SciForschen
Open HUB for Scientific Research

Journal of Clinical and Laboratory Medicine

ISSN 2578-9578 | Open Access

RESEARCH ARTICLE

Volume 3 - Issue 1 | DOI: http://dx.doi.org/10.16966/2578-9578.121

Effects of Laparoscopic Cholecystectomy on Apolipoproteins (ApoA1, ApoB100, ApoE) Status in Bangladeshi Patients with Cholelithiasis

Jhuma KA[1], Giasuddin ASM[2]*, Choudhury MAM[3] and Haq AMM[4]

[1]Professor of Biochemistry, Department of Biochemistry, Medical College for Women & Hospital (MCW&H) & Member Secretary, Medical Research Unit (MRU), MHWT, Dhaka, Bangladesh
[2]Professor of Biochemistry & Immunology & Director, MRU, MCW&H Building, MHWT, Dhaka, Bangladesh
[3]Associate Professor of Surgery, Department of Surgery, MCW&H, MHWT, Dhaka, Bangladesh
[4]Honorary Professor of Medicine, Dept of Medicine, MCW&H & Chairman, MRU, MCW&H Building MHWT, Dhaka, Bangladesh

***Corresponding author:** Prof Dr. ASM Giasuddin MSc PhD PGD CSci FIBMS MNYAS, Professor of Biochemistry & Immunology & Director, Medical Research Unit (MRU), MHWT, MCW&H Building, Plot-4 Road-9 Sector-1 Uttara Model Town, Dhaka-1230, Bangladesh Mobile-01787657685; Tel: 880-2-58953939 Ex-144, **E-mail:** mru.mhwt@gmail.com; asmgias@hotmail.com

Received: 03 Sep, 2018 | **Accepted:** 29 Oct, 2018 | **Published:** 05 Nov, 2018

Citation: Jhuma KA, Giasuddin ASM, Choudhury MAM, Haq AMM (2018) Effects of Laparoscopic Cholecystectomy on Apolipoproteins (ApoA1, ApoB100, ApoE) Status in Bangladeshi Patients with Cholelithiasis. J Clin Lab Med 3(1): dx.doi.org/10.16966/2578-9578.121

Abstract

Objectives: The present study was conducted on serum apolipoproteins (ApoA1, ApoB100, ApoE) status as scanty reports are available in Bangladeshi patients with cholelithiasis i.e. gallstone disease (GD).

Patients and Methods: Fifty five adult patients (Pts) with cholelithiasis and 40 normal controls (NC) were included in the study carried out from October 2016 to March 2018. The blood samples were taken from fasting patients before cholecystectomy (Serum-I^0), gall bladder biles during cholecystectomy (Bile-I^0) and blood samples again after 2-3 months at follow-up (Serum-II^0) and from fasting NC subjects. ApoA1, ApoB100 and ApoE levels were measured in serum and bile samples by enzyme immunoassay (EIA) method using commercially available research/diagnostic kits. The results were compared statistically by Student's t-test and Chi-squared test using SPSS programme.

Results: ApoA1 level (mg/dl) was reduced in the Pts which was raised significantly after 2-3 months of laparoscopic cholecystectomy, but not to the full extent similar to NCs (Mean ± SD → Serum I^0: 50.89 ± 14.01, Serum II^0: 60.75 ± 16.40) ($p<0.001$). ApoB100 level (mg/dl) was raised in Pts which was reduced significantly after cholecystectomy, but not to the full extent as NCs (Mean ± SD → Serum I^0: 202.84 ± 53.42, Serum II^0: 138.34 ± 30.63) ($p<0.001$). Similarly, ApoE level (mg/dl) was raised in Pts which was reduced significantly after laparoscopic cholecystectomy, but not to the full extent similar to NCs (Mean ± SD → Serum I^0: 69.97 ± 13.50, Serum II^0: 57.13 ± 17.38) ($p< 0.001$). Chi-squared (χ^2) test revealed that large proportions of Pts had lower ApoA1, higher ApoB100 and higher ApoE levels compared to NCs and the proportions were changed towards improvement similar to NCs significantly, but not to the full extent after laparoscopic cholecystectomy) ($p<0.001$).

