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ASSOCIATION OF LIFESTYLE AND METABOLIC RISK FACTORS WITH GRADES OF FATTY LIVER IN NON DIABETIC NAFLD PATIENTS.

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ABSTRACT

Introduction: Non Alcoholic Fatty Liver (NAFL) is a public health problem in India. **Objectives:** To study association of lifestyle and metabolic risk factors with grades of fatty liver (FL). **Materials and Methods:** NAFL (n=160) cases constituted the study group, FL was diagnosed by ultrasound of abdomen. Anthropometric and biochemical parameters were recorded. Lifestyle and metabolic risk factors were compared in different grades of FL. **Results:** Grade 1 FL was present in 69 % and grade 2 in 31 % of subjects. The prevalence of lifestyle risk factors were higher in grade 2 FL versus grade 1 FL but were non-significant: Low physical activity (57.1 % versus 55.8 %), snack intake (87.7 % versus 81.0 %) , saturated fatty acid >8% of total calories (18.3 % versus 16.2 %), edible oil >25 g for males and >20 g for females (93.8 % versus 90.9 %) alcohol intake (81.6 % versus 78.3 %). The prevalence of metabolic risk factors were significantly higher in grade 2 FL versus grade 1 FL: BMI > 25 (87.7 % versus 61.2%), waist circumference (75.5% versus 45.0 %) , WHR (91.8 % versus 76.5%), IGT (24.4 % versus 6.3%),triglycerides (53.0 % versus 28.8 %), Metabolic Syndrome (MetS) (46.9 % versus 22.5 %). On univariate regression analysis, grade 2 FL showed positive association with metabolic factors [BMI > 25 [OR (95% CI) [11.38 (1.46-18.37), waist circumference [3.7 (1.77-7.97), WHR [3.4 (1.13-4.47), IGT [4.8 (1.76-13.16), high serum triglycerides [2.7 (1.39-5.59) and MetS [3.0 (1.48-6.22). **Conclusions** The worsening grades of fatty liver are associated with metabolic risk factors and should initiate workup of patients to avoid progression of NAFL to severe forms.

Keywords: Non alcoholic fatty liver , grade of fatty liver, lifestyle risk factors, metabolic risk factors, non diabetic

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is stated to be the leading cause of liver disease globally (1) . It's a public health problem worldwide with a enormous burden on healthcare system. NAFLD includes a broad spectrum of its benign form in which there is infiltration of fats in the liver (Hepatic Steatosis), followed by inflammation (NASH), fibrosis, cirrhosis and hepato cellular carcinoma (HCC) in the absence of alcohol intake and other known causes of CLD (2).

The prevalence rate of NAFLD reported across the globe is estimated to be 25 %. The highest rates of prevalence are from South America (30.45 %), followed by Middle East & Australia (20-30 %), Asia (25 %) of which India is 8.7 -32.6%, USA & Europe (24%) & lowest rates are reported from Africa (13%) (3).

NAFLD is stated to be associated with Obesity, Insulin resistance, T2DM, hypertension, hyperlipidemia and MetS. It is also associated with lifestyle factors like faulty diets, lack of physical activity, use of alcohol, smoking, which contribute to deposition of fat in liver and increase mortality (4,5,6). Early diagnosis of underlying predisposing factors and treatment of NAFLD is important to avoid the progression of liver damage. Liver biopsy is the gold standard test for diagnosis, grading, and histological assessment of NAFLD. But due to its invasive nature and the risks and costs associated with the liver biopsy it is not used in routine practice.

The ultrasound examination is clinically easy-to-use, economical and non-invasive as a tool to detect NAFLD, compared to the gold standard, liver biopsy. To identify hepatic steatosis, USG has sensitivity from 60 to 94% and specificity from 84 to 95% (7) and sensitivity is more than 90% when liver biopsy shows > 20% steatosis (8).

To date, there has been no study in India to link lifestyle and metabolic risk factors and ultrasound-based severity grading of NAFLD. We aimed to study the association between lifestyle and metabolic risk factors and ultrasound-based grades of NAFLD in non diabetic subjects.

