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Effects of Laparoscopic Cholecystectomy on Mucin1 and Cholesterol Ester Transfer Protein Status in Bangladeshi patients with Cholelithiasis

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Abstract

Objective: Although it was reported that cholecystectomy had profound impact on lipid profile and lipoprotein (a) status, no studies were reported on Mucin1 and cholesterol ester transfer protein (CETP) in Bangladeshi patients with cholelithiasis i.e. gallstone disease (GD). The present study was done about effects of laparoscopic cholecystectomy on Mucin1 and CETP status in Bangladeshi patients with cholelithiasis.

Patients & Methods: Forty-four adult GD patients with cholelithiasis and 30 normal controls (NC) subjects were included in the study. The blood was taken from fasting patients before cholecystectomy (Serum-I°), gall bladder bile during cholecystectomy (Bile-I°) and blood again after 2-3 months at follow-up (Serum-II°) and from fasting NC subjects. Mucin1 and CETP levels were quantified in serum and bile by enzyme immunoassay (EIA) method using commercially available research kits. The results were compared by appropriate statistical tests using SPSS program.

Results: Serum levels of both Mucin1 and CETP were increased in Pt-I°(S) compared to NC-I°(S) which were reduced after cholecystectomy in Pts-II°(S). However, serum CETP mean level in Pts-I°(S) was not significantly higher than NC-I°(S).The Mucin1 and CETP levels in patients bile, i.e. Pt-I°(B), were lower compared to serum levels before, Pt-I°(S), and after, (Pt-II°(S), cholecystectomy [Mucin1 (ng/ml): Pts-I°(S): 10.77 ± 2.93, Pts-I°(B): 1.29 ± 1.21, Pts II°(S): 6.67 ± 2.03, NCs(I°): 4.63 ± 0.53; CETP (µg/ml): Pts-I°(S): 11.47 ± 5.04, Pts-I°(B): 1.16 ± 1.19, Pts-II°(S): 9.41 ± 2.42, NCs (I°): 9.57 ± 3.63] . A significantly large proportion of patients had higher levels of Mucin1, but large number of patients had CETP levels within the normal range in Pt-I°(S). Interestingly, this was changed after cholecystectomy that significant proportion of patients had higher CETP levels in Pt-II°(S).

Conclusions: Alterations in serum Mucin1 and CETP status were significant but complex and laparoscopic cholecystectomy had significant impact indicating an important function of gallbladder relevant to their metabolism. Further studies are needed on prevalence and frequency of metabolic syndrome, insulin resistance, cytokines and other relevant parameters in Bangladeshi patients with GD i.e. cholelithiasis.

Keywords: Cholelithiasis; Gallstone disease; Mucin1; CETP; Laparoscopic cholecystectomy

Introduction

The prevalence of gallbladder disease has advanced with the use of ultrasound surveys as opposed to previous studies based on clinical or necropsy evidence [1,2]. Of the gallbladder diseases, cholelithiasis i.e. gallstone disease (GD) is prevalent in about 10-15% of adults in the developed countries and one of the most common of all digestive disorders requiring hospital admissions [2-4]. Cholesterol gallstones account for 80-90% of all gallstones found during cholecystectomy [5]. Cholesterol gallstones are primarily made up of cholesterol crystals (70%) which are held together in an organic matrix of glycoproteins, calcium salts and bile pigments [6]. The etiology of cholesterol gallstones is multi-factorial, with interaction of genetic and/or environmental factors [7]. Some features such as genetics, advancing age and female gender cannot be modified, whereas others such as diet, physical activity, rapid weight loss and obesity are modifiable [2].