Conclusions: Cholelithiasis i.e. GD had profound influence on ApoA1, ApoB100 and ApoE status suggesting their probable causative and protective roles in the aetiopathogenesis of the disease. Investigations related to leptin and apolipoproteins levels and their associations with metabolic syndrome in GD are needed in our future research endeavors to substantiate the findings.

Short Title: Apolipoproteins (A1, B100, E) in Cholelithiasis.

Keywords: Cholelithiasis; ApoA1; ApoB100; ApoE; Laparoscopic cholecystectomy

Introduction

Lipoproteins are typically spherical particles with non polar neutral lipids, i.e. triglycerides (TG) and cholesterol ester (CE), in their core and more polar amphipathic lipids, i.e. phospholipids (PL) and free cholesterol (C), at their surfaces. Equally important is the fact that they also contain one or more specific proteins called apolipoproteins on their surfaces. Each class of lipoprotein, i.e. Chylomycron, VLDL, IDL, LDL, HDL & Lp(a), carries several apolipoproteins in different proportions such as ApoA1 in HDL and Chylomycron, ApoB100 in LDL, VLDL, IDL and Lp(a), ApoB48 in Chylomycron, and ApoE in VLDL and IDL. Structural features of apolipoproteins enable them to bind to lipid end still interact with the surrounding aqueous environment. They facilitate metabolism of lipoproteins and are involved in normal physiology [1,2]. Many lipoproteins such as Chylomycron, VLDL, LDL, LDL-C and Lp(a) have been reported to be atherogenic associated with coronary artery disease (CHD) in many epidemiological and clinical trial studies [3-7]. These study reports have implications that apolipoproteins are associated with CHD and in fact many studies reported that some of the apolipoproteins are

involved in patients with myocardial infarction (MI) [3,4]. Literature review indicated that some of the apolipoproteins such as ApoA1, ApoB100 and ApoE are involved in the process of Cholelithiasis i.e. gallstone disease (GD) as well [8-10]. GD is a metabolic problem which correlates with lipid abnormalities, adiposity, diabetes mellitus, hypertension and metabolic syndrome. Some studies reported that ApoA1, ApoB100 and ApoE were striking higher in serum of patients with GD compared to individuals with no gallstones [11-13]. Other factors such as cholesterol ester transfer protein (CETP) and mucin were also implicated in the aetiopathogenesis of GD. Suggestions were made that serum ApoA1, ApoB100 and ApoE levels would possibly be sensitive parameters as compared with serum lipids in distinguishing patients with gallstones from subjects without gallstones. The status of lipid profile, lipoprotein (a), mucin 1 and CETP in Bangladeshi patients with cholelithiasis i.e. GD had been published recently [13-15]. However, literature survey indicated that various apolipoproteins status in Bangladeshi patients with GD is not known. In the present article, we have therefore reported effects of laparoscopic cholecystectomy on serum levels of ApoA1, ApoB100 and ApoE in Bangladeshi patients with cholelithiasis i.e. GD.

Patients and Methods

Fifty five adult patients (Gender: 10 males, 45 females; Age range: 25-65 years, Mean age ± SD: 45.5 ± 12.2 years) with cholelithiasis i.e. GD and 40 healthy adult normal controls (Gender: 16 males, 24 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 hospital and patients not fulfilling the criteria for our study i.e. Patients with diabetic mellitus, renal disease, thyroid disease, liver disease, history of taking anti-hypertensive or anti-hyperlipidemic drugs and smoking habits were excluded from the study [14-16]. After obtaining consent, patient's demographic details and clinical findings such as pain (severity, duration, location), merphy'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 -80°C as first degree serum sample (I^0). At the time of cholecystectomy, gall bladder bile was also collected from the same patient, centrifuged, aliquoted and stored frozen at -80°C as first degree bile sample (I^0). 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 -80°C as second degree serum samples (II^0) until analyzed for the lipid profile (i.e. TG, TC, HDL-C, LDL-C), Lp(a) and apolipoproteins (ApoA1, ApoB100, ApoE) related to our research interest. All quantitative estimations in serum and bile were made by standard medical laboratory methods using research/diagnostic kits from internationally reputed companies and LDL-C calculated by Friedwald formula [14,16]. ApoA1, ApoB100 and ApoE levels in serum were determined by enzyme-linked immunosorbent assay (ELISA) research/diagnostic kits based on biotin double antibody sandwich technique taking optical density readings at 450 nm in a Microplate EIA Reader (Model: RE-4.0 Microplate Reader, IDCS Ltd Briloner Landstrasse 4-6, 34497 KORBACH-Germany). The apolipoprotein kits were obtained from Shanghai Yehua Biological Technology Co Ltd, Shanghai, China (Ref: ApoA1, Cat No.YHB3610Hu; ApoB100, Cat No.YHB0379Hu; ApoE, Cat No.YHB0371Hu). The results of laboratory analyses in biological specimens of patients (I^0,II^0) and controls (NCs) were compared statistically by Student's t-test and Chi-squared (χ^2) test using SPSS programme in computer [17]. The study was carried out at Medical Research Unit (MRU), The Medical and Health Welfare Trust (MHWT), Uttara, Dhaka-1230, Bangladesh in collaboration with Department of Surgery, Medical College for Women and Hospital (MCW and H), Uttara, Dhaka-1230, Bangladesh from October 2016 to March 2018.

