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Quantitative Analysis of Serum Lipid Profile in Gallstone Patients and Controls

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Abstract

The present study was undertaken to explore the possible role of serum lipid profile in gallstone formation. For this serum lipid profile such as total, free and bound cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols and total lipids were determined in 109 gallstone patients and 100 controls (matched for age, sex and with negative personal or family history of gallstones) treated at Liaquat University Hpspital, Jamshoro, Pakistan. Comparison for serum lipid profile between different groups of gallstone patients and controls revealed no significant variation except for the triacylglycerols and total lipids, which were differed significantly between females of up to 45 and above 45 years age. Comparison for serum lipid profile between pure cholesterol and mixed composition gallstone formers showed no significant difference ($p>0.05$) between the two groups. The serum lipid profile significantly varied between gallstone patients and controls except bound cholesterol level. Comparison of total cholesterol, free cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols and total lipids between gallstone patients and controls revealed that there was a significant difference between gallstone patients and controls for (a) females with or without gallstones, (b) females of up to 45 years age and (c) females having more than 3 children. HDL cholesterol is significantly decreased in all the groups of gallstone patients as compared to controls, whereas, bound cholesterol remained non significant in all the groups of gallstone patients when compared with controls. In conclusion, elevated serum total cholesterol, free cholesterol, LDL cholesterol, triacylglycerols and decreased levels of HDL cholesterolseem to play major contributing role in the pathogenesis of gallstones in females of upto 45 years age with more than three children..

Keywords: Gallstone disease; Cholelithiasis; Serum lipids; Lipid profile; Cholesterol; Triglycerides; Free cholesterol.

Introduction

In Pakistan, hypercholesterolemia is common finding in adults and pure cholesterol gallstones are more common as compared to other types of gallstones [1]. Cholesterol is water insoluble lipid, and is taken in mixed micelles and vesicles. Micelles are aggregates of phospholipids, bile salts, and cholesterol, and vesicles are closed spherical bilayers of phospholipids with associated cholesterol. There are three stages of gallstone formation, supersaturation, nucleation and aggregation [2]. Cholesterol crystals form on the surfaces of these vesicles and grow within the mucin gel. Cholesterol crystals are glued together by bile proteins to make gallstones [3 – 6].

The relative concentrations of cholesterol, bile salts and phospholipids determine the cholesterol solubility in bile [7]. Cholesterol precipitation results from an imbalance of these three components in bile; cholesterol, bile salts and

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phospholipids [8]. These changes in bile composition are closely related to the disorders of lipid metabolism in liver. However, during the formation of cholesterol gallstones, different links in the disturbance of lipoprotein cholesterol metabolism [9] and their effects in lithogenesis still have many controversies. Some investigators reported that gallstone patients had hyperlipidemia [10 – 12]. The paucity of information regarding the association between serum lipid profile and gallstone formation tempted us to undertake the present study.

Experimental

The materials for this study were 109 gallstone patients (98 females and 11 males, age range 23.7 to 64.7 years) and 100 control inpatients (90 females and 10 males, age range 24 to 65) with no personal or family history of gallstones. All the gallstone samples recovered from the patients were analyzed for composition by Fourier transform infrared spectroscopy. Total serum cholesterol was estimated by as per reported method [13], whereas, the method used for the estimation of free cholesterol was of Parkh and Jung [14]. The bound cholesterol was calculated from the difference between the total cholesterol and free cholesterol [15 – 16]. High density lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C) were estimated by CHOD-PAP method [15 – 17]. Triacylglycerol level was determined according to Rifai et al. and Cole et al. [17 – 18]. Total lipids were determined by Zollner and Kirch method [19]. All the chemicals and reagents used were of analytical reagent grade, supplied by E. Merck, W. Germany. Microlab 200, Merck was used for the analysis of serum total cholesterol, whereas double beam UV-visible spectrophotometer, L-2800 Hitachi was used for free cholesterol analysis. All the results for each sample were in triplicates for each parameter of lipid profile; the mean for each sample was used in statistical analysis.