The first step in the formation of gallstones is the secretion of bile supersaturated with cholesterol by the liver. The second step in gallstone formation is crystallization. The precipitation of cholesterol crystals initiates the formation of gallstones. When the gallbladder bile becomes abnormally supersaturated, inhibitors of crystallization are also important in the initiation of nucleation and crystal formation. The promoters and inhibitors are mostly proteins such as mucous glycoproteins. The growth of the crystal to macroscopic stones is further facilitated by the gallbladder mucus [7,8]. It soon became clear that other factors including nucleation of cholesterol crystal, binding together of these crystals with Mucin and hypomotility of the gallbladder played equally important roles in gallstone formation [3]. Controversy exists as to whether pro- or anti-nucleating factors are responsible for the development of cholelithiasis particularly cholesterol gallstones in humans. Experimental studies in animals and

humans indicated that gallbladder Mucin, a high molecular weight glycoprotein secreted by the gallbladder epithelium, is important for the initiation of gallstone formation [9]. The initial stage of gallstone formation, nucleation of cholesterol monohydrate crystals, occurs in mucus gel adherent to the gallbladder epithelium [9,10]. Aspirin, in doses that suppress Mucin hyper secretion, prevents cholesterol crystal nucleation and stone formation while not altering the super saturation of gallbladder bile with cholesterol [9,11]. Moreover, a mucin-bilirubin complex was reported to be present in the nonlipid matrix at the centre of cholesterol gallstones having a pigment composition like biliary sludge. These observations taken together suggest that gallbladder sludge consisting of mucus and bile pigments may serve as the core for gallstone development in man [12].

Exogenous, endogenous, intracellular-cholesterol transport and reverse-cholesterol transport are the major pathways of lipoprotein metabolism. These pathways are important and complex relevant to lipids and lipoproteins metabolism. The function of the reverse-cholesterol transport pathway is to remove excess cellular cholesterol from peripheral cells and return it to the liver for excretion and largely mediated by high density lipoprotein (HDL). Lecithin cholesterol acyltransferase (LCAT) which esterifies cholesterol on HDL, plays an important role in reverse-cholesterol transport. Cholesterol ester transfer protein (CETP) also plays a key role in this pathway because significant fraction of cholesterol that is removed from cells by HDL is transferred as cholesterol esters onto low-density lipoprotein (LDL) by CETP and eventually removed from the circulation by hepatic LDL-receptor [13-15]. In recent times cholesterol gallstone disease is considered as a metabolic syndrome which correlates with lipid abnormalities, particularly low HDL-cholesterol, hypertriglyceridemia and high homocysteine levels [16-21].

Considering these important observations and parameters and complex pathways, we have investigated effects of laparoscopic cholecystectomy on lipid profile, lipoprotein (a) [Lp(a)] status, Mucin1, CETP and Apolipoproteins (ApoA1, ApoB100, ApoE) levels in Bangladeshi patients with GD i.e. cholelithiasis. The results on lipid profile and Lp(a) status were published previously [22,23]. Therefore, the results on serum Mucin1 and CETP levels are reported in the present communication.

Patients and Methods

The patients and methods were the same as described previously in our earlier publications [22,23]. Forty-four adult patients (Gender: 8 males, 36 females; Age range: 25-65 years, mean age ± SD: 45.5 ± 12.2 years) with cholelithiasis i.e. GD and 30 healthy adult normal controls (Gender: 12 males, 18 females; Age range: 28-60 years; Mean age ± SD: 42.5+10.5 years) were included in this case-control prospective interventional study. The patients with GD were diagnosed as having cholelithiasis according to standard clinical and laboratory criteria as practiced in hospitals and patients not fulfilling the criteria for our study were excluded [22,23]. After obtaining consent, patient's demographic details and clinical findings such as pain (severity, duration, location), Murphy's sign, ultrasonogram (USG), etc were recorded as per 'PROFORMA' at diagnosis. The fasting blood samples were taken at diagnosis before cholecystectomy and conducted routine laboratory tests. The serum separated was aliquoted and stored frozen at -300°C to -80°C as first degree serum sample (I°). At the time of cholecystectomy, gall bladder bile was also collected from the same patient, centrifuged, aliquoted and stored frozen at -300°C to -80°C as first degree bile sample (I°). After Cholecystectomy, treatments/medications were given as required for the patients. After 2-3 months at follow-up, fasting blood samples were taken again from the same patient, serum separated, aliquoted and stored frozen at -300C to -80°C as second degree serum samples (II°) until analyzed for the special laboratory parameters, i.e. lipid profile (i.e. TG, TC, HDL-C, LDL-C), Lp(a), Mucin1, CETP and Apolipoproteins (ApoA1, ApoB100, ApoE) levels. All quantitative estimations in serum and bile were made by standard medical laboratory methods for lipid profile and Lp(a) using standard diagnostics kits from internationally reputed companies and LDL-C calculated by Friedwald formula [13-15]. The Mucin-1 and CEPT were quantitatively estimated by using Human Mucin-1 (MUC1) ELISA kit (Cat No YH B2055 Hu) and Human Cholesterol Ester Transfer Protein (CETP) ELISA kit (Cat No YH B0694 Hu) respectively from Shanghai Yehnua Biological Technology Co Ltd, China. The results of laboratory analyses in biological specimens of patients (I°, II°) and controls (NC) were compared statistically by ANOVA, Student's t-test and Chi-squared ($\chi 2$) test using SPSS program in computer [24]. The results of our studies on lipid profile i.e. TG, TC, HDL-C, LDL-C and Lp(a) were reported previously [22,23]. In the present article, the results on Mucin1 and CETP status and effects of Laparoscopic cholecystectomy on them in our Bangladeshi Patients with GD i.e. Cholelithiasis are reported.

