Role of Lipid profile and Biochemical markers in Non-alcoholic Fatty Liver Disease patients in tertiary care hospital, Lahore

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ABSTRACT

Objective: To determine biochemical markers and lipid profile in patients with and without nonalcoholic fatty liver disease, and also compare their possible association with degrees of nonalcoholic fatty liver disease.
Study Design: Descriptive, cross sectional study
Place and Duration: Department of Gastroenterology, Lahore general hospital from 15th May 2019 to 20th January 2020.
Methodology: Through sequential sampling 650 individuals were enrolled in the study who came to hospital for routine checkup. Individuals having history of acute viral or chronic hepatitis B, C or previous liver disease were excluded. Blood sample was taken for analysis of all liver enzymes (Gamma Glutamyl Transferase, Alanine Aminotransferase and Aspartate Aminotransferase, Alkaline Phosphatase) and Lipid profile. Also, abdominal ultrasonography was performed to assess Non-alcoholic Fatty Liver Disease and its grades.
Results: Out of the total 650 participants, 55.69% were females and 44.30% were males. Mean age of individuals having nonalcoholic fatty liver disease was 47.49±7.06. Significant association was observed between fasting blood Glucose, total Cholesterol, low density Lipoprotein to high density Lipoprotein ratio, Gamma Glutamyl Transferase, Alanine Aminotransferase and Aspartate Aminotransferase with individuals having Non-alcoholic Fatty Liver Disease ($p<0.001$). Results showed that there was a significant relationship between liver enzymes ($p < 0.001$), and the severity of fatty liver.
Conclusion: Mild elevations of biochemical markers like liver enzymes and lipid profile are associated with Non-alcoholic Fatty Liver Disease.
Keywords: Cholesterol, Fasting blood glucose, Gamma Glutamyl Transferase, Non-alcoholic Fatty Liver Disease, Obesity, Ultrasonography.

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INTRODUCTION

Nonalcoholic Fatty Liver Disease (NAFLD) is worldwide widespread and is mostly asymptomatic and develop slowly[1]. Nonalcoholic Fatty Liver Disease (NAFLD) is recognized as an important public health problem nowadays. NAFLD involves a whole variety of liver pathologies from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, and hepatocellular carcinoma[2].

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Prevalence of NAFLD has increased over the past 20 years. Prevalence of NAFLD is 25% in non-obese Asian population in the world[3]. The community prevalence of NAFLD in South Asia and South East Asia ranges from 5-30%[4]. In Pakistan prevalence of NAFLD is 14.5%[5]. NAFLD is recognized by abnormal results of liver tests, imaging reports and liver biopsy[6]. It acts as a most likely cause of liver transplantation in future. The most common method for screening fatty liver is ultrasound[7,8].

Generally, population visits gastroenterologists when they find high levels of serum alanine transaminases. That is the main reason in many studies it is found that NAFLD diagnosis is made on the levels of aspartame transaminase (AST) and alanine transaminase (ALT)[9,10]. In clinical settings blood lipid profile and serum AST, ALT levels are done along with impaired blood sugar fasting levels[11,12]. All such biomarkers have a significant role in the development of NAFLD. These biochemical markers help in understanding the pathogenesis and future outcome of the disease so that early intervention can be done to reduce the risk of cirrhosis, hepatocellular carcinoma[13].

In the past, liver biopsy was the primary investigation to

determine the severity of NAFLD. It also serves to exclude other liver diseases. However, it is an invasive procedure with a 0.3% risk of bleeding. Patient acceptability is low, and it is undesirable to perform liver biopsy repeatedly to assess disease progression and treatment response. More importantly, liver biopsy is not a real gold standard for the evaluation of the histological features of NAFLD[14]. The justification for this research was that if changes are observed in the biochemical markers of NAFLD then ultrasound should be done so that further complications can be prevented. The objective of the study is to determine association of biochemical markers and lipid profile in patients with and without Non-alcoholic Fatty Liver Disease, and also compare their possible association with degrees of Non-alcoholic Fatty Liver Disease.

