

IRON & CALCIUM DEFICIENCY – POTENTIAL RISK FACTOR IN THE FORMATION OF BILIARY CALCULI – A PROSPECTIVE STUDY CONDUCTED IN A TERTIARY CARE HOSPITAL OF EASTERN INDIA.

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ABSTRACT

Alteration in the serum level of trace elements may be an important causative factor in the formation of biliary calculi. Better understanding of the pathophysiology will help in prevention of this one of the commonest surgical problem in future. Deficiency of the trace elements especially iron & calcium interferes with the hepatic enzyme function that precipitates gall stone formation. This hypothesis is supported by few recent studies in animal & human model in India and abroad. This prospective study was planned to evaluate serum concentration of iron & calcium in 2 groups of patients, one group having cholelithiasis and another group undergoing other elective surgical procedures, named cases and controls respectively, over a period of one and half years. It was found that the level of serum iron of the patients with gallstone was significantly lower than that of the patients without gallstone [$p=0.04$]. The risk of gallstone formation was also 5.44 times more among the patients with serum iron deficiency as compared to the patients with normal level of serum iron and the risk was significant [OR-5.44(1.61, 48.39); $p=0.04$]. Moreover, further analysis in the case group shows that the mean level of serum iron for Pigment stone was the highest and that for Cholesterol stone was the lowest and the difference is statistically significant. Corrected Chi-square (χ^2) test showed that there was significant association between level of serum calcium and patients of the two groups ($p=0.026$). The risk of gallstone was 7.97 times more among the patients with serum calcium deficiency as compared to the patients with normal level of serum calcium and the risk was significant [OR-7.97(1.04, 67.45); $p=0.026$]. Also, t-test showed that there was significant difference in the level of serum calcium of the patients with gallstone than that of the patients without gallstone [$t_{98} = 1.98$; $p=0.04$]. Among the 3 stone groups in the Case arm the mean level of serum calcium for Pigment stone was the highest and that for Cholesterol stone was the lowest, but this difference is not statistically significant ($p=0.08$).

KEYWORDS

INTRODUCTION

The old axiom, “a typical gallstone sufferer, is a fatty, fertile, fair female of forty”, is only partially true as the disease has been frequently found in young, underweight & thin individuals. The prevailing opinion suggested that the formation of cholesterol calculi was the result of specific alterations in hepatic metabolism while the gallbladder was “a harmless spectator”. Recently added reports indicate that neither the liver nor gallbladder alone has an exclusive etiological role in the formation of calculi. It appears now that the calculi are formed as a result of dynamic interaction between the liver and the gallbladder. Though the exact etiology of cholelithiasis is still not well understood, various factors held responsible are infection, metabolic changes and bile stasis. Three conditions must be met to permit the formation of cholesterol gallstones:

- (1) Supersaturation of bile with cholesterol
- (2) Kinetically favorable nucleation
- (3) Gallbladder stasis

The established risk factors for causing this supersaturation of cholesterol are increased age, female sex, obesity with rapid weight loss, hepatic cirrhosis and dietary factors. While searching for other established factors, recent studies have defined the role of trace elements, specifically **iron & calcium** in the formation of gallstones. Out of these trace elements, **iron deficiency** has been shown to alter the activity of several hepatic enzymes leading to increased bile cholesterol saturation and promotion of crystal formation. Iron acts as a cofactor for **Nitric Oxide Synthase (NOS)**, which synthesizes Nitric Oxide (NO) and is important for the maintenance of basal gallbladder tone and normal relaxation. The NOS acts as a **calcium-calmodulin** dependent enzyme. Hence, a deficiency in serum calcium causes deranged function of NOS resulting in altered gallbladder motility, leading to biliary stasis and subsequently increased crystal formation in bile. So, Alteration in the level of trace elements, specifically Iron & Calcium can lead to increased risk of cholelithiasis.

MATERIALS & METHODS

This prospective study was conducted in the Departments of Surgery, Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences (VIMS), Kolkata, India, over a period of one and half years. There were 2 groups in the study. 50 patients were included in the CASE group who were diagnosed to have cholelithiasis on

ultrasonography and were scheduled for elective laparoscopic or open cholecystectomy. 50 other patients were included in the CONTROL group who were admitted for other elective operations. Individuals who were already on micronutrient (iron & calcium) supplementation were excluded from the study in both the groups. Patients who had any form of hemoglobinopathies, chronic renal failure or chronic liver disease were excluded from both the groups.

