16 patients (24%). Our study population was younger, mean age 40.8 years. Daradkeh *et al*. (2005) reported mean age of 47.2 years, whereas Bingener *et al*. (2002) reported 40 years. In present study, out of 67 patients 51(76%) patients were females and 16(24%) patients were males. So Male:Female ratio in present study was 1:3. This was supporting the old oxiom that gallstone formation is most common in the female population. This is in accordance with the previous studies which stated a high prevalence of cholelithiasis in females which may be due to the effects of estrogen and progesterone on the biliary tract. Estrogenic influences increase the effect of hepatic lipoprotein receptors and stimulate hepatic hydroxyl methyl glut aryl coenzyme A (HMG Co-A) reductase activity.

Table 5. Comparison of sex ratio and mean age between different reference studies

Study	Sample size	No. of patients		Male: female ratio	Mean Age
		Male	Female		
Misra *et al* 2014	100	18	82	1:4	39.7
Prasad *et al* 2012	100	16	84	1:4	40.3
Hamid Sarhan *et al* 2009	50	10	40	1:4	41.2
Sahu *et al* 2007	100	20	80	1:4	40.5
Kumar *et al* 2006	50	10	40	1:4	39.8
Present study 2016	67	16	51	1:3	40.8

Table 6. Comparison of serum cholesterol levels in different studies

Study	Serum Cholesterol (in mg/dl)		P-Value
	Iron deficiency group	Non iron deficiency group	
Misra *et al*. 2014	129.7±38	133.0±32.0	0.765
Prasad *et al* 2012	173±51	184±39	0.078
Hamid H Sarhan *et al* 2009	172±49	183±36	0.367
Sahu *et al* 2007	202±19.9	200.5±17.8	0.348
m Kumar *et al* 2006	172±49	183±36	0.367
Present Study 2016	179±16	188±21	0.0797

Table 7. Comparison of bile cholesterol levels in different studies

Study	Bile cholesterol (in gm/dl)		P-Value
	Iron deficiency group	Non iron deficiency group	
Misra *et al* 2014	0.747±0.115	0.212±0.204	<0.001
Prasad *et al* 2012	0.949±0.101	0.223±0.201	<0.001
hamid h sarhan *et al*[24] 2009	1.20±0.41	0.70±0.37	<0.001
S Sahu *et al*[23] 2007	0.375±0.086	0.214±0.040	<0.001
M Kumar *et al*[22] 2006	1.20±0.41	0.70±0.37	<0.001
Present Study 2016	1.4±0.13	0.7±0.85	<0.0001

Consequently, together cholesterol uptake and biosynthesis are increased leading to super-saturation of bile with cholesterol and helping in formation of gallstones. Progesterone alters the sphincter of Oddi and gallbladder function ultimately causing a derangement in bile flow dynamics. Even though, the effects of progesterone on the biliary tract have been implicated in the increased incidence of gallstones among the women, the specific effects of prolonged elevated levels of progesterone on the sphincter of Oddi and bile flow dynamics are still incompletely understood (Crawford, 1999). Pregnancy favours the formation of gallstones through the hormonal influence on bile composition (increased biliary cholesterol secretion, diminished and disturbed bile acid pool). Estrogen induces an increased input to the hepatic free cholesterol pool by up regulating the low density lipoprotein. Decreased gallbladder motility during third trimester of pregnancy and an altered function of gallbladder mucosa that may favour nucleation and growth of stones (Acalovschi, 2001). The serum cholesterol level of all the patients included in the study were determined. The mean serum cholesterol level for Group A was 179.3mg/dl ± 15.8 ranging from 163.5mg/dl to 195.1mg/dl. the mean serum cholesterol level for Group B was 187.5mg/dl ± 21.3 ranging from 166.2mg/dl to 208.8mg/dl. The serum cholesterol levels in group A and B is shown in Table 3. There was no significant difference(p-0.0797) in groups A and B and the mean serum cholesterol level was found to be within the normal range in both groups. This suggesting that serum cholesterol is not a significant criterion in the supersaturation of bile with respect to cholesterol. The bile cholesterol level of all the patients included in the study were determined. The mean bile cholesterol level for Group A was 1.397gm/dl ± 0.129 ranging from 1.268gm/dl to 1.526gm/dl.