METHODOLOGY

This is a cross-sectional study, in which 650 patients were enrolled from 15th May 2019 to 20th January 2020 after getting approval from ethical review committee. The study was conducted at Lahore General Hospital, Gastroenterology ward. All patients of both gender having age ranges between 35–65 years and who never treated with any antihyperglycemic, antihypertensive, lipid lowering drugs were included in the study. Patients suffering from chronic viral hepatitis B and C, hemochromatosis, autoimmune liver disease, alpha-1 antitrypsin deficiency, Wilson's disease, CKD, cancers, co-morbidities, pregnancy, Drug induced liver injury (DILI) were excluded from the study[15].

To counter the effects of bias, a structured proforma was provided to doctors (data collectors), to ensure uniformity among all participants recruited into the study. Clear definitions were provided to minimize biasing. BMI was determined by dividing the body weight by the square of height. Blood pressure (BP) was measured using the oscillometric method, in the sitting position. Blood samples were collected after an overnight fast[16]. The following biomarkers were investigated: High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), Triglyceride (TG), Glucose, Aspartate Aminotransferase (AST), Aaminotransferase (ALT), and Gamma-glutamyl Transpeptidase (G-GTP). Liver enzymes as well as the lipid profile was done by ELISA method using HITACHI automated biochemistry analyzer. Fatty liver was confirmed by doing abdominal ultrasound. On the basis of ultrasound findings three grades of fatty liver were made. Grade I is mild in which raised echogenicity of liver parenchyma with noticeable periportal and diaphragm. Grade II (moderate) with raised echogenicity of liver parenchyma having obstruction in the walls of the portal vein branches but diaphragm was not block. Grade III (Severe) in which elevated echogenicity of liver parenchyma with untraceable periportal echogenicity and diaphragm obstruction[17].

Data Analysis: SPSS 23 was used for analysis. Quantitative variables were expressed as mean ± standard deviation and qualitative variables like gender, frequency and percentages were calculated were expressed by number (%). For comparison of the two groups independent sample *t*-test was used. One-way ANOVA was applied for describing association of biochemical markers with ultrasound-based grading of NAFLD. *p* value less than 0.05 was considered significant.

RESULTS

A total of 650 subjects participated in the study out of which 362 (55.70%) were female and 288 (44.30%) were male. Among them, 183 (50.55%) females had NAFLD. The minimum age was 35 and the maximum was 65 years, Majority patients, 302 (46.46%) were in age group 46-55 years in which 199 (65.89%) had NAFLD. Descriptive variables of patients with NAFLD present and NAFLD absent is reported in Table-I

Table-I: Descriptive variables of patients with NAFLD present and NAFLD absent (N=650)

Parameter		Total N=650(%)	NAFLD present n=348 (%)	NAFLD absent n=302(%)
Gender	Male	288(44.30%)	165(57.29%)	123(42.70%)
	Female	362(55.70%)	183 (50.55%)	179(49.44%)
Age years	35-45	185(28.46%)	81(43.78%)	104(56.22%)
	46-55	302(46.46%)	199(65.89%)	103(34.11%)
	56-65	163(25.08%)	68(41.72%)	95(58.28%)

Table-II: Comparison of biochemical markers and lipid profile in NAFLD present and NAFLD absent patient by Independent-Samples T-test (N=650)

Variables	NAFLD present n= 348	NAFLD absent n= 302	p value
Age (years)	47.49±7.06	45.49±7.54	0.43
BMI kg/m^2	26.24±4.32	23.43±3.42	<0.001
Systolic BP (mmHg)	125.64±18.84	115.41±15.50	<0.001
Diastolic BP(mmHg)	85.74±12.15	78.12±11.61	<0.001
FBS (mg/dl)	110.02±41.05	95.61±25.78	<0.001
AST (U/L)	23.18±12.47	19.45±8.75	<0.001
ALT (U/L)	28.35±18.11	18.25±14.28	<0.001
ALP (U/L)	192.21±63.81	185.02±21.29	0.27
GGT (U/L)	42.31±34.65	24.34±19.71	<0.001
AST/ALT ratio	0.81±1.45	0.93±1.49	<0.001
Triglycerides mg/dl	204.21±98.91	151.17±86.22	<0.001
Total cholesterol mg/dl	208.70±40.44	195.68±53.82	0.006
HDL-C (mg/dl)	43.57±7.65	45.67±7.95	<0.001
LDL-C (mg/dl)	122.28±23.50	121.18±14.81	0.72
LDL-C/HDL-C (ratio)	2.80±3.09	2.65±1.86	0.003

(FBS Fasting blood sugar, AST Aspartate aminotransferase, ALT Alanine aminotransferase, ALP Alkaline phosphatase, GGT gamma glutamyl transferase, HDL-C High density lipoprotein cholesterol, LDL-C Low density lipoprotein cholesterol).