Patient's particulars including Name, Age, Sex, Registration number, Co-morbidity, Drug history were registered in the case taking proforma. Serum levels of Calcium & Iron were measured in all patients. Blood for calcium was drawn without applying tourniquet. Blood samples were sent to Department of Biochemistry for analysis. Serum Iron & Calcium were measured by Dry Chemistry method. Values were collected and were registered in the proforma for analysis. In the Case group, gallstones were examined after cholecystectomy.

Based on their gross physical appearance, stones were labelled as cholesterol, pigment or mixed type. Stone numbers were also noted. Serum levels of iron & calcium were compared between 2 groups.

Type Of Study: Prospective study

Time Line: January 2016 to June 2017

Sample Size: 50 cases & 50 control.

Normal Reference Values Used In The Study

Calcium 8.4-10.2 mg/dl

Iron - Male : 49-181 mcg/dl, Female : 37- 170 mcg/dl

Cases: Inclusion Criteria: All patients suffering from cholelithiasis confirmed by ultrasonography

Exclusion Criteria:

- (1) Patients who are on micronutrient supplementation
- (2) Patients who have any hemolytic disease or hemoglobinopathies
- (3) Patients who have chronic renal failure
- (4) Patients who have chronic liver disease

Control: Inclusion Criteria:

Patients of any age & sex who are admitted in General Surgery

Department in this hospital for other elective surgery for benign conditions in the same time period.

Exclusion Criteria:

- 1) Patients who are on micronutrient supplementation
- 2) Patients who have any hemolytic disease or hemoglobinopathies
- 3) Patients who have chronic renal failure.
- 4) Patients who have chronic liver disease

Statistical Analysis

Statistical Analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC). Using this software, basic cross-tabulation, inferences and associations were performed. Means along with the standard deviations were calculated under descriptive analysis.

χ^2 test was used to test the association of different study variables with the study groups. Z-test (Standard Normal Deviate) was used to test the significant difference between two proportions. t-test was used to compare the means. Odds ratio (OR) with 96% confidence intervals was calculated to find the risk factors. $p < 0.05$ was considered statistically significant.

Table-1: Distribution Of Level Of Serum Iron Of The Patients Of The Two Groups

Level of serum iron	With Gallstones (n=50)	Without Gallstones (n=50)	Total
Deficient	5	1	6
Row%	83.3	16.7	100.0
Col %	10.0	2.0	6.0
Normal	45	49	94
Row%	47.9	52.1	100.0
Col %	90.0	98.0	94.0
TOTAL	50	50	100
Row%	50.0	50.0	100.0
Col %	100.0	100.0	100.0
Mean±s.d.	86.54±39.34	126.78±43.14	
Median	84.00	126.00	
Range	23 - 174	43 - 179	

$\chi^2=4.83$; $p=0.04$ S-Significant

Corrected Chi-square (χ^2) test showed that there was significant association between level of serum iron and patients of the two groups ($p=0.04$). The risk of gallstone was 5.44 times more among the patients with serum iron deficiency as compared to the patients with normal level of serum iron and the risk was significant [OR=5.44(1.61, 48.39); $p=0.04$]. Also, t-test showed that level of serum iron of the patients with gallstone was significantly lower than that of the patients without gallstone ($t_{98}=4.87$; $p<0.001$).

Table-2: Distribution Of Level Of Serum Calcium Of The Patients Of The Two Groups

Level of serum calcium	With Gallstones (n=50)	Without Gallstones (n=50)	Total
Deficient	7	4	11
Row %	63.6	36.4	100.0
Col %	14.0	8.0	11.0
Normal	43	46	89
Row %	48.3	51.7	100.0
Col %	86.0	92.0	89.0
TOTAL	50	50	100
Row %	50.0	50.0	100.0
Col %	100.0	100.0	100.0
Mean±s.d.	9.36±0.78	9.56±0.64	
Median	9.45	9.50	
Range	7.5 - 10.9	8.3 - 10.8	

$\chi^2=4.89$; $p=0.026$ S-Significant

Corrected Chi-square (χ^2) test showed that there was significant association between level of serum calcium and patients of the two groups ($p=0.026$). The risk of gallstone was 7.97 times more among the patients with serum calcium deficiency as compared to the patients with normal level of serum calcium and the risk was significant [OR=7.97(1.04, 67.45); $p=0.026$].

