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ORIGINAL RESEARCH

A CROSS SECTIONAL ANALYTICAL STUDY TO EVALUATE THE PREVALENCE AND EARLY DIAGNOSIS OF NAFLD IN VARIOUS COMORBIDITIES USING LIPID PROFILE AND HEPATIC ENZYMES AS BIOMARKERS

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is a global epidemic that is often asymptomatic and silent, and progresses slowly and is one of the most common causes of chronic liver disease. NAFLD affects a third of the world population with very much high prevalence among patients with diabetes mellitus, obesity, hypertension, dyslipidemia, hypothyroidism etc. The natural history of NAFLD ranges from pure steatosis to steatohepatitis (NASH) to cirrhosis and in some patientsto hepatocellular carcinoma (HCC). NAFLD has been found to be the hepatic components of metabolic syndrome which is one of the leading causes of chronic liver disease. This study aimed to determine the biochemical hepatic markers and lipid profile among NAFLD patients and their possible relationship with degrees of fatty liver and other comorbidities to aid the clinician tointervene early in order to delay the occurrence of complications associated with NAFLD.

Materials and Methods: In this analytical cross sectional study, 145 individuals aged 20–69 years referred to the Govt Medical College/ GGH Hospital Suryapet during the period from June 2021 to May 2022, were included through sequential sampling method after meeting inclusion and exclusion criteria and after taking informed consent and ethical committee approval. Serum lipid profile and Serum liver enzymes was estimated on ERBA EM 360 auto analyzer.

Results: We found significant increase in lipid parameters (TC, LDL-C, VLDL-C, and TG), liver enzymes (AST, ALT, and GGT) and decrease in HDL-C and AST/ALT (Deritis ratio) in NAFLD with type 2 DM compared to controls.

Conclusion: We conclude from our study that in obesity, dyslipidemia, hypertension, hypothyroidism and Type 2 DM the elevated liver enzymes and lipid profile could be biomarkers for the early diagnosis of NAFLD with Type 2 DM and other comorbidities.
Keywords: ALT, AST, GGT, HCC, NAFLD, Type 2 DM, IR, Oxidative stress.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disorder sharing the same factors with metabolic syndrome in pathogenesis and insulin resistance. NAFLD involves a whole spectrum of liver pathologies from simple steatosis to non‑alcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).[1] Prevalence of NAFLD has doubled over the past 20 years, while prevalence of other chronic diseases of the liver has remained constant and even diminished.[2] Prevalence of NAFLD in the world is about 25%,[3] in non‑obese Asian‑Pacific individuals, it is 15–21%.[4]

The etiology of NAFLD reflects complex interactions between genetic, neurohumoral, metabolic and stress-related factors, altered food habits and sedentary life style has led to the development of fatty liver, a marker of metabolic syndrome, leading to cardiovascular disease and Type 2 DM at a younger age which are more commonly found in Asian countries.[5]

NAFLD is identified by abnormal liver tests, imaging studies, and liver biopsy, and has the potential to become the most common cause of liver transplantation in the future.[6] Ultrasonography of the liver is the most common technique for screening fatty liver in the general population.[7]

There is increase in the incidence of Type 2 DM, central obesity, hypothyroidism, hypertension and dyslipidemia in India in last two decades and hence it is logical to expect increase in incidence of NAFLD in India.

There is limited data emphasizing the causes underlying NAFLD among the susceptible individuals from India. Therefore, this study was undertaken to estimate BMI, Serum lipid profile and liver enzymes changes along with USG finding of fatty liver and in turn aid the clinician to intervene early in order to delay the occurrence of complications associated with NAFLD and normal healthy controls with various above mentioned comorbidities.

Aims and objectives

To studyand compare blood levels of glucose (FBS, PPBS) HbA1c, liver enzymes SGOT (AST), SGPT (ALT) and GGT and lipid profile in type 2 diabetes mellitus with NAFLD and control group to study BMI in cases of obesity, type 2 diabetes mellitus and others with NAFLD and controls.

MATERIALS & METHODS

In this analytical cross-sectional study, 145 individuals aged 20–69 years referred to the Govt Medical College/GGH Suryapet during the period from June 2021 to May 2022, were included through sequential sampling method after meeting inclusion and exclusion criteria and after taking informed consent and ethical committee approval.

Inclusion criteria

1. Type 2 DM Patients diagnosed to have Fatty liver by USG, belonging to both sexes and with age between 30 and 70 years in the Department of Medicine at GGH Suryapet were included in the study.
2. Fifty normal age and sex matched healthy volunteers in the age group of 30 to70 years.

Exclusion criteria: History of alcohol consumption, Viral and infective Hepatitis, Drug induced Hepatitis, Toxins, History of Gastrointestinal bypass surgeries, Pregnancy, Autoimmune Hepatitis.

RESULTS

The Mean FBS level among male cases was 180.81 ±53.45 as compared to 86.94 ± 8.14 among controls. The mean PPBS level among cases is 300.48 ±77.55 as compared to 120.11 ± 6.03 among controls and HbA1C was 8.01 ± 1.87 in comparison with controls 4.96 ± 0.27 (P =0.0001). In case of females the mean values have shown slight increase in FBS, PPBS and HbA1C as compared to males.