MATERIALS AND METHODS

A hospital based, case control study was undertaken in the period of September 2015 to September 2016 at AIIMS, N Delhi. The study was approved by the Institutional Ethics Committee of Lady Irwin College and AIIMS. Adults of both genders (18 - 60 years) were included in the study. A total of 160 NAFLD cases who fulfilled the inclusion criteria for Cases were enrolled from the Gastroenterology & Medicine OPDs of AIIMS.

Eligibility Criteria for Cases included (i) Fatty liver on radiological examination (ultrasound) in the previous month (ii) By interview-cum-questionnaire method that the current /recent alcohol consumption is less than 21 drinks on average per week in males and less than 14 drinks on average in females per week(9). Exclusion criteria for Cases included (i) Past history of CLD (Chronic Liver Disease) and (ii) history of intake of drugs leading to fatty liver like steroids, tetracycline, tomoxifen, valproic acid, oral contraceptives or corticosteroids (iii) Patients with Type 2 Diabetes, CVD,IBD, HIV infection, pregnant and lactating women (iv) Patients with presence of HBs Ag (Hepatitis B Surface Antigen),anti-HCV (Antibody to Hepatitis C Virus) and anti HIV antibody (vi) Any use of medications for weight loss /participation in weight loss programs. (vii) Providing no consent for the study.

Detailed sample size calculation is published in study by Chaturvedi et al, 2017(10). The data on the sub group of NAFLD Cases (n=160) from above referred study data are presented in this research article. Equal number of males (n=80) and equal number of females (n=80) comprised the NAFLD Cases.

A pretested questionnaire cum interview schedule was used to collect demographic and household information, based on educational status, occupation and income (11). Anthropometric measurements using standard WHO techniques were undertaken (12). The height was measured to the nearest 0.1 cm using a stadiometer. Weight was measured to the nearest 100 grams with electronic scale (Seca Model 803). Waist circumference and hip circumference were measured to the nearest 0.1 cm and at a level midway between the lowest rib and the iliac crest and at the level of the great trochanter respectively. Quetelet's ratio was used for calculation of BMI (Weight in kilograms divided by the square of height in meters). Cut offs as per Asian Indian population were used for BMI and Waist - Hip Ratio (13). Body circumferences (Waist and hip circumfererence) were measured by non elastic, non stretchable fiber glass tape. Clinical parameters included measurement of Systolic blood pressures (SBP) and Diastolic blood pressures (DBP) which were recorded in a sitting position. An average of three readings was noted after a gap of five minutes using an automated blood pressure instrument (Omron HEM-7203, Kyoto, Japan). One single trained radiologist performed trans-abdominal ultrasound of the liver using a 1-5 MHz curvilinear transducer (iU22, Philips, Netherlands) after an overnight fast. The radiologist was blinded for laboratory and anthropometric data of the participants. Brightness and posterior attenuation were considered indices of the extent of fatty infiltration.

The ultrasound-based grades of NAFLD consisted of grade 0 (no NAFLD), grade 1 (increased liver echogenicity with normal images of intrahepatic vessel lines and diaphragm), grade 2 (blurred image of intrahepatic vessel lines) and grade 3 (blurred images of intrahepatic vessel lines and diaphragm) (14)**.**

Data was analyzed with help of statistical software STATA 14.0. The continuous descriptive data is presented as means and standard deviation for normally distributed data and as medians and Inter quartile range (IQR) for non normally distributed data. Categorical variables are reported as percentages. Differences in continuous a variable was computed with independent t test for normally distributed data and with nonparametric analysis (Mann Whitney U test) for none normally distributed data. Differences between categorical variables were determined by Pearson chi square test or Fishers exact test. All p values were interpreted at the 5 % level of significance. Logistic regression analysis was used to estimate Odds Ratio. Statistical p-value was significant at ≤ 0.05.