Also, t-test showed that there was significant difference in the level of

serum calcium of the patients with gallstone than that of the patients without gallstone ($t_{98}=1.98$; $p=0.04$).

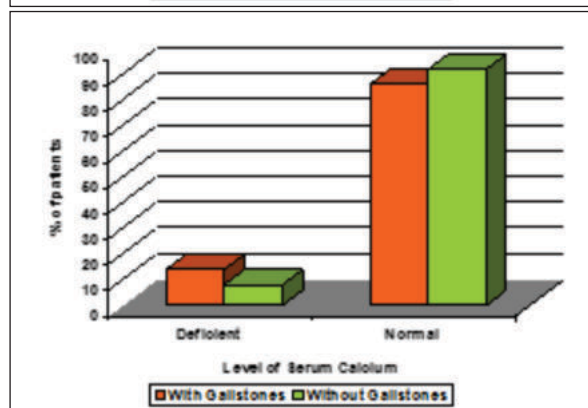
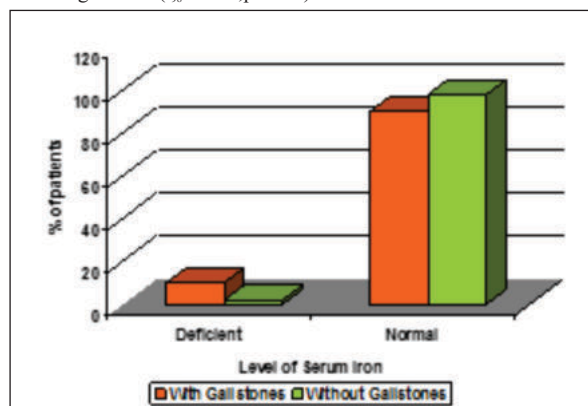


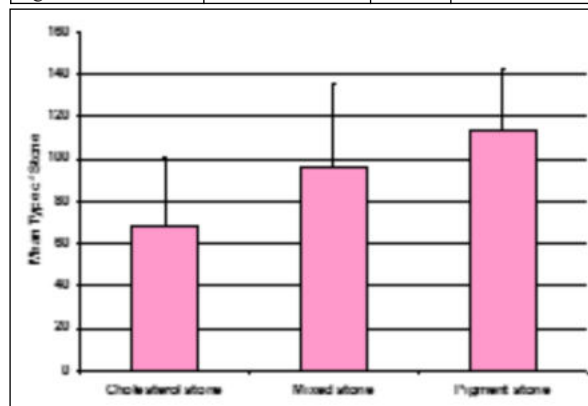
Table-3: Distribution Of Number Of Stones Found In The Patients With Gallstone

Number of stones	Number	%
Multiple	33	66.0%
Single	17	34.0%
Total	50	100.0%

Most of the patients had multiple stones (66.0%) which was significantly higher than single stone (34.0%) ($Z=4.52$; $p<0.0001$).

Table-4: Comparison Of Level Of Serum Iron Of The Patients With Gallstone According To Type Of Stone

Type of stone	Mean±s.d.	Median	Range
Cholesterol stone	68.21±31.89	64	23 - 137
Mixed stone	95.50±40.57	86	63 - 174
Pigment stone	113.38±28.95	113.5	61 - 165



The mean level of serum iron for Pigment stone was the highest and that for Cholesterol stone was the lowest.

Table-5: ANOVA Table For Comparison Of Level Of Serum Iron

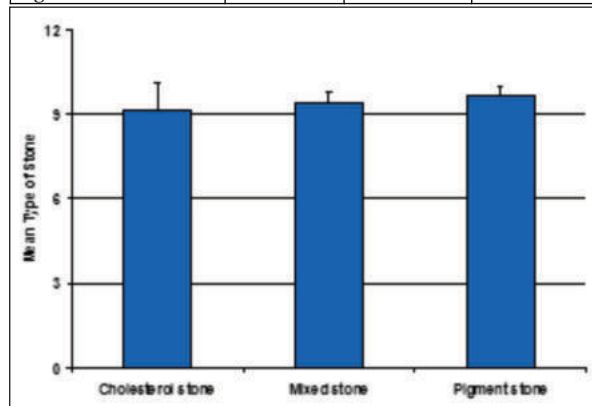
ANOVA					
Source of Variation	SS	Df	MS	F	P-value
Between Groups	21388.86	2	10694.43	10.41	0.0001*

Within Groups	48263.96	47	1026.89		
Total	69652.82	49			

One way ANOVA showed that there was significant difference in mean level of serum iron for three types of gallstone ($F_{2,47}=10.41$; $p<0.0001$).