When the changes in biochemical parameters were compared with different degrees of NAFLD, the research results showed that there was a significant relationship between GGT p = 0.004, ALT p < 0.001 and AST p < 0.001 and the severity of fatty liver.

In this study, the mean age of the subjects with NAFLD was 47.49 ± 7.06 years, and with non NAFLD was 45.49±7.54 years. The mean BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) was, 26.24±4.32, 125.64±18.84 and 85.74±12.15 in NAFLD present cases. The total mean of the results of lipid profile and level of hepatic enzymes is presented in Table-II. The results showed raised levels of FBS, TC, LDL/HDL ratio, AST/ALT ratio, GGT, ALT, and AST and reduction of HDL in the patients having NAFLD and a significant relationship was observed (p < 0.05). However, the level of LDL and ALP was not significant between the groups.

Table-III: Association of biochemical markers with ultrasound-based grading of NAFLD using one-way ANOVA (N=650)

Marker	Grade I	Grade II	Grade III	p value
AST U/L	21.37±11.08	24.42±15.56	27.36±9.19	<0.001
ALT U/L	26.26±18.59	36.79±8.26	34.19±18.64	<0.001
ALP U/L	193.24±64.21	198.65±54.16	201.37±24.55	0.52
GGT U/L	30.52±23.65	40.77±36.13	32.59±11.88	0.004
FBS mg/dl	107.16±35.35	113.76±44.88	114.27±30.34	0.26
Triglycerides mg/dl	167.88±99.11	208.85±98.09	197.00±46.63	0.81
Total cholesterol mg/dl	203.51±27.38	209.37±63.85	206.63±46.44	0.95
HDL-C mg/dl	43.42±9.08	43.28±6.47	45.73±6.87	0.44
LDL-C mg/dl	119.74±21.70	122.30±23.66	136.65±59.69	0.85

DISCUSSION

NAFLD is a silent disease and this study helps in expressing the relationship between clinical sign symptoms with biochemical markers. Our study shows no significant relation between age and NAFLD and results are consistent with other study done by Pardhe *et al*. However, two studies reported stated significant relation between age and NAFLD[18]. With aging, the liver undergoes substantial changes and more risk factors come for development of NAFLD. One study showed age as confounding variable for accurate non-invasive diagnosis of NAFLD[19].

In the present study, the mean DBP and SBP in the NAFLD group was found raised than the non-NAFLD group, a significant relationship was observed between BP and NAFLD. This result is in accordance with the findings of a number of studies. It is reported that carotid artery wall thickness is increased in patients with NAFLD and that is the reason of high blood pressure among them. The present study showed that with elevation of FBS level, the possibility of developing NAFLD increases and there is a significant relationship between them. Various studies also confirmed this finding because people who are overweight and have fatty liver are at risk of developing insulin resistance[20].

Dyslipidemia is known as a risk factor for NAFLD. In this study, individuals in the NAFLD group had abnormal lipid profile showing a higher TC, LDL/HDL ratio and lower HDL as compared to those individuals in which NAFLD is absent. Further, in the individuals having NAFLD, a significant relationship was observed with TG, but no significant relationship was seen between LDL and NAFLD. In a study conducted by McPherson S *et al*. patients with NAFLD had higher TC, LDL, and TG, and lower HDL as compared to the control group[21]. In another study, again, mean LDL, and TC was higher than the normal range among NAFLD subjects[22]. Further, Novakovic et al, compared chemical parameters with NAFLD and found that there is a significant relationship between TG, LDL, TC, and HDL, and an inverse relationship with HDL in the group. Various studies indicated similar results.

