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Comparative Analysis Of Biliary Cholesterol Levels In Iron Deficient And Non-Iron Deficient Patients Operated For Gall Stone Disease

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Abstract

Background :Gall Stone disease is a common clinical entity affecting adult population of both sexes. The old saying, that gall stone sufferer is a fat, fertile, female of fifty is only partly true, as the disease has been observed in women after their first delivery and also in underweight and thin people. Several etiological factors have been studied in the formation of gall stones. There has been recent interest in establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease.Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. Establishing the role of Serum Iron in the etiology of Gall stones is of special importance in our population group because of the huge prevalence of nutritional deficiencies. It also provides scope for early detection, treatment and risk modification if its role as an etiologic/risk factor is clearly defined.

Aim: The study is aimed at establishing the role of iron deficiency in the super saturation of bile with respect to cholesterol, which leads to gall stone formation.

Methods:A study was done in 2023 at the department of General Surgery. ACS Medical College & Hospital, Poonamallee High Rd, Velappanchavadi, Chennai, Tamil Nadu, India.50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Serum Iron, Biliary Cholesterol and Serum Cholesterol of all the patients was obtained. The Biliary cholesterol levels and Serum cholesterol levels of both the groups was analysed by using a student t-test.

Results: Out of the 50 Patients, 40 (80%) were female and 10 (20%) were males. The female to male ratio was 4:1. The Biliary Cholesterol values for Group A and B respectively were 754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl. The Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of <0.0004. Similarly, an independent t-test comparing Serum Cholesterol levels in Non-Iron Deficient (184.8 ± 35 mg/dl) and Iron Deficient subjects (171 ± 49.3 mg/dl) did not find a statistically significant difference. (p=0.2544).

Conclusion: These results suggest that Iron Deficiency has an association with Biliary Cholesterol Levels. According to our research, cholelithiasis affects people most frequently between the ages of 30 and 40, and it is more common in women than in men. Gallbladder stone development is significantly influenced by low serum iron levels. Raised serum cholesterol and cholelithiasis were not significantly correlated. Therefore, it can be assumed that low serum iron levels are producing biliary stasis, which in turn is increasing the prevalence of

cholelithiasis. In our investigation, low serum iron levels with cholelithiasis were related with elevated bile cholesterol levels

Keywords: Biliary Cholesterol, Iron Deficiency, Gall Stone Disease

Introduction

Gall Stone disease is a common clinical entity affecting adult population of both sexes. The old saying, that gall stone sufferer is a fat, fertile, female of fifty is only partly true, as the disease has been observed in women after their first delivery and also in underweight and thin people[1]. Several etiological factors have been studied in the formation of gall stones.There has been recent interest in establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease.[2]Cholesterol gallstones occur most commonly in multiparous women, but the causes for this phenomenon remain unclear[3]. This same patient population is prone to chronic iron deficiency anemia. With this as the background, Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. In experimental data from adult prairie dogs,[4] 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. 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[5]. It was found that iron deficiency resulted in altered motility of gall and sphincter of oddi (SO), leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile.[6]Therefore, we tested the hypotheses that iron deficiency would alter hepatic cholesterol metabolism causing increased biliary cholesterol saturation and hence enhance gallstone formation. Establishing the role of Serum Iron in the etiology of Gall stones is of special importance in our population group because of the huge prevalence of nutritional deficiencies.[7] It also provides scope for early detection, treatment and risk modification if its role as an etiologic/risk factor is clearly defined. The present study was conducted on the randomly selected individuals of the South Indian Population, suffering from gall stone formation, to study the role of iron deficiency anemia in gall stone formation.

Methods:A study was done in 2023 at the department of General Surgery. ACS Medical College & Hospital, Poonamallee High Rd, Velappanchavadi, Chennai, Tamil Nadu, India.50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Serum Iron, Biliary Cholesterol and Serum Cholesterol of all the patients was obtained.50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Gall bladder Bile cholesterol and serum cholesterol of both the groups are compared.The patients were selected, based only on the USG confirmation of their gall stones, irrespective of their age, sex, physique, parity, etc. Only those patients were included, whose serum as well as bile could be procured for analysis. Patients with empyema and mucocele of gall bladder were excluded.All the patients, who were included in the study were given a serial number1 to 50, in the order of their admission to the surgery department for Cholecystectomy. Thus their bile and serum samples were also labeled 1 to 50. accordingly. The numbered samples were sent to the Biochemistry department for analysis. All the numbered samples with less than normal serum iron (n=23) were put in the anaemic group, B and all the samples with normal serum iron (n=27) were put in the non anaemic group, Group A.Serum iron was estimated by Ferrozine kit method for determination of iron. The normal reference values supplied with the kit, for males (60-160 µg/dl) and for females (35-145 g/dl), were used to label the patients as anaemic and non- anaemic i.e. males with serum iron < 60 g/dl and females with serum iron <35 µg/dl were labeled as anaemic. During the operation for open cholecystectomy, bile was aspirated with an aspiration needle mounted on a