The mean bile cholesterol level for Group B was 0.767gm/dl ± 0.231 ranging from 0.536gm/dl to 0.998gm/dl. The bile cholesterol levels in group A and B is shown in Table 4. There was significant difference ($p<0.0001$) in groups A and B. These findings were in accordance to those of Tierney *et al*. (1999) who contributed increased levels of biliary cholesterol to the effects of hormones like progesteron. The difference in values was statistically significant indicating that the mean bile cholesterol of patients with iron deficiency was higher than patients with normal serum iron ($p< 0.05$). These findings were similar to those that observed that iron deficient diet altered hepatic enzyme metabolism which in turn increase gallbladder bile cholesterol and promoted cholesterol crystal formation (Johnston *et al*., 1997). The comparison between the levels of serum iron and bile cholesterol in group A and group B patients showed statistically significant difference in values of both the groups showing that group A patients had low serum iron and high bile cholesterol levels indicating super saturation of bile in iron deficient patients. These findings were in accordance with those of Kumar *et al*. (2006) who also observed in their study that iron deficiency altered the activity of several hepatic enzymes leading to increased gallbladder bile supersaturation and promotion of cholesterol crystals.

Iron deficiency alters the activity of several hepatic enzymes, leading to increased gallbladder bile cholesterol saturation and promotion of cholesterol crystal formation (Kumar *et al*., 2006). It is also suggested that iron deficiency alters the activity of several hepatic enzymes (Roslyn *et al*., 1987). They concluded that consumption of diet rich in carbohydrates but deficient in iron altering hepatic metabolism of cholesterol that might be important in gallstone formation.

Researchers concluded that iron deficient diet altered hepatic enzyme metabolism which in turn increased gallbladder bile cholesterol and promoted cholesterol crystal formation (Johnston *et al*., 1997). Researchers have observed the same parameters in their study (Salomons et al., 1997). Researchers have demonstrated that diminished gallbladder neuronal nitric oxide synthase contributed to the gallbladder stasis that occurred with iron deficiency (Swartz-Basile *et al*., 2000). Iron, a cofactor for nitric oxide synthase, plays a key role in normal relaxation of gallbladder. It has been reported that iron deficiency resulted in altered motility of gallbladder and sphincter of Oddi and thus increased cholesterol crystal formation in the gallbladder bile (Swartz-Basile *et al*., 2000; Goldblatt *et al*., 2001). Thus, iron deficiency was found to have a major role in gallstone formation. A comparative comparison of the results with other studies as mentioned above shows the same result of deficiency of both iron is associated with increased chances of super-saturation of bile in gallbladder followed by increase in incidence of gallstone formation.

Conclusion

47.8% patients with gallstone disease in this study had Iron Deficiency Anemia. The gall bladder disease was more common in females as compared to males with a ratio of 1:3 (76% of the patients were females) in this study. The commonest age of presentation of gall bladder diseases is 31-40 years (25% of the patients presented in this age group). The mean serum cholesterol level for Group A was 179.3mg/dl ± 15.8 ranging from 163.5mg/dl to 195.1mg/dl. The mean serum cholesterol level for Group B was 187.5mg/dl ± 21.3 ranging from 166.2mg/dl to 208.8mg/dl. There was no significant difference (p-0.0797) in groups A and B. This suggesting that serum cholesterol is not a significant criterion in the supersaturation of bile with respect to cholesterol. The mean bile cholesterol level for Group A was 1.397gm/dl ± 0.129 ranging from 1.268gm/dl to 1.526gm/dl. The mean bile cholesterol level for Group B was 0.767gm/dl ± 0.231 ranging from 0.536gm/dl to 0.998gm/dl. There was significant difference ($p<0.0001$) in groups A and B. This suggesting that bile cholesterol is one of a significant criterion in the supersaturation of bile with respect to cholesterol. Low serum iron levels lead to bile supersaturation with respect to cholesterol, which leads to gallstone formation.

LIST OF ABBREVEATIONS:

- NOS : Nitric Oxide Synthetare
- SO : Sphincter of Oddi
- SAGES : South Africa Gasteroenteroloy Society
- CCK : Choleeystokmin
- OCP : Oral Contraceptive Pills
- RBC : Red Blood Cell
- NASAID :Non Steroidal Anti Inflammatory Drug
- MCV : Mean Corpuscular Volume
- TIBC : Total iron binding capacity
- RDW : Red Blood Cell Distribution Width
- HB : Hemoglobin
- CBC : Complete Blood Count
- USG : Ultrasonography
- µg/dL : Microgram Per Deciliter
- mg/dL : Miligram Per Deciliter
- gm/dL : Gram Per Deciliter
- GB : Gall Bladder
- SD : Standard Deviation
- HMG Co-A : Hydroxy Methyl Glutaryl Coenzyme A

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