Statistical analysis

Results were expressed as mean ± SD and mean ± SEM. Student's *t* test was used to compare the data between patients and control groups and between the patients distributed in different groups, ($p<0.05$ was considered statistically significant). Minitab software, version 13.2 was used for statistical analysis.

Results and Discussion

Comparison of serum lipid profile between different groups of gallstone patients and controls showed no significant variation except for the triacylglycerols and total lipids, which were significantly raised in females of up to 45 years age than the females above 45 years (Table 1). Comparison for serum lipid profile between pure cholesterol and mixed composition gallstone formers (Table 2) showed no significant difference ($p>0.05$) between the two groups. The serum lipid profile significantly differed ($p<0.05$) varied between gallstone patients and controls except for bound cholesterol level (Fig. 1).

Interestingly, the association seen between serum cholesterol levels and mixed composition gallstones in the present study was of the same pattern as that with cholesterol gallstones (Table 2). This suggests that serum lipids do play just as big a role in the pathogenesis of mixed composition gallstones as in that of pure cholesterol gallstones. As a corollary, mixed composition and cholesterol gallstones may share more causal determinants than previously suggested. This is supported by the finding of a similar serum cholesterol pattern in mixed and cholesterol gallstone formers and is consistent with the hypothesis that the association of gallstones with serum cholesterol is merely a consequence of an influence of the presence of gallstones on serum cholesterol levels. Because the present study cannot distinguish between cause and effect and also because of the limited power of this study to detect subtle differences between mixed and cholesterol gallstones with respect to serum lipids, the results of the present study need to be confirmed by further studies.

Figures 2, 3, 6, 7 and 8 showed the comparison of total cholesterol, free cholesterol, LDL cholesterol, triacylglycerols, and total lipids between gallstone patients and controls. A closer look at those figures revealed that these parameters in comparison to corresponding controls were

Table 1. **Comparison of serum lipid levels between different groups of gallstone patients.**

Groups	Total Cholesterol (≤200 mg/dl) Mean ± SEM	Free Cholesterol (5-40 mg/dl) Mean ± SEM	Bound Cholesterol (195-60 mg/dl) Mean±SEM	HDL-Cholesterol (35-55mg/ dl males) (45-65mg/dl females) Mean±SEM	LDL-Choles-terol (≤150mg /dl) Mean±SEM	Triacylglycerols (<150mg/dl) Mean ± SEM	Total lipids (450 – 1000 mg/dl) Mean ± SEM
Females (N=98)	199.3 ± 5.4	44.8 ± 2.5	154.1 ± 4.0	23.9 ± 0.28	118.7 ± 3.8	191.4 ± 10.0	948.0 ± 34.0
Males (N=11)	183.4 ± 13	40.3 ± 6.4	143.1 ± 9.9	21.3 ± 1.2	108.3 ± 9.2	173.0 ± 21.0	899.0 ± 53.0
p value	0.275	0.525	0.324	0.085	0.315	0.437	0.441
Females of upto 45 years age group (N=71)	204.4 ± 6.8	47.1 ± 3.2	156.9 ± 4.9	23.77 ± 0.9	121.4 ± 4.7	202.0 ± 13.0	993.0 ± 41.0
Females of above 45 years age group (N=27)	185.7 ± 7.9	38.7 ± 3.3	146.6 ± 6.7	24.2 ± 1.6	111.6 ± 6.1	162.6 ± 14.0	828.0 ± 50.0
p value	0.077	0.075	0.222	0.811	0.208	0.036*	0.013*
Females having up to 3 children (N = 81)	198.6 ± 5.7	44.5 ± 2.8	153.6 ± 4.2	24.09 ± 0.9	117.8 ± 4.1	195.2 ± 11.0	971.0 ± 39.0
Females having more than 3 children (N = 17)	202.3 ± 16.0	46.1 ± 5.7	156.2 ± 12.0	22.9 ± 1.4	122.8 ± 11.0	173.0 ± 25	838.0 ± 59.0
p value	0.827	0.808	0.837	0.472	0.669	0.428	0.060