Results

The results of ApoA1, ApoB100 and ApoE status in our study subjects and their statistical analyses are stated in (Tables 1-4). ApoA1 level was significantly reduced in our Pts which was raised significantly after 2-3 months of laparoscopic cholecystectomy, but not to the full extent similar to NCs and bile (Pts) contained lower level of ApoA1 ($p<0.001$) (Table 1). ApoB100 level, on the other hand, was significantly raised in Pts which was reduced significantly after cholecystectomy, but not to the full extent as NCs and bile (Pts) contained lower levels of ApoB100 (p 0.001) (Table 2). Similarly, ApoE was significantly raised in Pts which was reduced significantly after 2-3 months of laparoscopic cholecystectomy, but not to the full extent similar to NCs and bile (Pts) contained lower level of ApoE ($p<0.001$) (Table 3). The analysis by Chi-squared (χ^2) test revealed that large proportion of Pts had lower ApoA1, higher ApoB100 and higher ApoE levels compared to NCs and the proportions were changed towards improvement significantly similar to NCs, but not to the full extent in 2-3 months after laparoscopic cholecystectomy ($p<0.001$) (Table 4).

Discussion

Apolipoproteins were reported to be associated with CHD showing that some of them are involved in MI in many studies [3-5,18-20]. Multiple associations between gallstones and dislipidaemias, CHD and stroke have been reported [21-24]. Our findings on serum levels of ApoA1 (Table 1) and ApoB100 (Table 2) in Bangladeshi patients with cholilithiasis were similar to those reported by Sarac, et al. [8]. These results were consistent with those of other previous studies showing lower ApoA1 and higher ApoB100 serum levels in patients with cholilithiasis [25,26]. These findings could be useful in understanding the pathophysiology of GD. Leptin, a hormone produced by adiposities, was shown to modulate the activity of enteric inhibitory and excitatory neurons in the proximal colon and it seems to have a regularly function in gastrointestinal motility [8,27]. Sarac et al showed further that levels of ApoA1 were negatively correlated while ApoB100 levels were positively corrected with leptin [8]. Our findings on ApoE levels in patients with GD had similar trends as ApoB100 i.e. ApoE was increased in serum I^0, followed by decrease in Serum-II^0, significantly, but not to the full extent as NCs ($p<0.001$) (Table 3).

ApoE is one of the key regulatory protein in cholesterol and lipoprotein metabolism [1,27]. ApoE has three common isoforms, E2, E3, and E4, which are coded by the alleles e2, e3 and e4, respectively, at a single locus in chromosomal region 19q13. These alleles define six apo E phenotypes: E2/2, 2/3, 2/4, 3/3, 4/3, and 4/4. Apo E4 has been associated with several diseases, such as coronary heart disease and Alzheimer's disease [28-30]. The E2 allele is associated with low and the E4 allele with high serum total and LDL cholesterol concentrations in various populations and with altered enterohepatic metabolism of cholesterol and bile acid. It was reported that the cholesterol content of gallstones tends to be low in subjects with the E2 allele and high in subjects with the E4 allele suggesting that ApoE2 is a genetic factor providing protection against GD, while ApoE4 isoform may be a risk factor for cholilithioasis [28]. Our observation of increased serum

Table 1: Apolipoprotein A1 levels in patients (Pts) and controls (NCs) and their statistical analyses by student's t-test.