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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. Similarly during laparoscopic cholecystectomy, bile was aspirated under vision through a long venflon needle or a veress needle just before delivering out the gallbladder at the port site. Bile was kept in a sterile labeled container and sent for analysis. Serum cholesterol and gall bladder bile cholesterol of all the patients were estimated. Bile was first subjected to the Folch method to extract lipids and then the cholesterol contents were estimated as for serum cholesterol. In the Folch method, lipids from bile were extracted by using water, Methanol and Chloroform mixture in the ratio of 3:4:8 v/v and from the extracted lipids, cholesterol was estimated by Enzopak kit, based on the cholesterol oxidase/peroxidase method. The enzymes used only the cholesterol as substrate and hence Bilirubin is automatically eliminated, from the procedure of cholesterol estimation.

Statical Analysis: The biliary cholesterol levels in the 2 groups of patients A and B were compared using a student t-test to detect any statistically significant difference.Similarly, the serum cholesterol levels of these 2 groups were analyzed with a student t-test.Also, the age, sex and parity distributions of serum iron and biliary cholesterol in the study population was analyzed.

Results

TABLE 1. AGE DISTRIBUTION OF THE STUDY GROUP:

Age Range	**Frequency**	**Percentage**
<20 yrs	2	4%
20-30 yrs	11	22%
30-40 yrs	13	26%
40-50 yrs	14	28%
50-60 yrs	8	16%
60-70 yrs	2	4%
Total	50	100%

Table 2:Distribution of patients into Group A and Group B:

Group A **(Non-Iron Deficient)**	**Group B (Iron Deficient)**
27	23

Table 3 Serum Iron Levels in Study Group Patients

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Patients	No.	%	Range of Serum Iron (µg/dl)	Mean ±S.D
Males				
• Non-Iron Deficient	9	90	95.5-163	119.8 ± 25.3
• Iron Deficient	1	10	40	-
• Total	10	100	95.5-163	111.8 ± 34.7
Females				
• Non-Iron Deficient	18	45	36.3-140.1	79.8 ± 31.1
• Iron Deficient	22	55	7.4-34.6	26.2 ± 8.3
• Total	40	100	7.4-140.1	50.4 ± 34.5

Table 4. Serum Iron Contents in Group A & Group B Patients

Group	No. of Patients	Serum Iron Range µg/dl	Serum Iron Mean ±S.D
A (Non-Iron Deficient)	27	36-163	93.2 ± 34.6
B (Iron deficient)	23	7-40	26.8 ± 8.6
P-Value			<0.0001

Table 5. Biliary Cholesterol Levels in Group A & Group B Patients

Group	No. of Patients	Biliary Cholesterol Range mg/dl	Biliary Cholesterol Mean ±S.D
A (Non-Iron Deficient)	27	3-147	754.5 ± 398.3
B (Iron deficient)	23	26-181	1184.7 ± 405.2

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P-Value			<0.0004

Table 6. Serum Cholesterol Levels in Group A & Group B Patients

Group	No. of Patients	Serum Cholesterol Range mg/dl	Serum Cholesterol Mean ±S.D
A (Non-Iron Deficient)	27	93-254	184.8 ± 35
B (Iron deficient)	23	79-270	171 ± 49.3
P-Value			0.2544

Table 7 Serum Iron, Biliary Cholesterol and Serum Cholesterol Levels with P- Values after T- Test Analysis

Groups	Serum Iron (µg/dl)	Biliary Cholesterol (mg/dl)	Serum Cholesterol (mg/dl)
A=27 (Non- Iron Deficient)	93.2 ± 34.6	754.5 ± 398.3	184.8 ± 35
B=23 (Iron Deficient)	26.8 ± 8.6	1184.7 ± 405.2	171 ± 49.3
P-Value	<0.0001 ***Extremely Significant***	<0.0004 ***Extremely Significant***	0.2544 ***Not Significant***

