COMPARISON OF SERUM LIPID PROFILE IN NON ALCOHOLIC FATTY LIVER DISEASE

Atif Latif, Qurrat-Ul-Ain, Syed Raza Jafer, Nisar Ahmed, Mazhar Shafiq, Kumari Sapna

Pakistan Naval Ship (PNS) Shifa Hospital, Karachi Pakistan

ABSTRACT

Objective: To compare serum lipid profile in different ultrasonographic grades of non alcoholic fatty liver disease (NAFLD).

Study Design: Cross sectional study.

Place and Duration of Study: PNS SHIFA hospital, Karachi, from Oct 2015 to Jul 2016.

Material and Methods: Seventy three adults of either gender were consecutively inducted after diagnosis of non alcoholic fatty liver disease (NAFLD) on ultrasonography (USG). These individuals were further classified into grade I, II and III of NAFLD depending on US findings. Fasting blood sample of all the subjects was analyzed for serum fasting lipid profile comprising of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Serum non HDL cholesterol (nonHDL-C) was calculated by subtracting HDL-C from TC.

Results: Among 73 subjects with NAFLD, 42.5%, 37% and 20.5% had grade I, II and III NAFLD respectively. All parameters showed significant increase in frequency of abnormal results with increasing grade of NAFLD except TG. Significant difference was found in mean TC (p=0.000), LDL-C (p=0.000), HDL-C (p=0.005) and nonHDL-C (p=0.000) between grades of NAFLD. Post hoc analysis revealed that only mean nonHDL-C was significantly different amongst all the grades of NAFLD.

Conclusion: The increasing severity of NAFLD was found associated with increased frequency of dyslipidemia. Though most frequent dyslipidemia in NAFLD was low serum HDL-C followed by hypertriglyceridemia, only serum nonHDL-C was statistically different amongst all the grades of NAFLD.

Keywords: Lipid profile, Non-alcoholic fatty liver disease (NAFLD), NonHDL cholesterol, Ultrasonography.

INTRODUCTION

Non alcoholic fatty liver disease is the most emergent liver disease that encompasses a complete spectrum of liver pathologies ranging from simple steatosis characterized by hepatic lipid accumulation in the form of TG to nonalcoholic steatohepatitis (NASH) that may lead to cirrhosis and finally hepatocellular carcinoma[1]. The prevalence of NAFLD has doubled during last 20 years owing to the current epidemic of obesity and its sub-sequent metabolic derangements[2]. The median worldwide prevalence of NAFLD is 20% (range: 6.3%-33%)[1]. There is no community based study from Pakistan but a hospital based study showed frequency of approximately 14%[3,4]. NAFLD can be diagnosed either by imaging or by histology and absence of secondary hepatic fat accumulation[1]. A liver biopsy remains the only method to distinguish NASH from simple steatosis and reference method to establish the extent of liver damage and fibrosis[5]. But it is a painful and invasive procedure with low but definite risk of potentially life threatening complications like bleeding and need expert hands to avoid sampling errors[5,6]. An ultrasonographic classification system comprising of three grades has been proposed that correlates certain histologic features with the long-term prognosis[6].

NAFLD is associated with metabolic risk factors such as obesity, metabolic syndrome, dyslipidemia, insulin resistance (IR) and type 2 diabetes[7]. Exact pathogenesis of NAFLD and factors that determine the severity are still to be clearly understood[1,4]. Though conventional

Correspondence: Dr Atif Latif, Department of Radiology PNS Shifa Karachi Pakistan *(Email: atif_latif98@hotmail.com)*

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paradigm considered IR as the root cause for the development and progression of NAFLD, recent studies have emphasized novel pathophysiologic mechanisms involving environmental and genetic factors that result in development of necroinflammation and fibrosis[8,9]. Several studies have contributed to the evidence that NAFLD patients have increased cardiovascular mortality[10-14]. NAFLD is associated with increased serum LDL, very low density lipopro-tein (VLDL), small dense LDL (sdLDL) and TG, combined with decreased HDL that represents a threat for cardiovascular disease (CVD) development[15,16]. Different studies have revealed the association of various components of lipid profile with NAFLD as compared with healthy control[17,18]. This study aims at finding association of serum lipid profile with ultrasound grades of increasing severity of NAFLD to elucidate the factors involved in the progression of disease and thus may be used to predict severity of NAFLD.

PATIENTS AND METHODS

This cross sectional study was conducted in the Department of radiology and chemical pathology, PNS SHIFA hospital Karachi after approval by the Institutional review board. The sample size for analysis of variance was calculated based on a mean (standard deviation) value for TC of 185.2 (36.5) and 251.4 (53.9) for grade I and II NAFLD respectively while keeping probability of type I error at 0.05 and power of test at 0.9. Using these assumptions, a sample size of at least 15 in each group was obtained.