RESULTS

A total of 160 NAFLD cases, out of which 80(50%) were males and 80(50%) were females. In the present study 69.4 % (n=111) had Grade 1 Fatty Liver and 30.6% (n=49) had Grade 2 Fatty Liver (Figure 1).

There were 47 (42.3 %) males and 64(57.7 %) females in grade 1 fatty liver and 33(67.3 %) males and 16(32.7 %) females in grade 2 fatty liver. There was a significant higher percentage of males in Grade 2 fatty liver, compared to females ($p<0.004$).

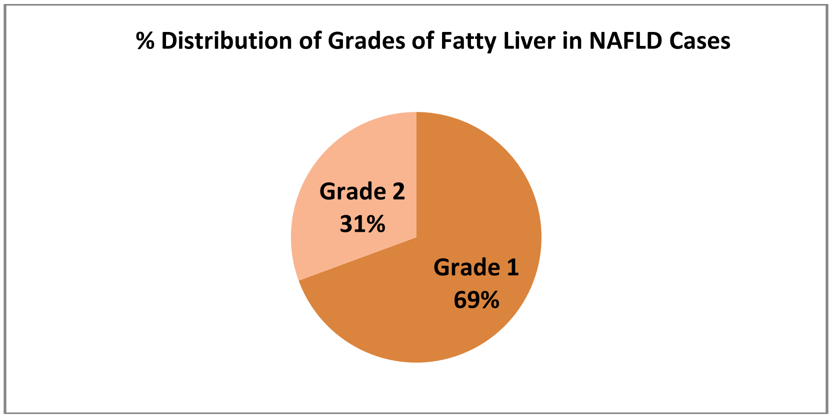

Figure 1. Percent Distribution of Grades of Fatty Liver in NAFLD Cases

The anthropometric and biochemical characteristics of NAFLD Cases are presented in Table 1. The characteristics included gender, age, BMI, waist circumference, WHR, Waist Height Ratio and biochemical parameters like fasting blood glucose, IGT, total cholesterol, triglycerides, LDL, HDL, liver enzymes, blood pressure and presence of metabolic syndrome.

The mean age of the NAFLD cases was 39.8 ± 8.4 years. The maximum number of cases (93/160) occurred in 30-45 age group. The mean BMI of NAFLD cases was 26.9±3.5.

The distribution of NAFLD cases in grades of obesity based on BMI revealed that 11.9 % had BMI in normal range (BMI:18-22.9), 18.7 % were overweight(BMI: 23-24.9) and 69.3 % were obese (BMI>25).

Table 1- Anthropometric and biochemical characteristics of NAFLD Cases

Variables	**Cases (Mean)(n=160)**	**Range**
Age	39.8 ± 8.4 years (160)	18-60
Height	162.0±8.64	(139.5-183.5)
Weight	70.89±11.66	(44.7-102.3)
BMI	26.98±3.48	(19.6-37.1)
Waist circumference	86.57±8.87	(64-112)
Hip circumference	100.53±8.89	(56-120)
WHR	0.86±0.09	(0.68-1.3)
WHtR	0.53±0.04	(0.39-0.66)
Fasting Glucose	94.98 ±10.0	(67-130)
Total Cholesterol	190.23 ± 40.68	(81-322)
Triglycerides * (mg/dl)	125.5	(90.5-171.5)
HDL	43.46 ± 7.25	(20-62)
LDL	122.32 ± 32.63	(45-215)
SGOT (IU/L)	28.05±10.81	(13-73)
SGPT *(IU/L)	29	(19-44)

Independent t test applied. Figures in parenthesis denotes ranges.

***Represents data represented as Median (IQR) & Wilcoxon Rank sum test applied.**

Table 2 shows the Lifestyle Risk Factors as defined by presence /absence and cutoffs based on guidelines were assessed and compared in different grades of fatty liver in NAFLD cases by the frequency distribution, followed by logistic regression of the individual lifestyle risk factors The prevalence of lifestyle risk factors were higher in grade 2 FL versus grade 1 FL but were non-significant: Low physical activity (57.1 % versus 55.9 %), snack intake (87.8 % versus 81.1 %) , saturated fatty acid >8% of total calories (18.4 % versus 16.2 %), edible oil >25 g for males and >20 g for females (93.9 % versus 90.9 %) alcohol intake (81.6 % versus 78.4 %).

