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RESEARCH ARTICLE

CAN SERUM FERRITINBE A MARKER FOR LITHOGENIC BILE & GALLSTONES ? A PROSPECTIVE STUDY

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ABSTRACT

The study is focused to establish correlation between Serum Ferritin, Serum Cholesterol and Biliary Cholesterol. The study was performed on a group of 118 patients who were admitted in department of surgery for cholecystectomy (laparoscopic / open) in multi-speciality hospital at Lucknow. Based on the serum iron content, the patients with cholelithiasis were divided into two groups. Group A patients were with normal serum iron and Group B iron deficient patients. Serum and Biliary cholesterol contents of both groups will be analysed and comparison were done with each other. The mean bile cholesterol level in Group I and in Group II will be measured. The difference in values in both the groups were analysed statistically for p value. The result was analysed and formulated. There was no significant variation (P =0.367) in the serum cholesterol of the two groups in previous studies. Similar results of p value in the serum cholesterol of the two groups in present study were obtained i.e. (p=0.394), whereas gall bladder bile cholesterol was significantly increased ($P < 0.0001$) in the serum iron deficient (Group II) than in the normal serum iron (Group I), thus suggesting that iron deficiency may be contributing to the super saturation of gall bladder bile with respect to cholesterol independent of serum cholesterol levels

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INTRODUCTION

Gallstones are small deposits of variable consistency that are formed in the gallbladder, a sac-like organ that lies under the liver on the right side of the abdomen. Gallstones are the most common biliary pathology. Autopsy reports have shown a prevalence of gall stones from 11-36% (Brett, 1976). Among gastroenterological diseases, Gallstone disease is one of the world's most expensive medical conditions (Bagaudinov, 2002). In the United States, there are more than 500 000 cholecystectomies, the total cost of which exceeds 5 billion dollars (Doggrell, 2006). The gallstones in 75% of patients are composed predominantly (70-95%) of cholesterol or mixed stones. The remaining 25% are pigment stones. Regardless of composition, all gallstones give rise to similar clinical sequel (Novacek, 2006). Cholesterol is not very soluble, so in order to remain suspended in fluid it must be transported within clusters of bile salts called *micelles*. The imbalance between these bile salts and cholesterol leads to sludge formation. This thickened fluid consists of a mucus gel containing cholesterol and calcium bilirubinate. This further leads to *super saturation* of cholesterol which is caused by cholesterol hyper secretion rather than by a reduced secretion of phospholipids or bile salts (Doggrell, 2005). In mixed stones, there is a significantly a positive correlation between oxalate and iron content of bile and total bilirubin, oxalate and sodium of serum and bile. This is supported by positive correlation between oxalate and bilirubin contents of serum and gallstones (Klein *et al*., 1996). Four major groups of factors that contribute to the formation of cholesterol gallstones identified are(1) cholesterol super saturation in bile; (2) cholesterol precipitation and crystallization core formation ;(3) impairment of basic gallbladder functions (contraction, absorption and secretion, etc); and (4) impairment of the enterohepatic circulation of bile acids (Strasberg *et al*., 1998). Vlahcevic and associates[7, 8] have recently shown that patients with lithogenic bile and cholesterol gallstones generally have reduced pool sizes of bile acids. Thus, they have postulated that lithogenic bile is primarily due to a deficiency of bile acids relative to cholesterol. The study is focused to establish correlation between Serum Ferritin ,Serum Cholesterol and Biliary Cholesterol.

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MATERIAL AND METHODS

This prospective study was conducted in the Department of Surgery in collaboration with Department of Pathology in a multispeciality hospital, Lucknow, India. The ethical

Table 1. Group-wise distribution of subjects

S.No.	Group	Description	No. of cases	Percentage
1.	I	Normal serum ferritin patients (Serum ferritin level ≥60 µg/dl for males, ≥35 µg/dl for females)	86	72.9
2.	II	Serum ferritin deficient patients (Serum ferritin level <60 µg/dl for males, <35 µg/dl for females)	32	27.1

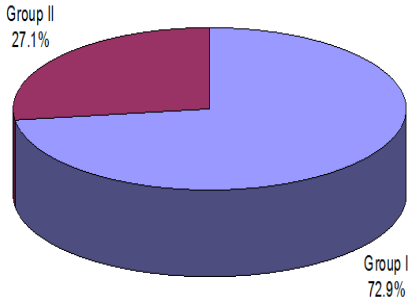

Fig. 1.Group-wise distribution of subjects

committee of the institute approved the study protocol. The study was performed on a group of 118 patients who were admitted in department of surgery for cholecystectomy (laproscopic/ open) during period of 1 year and 6 months Patients suffering from cholelithiasis confirmed by Ultrasonography and admitted in the surgical ward for cholecystectomy were included in the study irrespective of their age, sex and parity. Both laparoscopic and open cholecystectomies were the procedures advocated for treatment.