Group Compared*	ApoA1 (mg/dl)*			
	Pts			NCs
	Serum (I^0)	Bile (I^0)	Serum (II^0)	Serum (I^0)
n	55	40	45	40
Observed Range	32.17-88.54	10.36-22.59	42.47-110.60	75.55-120.70
Mean ± SD	50.89 ± 14.01	19.9 ± 4.18	60.75 ± 16.40	92.02 ± 14.62
Std Error (SEM)	1.89	0.66	2.44	2.31
95%CIM	47.11-54.68	17.85-20.52	55.82-65.68	87.34-96.69
Student's t-test	t-value		df	P-value
NCs-I^0(S) *vs* Pts-I^0(S)	13.87		93	<0.001
NCs-I^0(S) *vs* Pts-II^0(S)	9.23		83	<0.001
Pts-I^0 (S) *vs* Pts-II^0(S)	-3.24		98	0.002

***n: Number of participants, NCs-I^0(S):** Normal Control Serum (**I^0**), **Pts-I^0(S):** Patients Serum (**I^0**), **Pts-I^0(B):** Patients Bile (**I^0**), **Pts-II^0(S):** Patients Serum (**II^0**); $p \leq 0.05$: Significant, P>0.05: Not significant.

Table 2: Apolipoprotein B100 levels in patients (Pts) and controls (NCs) and their statistical analyses by student's t-test.

Group Compared*	ApoB100 (mg/dl)*			
	Pts			NCs
	Serum (I^0)	Bile (I^0)	Serum (II^0)	Serum (I^0)
n	55	40	45	40
Observed Range	123.50-409.10	10.21-42.03	110.20-219.3	48.52-172.00
Mean ± SD	202.84 ± 53.42	19.64 ± 8.28	138.34 ± 30.63	104.77 ± 33.57
Std Error (SEM)	7.2	1.31	4.57	5.31
95%CIM	188.40-217.28	16.99-22.29	129.1-147.5	94.03-115.51
Student's t-test	t-value		df	P-value
NCs-I^0(S) *vs* Pts-I^0(S)	-10.22		93	<0.001
NCs-I^0(S) *vs* Pts-II^0(S)	-4.82		83	<0.001
Pts-I^0 (S) *vs* Pts-II^0(S)	7.19		98	<0.001

*n: Number of participants, NCs-**I^0**(S): Normal Control Serum (**I^0**), Pts-**I^0**(S): Patients Serum (**I^0**), Pts-**I^0**(B): Patients Bile (**I^0**), Pts-**II^0**(S): Patients Serum (**II^0**); $p \leq 0.05$: Significant, P>0.05: Not significant.

Table 3: Apolipoprotein E levels in patients (Pts) and controls (NCs) and their statistical analyses by student's t-test.

Group Compared*	ApoE (mg/dl)*			
	Pts			NCs
	Serum (I^0)	Bile (I^0)	Serum (II^0)	Serum (I^0)
n	55	40	45	40
Observed Range	55.48-112.30	23.37-40.12	39.88-94.76	28.19-57.54
Mean ± SD	69.97 ± 13.50	28.22 ± 6.59	57.13 ± 17.38	35.66 ± 6.80
Std Error (SEM)	1.82	1.04	2.59	1.08
95%CIM	66.32-73.62	26.12-30.33	51.91-62.35	33.48-37.83
Student's t-test	t-value		df	P-value
NCs-I^0(S) *vs* Pts-I^0(S)	-14.75		93	<0.001
NCs-I^0(S) *vs* Pts-II^0(S)	-7.33		83	<0.001
Pts-I^0 (S) *vs* Pts-II^0(S)	4.16		98	<0.001

*n: Number of participants, NCs-**I^0**(S): Normal Control Serum (**I^0**), Pts-**I^0**(S): Patients Serum (I0), Pts-**I^0**(B): Patients Bile (**I^0**), Pts-**II^0**(S): Patients Serum (**II^0**); $p \leq 0.05$: Significant, P>0.05: Not significant.