Individuals of either gender, aged more than 18 years who were diagnosed as having hepatic steatosis on USG abdomen were included by non probability consecutive sampling technique. The USG examinations were performed on GE Logic C5 premium using 5 MHz probe[6]. The hepatic steatosis was graded according to following criteria, Grade I: increased echogenecity of liver with visible periportal and diaphragmatic echogenecity; Grade II: increased liver echogenecity with imperceptible periportal echogenecity without obscuration of diaphragm; Grade III: increased echogenecity of liver with imperceptible periportal echogenecity and obscuration of diaphragm. Overall seventy three subjects with ultrasonographic evidence of NAFLD were

Table-I: Demographic, clinical and laboratory features of study population (n=73).

Characteristic	Overall Mean ± SD	Female (n=46) Mean ± SD	Male (n=27) Mean ± SD	*p*-value*
Age (years)	45.9 ± 11.1	45.4 ± 10.9	46.7 ± 11.5	0.631
Body mass index (kg/m^2)	25.6 ± 3.55	25.9 ± 3.8	25.1 ± 2.98	0.352
Waist circumference (cm)	91.1 ± 6.78	90.9 ± 7.34	91.3 ± 5.83	0.812
Serum Total Cholesterol (mmol/L)	4.97 ± 0.93	4.99 ± 0.9	4.95 ± 0.97	0.849
Serum Triglycerides (mmol/L)	2.13 ± 0.72	2.2 ± 0.7	1.98 ± 0.7	0.166
Serum HDL-Cholesterol (mmol/L)	0.99 ± 0.25	0.97 ± 0.28	1.01 ± 0.19	0.513
Serum NonHDL-Cholesterol (mmol/L)	3.98 ± 0.99	3.99 ± 0.99	3.97 ± 0.99	0.933
Serum LDL-Cholesterol (mmol/L)	3.07 ± 0.94	3.01 ± 1.0	3.16 ± 0.8	0.509

*Independent sample t-test was applied.

finally selected after excluding individuals with hepatitis, chronic liver disease, use of lipid lowering medicine and history of significant alcohol intake (more than 30 g/d in males and more than 20 g/d in females).

BMI was calculated for all the subjects by using the formula [weight (kg)/height (meter^2)][16]. WC was also noted. Three ml of blood sample were taken after an overnight fast of 12-16 hours by venipuncture in gel tube for serum lipid profile. All samples were analyzed for serum TC, TG, HDL and LDL using routine Spectrophotometric methods on Roche Modular p800, fully automated chemistry analyzer. Dyslipidemias were defined according to American association of clinical endocrinologists' guidelines for management of dyslipidemia and prevention of atherosclerosis 2012 as follows: High TC ≥5.2 mmol/L, high TG ≥ 1.7 mmol/L high LDL-C ≥3.4 mmol/L, high NonHDL-C ≥ 4.2 mmol/L and low HDL-C < 1.04 mmol/L in males while <1.3 mmol/L in females[19]. All data including demographic and biochemical parameters was analyzed by Statistical Package for Social Sciences version 20 (SPSS Inc, Chicago, IL, USA). Results were reported as the mean ± standard deviation (SD) or n (%) for continuous variables and as frequencies for categorical variables and were compared between both genders using independent t-test. Frequencies of various dyslipidemias in different grades of NAFLD were compared using chi-square test. One-way ANOVA analysis was utilized with Post hoc Bonferroni correction for multiple comparisons as appropriate to compare different variables (such as BMI, WC, TG, TC, HDL-C, LDL-C, and non-HDL-C) with grading of NAFLD. At 95% confidence interval, *p*-value less than 0.05 was considered as significant.

RESULTS

Out of 73 cases which were diagnosed as NAFLD on ultrasonography, grade I NAFLD cases were 42.5%, grade II were 37% and grade III were 20.5%. The mean age of the patients was 45.9 years. The mean body mass index (BMI) and waist circumference (WC) were 25.6 kg/m^2 and 91.1 cm respectively. 27 (37%) were males while 46 (63%) females with male to female ratio of 3:5. There is no significant difference in mean age, BMI, WC and serum lipid profile between two genders as shown in table-I.

Table-II: Frequency of dyslipidemia in various grades of NAFLD.

Variable	Overall (n=73) N (%)	Grade I (n=31) N (%)	Grade II (n=27) N (%)	Grade III (n=15) N (%)	*p*-value*
Serum Total Cholesterol (≥ 5.2 mmol/L)	27 (37)	4 (12.9)	11 (40.7)	12 (80)	0.000
Serum Triglycerides (≥1.7 mmol/L)	54 (74)	19 (61.3)	22 (81.5)	13 (86.7)	0.098
Serum HDL-Cholesterol (male: <1.04mmol/L; female: <1.3mmol/L)	67 (91.8)	25 (80.6)	27 (100)	15 (100)	0.012
Serum NonHDL-Cholesterol (≥ 4.2mmol/L)	26 (35.6)	4 (12.9)	10 (37)	12 (80)	0.000
Serum LDL-Cholesterol (≥ 3.4mmol/L)	28 (38.4)	6 (19.4)	12 (44.4)	10 (66.7)	0.006

*Chi-square test was applied.