Patients suffering from empyema and mucocele of the gall bladder were excluded from this study. Serum iron, serum cholesterol and biliary cholesterol were estimated in all the patients. Serum iron was estimated by *time end point method (with ferroZine reagent* (Strasberg, 1984). The normal reference values were supplied with the kit, for males (60-158 µg/dl) and for females (35-145 µg/dl). Serum cholesterol was estimated by *time end point method (using chol reagent)* Biliary cholesterol was estimated after extraction of biliary lipids from bile from the gallbladder specimen of the patients which was followed by the procedure similar to the analysis of serum cholesterol *time end point method using chol reagent)* (Roslyn *et al*., 1987). Based on the serum iron content, the patients with cholelithiasis were divided into two groups. Group A patients were with normal serum iron and Group B iron deficient patients. Serum and biliary cholesterol contents of both groups will be analysed and comparison were done with each other. The mean bile cholesterol level in Group I and in Group II will be measured. The difference in values in both the groups were analysed statistically for p value. The result was analysed and formulated.

RESULTS OBSERVATION

The present study was carried out for studying the correlation of serum ferritin with bile cholesterol level in cholelithiasis. A total of 118 subjects with gall bladder stones were enrolled in the study. On the basis of their serum iron levels, they were divided into two groups as follows: A total of 86 (72.9%) patients comprised Group I which comprised of patients having Serum ferritin levels within normal range (≥60 µg/dl for males and ≥35 µg/dl for females). Remaining 32 (27.1%) subjects were anemic subjects with serum ferritin levels below normal range (<60 µg/dl for males and <35 µg/dl for females). Thus the ratio of iron deficient to non iron deficient patients in present study was 0.37:1. Table 2 shows the comparison of Serum cholesterol, S. ferritin and Bile cholesterol levels in two groups: A significant difference in mean Serum ferritin and Bile cholesterol was observed between two groups (p≤0.001). However, no significant difference was observed in Hb and S. cholesterol levels of two groups (p>0.05). The association

Table 2. Comparison of Mean Hb, S. cholesterol, Serum ferritin and Bile cholesterol in two groups

S.No.	Parameter	Group I (n=86)		Group II (n=32)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	Hb (gm/dl)	11.15	1.48	10.82	1.37	1.102	0.273
2.	S. cholesterol (gm/dl)	157.72	47.05	149.97	33.11	0.855	0.394
3.	S. ferritin (µg/dl)	90.40	73.40	40.06	13.21	3.581	<0.001
4.	Bile cholesterol (mg/dl)	301.20	212.89	470.97	268.01	3.581	0.001

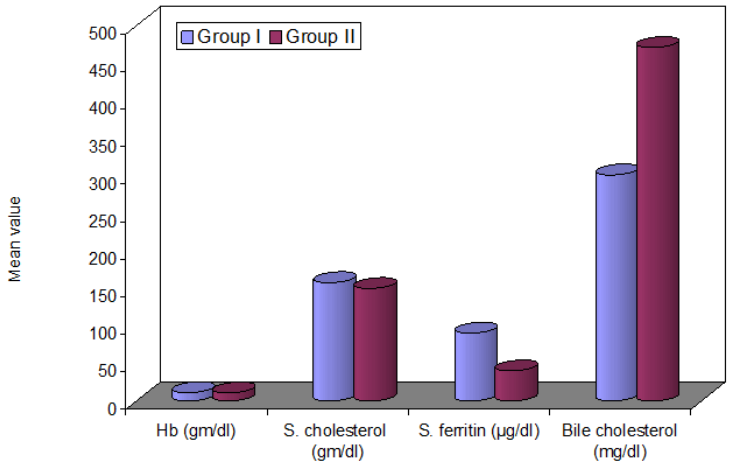

Fig. 2.Comparison of Mean Hb, S. cholesterol, Serum ferritin and Bile cholesterol in two groups

between serum ferritin levels and bile cholesterol levels was also traced by dividing the bile cholesterol in four quartiles and then comparing the proportion of subjects in two groups in these four quartiles. It was assumed that given the high range of bile cholesterol, some of the values might affect the mean values; hence a quartile-wise distribution will take care of the extreme values.