In this study, the mean levels of hepatic enzymes were higher in the NAFLD group, and apart from ALP p = 0.27. ALT, GGT, AST are markers of liver injury and may be useful surrogate measures of NAFLD. ALT is located in the hepatocellular cytosol, whereas AST is mostly within the mitochondria. In fact, NAFLD and NASH have been reported to be most common causes of chronically elevated liver enzymes and is often the tipping point for further diagnostic evaluation[23]. In the study of Novakovic et al[20] a significant relationship was observed between hepatic enzymes (ALT, GGT, AST/ALT ratio) apart from AST and NAFLD. Most previous studies have shown that there is a significant relationship between NAFLD and AST, ALT and ALP. Zakeri and colleagues has stated that ALT and dyslipidemia might be involved in the prevalence and development of NAFLD[24].

In this study, a significant relationship was observed between hepatic enzymes GGT, ALT and AST and NAFLD degrees. For preliminary diagnosis of NAFLD, ultrasonography can be used. It can be posited that sonography with the minimum cost and complications is the cheapest method for identifying NAFLD-associated changes. A study reported the investigation of fatty liver degrees through sonography indicated that AST, ALT, TG and FBS had a relationship with NAFLD grades, though it did not have any relationship with age, LDL, HDL and TG. Further, Mahaling *et al*. showed increased degrees of NAFLD with elevation of TC p =0.001, LDL p = <0.001 and VLDL p = 0.003 and reduction of HDL p =<0.001 but no significant relationship was observed between TG and NAFLD degrees[25]. A study also showed a significant relationship between hepatic enzymes (ALT, ALP) and dyslipidemia (TG, HDL), and different grades of NAFLD which is in accordance with our study[26].

CONCLUSION

Mild elevations of biochemical markers like liver enzymes and lipid profile are associated with nonalcoholic fatty liver disease.

Limitations of study: The limitation of this study was the use of ultrasonography to detect NAFLD. Liver biopsy is a golden standard for diagnosing fatty liver but due to high risk of complications and high cost, it is not recommended in general population. On the other hand, abdominal ultrasonography is a noninvasive, low risk, simple, relatively low-cost and easily available method. In this study, to control this limitation, the comments of two radiologists were used concurrently. Secondly, confounding variables effecting NAFLD must be analyze

statistically by multiple regression in future studies.

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AUTHOR'S CONTRIBUTION

Kiran N: Conceived Idea, Designed Research Methodology, Statistical Analysis, Manuscript final reading and approval
Waqas S: Data Collection, Literature Search, Data Interpretation, Manuscript Writing