ApoE level could mean either a protective or a susceptibility/risk factor role in GD. To resolve this vital issue, study on ApoE polymorphism is required to be done in patients with GD. Also in recent times, antioxidant defense system has been implicated with the prevalence of GD and thought to play a role in the pathophysiology of GD [31-35]. Among them, higher circulating vitamin E and vitamin C levels have been suggested that might provided protection against GD, and area of research that needs to be further investigated in longitudinal studies [34,35].

Our findings were important and interesting in the sense that serum levels of significantly large proportion of patients were altered in GD which were reversed towards NCs after 2-3 months of laparoscopic cholecystectomy, but not to the full extent ($p<0.001$) (Table 4). The reasons for this situation need some careful, relevant and logical explanations. In recent times, GD is considered to be a metabolic disease as Mendez-Sanchez, et al. and others reported a strong relationship between GD and metabolic syndrome [8-10,23]. They reported that leptin dysfunction and insulin resistance might

Table 4: Proportion of patients (Pts) with abnormal serum apolipoprotein levels before and after laparoscopic cholecystectomy and their statistical analysis by Chi-squared (χ^2) test.

Apolipo- proteins	Patients (Pts) and Normal Controls (NCs)								
	NCs-I^0	Serum-I^0	Total	NCs-I^0	Serum-II^0	Total	Serum-I^0 (Pts)	Serum-II^0 (Pts)	Total
ApoA1 (mg/dl)									
<62.78	1	42	43	1	18	19	42	18	60
≥ 62.78	39	13	52	39	27	66	13	27	40
Total	40	55	95	40	45	85	55	45	100
Chi-squared (χ^2) test	χ^2=51.0, df=1, p <0.001			χ^2=17.16, df=1 , p<0.001			χ^2=13.64, df=1, p <0.001		
ApoB100 (mg/dl)									
≤171.91	36	14	50	36	36	72	14	36	50
>171.91	4	41	45	4	9	13	41	9	50
Total	40	55	95	40	45	85	55	45	100
Chi-squared (χ^2) test	χ^2=37.70 , df=1, p <0.001			χ^2=1.63, df=1, p=0.201			χ^2=29.45, df=1 , p <0.001		
ApoE (mg/dl)									
≤ 49.26	38	1	39	38	17	55	1	17	18
>49.26	2	54	56	2	28	30	54	28	82
Total	40	55	95	40	45	85	55	45	100
Chi-squared (χ^2) test	χ^2=83.09, df=1, p <0.001			χ^2=30.36, df=1, p <0.001			χ^2=29.22, df=1, p <0.001		

*n: Number of participants, NCs-I^0(S): Normal Control Serum (I^0), Pts-I^0(S): Patients Serum (I^0) Pts-II^0(S): Patients Serum (II^0); p ≤ 0.05: Significant, P>0.05: Not significant.

ApoA1 (NCs) (Mean-2SD)=92.02-29.24=62.78;

ApoB100 (NCs) (Mean+2SD)=104.77+67.14=171.91;

ApoE (NCs) (Mean+2SD)=35.66+13.60=49.26.

be responsible for reduced gallbladder motility, which in turn may contribute to cholesterol gallstone formation. In the light of these reports, we are now in the process of evaluating our results for the association between Lipid profile and ApoA1, Lipid profile and ApoB100, Lipid profile and ApoE, Lp(a) and ApoA1, Lp(a) and ApoB100 and Lp(a) and ApoE.

In conclusion, cholelithiasis i.e. GD had profound influence on ApoA1, ApoB100 and ApoE status suggesting their cause and effect roles in the aetiopathogenesis of the disease. It is important to further investigate leptin and apolipoproteins levels and their associations and also to study metabolic syndrome in Bangladeshi patients with GD in our future research endeavors to substantiate the findings.

Acknowledgements

The authors appreciate Mr. TK Datta, Medical Technologist, for laboratory analysis, Mr. SMN Ali for computer composing and Mr. AHM Salman for statistical analysis. The authors gratefully acknowledge the financial support from Ministry of Science and Technology (MOST), Government of the Republic of Bangladesh, Dhaka-1000, Bangladesh (Project Serial No.240, Group Serial No.6 MEDI'S, Year 2016-2017) for the research project.