Results

The results on serum Mucin1 and CETP levels in cholelithiasis are stated in Table 1 and Table 2. Serum levels of both Mucin1 and CETP were increased in Pt-I°(S) compared to NC-I°(S) which were reduced after cholecystectomy in Pts-II°(S). However, serum CETP mean level in Pts-I°(S) was not significantly higher than NC-I°(S). The Mucin1 and CETP levels in patient's bile, i.e. Pt-I°(B), were lower compared to serum levels before, Pt-I°(S), and after, (Pt-II°(S), cholecystectomy (Table 1). A significantly larger proportion of patients had higher levels of Mucin1, but larger number of patients had CETP levels within the normal range in Pt-I°(S). Interestingly, this was changed after cholecystectomy that significant proportion of patients had higher CETP levels in Pt-II°(S) (Table 2).

Groups compared	Mucin 1 (ng/ml)*				CETP (µg/ml)*			
	Pts		NCs		Pts		NCs	
	Serum (I°)	Bile (I°)	Serum (II°)	Serum (I°)	Serum (I°)	Bile (I°)	Serum (II°)	Serum (I)
N	55	40	45	40	55	40	45	40
Obs Range	4.39-15.31	0.21-4.38	2.85-10.21	3.73-5.89	3.53-18.91	0.23-4.96	3.52-13.14	5.18-20.12
Mean ± SD	10.7 ± 2.93	1.29 ± 1.20	6.66 ± 2.03	4.63 ± 0.53	11.47 ± 5.04	1.16 ± 1.19	9.40 ± 2.42	9.57 ± 3.62

Std Error(SEM)	0.4	0.19	0.3	0.08	0.68	0.19	0.36	0.57
95%CIM	9.97-11.56	0.91-1.68	6.05-7.26	4.68-4.80	10.11-12.84	0.78-1.54	8.68-10.13	8.41-10.72
ANOVA (NCs-I°,Pts-I°, Pts-I°(B) Pts-II°)	df(Bg,Wg,Total)		F-ratio	P –value*	df(Bg,Wg, Total)		F-ratio	P –value*
	3		182.63	<0.001	3		72.36	<0.001
Student's t- test	t-value		Df	P-value*	t-value		df	P-value*
NCs-I°(S) vs Pts-I°(S)	-13.06		93	<0.001	-2.04		93	0.044
NCs-I°(S) vs Pts-I°(B)	16.08		78	<0.001	13.94		78	<0.001
NCs-I°(S) vs Pts-II°(S)	-6.13		83	<0.001	0.246		83	0.806
Pts-I°(S) vs Pts-I°(B)	19.27		93	<0.001	12.66		93	<0.001
Pts-I°(B) vs Pts-II°(S)	-14.61		83	<0.001	-19.54		83	<0.001
Pts-I°(S) vs Pts-II°(S)	7.97		98	<0.001	1042		98	0.013

*NCs-I°(S): Normal Control Serum (I°), Pts- I° (S): Patients Serum (I°), Pts- I0(B): Patients Bile (I°), Pts- II° (S): Patients Serum (II°); df (Bg, Wg,Total): df (Between groups, Within groups, Total); *P≤0.05:Significant, *P>0.05: Not significant

Table 1: Mucin1 and CETP levels in patients (Pts) and controls (NCs).