Table 2 -Association of Lifestyle risk factors as per grades of fatty liver in NAFLD cases

Lifestyle Factors	**Grade1(n=111) n (%)**	**Grade 2(n=49) n (%)**	**p Value**	**OR(95% CI)**	**p value**
Low Physical Activity					
Absent	49 (44.1)	21(42.9)			
Present	62 (55.9)	28(57.1)	0.880	1.05(0.53-2.07)	0.880
Snack intake					
Absent	21(18.9)	6(12.2)			
Present	90(81.1)	43(87.8)	0.299	1.67(0.62-4.44)	0.302
Saturated fatty acid >8% of total calories					
< 8 %	93(83.8)	40(81.6)			
>8 %	18(16.2)	9(18.4)	0.738	1.16(0.48-2.80)	0.738
Edible oil >25 g for males and >20 g for females					
<25 g	10(9.1)	3(6.1)			
>25 g	101(90.9)	46(93.9)	0.538	1.51(0.39-5.77)	0.540
Alcohol intake					
Absent	24(21.6)	9(18.4)			
Present	87(78.4)	40(81.6)	0.639	0.81(0.34-1.91)	0.639

Categorical variables are presented as sum and percentages/ Data are presented as n (%).
Table 3 depicts the metabolic risk factors as defined by cutoffs based on guidelines were assessed and compared in different grades of fatty liver in NAFLD cases by the frequency distribution, followed by logistic regression of the individual metabolic risk factors

The prevalence of metabolic risk factors in NAFLD cases in Grade 2 versus Grade 1, for BMI > 25, was (87.8 % versus 61.3 %) versus Waist Circumference of >80 for Females/90 for Males was (75.5 % versus 45.0 %) versus WHR >0.80 for Females/0.88 for Males (91.9 % versus 76.6 %) versus IGT 2 h PP ≥140 <200 & FG <126 (24.5 % versus 6.3 %) Triglycerides ≥150 mg/dl (53.0 % versus 28.8 %) presence of MetS (46.0 % versus 22.5 %), ($p<0.05$) for all variables.

The odds for Grade 2 fatty liver were 11 times higher compared to those when BMI was >25 [OR(95 % CI) 11.38(1.46-88.37),p=0.020. The change in BMI was statistically significant in different grades of fatty liver where BMI was found to increase with steatosis.

The risk of developing Grade 2 fatty liver was 3.7 times higher when WC was above the cutoff range [OR(95 % CI) 3.7(1.77-7.97), (p=0.001).The odds for Grade 2 fatty liver were 3.4 times higher compared to those when WHR was below the standard cutoffs [OR(95 % CI) 3.4(1.13-0.47), (p=0.030). The change in Central obesity (WC and WHR) was statistically significant in different grades of fatty liver and was found to increase with steatosis.

The risk of developing Grade 2 fatty liver was 4.8 times higher when IGT is above the cutoff range [OR(95 % CI): 4.8 (1.76-13.16)(p=0.002). The risk of developing Grade 2 fatty liver was almost 3 fold higher when triglycerides was above the cutoff range [OR(95 % CI): 2.7 (1.39-5.59) (p=0.004). The change in triglycerides was statistically significant in different grades of fatty liver where triglycerides were found to increase with steatosis.

There was a significant association of ultra sound grade of fatty liver with MetS. The risk of developing Grade 2 fatty liver was 3 fold higher when Metabolic Syndrome was present using ATP 3 criteria [OR(95 % CI): 3.0(1.48-6.22)(p=0.002).

There was no significant change in Total cholesterol. HDL-C and LDL-C between different grades of NAFLD. Similarly there were no significant changes in high blood pressure, liver enzymes between the grades.