* $p<0.05$ (level of significance)

Table 2. **Comparison of serum lipid levels between pure cholesterol and mixed composition gallstone formers.**

Serum Cholesterol levels (mg/dl)	Pure Cholesterol stone formers (n=74) Mean ± SEM	Mixed composition stone formers (n=23) Mean ± SEM	P (<0.05)
Total Cholesterol (≤200mg/dl)	195.8 ± 14.0	195.2 ± 8.5	0.97
Free Cholesterol (5-40mg/dl)	39.34 ± 5.3	42.3 ± 4.7	0.68
Bound Cholesterol (195-160mg/dl)	156.45 ± 9.5	152.3 ± 6.1	0.72
HDL-Cholesterol (35-55mg/dl males) (45-65mg/dl females)	22.35 ± 1.4	23.79 ± 1.5	0.48
LDL-Cholesterol (≤150mg/dl)	121.23 ± 9.9	118.7 ± 5.8	0.82
Triacylglycerols (<150mg/dl)	220.91 ± 25.0	194.9 ± 18.0	0.40
Total Lipids (450 -1000mg/dl)	1060 ± 95.0	1014 ± 72.0	0.69

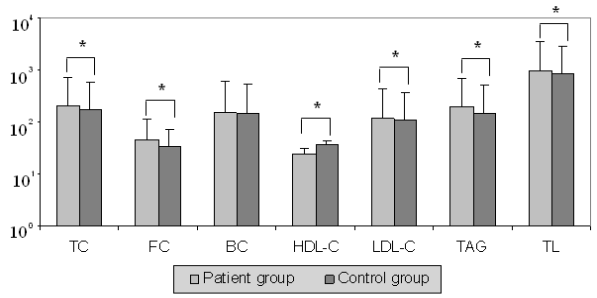

Figure 1. **Comparison of serum lipid profile between gallstone patients and control subjects. * p<0.05. Each value is mean ± SEM**

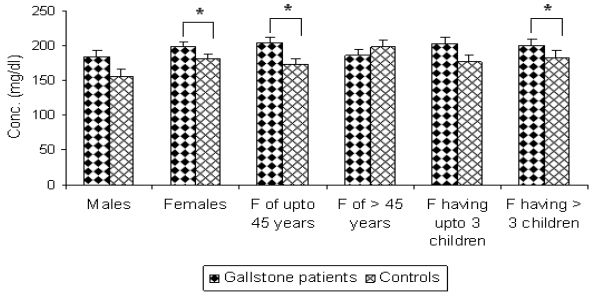

Figure 2. **Comparison of serum total cholesterol between different groups of patients and controls. *p<.05. Each value is mean ± SEM.**

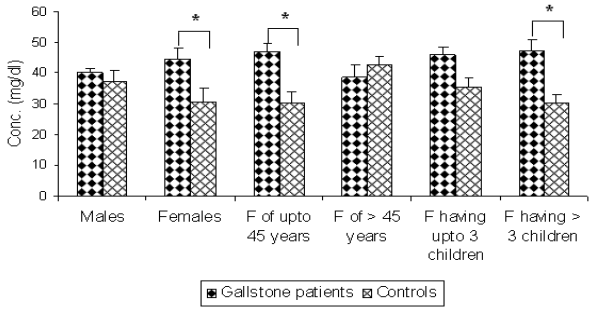

Figure 3. **Comparison of serum free cholesterol between different groups of patients and controls. *p<.05. Each value is mean ± SEM.**