Laboratory Parameter	**Patients (Pts) & Normal Controls (NCs)***								
	NCs- I°	**Serum-I°**	**Total**	**NCs-I°**	**Serum- II°**	**Total**	**Serum-I° (Pts)**	**Serum-II0 (Pts)**	**Total**
Mucin 1 (ng/ml)									
≤ 5.69	36	3	39	36	17	53	3	17	20
>5.69	4	52	56	4	28	32	52	28	80
Total	40	55	95	40	45	85	55	45	100
Chi-squared (χ2) test*	χ2= 68.40, df=1, p <0.001			χ2 = 43.24, df=1, p <0.001			χ2 = 16.16, df=1, p <0.001		
CETP (µg/ml)									
≤ 16.18	37	47	84	37	44	81	47	41	88
>16.18	3	8	11	3	1	4	8	4	12
Total	40	55	95	40	45	85	55	45	100
Chi-squared (χ2) test*	χ2= 1.12, df=1, p=289			χ2= 1.32, df=1, p =0.251			χ2= 0.75, df=1, p =0.386		

*NCs-I0(S): Normal Control Serum (I0), Pts- I0 (S): Patients Serum (I0), Pts- I0(B): Patients Bile (I0), Pts- II0 (S): Patients Serum (II0); *P≤0.05: Significant, *P>0.05: Not significant

Table 2: Proportion of patients (Pts) with abnormal serum Mucin 1and CETP levels before and after laparoscopic cholecystectomy and their statistical analysis by Chi-squared (χ2) test.

Discussion

Multiple case-control studies, comparing those with gallstone versus those without, have shown that pathogenesis of GD is multi-factorial associated with old age, obesity, specific dietary habit, resistance, pregnancy, ethnicity and genetic background [5,25-28]. It is thought that the GD probably develops from complex interactions among multiple genetic and environmental factors [5,27,28]. Recently, it was reported that metabolic syndrome is associated as one of the important components in GD [25,28-30]. GD is a metabolic problem which correlates with lipid abnormalities such as low HDL-cholesterol, hypertriglyceridemia carrying an increased risk of developing stones [22,23,30]. More recently, we have reported the lipid abnormalities in our patients with GD earlier in 2016 [22,23]. In the present article, we have reported our study results on Mucin1 and CETP levels in the same patients with GD. This study report is the first of its kind to demonstrate and document abnormal levels of Mucin1 and CEPT in Bangladeshi patients with GD. We found that both Mucin1 and CETP levels were raised in Pts-I°(S) significantly (Table 1). A significantly

large proportion of patients had higher levels of Mucin1, but large number of patients had CETP levels within the normal range in Pt-I° (S). Interestingly, this was changed in Pts-II°(S) that significant proportion of patients had higher CETP levels after cholecystectomy in Pt-II°(S) (Table 2).

The metabolic syndrome is defined by the presence of at least 3 features out of: abdominal obesity, high blood pressure, high fasting glucose, insulin resistance, increased triglyceride and reduced HDL levels [16,20,21]. Both the metabolic syndrome and diabetes mellitus are risk factors for GD [25]. Insulin resistance predisposes to cholesterol gallstone formation suggesting altered cholesterol and bile salt metabolism [31,32]. Also, hepatic insulin resistance may act by enhancing hepatic cholesterol secretion, depressing bile salt synthesis and impairing gallbladder motility [33,34]. Bile plays important role in fat/lipid digestion and absorption and gallbladder bile is secreted due to contraction of it and relaxation of sphincter of oddi by various physiological mechanisms [35]. It is quite possible that abnormal Mucin1 and CETP levels are involved in the process of developing metabolic syndrome through dyslipidemia causing impaired gallbladder function, particularly reduced motility. This is probably one the ways which create favorable environment leading to stone formation in the gallbladder. Hence, it is important to investigate the prevalence and extent of metabolic syndrome and its components in our patients with GD i.e. cholelithiasis.

In conclusion, Mucin1 and CETP levels have implications in the process of gallstone formation through dyslipidemia causing possibly metabolic syndrome. It would, therefore, be worthwhile and interesting to conduct further studies along this line in Bangladeshi patients with GD i.e. cholelithiasis soon.

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