Table-6: Comparison Of Level Of Serum Calcium Of The Patients With Gallstone According To Type Of Stone

Type of stone	Mean±s.d.	Median	Range
Cholesterol stone	9.15±0.96	9.35	7.5 - 10.9
Mixed stone	9.43±0.36	9.5	8.8 - 9.8
Pigment stone	9.69±0.28	9.65	9.1 - 10.1



The mean level of serum calcium for Pigment stone was the highest and that for Cholesterol stone was the lowest.

Table- 7: ANOVA Table For Comparison Of Level Of Serum Calcium

ANOVA					
Source of Variation	SS	Df	MS	F	P-value
Between Groups	2.94	2	1.47	2.57	0.08 NS
Within Groups	26.86	47	0.57		
Total	29.80	49			

One way ANOVA showed that there was no significant difference in mean level of serum calcium for three types of gallstone ($F_{2,47}=2.57$; $p=0.08$).

DISCUSSION

In this prospective study, Case and Control groups have been compared in respect of the different parameters.

- Considering the demographic pattern of the 2 groups, it has been found that there was no significant association between either the age or sex and patients of the two groups. The risk of gallstone was 2.68 times more among the patients with age >45 years as compared to the patients with age ≤45 years and it was 3.08 times more among the female patients as compared to the male patients. This finding is quite in accordance to the known epidemiology of gallstone disease.
- There was no significant association between either the presence of co-morbidity or the history of drug use and patients of the two groups. Thus the patients of the two groups were matched for co-morbidity & routine drugs.
- Distribution of level of serum iron of the patients of the two groups show that the level of serum iron of the patients with gallstone was significantly lower than that of the patients without gallstone [$p=0.04$]. The risk of gallstone formation was also 5.44 times more among the patients with serum iron deficiency as compared to the patients with normal level of serum iron and the risk was significant [OR-5.44(1.61, 48.39); $p=0.04$]. (Table 5). Moreover, further analysis in the case group shows that the mean level of serum iron for Pigment stone was the highest and that for Cholesterol stone was the lowest (Table 14) and the difference is statistically significant (Table 15).
- Statistical analysis of distribution of level of serum calcium of the patients of the two groups show that the association is significant. Corrected Chi-square () test showed that there was significant association between level of serum calcium and patients of the two groups ($p=0.026$). The risk of gallstone was 7.97 times more among the patients with serum calcium deficiency as compared to the patients with normal level of serum calcium and the risk was

significant [OR-7.97(1.04, 67.45); $p=0.026$]. Also, t-test showed that there was significant difference in the level of serum calcium of the patients with gallstone than that of the patients without gallstone [$t_{98}=1.98$; $p=0.04$] (Table 7). Among the 3 stone groups in the Case arm the mean level of serum calcium for Pigment stone was the highest and that for Cholesterol stone was the lowest, but this difference is not statistically significant ($p=0.08$) (Table 18 & 19).

CONCLUSION

In this study it has been observed that there is significant correlation between deficiency of trace elements like Iron & Calcium with the formation of gallstones. Among the three stone groups iron deficiency was maximum in the cholesterol stone group.

Iron deficiency alters the activity of several hepatic enzymes leading to increased gallbladder cholesterol saturation and promotion of cholesterol crystal formation, it also acts as a coenzyme for nitric oxide synthase which synthesises nitric oxide important for the maintenance of gallbladder tone and normal relaxation.

Calcium deficiency causes deranged function of nitric oxide synthase which acts as a calcium calmodulin dependent enzyme producing relaxation of gallbladder.

Hence, gallbladder stasis occurs which subsequently increases crystal formation in bile so deficiency in both iron & calcium is associated with increased risk of gallstone formation. In this study, lipid profile & bile analysis were not done. Measurement of biliary cholesterol could prove the hypothesis of supersaturation of bile more vividly. Chemical analysis of calculi for its composition including trace elements can give new relevant informations. Apart from Iron and Calcium, other trace elements may play vital role in formation of gallstone that can be studied in future.

There is no nationwide study available regarding role of trace elements in the causation of biliary calculi in India. A metaanalysis, taking account of all the studies done so far in different geographic areas, can profoundly establish the correlation between deficiency of trace elements and formation of gallstone.

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