References

1. Remaley AT, Rifai N, Warnick GR (2015) Lipids, Lipoprotein, apolipoproteins and other cardiac risk factors. In: Bruns DE, Butris CA (Eds). Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics, 7th Edition; St. Louis, Missouri: Elsevier sounders, USA 388-411.
2. Ferrier DR (Ed) (2014) Lippincott's Illustrated Reviews: Biochemistry, 6th Edition; Baltimore, USA: Lippincott Williams&Wilkins 219-244.
3. Jhuma KA, Hoque MM (2008) Serum Apoprotein (ApoA1 and ApoB) in Myocardial infarction. J Bangladesh Coll Phys Surg 26: 62-66.
4. Hoque MM, Jhuma KA, Sultana P, Alam MM (2008) Apoproteins (B100 and A1) *vs* standard lipid measures (lipid profile) as indices of coronary heart disease risk. Should apoproteins replace the lipid profile? Bangladesh J Pathol 23: 4-7.
5. Genest J, Mcnamara JR, Ordovas JM, Jenner JL, Silberman SR, et al. (1992) Lipoproteins cholesterol, Apolipoprotein A1 and B and lipoprotein (a) abnormalities in men with premature coronary artery disease. J Am Coll Cardiol 19: 792-802.
6. Vanstiphout WA, Hofman A, Kruijssen HA, Vermeeren R (1986) Is the ratio of ApoB/apoA1 an early predictor of coronary Atherosclerosis? Atherosclerosis 62: 179-182.
7. Al-Muhtaseb N, Hayet N, Al-Khafaji M (1989) Lipoproteins and apolipoproteins in young male servivors of myocardial infection. Atherosclerosis 77: 131-138.
8. Sarac S, Atamer A, Atamer Y, Can AS, Bilici A, et al. (2015) Leptin levels and lipoprotein profiles in patients with cholelithiasis. J Int Med Res 43: 385-392.
9. Chen LY, Qiao QH, Zhang SC, Chen YH, Chao GQ, et al. (2012) Metabolic syndrome and gallstone disease. World J Gastroenterol 18: 4215-4220.
10. Stender S, Frikke-Schmidt R, Benn M, Nordestgaard BG, Tybjærg-Hansen A (2013) Low-density lipoprotein cholesterol and risk of gallstone disease: a Mendelian randomization study and meta-analyses. J Hepatol 58: 126-133.
11. Davidson MH, Ballantyne CM, Jacobson TA, Bittner VA, Braun LT, et al. (2011) Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. J Clin Lipidol 5: 338-367.

12. Vesper HW, Wilson PW, Rifai N (2012) A message from the laboratory community to the National Cholesterol Education Program Adult Treatment Panel IV. Clin Chem 58: 523-527.

13. Haq AMM, Giasuddin ASM, Jhuma KA, Choudhury MAM (2015) Effect of cholecystectomy on lipid profile in Bangladeshi patients with Cholelithiasis. J Metabolic Synd 5: 192.

14. Giasuddin ASM, Jhuma KA, Choudhury MAM, Haq AMM (2016) Lipoprotein (a) status and effect of laparoscopic cholecystectomy on it in Bangladeshi patients with Cholelithiasis. J Metabolic Synd 5: 216.

15. Choudhury MAM, Giasuddin ASM, Jhuma KA, Haq AMM (2018) Effects of Laparoscopic Cholecystectomy on Mucin1 and Cholesterol Ester Transfer Protein Status in Bangladeshi patients with Cholelithiasis. J Metabolic Synd 7: 240.

16. Anstee QM, Jones DEJ (2014) Liver and biliary tract disease. In: Walker BR, Colledge NR, Ralston SH, Penman ID (Eds). Davidson's Principles&Practice of Medicine, 22nd Edition, Edinburgh: Churchill Livingstone (Elsevier): 921-988.

17. Kirkwood BR, Sterne JAC (2003) Essential Medical Statistics, 2nd Edition (Reprinted), Oxford: Blackwell Science Ltd.

18. Al-Muhtaseb N, Hayet N, Al-Khafaji M (1989) Lipoproteins and apolipoproteins in young male survivors of myocardial infarction. Atherosclerosis 77: 131-138.