Table 8 Parity specific distribution of Serum Iron Levels in Female Patient

Parity		Group A	*Non Iron-Deficient*		Group B	*Iron Deficient*
	No of Pts	**%**	**Mean Serum Iron**	**No. of Pts**	**%**	**Mean Serum Iron**

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Nulliparous	2	11	120.6 ± 27.5	1	4.5	34
Primiparous	4	22	89.3 ± 25.2	2	9	33 ± 1
Multiparous	12	67	69.9 ± 28.5	19	86.5	25.1 ± 8.4
Total	18	100	79.8 ± 31.1	22	100	26.2 ± 8.3

Discussion

Cholelithiasis is a very common condition encountered in surgical practice. Though most cases may be asymptomatic, a definitive percentage of these patients may develop symptoms of Gall Stone disease. A few will develop complications that may even be life threatening.[8] Over the past century the mortality and morbidity related to Gall Stone Disease has decreased due to the early recognition and treatment of symptomatic cholelithiasis. By and large, surgical management has been the treatment of choice, and with the advent of laparoscopy, Laparoscopic cholecystectomy has replaced Open Cholecystectomy as the Gold Standard in the surgical management of uncomplicated gall stone disease.[9]There have been numerous studies done to assess the risk factors for Cholelithiasis. These have been discussed earlier in this study. Also its of paramount importance to identify easily modifiable risk factors as these can help in risk stratification and also prevention.[10]The exact mechanism of Gall Stone formation is a complex process that is not solely dependent on any single factor. Thus Gall stone disease has a multi factorial etiology. Newer risk factors/ etiological agents have been studied time and again, with some showing to have a significant impact on the disease process.As stated already, animal studies had suggested that Iron may have a role to play in the pathophysiology of gall stone disease. More importantly the finding that iron deficient mammals were more prone to develop gall stones, has sparked interest in its probable role in humans.[11] Also the classical teaching is that a females, in her forties, obese and multiparous is more prone for gall stone disease. When this is viewed, keeping the recent findings in perspective, it may suggest that apart from the other risk factors in this group, these are also the patients who are most iron deficient. Hence, it makes perfect sense, more so in the Indian population, to define the role of Iron or its deficiency in the pathophysiology of gall stone disease.[12]As data from other studies suggest, Iron deficiency changes the activity of many liver enzymes. This may be a factor in promoting cholesterol supersaturation and increased cholesterol crystal nucleation. Also Iron acts as a coenzyme for nitric oxide synthase. [13]Nitric oxide helps in the normal gall bladder tone and relaxation. An alteration in this fine balance may promote gallbladder stasis which results in cholesterol stone formation.The non-Iron deficient group (Group A) had an above average value of 93.2 ± 34.6 micro gm/dl as compared to the Iron deficient group (Group B) which had a value of 26.8 ± 8.6 micro gm/dl.The Biliary Cholesterol values for Group A and B respectively were754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl.The Serum Cholesterol values for Group A and B respectively were 184.8.± 35 and 171 ± 49.3 mg/dl. In our study the Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of <0.0004.[14] Also, there was no significant difference between the values of serum cholesterol levels in the Iron Deficient (Group B) and Non-Iron Deficient Group. (Group A). P value of 0.2544.[15]The current study suggests that deficiency in serum iron could play a role in the increased saturation of biliary cholesterol. Biliary cholesterol supersaturation is an independent factor in the formation of cholesterol gall stones. As mentioned previously, the probable explanation for this is the defective cholesterol metabolism and gall bladder stasis promoted a deficiency in serum iron.[16,17]

Conclusion

To conclude, there was a significant difference in the Biliary Cholesterol Levels for Non-Iron Deficient

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(M=754.5 mg/dl , SD=398.3) and Iron Deficient Subjects(M=1184.7 mg/dl, SD=405.2) t=3.77, p =<0.0004.Similarly, an independent t-test comparing Serum Cholesterol levels in Non-Iron Deficient (184.8 ± 35 mg/dl) and Iron Deficient subjects (171 ± 49.3 mg/dl) did not find a statistically significant difference. (p=0.2544) These results suggest that Iron Deficiency has an association with Biliary Cholesterol Values.

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