Table 3 Comparison of Metabolic Risk Factors as per Grades of Fatty Liver in NAFLD Cases by Logistic Regression

Abnormalities	**Grade1(n=111) n (%)**	**Grade 2(n=49) n (%)**	**p Value**	**OR(95% CI)**	**p value**
BMI					
Normal(18-22.9)	18(16.2)	1(2.0)		1	
Overweight(23-24.9)	25(22.5)	5(10.2)		3.6(0.38-33.50)	0.260
Obese(>25)	68(61.3)	43(87.8)	**0.003**	11.38(1.46-88.37)	**0.020**
Abdominal Obesity WC (cms)					
≤80 for Females/90 for Males	61(54.0)	12(24.5)		1	
>80 for Females/90 for Males	50(45.0)	37(75.5)	**<0.001**	3.7(1.77-7.97)	**0.001**
WHR					
≤ 0.80 for Females/0.88 for Males	26(23.4)	4(8.1)		1	
>0.80 for Females/0.88 for Males	85(76.6)	45(91.9)	**0.023**	3.4(1.13-0.47)	**0.030**
Fasting Blood Glucose(mg/dl)					
≤100	84(75.7)	31(63.3)		1	
>100	27(24.3)	18(36.7)	0.108	1.8(0.87-3.72)	0.110

IGT					
2 h PP ≤140 >200	104(93.7)	37(75.5)		1	
2 h PP ≥140 <200 & FG <126	7(6.3)	12(24.5)	**0.001**	4.8(1.76-13.16)	**0.002**
Hypercholesterolemia(mg/dl)					
≤ 200	74(66.7)	29(59.1)		1	
> 200	37(33.3)	20(40.9)	0.362	1.3(0.68-2.75)	0.363
HDL-C(mg/dl)					
≥40 for Males/50 for Females	61 (54.0)	23(46.0)		1	
<40 for Males/50 for Females	50(45.0)	26(53.0)	0.349	1.4(0.70-2.70)	0.350
Hypertriglyceridemia (mg/dl)					
<150	79(71.7)	23(46.94)		1	
≥150	32(28.83)	26(53.06)	**0.003**	2.7(1.39-5.59)	**0.004**
LDL(>130) (mg/dl)					
≤130	69(62.2)	30(61.2)		1	
>130	42(37.8)	19(38.8)	0.910	1.0(0.52-2.07)	0.910
High Blood Pressure (mmHg) (≥130/85)-					
No	83(74.8)	35(71.4)		1	
Yes	28(25.2)	14(28.6)	0.479	1.3(0.64-2.53)	0.479
Liver Enzymes					
SGOT (<40 U/L)	108 (97.3)	45(91.9)		1	
SGOT (>40 U/L)	3 (2.7)	4(8.1)	0.120	3.1(0.68-4.87)	0.138
SGPT (<40 U/L)	97(87.4)	38(77.6)		1	
SGPT (>40 U/L)	14(12.6)	11(22.4)	0.114	2.0(0.83-4.80)	0.119
Metabolic Syndrome					
Absent	86(77.5)	26(53.1)		1	
Present	25(22.5)	23(46.9)	**0.002**	3.0(1.48-6.22)	**0.002**

Categorical variables are presented as sum and percentages/ Data are presented as n (%).

DISCUSSION

The majority of NAFLD cases were in 30-45 years age group. The younger age of presentation is due to the Westernized lifestyle followed by the younger generation. Lifestyle with a high calorie intake and low physical activity predisposes to obesity which is an underlying factor in etiology of NAFLD. There was a significant higher percentage of males in Grade 2 Fatty liver, compared to females (p<0.004). Similar results were reported by Mohammadi et al. (15) wherein male patients had severe grades of NAFLD. More men have NAFLD than women in a meta-analysis study based on epidemiologic research (4) Interesting results of gender as the risk factor come from a systematic review about NAFLD in Asia.