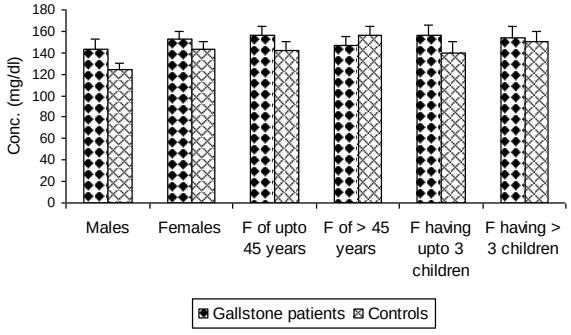

Figure 4. **Comparison of serum bound cholesterol between different groups of patients and controls. Each value is mean ± SEM.**

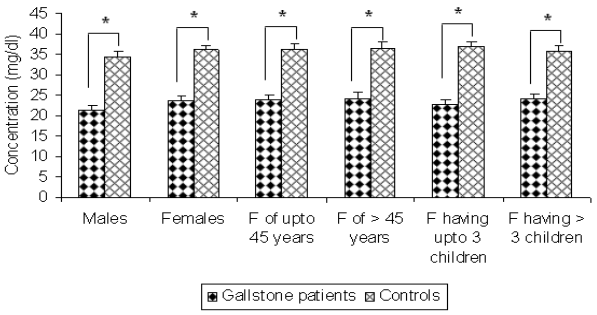

Figure 5. **Comparison of serum HDL- cholesterol between different groups of patients and controls. * p<.05. Each value is mean ± SEM.**

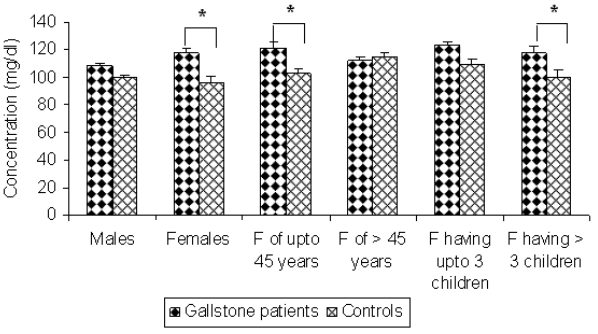

Figure 6. **Comparison of serum LDL- cholesterol between different groups of patients and controls. * p<.05. Each value is mean ± SEM**

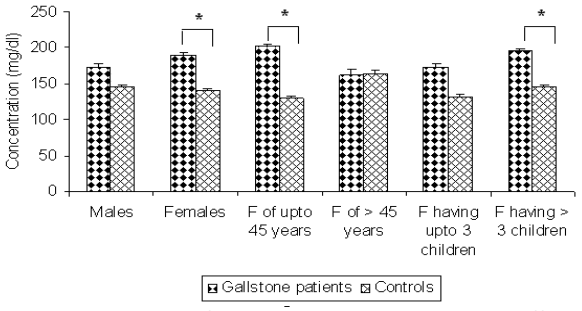

Figure 7. **Comparison of serum Triacylglycerols between different groups of patients and controls. * p<.05. Each value is mean ± SEM.**

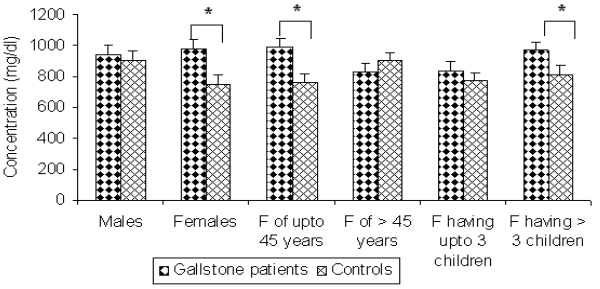

Figure 8. **Comparison of serum total lipids between different groups of patients and controls. * p<.05. Each value is mean ± SEM.**

significantly raised in: (a) female gallstone patients, (b) female gallstone patients of up to 45 years age and (c) female gallstone patients having more than three children.