Table 3. Distribution of Subjects in different quartiles of bile cholesterol levels in two groups

S.No.	Quartile of Bilirubin cholesterol (mg/dl)	Group I (n=86)		Group II (n=32)	
		No.	%	No.	%
1.	First – (≤148.75 mg/dl)	14	16.3	2	6.3
2.	Second – (148.75-244.50 mg/dl)	34	39.5	9	28.1
3.	Third – (244.50-537.00 mg/dl)	25	29.1	5	15.6
4.	Fourth – (>537 mg/dl)	13	15.1	16	50.0

χ^2=15.769 (df=3); p=0.001

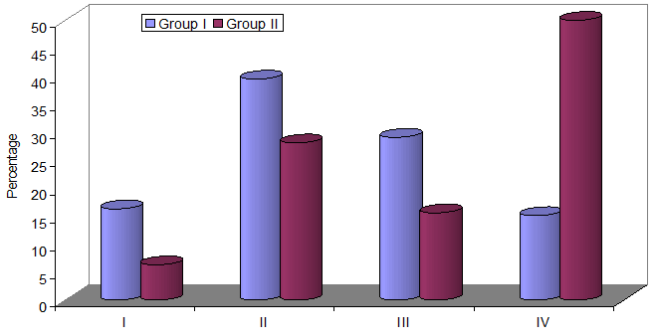

Fig. 3. Distribution of Subjects in different quartiles of bile cholesterol levels in two groups

Distribution of subjects according to different quartiles of bile cholesterol in two groups has been shown in Table 3 below:
In Group II, maximum number of subjects had bile cholesterol values in the fourth quartile whereas in Group I, maximum number of subjects had bile cholesterol values in the second quartile. In Group II, minimum number of subjects had bile cholesterol values in first quartile whereas in Group I minimum number of subjects had values in fourth quartile. The significance of difference in proportion of patients in quartiles of bile cholesterol in two groups was also found to be significant statistically (p=0.001).

DISCUSSION

The study was performed on a group of 118 patients who were admitted in department of surgery for cholecystectomy (laproscopic/ open) for the period of 1 year and 6 months. A significant difference in mean Serum ferritin and Bile cholestesrol was observed between two groups ($p \leq 0.001$). However, no significant difference was observed in Hb and Serum. cholesterol levels of two groups ($p>0.05$). The association between serum ferritin levels and bile cholesterol levels was also traced by dividing the bile cholesterol in four quartiles and then comparing the proportion of subjects in two groups in these four quartiles. It was assumed that given the high range of bile cholesterol, some of the values might affect the mean values; hence a quartile-wise distribution will take care of the extreme values. Distribution of subjects in different quartiles of bile cholesterol in two groups has been shown in Table 3. The study by Roslyn *et al* (1987) suggested that dietary factors may be responsible for the increasing incidence of pigment gallstones. Although iron deficiency alters the activities of several hepatic enzymes, its effect on biliary lipid metabolism is not known. This study indicate that consumption of diets rich in carbohydrates but deficient in iron alters hepatic metabolism of cholesterol and may be an important etiologic factor in pigment gallstone formation. Iron supplementation may prevent pigment gallstones in certain high-risk groups.
In other study by Johnson *et al* (1997) animals receiving the iron-deficient diet were more likely to have cholesterol crystals in their bile than animals on the control diet (80% vs. 20%; $p < 0.05$). The study suggested that an iron-deficient di*et al*ters hepatic enzyme metabolism, which, increases gallbladder bile cholesterol and promotes cholesterol crystal formation. It was concluded that iron deficiency plays a previously unrecognized role in the pathogenesis of cholesterol gallstone formation in women (Roslyn, 1997). Few studies found a higher frequency of gallstones in female patients with Iron deficiency anemia, although this was not significant (11.4%). Various studies claimed that this condition was mediated via the effects of estrogens and/or progesterone on bile saturation (Hiroe Yanagida, 2007; Hiroe Yanagida, 1997 AND Swartz-Basile, 2000). There was a trend towards a higher frequency of gall stones in male subjects (21.7%) with Iron deficiency anemia. However, the number of male Iron deficiency anemia patients was not high enough. In another study it was found that gallbladder emptying in iron deficiency anemia patients was impaired. Iron is known to have an important role in hepatic enzyme metabolism (Bailey-Wood, 1975). Iron-containing cofactors are fundamental components of the nitric oxide synthase complex (Billiar, 1995). Nitric oxide acts as a putative inhibitory neurotransmitter and it are present throughout the gastrointestinal system (Li, 1990). In addition, the motilities of gall bladder and sphincter of oddi was suppressed acutely in iron deficiency because of decreased neuronal nitric oxide synthase levels and compensatory mechanisms return neuronal nitric oxide synthase to baseline levels while cholesterol crystal formation increases over time. Nitric oxide synthase was demonstrated to be present in neurons of the gall bladder in humans (Uemura, 1997). Nevertheless; the effect of iron deficiency on nitric oxide synthase in humans has not been studied yet. Impaired gallbladder emptying in these patients might have contributed to the higher frequency of gallstones in iron deficiency anaemia (Pamuk, 2009).