In the present study the higher BMI is associated with the increase of ultra based NAFLD grades. Similar results were reported by published studies (16,17,18). BMI is a predictor of NAFLD severity and ultra sound grade. The prevalence of obesity increased grade wise implying that there is a linear relationship of higher BMI with higher prevalence of NAFLD (19).

Central obesity (WC and WHR) were both associated with grades of NAFLD. With increase in waist circumference, the grade of fatty liver increased. The results were similar to studies by Kwon et al. and Abangah et al (20,21). The cut off points of the 2 measurements (WC and WHR) as per the guidelines to predict whether individuals are at risk to develop NAFLD may vary from amongst different population (22).

Glycemic control is important to prevent complications in T2DM.There was a significant association of IGT with grades of fatty liver. It implies that the presence of NAFLD can be in prediabetic stage also (23).

In the present study, there was no significant association between hypercholesterolemia and grades of NAFLD. This was similar to published studies (24,25, 26) where no significant association of **t**otal cholesterol with increasing grades of fatty liver was reported. However Mahaling et al (27) showed significant association of increasing grades of fatty liver with changes in Total Cholesterol and LDL-C. The only component of lipid profile associated with grades of NAFLD was serum triglycerides.

The ultrasound grade of fatty liver was significantly associated with presence of hypertriglyceridemia. Hypertriglyceridemia can lead to hepatic uptake or synthesis of TGs resulting in hepatic steatosis The findings were similar to published studies 28,29,21,24,30).However , Kirovski et al (26) & Mahaling et al (27) showed no significant association of increasing grades of fatty liver with changes in triglycerides.

No significant associations were observed in LDL-Cholesterol and Low HDL-C and grades of Fatty liver. However, Pardhe et al (24) reported that change in HDL-C was statistically significant in different grades of NAFLD where HDL-C decreased with steatosis.

No significant associations were observed in Liver enzymes and grades of fatty liver. Likewise no significant association of liver enzymes and different grades of fatty liver has been reported by other studies also (16, 17) and thus liver enzymes are not of any use for predictive purpose. However a significant association was reported with elevated liver enzymes and higher degrees of fatty liver in published studies (24,30, 31).

The risk of developing Grade 2 fatty liver was 3 fold higher when Metabolic Syndrome was present using ATP 3 criteria .The results were similar to Mustapic et al (32) from Canada.

In brief it was observed that the worsening grades of fatty liver as diagnosed by ultrasound have been accompanied by a higher prevalence of metabolic derangements/risk factors -Obesity, Central Obesity, IGT, hypertriglyceridemia and Metabolic Syndrome in NAFLD Cases. Also association of grades of fatty liver with metabolic parameters for BMI > 25, waist circumference ,WHR, IGT ,triglycerides , MetS [3.0 (1.48-6.22) was observed. When higher grades of NAFLD are detected, it should initiate a detailed diagnostic workup of patients to avoid more complex co morbidities associated with NAFLD.

LIMITATIONS OF THE STUDY

Liver biopsy is the gold standard for diagnosis of NAFLD. The risk and cost factor and invasive nature is the drawback associated. However ultra sonography is the tool of choice for detection of fatty liver as it is cost effective, noninvasive. Ultrasound is observer dependent and there is significant intra- and inter-observer variability (33, 34). We have tried to overcome this limitation by providing a single observer.

CONCLUSION

The risk factors associated with NAFLD grades based on ultra sonography are metabolic risk factors (higher BMI, waist circumference, WHR, IGT, hypertriglyceridemia and Metabolic syndrome). These results suggest that the importance of lifestyle modification (healthy diet and exercise) to prevent NAFLD progression should be promoted.