19. Durrington PN, Hunt L, Ishola M, Arrol S (1988) Apolipoproteins(a) A1 and B and parental history in men with early onset ischemic heart disease. The Lancet 14: 1070-1073.

20. Zunic G, Jelic-Ivanovic Z, Spasic S, Stojiljkovic A, Singh NM (1992) Reference Values for Apolipoproteins A-I and B in Healthy Subjects, by Age. Clin Chem 38: 566-569.

21. Damnjanovic Z, Jovanovic M, Nagorni A, Radojković M, Sokolović D, et al. (2013) Correlation of inflammation parameters and biochemical markers of Cholestasis with the intensity of lipid peroxidation in patients with choledocholithiasis. Vojnosanit Pregl 70: 170-176.

22. Chavez-Tapia NC, Kinney-Novelo IM, Sifuentes-Rentería SE, Torres-Zavala M, Castro-Gastelum G, et al. (2012) Association between cholecystectomy for gallstone disease and risk factors for cardiovascular disease. Ann Hepatol 11: 85-89.

23. Mendez-Sanchez N, Zamora-Valdes D, Flores-Rangel JA, Pérez-Sosa JA, Vásquez-Fernández F, et al. (2008) Gallstones are associated with carotid atherosclerosis. Liver Int 28: 402-406.

24. Mendez-Sanchez N, Chavez Tapia NC, Motola-Kuba D, Sanchez-Lara K, Ponciano-Rodriguez G, et al. (2005) Metabolic syndrome as a risk factor for gallstone disease. World J Gastroenterol 11: 1653-1657.

25. Mendez-Sanchez N, Bermejo-Martinez LB, Vinals Y, Chavez-Tapia NC, Graff IV, et al. (2005) Serum leptin levels and insulin resistance are associated with gallstone disease in overweight subjects. World J Gastroenterol 11: 6182-6187.

26. Wang SN, Yeh YT, Yu ML, Dai CY, Chi WC, et al. (2006) Hyperleptinaemia and hypoadiponectinaemia are associated with gallstone disease. Eur J Clin Invest 36: 176-180.

27. Florin V, Caroline F, Francis C, Camille S, Fabielle A (2013) Leptin modulates enteric neurotransmission in the rat proximal colon: An *in vitro* study. Regul Pept 185: 73-78.

28. Niemi M, Kervinen K, Rantala A, Kauma H, Paivansalo M, et al. (1999) The role of apolipoprotein E and glucose intolerance in gallstone disease in middle aged subjects. Gut 44: 557-562.

29. Bertomeu A, Ros E, Zambon D, Vela M, Pérez-Ayuso RM, et al. (1996) Apolipoprotein E polymorphism and gallstones. Gastroenterology 111: 1603-1610.

30. Van Erpecum KJ, Carey MC (1996) Apolipoprotein E4: another risk factor for cholesterol gallstone formation? Gastroenterology 111: 1764-1767.

31. Shaffer EA (2006) Gallstone disease: Epidemiology of gallbladder stone disease. Best Pract Res Clon Gastroenterol 20: 981-996.

32. Zhou JF, Chen P, Yang JL, Zhu YG, Peng CH, et al. (2000) Oxidative stress before and after operation in patients with chronic cholecystitis containing gallstone. Biomed Environ Sci 13: 254-262.

33. Koch M, Borggrefe J, Barbaresko J, Groth G, Jacobs G, et al. (2014) Dietary patterns associate with magnetic resonance imaging-determined liver fat content in a general population study. Am J Clin Nutr 99: 369-377.

34. Ginsberg HN and Goldberg IJ(2001) Disorder of intermediary metabolism; In: Braunwald E, Fauci AS and Kasper DL (eds) Harrisons principles of internal medicine. 13th edition, McGraw Hill publishers, USA 1377-1387.

35. Waniek S, Giuseppe RD, Esatbeyoglu T, Ratjen I, Enderle J, et al. (2018) Association of Circulating Vitamin E (α-and γ-Tocopherol) Levels with Gallstone Disease. Nutrients 10: 133.