Significantly high concentrations of total cholesterol in serum found in females of up to 45 years age and having more than three children in present study (Fig. 2) seem to be in accordance with the findings of other investigators who had reported a positive association of total cholesterol with gallstone disease in females [20 – 22]. Interestingly, this positive association between total cholesterol and gallstones or gallstone disease (including cholecystectomy) had been seen not only in females but also in males [12, 23, and 24]. The present finding that females of up to 45 years age have high levels of serum total, free and bound cholesterol (Fig. 2, 3 and 4) seems to be in line with the reports of other investigators who found that positive association between serum cholesterol levels and gallstone disease is confined to multiparous female patients less than 50 years age [24]. One of the case-control studies reported lower concentrations for total cholesterol in gall-stone patients than in control subjects in both genders separately [24] or collectively [25]. Population studies based on gallbladder screening had reported a positive relation [26], an inverse relation [27], or no relation [28, 29] between total cholesterol and gallstone disease in both genders combined. Some similar studies had found an inverse association with prevalent gallstones in females [23] but not in males [23, 30]. This may be due to random selection of patients due to which more female patients of up to 45 years age can be recruited (in which the serum LDL cholesterol is significantly high) or it may be because of genetics [31 – 38] as well as age and gender [39 – 40] difference, since all these variables affect on serum lipid profile of patients.

In present study serum bound cholesterol remained non significant the groups of gallstone patients when compared to controls (Fig. 4). Kritchevsky had reported that the free and bound cholesterol fractions have distinct and separate metabolic functions. Free cholesterol may be esterified to form bound cholesterol, in doing so the specific metabolic function may change. The major function of free cholesterol is to serve as a substrate for steroid synthesis and for the renewal of blood cells and tissues and the bound cholesterol as part of the fatty acid transport mechanism [41]. Hence, in present study free cholesterol increases steroid synthesis, this in turn decreases the production of bile acids and hence of bile salts which keep the cholesterol soluble in bile. Decreased concentration of bile salts is responsible for the precipitation of cholesterol in bile, which is prerequisite for gallstone formation [2].

Some investigators reported a positive association between gallstone and serum triacylglycerol levels (in Spanish men [42]) [43], whereas, others found no such association [44, 45]. Present study has shown significant variations in triacylglycerol levels though in normal range (<150mg/dl) in the same groups as in case of total cholesterol (Figs. 2 and 7). This suggests a positive association between gallstone disease and serum cholesterol and triacylglycerol levels.

HDL cholesterol is significantly decreased in all the groups of gallstone patients as compared to controls (Fig. 3). Previous investigators had reported that high serum cholesterol levels are caused by the presence of an abnormal LDL class (LP-X) [46 – 48] in patients of intrahepatic or extrahepatic cholestasis and low HDL levels with an abnormal HDL component [49]. This suggests that changes in serum lipid profile are a possible consequence of the presence of gallstones, especially through biliary obstruction. In present study, because only few gallstone patients had experienced symptoms of biliary obstruction in the past (jaundice, pale stools, or dark urine), it is believed that only few of them would have had cholestasis at the time of the study. Nevertheless, more subtle changes in lipid profile as a consequence of the presence of gallstones cannot be excluded. In this respect, it had been reported that serum lipid profile in subjects with a history of cholecystectomy was more similar to controls than to gallstone patients [23].

Some investigators found a positive association between gallstone disease and increased levels of serum triacylglycerols, LDL cholesterol and decreased HDL cholesterol [50, 51]. Present study also suggests the same

associations as indicated by above investigators. A brief review of previous studies had not clearly indicated any existence of the relation between serum lipid profile and gallstone disease. Serum lipid profile in present study, however, is comparable with the findings of other investigators from Europe.

Conclusion

In conclusion, elevated serum total cholesterol, free cholesterol, LDL cholesterol, triacylglycerols and decreased levels of HDL cholesterolseem to play major contributing role in the pathogenesis of gallstones in females of upto 45 years age with more than three children.

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