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Conflicts of interest

There are no conflicts of interest

REFERENCES

1. Perumpail, BJ, Khan, MA, Yoo, ER, Cholankeril, G, Kim, D & Ahmed, A 2017, 'Clinical epidemiology and disease burden of nonalcoholic fatty liver disease', *World journal of gastroenterology*, vol. 23, no. 47, pp. 8263–8276.
2. Rinella, ME & Sanyal, AJ 2016, 'Management of NAFLD: a stage-based approach', *Nature Reviews Gastroenterology &Amp; Hepatology*, vol. 13, p. 196.
3. Younossi, ZM, Marchesini, G, Pinto-Cortez, H & Petta, S 2019, 'Epidemiology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis: Implications for Liver Transplantation', *Transplantation*, vol. 103, no. 1.
4. Vernon G, Baranova A , Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steato hepatitis in adults. Alimentary Pharmacology & Therapeutics. 2011; 34(3): 274–285.
5. Adriano LS, de Carvalho Sampaio HA, Arruda SPM, Portela CLM, Melo MLP , Carioca AAF et al. Healthy dietary pattern is inversely associated with non-alcoholic fatty liver disease in elderly. British Journal of Nutrition.2016; 115: 2189-2195.
6. Miyake T, Kumagi T, Hirooka M, Furukawa S, Yoshida O, Koizumi M, et al. Low alcohol consumption increases the risk of impaired glucose tolerance in patients with non-alcoholic fatty liver disease. Journal of Gastroenterology. 2016 ;51(11):1090-1100.
7. Joseph AE, Saverymuttu SH, al-Sam S, et al. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radiol 1991; 43:26-31.
8. Dasarathy S, Dasarathy J, Khiyami A et al (2009) Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 51:1061–1067
9. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55(6):2005-23.

10. Chaturvedi, S, Bhatia, N, Vikram, N K, Pandey, RM, Madhusudan, KS. Low and Moderate level of Physical Activity is a risk factor for NAFLD. Indian Journal of Applied Research. 2018; 8 (1):314–316.
11. Saleem, SM . Modified Kuppuswamy socioeconomic scale updated for the year 2019: April 2–5.
12. WHO Technical Report Series No. 854. (WHO, Geneva, 1995.) Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee
13. Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, et al. Consensus Group. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. J Assoc Physicians Indi 2009; 57:163-70.
14. Mottin CC, Moretto M, Padoin AV, Swarowsky AM, Toneto MG, Glock L, et al. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. Obes Surg 2004;14(5):635-7.
15. Mohammadi, A, Bazazi, A, Maleki-Miyandoab, T & Ghasemi-rad, M 2012, 'Evaluation of relationship between grading of fatty liver and severity of atherosclerotic finding', International Journal of Clinical and Experimental Medicine.
16. Sogabe, M, Okahisa, T, Nakagawa, T, Fukuno, H, Nakasono, M, Tomonari, T, Tanaka, T, Tanaka, H, Taniguchi, T, Muguruma, N & Takayama, T 2016, 'Influence of light alcohol consumption on lifestyle-related diseases: a predictor of fatty liver with liver enzyme elevation in Japanese females with metabolic syndrome', BMC Gastroenterology, vol. 16, no. 1, p. 17.
17. Miksztowicz, V, Lucero, D, Zago, V, Cacciagiú, L, Lopez, G, Gonzalez Ballerga, E, Sordá, J, Fassio, E, Schreier, L & Berg, G 2012, 'Hepatic lipase activity is increased in non-alcoholic fatty liver disease beyond insulin resistance', Diabetes/Metabolism Research and Reviews.
18. Novakovic, T, Mekic, M, Smilic, L, Smilic, T, Inić-Kostic, B, Jovicevic, L, Mirkovic, Z & Milinic, S 2014 , 'Anthropometric and biochemical characteristics of patients with nonalcoholic fatty liver diagnosed by non-invasive diagnostic methods.', Medicinski arhiv, vol. 68, no. 1, pp. 22–26.
19. Ahmed, MH, Barakat, S & Almobarak, AO 2012, 'Nonalcoholic fatty liver disease and cardiovascular disease: Has the time come for cardiologists to be hepatologists?' Journal of Obesity.
20. Kwon, Y-M, Oh, S-W, Hwang, S, Lee, C, Kwon, H & Chung, GE 2012, 'Association of Nonalcoholic Fatty Liver Disease With Components of Metabolic Syndrome According to Body Mass Index in Korean Adults', American Journal of Gastroenterology, vol. 107, no. 12.
21. Abangah, G, Yousef, A, Asadollahi, R, Veisani, Y, Rahimifar, P & Alizadeh, S 2014, 'Correlation of body mass index and serum parameters with ultrasono-graphic grade of fatty change in non-alcoholic fatty liver disease', Iranian Red Crescent Medical Journal.
22. Singh, Ayaskant & Nath, Preetam & Parida, Sasmita & Narayan, Jimmy & Padhi, Pradeep & Pati, Girish & Parida, Prasanta & Meher, Chudamani & Agrawal, Omprakash & Singh, Shivaram Prasad. Simple Anthropometric Indices are Useful for Predicting Non-alcoholic Fatty Liver Disease [NAFLD] in Asian Indians. Journal of Clinical and Experimental Hepatology | December 2017 | Vol. 7 | No. 4 | 310–315.
23. Mohan, V, Farooq, S, Deepa, M, Ravikumar, R & Pitchumoni, CS 2009, 'Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome', Diabetes Research and Clinical Practice
24. Pardhe, BD, Shakya, S, Bhetwal, A, Mathias, J, Khanal, PR, Pandit, R, Shakya, J, Joshi,