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REFERENCES

1. Ahmed M. Non-alcoholic fatty liver disease in 2015. World J Hepatol. 2015; 7(11):1450–1459. doi: 10.4254/wjh.v7.i11.1450
2. Jain P, Parate R, Dubey T, Jain R. Prevalence of NAFLD (non-alcoholic fatty liver disease) in metabolic syndrome and their correlation with various biochemical and serologic parameters for early detection and detecting patients of lean Nash (Non-alcoholic steatohepatitis) Prevalence. 2018;3:24–28. doi: 10.4103/jfmpc.jfmpc_243_18
3. LaBrecque DR, Abbas Z, Anania F, Ferenci P, Khan AG, Goh K-L, et al. World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J of Clin Gastroenterol. 2014;48:467–473
4. Shah AS, Khan S, Rahim H, Chishti KA, Ghafoor Khan AG. Prevalence of non alcoholic fatty liver and Non alcoholic Steatohepatitis in Peshawar Cantonment, Khyber Pakhtunkhwa, Pakistan. Pak J of Pharma Sci. 2018;31:193-198.
5. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterol. 2011;140(1):124-131. https://doi.org/10.1053/j.gastro.2010.09.038
6. Moghaddasifar I, Lankarani K, Moosazadeh M, Afshari M, Ghaemi A, Aliramezany M, et al. Prevalence of non-alcoholic fatty liver disease and its related factors in Iran. Int J Organ Transplant Med. 2016;7:149–160
7. Chun-Jen L. Prevalence and risk factors for non-alcoholic fatty liver disease in Asian people who are not obese. J Gastroenterol Hepatol1. 2012;27:1555–1560.
8. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010;28:155-161. https://doi.org/10.1159/000282080
9. Wai JW, Fu C, Wong VW. Confounding factors of non-invasive tests for nonalcoholic fatty liver disease. J of Gastroenterol. 2020;55:731-741.
10. McLernon DJ, Donnan PT, Sullivan FM, Roderick P, Rosenberg WM, Ryder SD, et al. Prediction of liver disease in patients whose liver function tests have been checked in primary care: Model development and validation using population-based observational cohorts. BMJ Open. 2014;4:e004837.
11. Swain M, Nath P, Parida PK, Narayan J, Padhi PK, Pati GK, et al. Biochemical profile of nonalcoholic fatty liver disease patients in eastern India with histopathological correlation. Indian J Clin Biochem. 2017;32:306–314.
12. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. PloS One. 2015;10:e0140908. https://doi.org/10.1371/journal.pone.0140908
13. Alterio A, Alisi A, Liccardo D, Nobili V. Non-alcoholic fatty liver and metabolic syndrome in children: A vicious circle. Horm Res Paediatr. 2014;82:283–289.
14. Das K, Das K, Mukherjee PS, Ghosh A, Ghosh S, Mridha AR, et al. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. Hepatology. 2010;51:1593–1602. https://doi.org/10.1002/hep.23567
15. Miele L, Zocco MA, Pizzolante F, De Matthaeis N, Ainora ME, Liguori A, Gasbarrini A, Grieco A, Rapaccini G. Use of imaging techniques for non-invasive assessment in the diagnosis and staging of non-alcoholic fatty liver disease. Metabolism. 2020;9:154355.
16. Seetlani NK, Memon AR, Tanveer S, Ali A, Ali P, Imran K, Haroon H. Frequency of Non-Alcoholic Steatohepatitis on Histopathology in Patients of Type 2 Diabetes Mellitus with Duration of More than 5 Years. J Coll Physic Surg Pak. 2016;1;26(8):643.
17. Aneni EC, Oni ET, Martin SS, Blaha MJ, Agatston AS, Feldman T, et al. Blood pressure is associated with the presence and severity of nonalcoholic fatty liver disease across the spectrum of cardiometabolic risk. J of Hypertension. 2015;33:1207–1214. DOI**:** https://doi.org/10.1097/HJH.0000000000000532
18. Pardhe BD, Shakya S, Bhetwal A, Mathias J, Khanal PR, Pandit R, et al. Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal. BMC Gastroenterol. 2018;18:109.
19. Uppalapati GP, Harish K. A study of clinical, biochemical and sonological profile of non-alcoholic fatty liver disease in type 2 diabetes patients. J Evid Based Med Health. 2017;4:5414–5417.
20. Novakovic T, Mekic M, Smilic L, Smilic T, Inić-Kostic B, Jovicevic L, et al. Anthropometric and biochemical characteristics of patients with nonalcoholic fatty liver diagnosed by non-invasive diagnostic methods. Med Arch. 2014;68:22–26. doi: 10.5455/medarh.2014.68.22-26
21. McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez

M, Allison M, Oliveira CP, Francque S, Van Gaal L, Schattenberg JM, Tiniakos D. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. The Am J of Gastroenterol. 2017;112(5):740.

22. Santhoshakumari TMJ, Radhika G, Kanagavalli P. A study of anthropometric and lipid profile parameters in non-alcoholic fatty liver disease patients attending a tertiary care hospital at Puducherry. IOSR J Dent Med Sci. 2017;16:33–37.
23. Cuenza LR, Razon TLJ, Dayrit JC. Correlation between severity of ultrasonographic nonalcoholic fatty liver disease and cardiometabolic risk among Filipino wellness patients. J Cardiovasc Thorac Res. 2017;9:85–89.
24. Zakeri A, Karamat-Panah S. Prevalence of non-alcoholic fatty liver disease and its risk factors in patients referred to Ardabil city hospital during 2015-2016. Int J Community Med Pub Health. 2018;5:917–921. DOI: http://dx.doi.org/10.18203/2394-6040.ijcmph20180747
25. Mahaling DU, Basavaraj MM, Bika AJ. Comparison of lipid profile in different grades of non-alcoholic fatty liver disease diagnosed on ultrasound. Asian Pac J Trop Biomed. 2013;3:907–912. https://doi.org/10.1016/S2221-1691(13)60177-X
26. Ghani RA, Saqlain M, Zafar MM, Jabeen S, Naqvi SM, Raja GK. Identification of Metabolic risk phenotypes predisposing to Non-Alcoholic Fatty Liver Disease in a Pakistani Cohort. Pak J of Med Sci. 2017;33(1):121.