Serum TG, TC, LDL-C and nonHDL-C levels were abnormally raised in 74%, 37%, 38.4% and 35.6% of subjects respectively while low serum HDL-C levels were seen in 90.5% of subjects. Chi-square test revealed significant difference among various grades of NAFLD for frequency of dyslipidemia in all parameters of lipid profile except serum TG (*p*-value=0.098) as shown in table-II. ANOVA test showed significant

difference for mean TC (p=0.000), LDL-C (p=0.000), HDL-C (p=0.005) and nonHDL-C (p=0.000) among different grades of NAFLD. However there was no significant difference for mean serum TG (p-value=0.224). The Bonferroni post hoc analysis revealed significant difference in mean TC, LDL-C, HDL-C levels of grade I and III of NAFLD while mean nonHDL-C is significantly different amongst all the grades of NAFLD as shown in table-III.

DISCUSSION

Alarming rise in prevalence of NAFLD demands to fully elucidate its pathogenesis and understand the risk factors responsible for progression of the disease[2,20]. The current study compared lipid profile among the patients with various ultrasound grades of NAFLD to evaluate the role of dyslipidemia in disease progression. A total of 73 nondiabetic, non-alcoholic subjects of both gender free from hepatitis participated in the present study. There was no statistically significant difference between two genders for mean age, BMI, WC and all parameters of lipid profile. Same findings were revealed by another Pakistani study by Bano et al except for the significantly raised BMI in their female subjects compared with males[21]. Seventy-four percent of all NAFLD patients had hypertriglyceridemia but chi-square test revealed no statistically significant difference among NAFLD grades in this regard. However, raised serum TC, LDL-C, nonHDL-C and low serum HDL-C levels were seen in 37%, 38.4%, 35.6% and 90.5% of all NAFLD subjects with statistically significant difference among NAFLD grades on chi-square test. Dyslipidemias among NAFLD subjects had been reported in several studies. Bano et al (2008) and Mahaling et al (2013) revealed that the most frequent dyslipidemia was hypertriglyceridemia followed by low HDL-C and hypercholesterolemia[6,21]. However, low HDL-C was the commonest dyslipidemia in our study. We also found that serum TC, HDL-C, LDL-C and nonHDL-C show statistical significance among different grades of NAFLD (p<0.05) while serum TG shows no statistical significance with increasing grades of NAFLD (p=0.05). An Indian cross-sectional study also revealed significant change in serum TC,

Table-III: Comparison of serum lipid profile among subjects with different grades of NAFLD (n=73).

Variables	Grade I (n=31) Mean ± SD	Grade II (n=27) Mean ± SD	Grade III (n=15) Mean ± SD	ANOVA p-value	Bonferroni post hoc test p-value		
					Grade I vs II	Grade I vs III	Grade II vs III
Serum Total Cholesterol (mmol/L)	4.5 ± 0.7	5.04 ± 1.01	5.7 ± 0.62	0.000	0.078	0.000	0.031
Serum Triglycerides (mmol/L)	1.98 ± 0.68	2.2 ± 0.66	2.4 ± 0.88	0.224	-	-	-
Serum HDL-Cholesterol (mmol/L)	1.09 ± 0.35	0.94 ± 0.08	0.86 ± 0.1	0.005	0.051	0.009	0.965
Serum NonHDL-Cholesterol (mmol/L)	3.46 ± 0.74	4.10 ± 1.05	4.88 ± 0.06	0.000	0.015	0.000	0.017
Serum LDL-Cholesterol (mmol/L)	2.65 ± 0.69	3.17 ± 1.02	3.76 ± 0.82	0.000	0.074	0.000	0.106

HDL-C and LDL-C and no relation of serum TG with increasing grade of NAFLD[6].

We found that serum nonHDL-C was the only parameter that significantly differs amongst all grades of NAFLD thus emphasizing upon its role in progression of NAFLD and making it a promising marker to predict the severity of NAFLD. This finding corroborates with several recent studies which have revealed that fatty liver-associated dyslipidemic profile was characterized by large VLDL, sdLDL, increased apolipoprotein B (ApoB) and decreased HDL-C and it has well correlated with the intrahepatic steatosis[22]. NonHDL-Cis a very promising calculated parameter as it includes all atherogenic lipoproteins including LDL-C, VLDL-C, intermediate density lipoprotein cholesterol (IDL-C) and lipoprotein (a) and is considered the surrogate marker for ApoB[7,18].

There are certain limitations in our study as the diagnosis and grading of NAFLD were based on ultrasonography and were not confirmed by liver biopsy, and observational study design that makes it nearly impossible to ascertain the causal relationship between various dyslipidemias and NAFLD.

CONCLUSION

Increasing severity of NAFLD was associated with increased frequency of dyslipidemia. The most frequent dyslipidemia in adults with NAFLD was low serum HDL-C followed by hypertriglyceridemia. It also reveals that only mean serum nonHDL-C was statistically different amongst all grades of NAFLD.

RECOMMENDATION

Further evaluation in terms of prospective and case control studies using reference method of liver biopsy for diagnosing and grading NAFLD are needed to support the importance of serum nonHDL-C as a predictive marker of severity of NAFLD.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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