HO & Marahatta, SB 2018. 'Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal', *BMC Gastroenterology*, vol. 18, no. 1, pp. 1–8.

25. Nakhjavani, M., Khalilzadeh, O., Khajeali, L., Esteghamati, A., Morteza, A., Jamali, A. and Dadkhahipour, S. (2010), Serum Oxidized- LDL is Associated with Diabetes Duration Independent of Maintaining Optimized Levels of LDL- Cholesterol. Lipids, 45: 321-327. https://doi.org/10.1007/s11745-010-3401-8
26. Kirovski, G, Schacherer, D, Wobser, H, Huber, H, Niessen, C, Beer, C, Sch??lmerich, J & Hellerbrand, C 2010, 'Prevalence of ultrasound-diagnosed non-alcoholic fatty liver disease in a hospital cohort and its association with anthropometric, biochemical and sonographic characteristics', *International Journal of Clinical and Experimental Medicine*, vol. 3, no. 3, pp. 202–210.
27. Mahaling, DU, Basavaraj, MM & Bika, AJ 2013, 'Comparison of lipid profile in different grades of non-alcoholic fatty liver disease diagnosed on ultrasound', *Asian Pacific Journal of Tropical Biomedicine*
28. Leite, NC, Salles, GF, Araujo, ALE, Villela-Nogueira, CA & Cardoso, CRL 2009, 'Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus', *Liver International*
29. Razavizade, M, Jamali, R, Arj, A & Talari, H 2012, 'Serum parameters predict the severity of ultrasonographic findings in non-alcoholic fatty liver disease', *Hepatobiliary and Pancreatic Diseases International*
30. 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;9:85-9.
31. Mansour-Ghanaei, et al.: Biochemical markers and lipid profile in nonalcoholic fatty liver disease patients. Mansour-Ghanaei R, Mansour-Ghanaei F, Naghipour M, Joukar F. Biochemical markers and lipid profile in nonalcoholic fatty liver disease patients in the PERSIAN Guilan cohort study (PGCS), Iran. J Family Med Prim Care 2019;8:923-8.
32. Mustapic, S, Ziga, S, Matic, V, Bokun, T, Radic, B, Lucijanic, M, Marusic, S, Babic, Z & Grgurevic, I 2018, 'Ultrasound Grade of Liver Steatosis Is Independently Associated with the Risk of Metabolic Syndrome', *Canadian Journal of Gastroenterology and Hepatology*.
33. Falck – Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin Liver Dis. 2001;21:17–26.
34. Mishra P, Younossi ZM. Abdominal ultrasound for diagnosis of nonalcoholic fatty liver disease (NAFLD). AM J Gastroenterol. 2007;102:2216–7.