A study was conducted on the randomly selected individuals of the Punjabi population, suffering from gall stone formation, to decipher the facts on the current divided opinion available in literature regarding the aetiology of gall stone formation and the role of iron deficiency anaemia in gall stone formation (Kumar Muneesh, 2006). Another study state that excess of iron is also causative factor of gall stones. Body iron stores accumulate by the absorption of dietary iron, including heme iron and non-heme iron. In experimental studies that used controlled meals, the absorption of heme iron was shown to be more complete and less regulated than that of nonheme iron (Kumar Muneesh, 1990). Heme iron, which is mainly present in red meat, fish, and poultry, is highly bioavailable, and its absorption is substantially higher than that of non-heme iron. Non-heme iron absorption is more likely to be influenced by various dietary enhancers and inhibitors, and its bioavailability varies significantly (Hallberg, 2000). Experimental studies have shown that a high dietary iron intake can induce lipid per oxidation (Brunet, 1999) and stimulate generation of hydroxyl radicals (Kadiiska, 1995), which in turn may stimulate mucous

glycoprotein secretion in the gallbladder (Hale, 1987 and LaMont, 1989), and promote cholesterol crystal formation in bile (LaMont, 1996) both of which may promote the formation of gallstones. An increased iron intake can alter blood lipids and increase the ratio of saturated to unsaturated fatty acids (LaMont, 2001) and thus may enhance cholesterol gallstone formation (Jonnalagadda, 1995) Also, consumption of a high iron diet can elevate plasma triacylglycerol concentrations, (Fields, 1999) and thus may increase the risk for gallstones. In conclusion, they suggested that a higher consumption of heme iron was associated with an increased risk of gallstone disease among men (Morris, 1995).

Iron is known to be necessary in the functioning of many intracellular enzyme systems, including hepatic enzymes involved in cholesterol and bile salt regulation. Iron also may play a role in normal gallbladder and sphincter of Oddi functions because of its interaction with nitric oxide, which, in turn, affects smooth muscle function (Portincasa, 2004). Iron deficiency is further associated with raised serum transferrin. Therefore, iron deficiency may enhance cholesterol gallstone formation by altering hepatic enzyme function, biliary motility, cholesterol crystal nucleation, or some combination of the three. This study was undertaken to test the hypothesis that iron deficiency would alter hepatic cholesterol metabolism and enhance cholesterol gallstone formation. Above study had mentioned that all normal serum iron (Group I) gall stone sufferers (n=27) had a high average serum iron content of 91 ± 35 µg/dl, as compared to serum iron deficient (Group II) ones (n=23), where average serum iron was 26 ± 9.5 µg/dl as compared to present study normal serum iron (Group I) gall stone sufferers (n=86) had serum iron content of 90.4±73.4 µg/dl as compared to serum iron deficient (Group II) ones (n=32), where average serum iron was 40.0 ± 13.2 µg/dl.

There was no significant variation (P =0.367) in the serum cholesterol of the two groups in previous study. Similar results of p value in the serum cholesterol of the two groups in present study were obtained i.e. (p=0.394), whereas gall bladder bile cholesterol was significantly increased ($P <$ 0.0001) in the serum iron deficient (Group II) in previous study and was also increased significantly in present study with (p<0.001) than in the normal serum iron(Group I), thus suggesting that iron deficiency may be contributing to the super saturation of gall bladder bile with respect to cholesterol independent of serum cholesterol levels. Similar study Tikrit Medical Journal 2009, Serum total cholesterol of gall stone formers was not different from that of the general population. There were no significant variations in the serum cholesterol contents of both groups (P =0.367, t=0.91). Also, there was no significant variation of the above parameter in the male and female patients (P =0.082, t=1.77).The gall bladder bile cholesterol was significantly higher in the anaemic individuals, as compared to that of the non-anemic ones (P <0.0001, t=4.53) (Hamid, 2009). A high level of cholesterol as a cause is getting closer, but it's regulation of cholesterol, not cholesterol per se that's responsible for gallstones.

Conclusion

Iron deficiency and low serum ferritin are probably independent risk factors operating for the causation of gallstones .In the study correlation between serum ferritin deficiency and increase in cholesterol level in bile was established. This result gives impression that in reference to serum cholesterol, deficiency of serum ferritin will lead to increase in saturation of biliary cholesterol which may enhance Gallstone formation. The scope of this study can be further advanced in the field of enzymes controlling gall bladder tone, motility and relaxation and cofactors affecting these enzymes. The study also raises a question if serum levels of ferritin can be used as a marker for lithogenic bile with high levels of biliary cholesterol level which cannot be measured in a normal healthy person. Do prevention of iron defeciency anemia also prevents the possible development of gallstones in a healthy person.

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