Table 1: Gender wise mean values of various biochemical parameters in NAFLD

		(N=50) Males			**(N=95) Females**		
		Control Mean Sd	**Case Mean Sd**	**P Value**	**Control Mean Sd**	**Case Mean Sd**	**P Value**
Age		45.61±1.62	54.80±1.96	>0.005	46.22±1.25	57.48±1.87	> 0.005
Bmi		24.17 ± 3.34	26.14 ± 4.59	0.001	23.61 ± 1.34	29.13 ± 3.59	0.001
Glucose Levels	FBS	86.94 ± 8.14	180.81 ±53.45	<0.0001 ***	87.94 ± 5.14	182.65 ± 41.17	<0.0001 ***
	PPBS	120.1 ± 16.03	300.48 ±77.55	<0.0001 ***	119.44 ± 10.21	310.66 ± 75.33	<0.0001 ***
	HBA1C	4.96 ± 0.27	8.01 ± 1.87	< 0.0001 ***	5.33 ± 0.36	8.68 ± 1.87	< 0.0001 ***
Lipid Profile	LDL C	95.3 ± 16.24	132.8 ± 21.16	< 0.0001 ***	96.3 ± 19.14	136.8 ± 21.06	< 0.0001 ***
	VLDL	21.15	48.12 ± 30.56	< 0.0001 ***	23.46 ± 11.10	49.02 ± 31.47	< 0.0001 ***
	HDL C	43.22 12.53	26.89 ± 12.11	0.582	45.12 ± 15.53	30.68 ± 10.01	0.582
	TC	159.9 ±	222.28 ±	< 0.000	160.92 ±	229.82 ±	< 0.0001

		2 21.14	48.96	** 1 *	25.16	50.96	***
	TG	108.5	240.24 ± 100.52	< 0.000 ** 1 *	118.5 ± 22.4	250.24 ± 134.52	< 0.0001 ***
	LDL/HDL	2.20	4.93		2.13	3.03	
	TC/HDL	3.41	8.26		3.76	7.49	
Hepatic Enzyme Profile	ALT	18.55 ± 6.02	33.69 ± 12.44	< 0.000 ** 1 *	18.46 ± 5.02	34.58 ± 13.34	< 0.0001 ***
	AST	18.01 ± 2.16	26.08 ± 12.14	< 0.000 ** 1 *	18.12 ± 5.16	29.18 ± 10.34	< 0.0001 ***
	ALP	51.25±9.32	198.98±26.75	< 0.0001	49.02±6.25	211.95±59.27	< 0.001
	GGT	12.99 ± 3.18	34.11 ± 10.54	< 0.000 ** 1 *	13.52 ± 3.21	35.22 ± 9.94	< 0.0001 ***
	AST/ALT	0.99 ± 0.19	0.77 ± 1.34	0.0002 ***	0.98 ± 0.26	0.84 ± 1.39	0.0002 ***
	Amylase	69.32±24.3	111.23±12.69	0.0002***	75.26±12.36	124.37±16.98	0.0002***
	Uric acid	5.1±2.36	6.99±3.59	0.05	5.26±3.29	7.64±4.58	0.05
	Creatine kinase	89±39.25	310±185	0.001	92.56±56.32	329±62.96	0.001
	Albumin	4±0.5	4.3±1.2	>0.05	4.12±.086	4.39±1.02	>0.05
	5’NT	10±12.03	28.23±14.28	0.005	11.81±11.42	32.96±19.25	0.005
	Choline esterase	7.99±1.23	22.35±10.32	0.001	8.69±26.47	26.14±27.61	0.001

The Mean TC level among male cases was 222.28 ± 48.96 as compared to 159.92 ± 21.14 among controls. Whereas in females it was 229.82 ± 50.96 and 160.92 ± 25.16 respectively in cases and controls. The Mean HDL-C level among cases is 26.89 ± 12.11as compared to 43.22 ± 12.53 among controls and values 30.68 ± 10.01, 45.12 ± 15.53 were in female cases and controls respectively. The Mean LDL-C level among cases is 132.8 ± 21.16 as compared to 95.3 ± 16.24 among controls the estimates 136.8 ± 21.06 and 96.3 ± 19.14 were in female cases and control subjects. The Mean VLDL-C level among cases is 48.12 ± 30.56 as compared to 21.15 ± 11.11 among controls and the assessment in female cases and controls were 49.02 ± 31.47, 23.46 ±11.10 individually.

Table 2: Age and gender wise analysis of NAFLD

Age	Male (n=50)			Female (n=95)		
	Elevated AST	Elevated ALT	Elevated AST & ALT	Elevated AST	Elevated ALT	Elevated AST & ALT
20-29	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
30-39	1 (2%)	1 (2%)	3 (6%)	1 (1.05%)	2 (2.10%)	5 (5.26%)
40-49	3 (6%)	2 (4%)	12 (40%)	2 (2.10%)	5 (5.26%)	34 (35.78%)
50-59	2 (4%)	2 (4%)	8 (16%)	6 (6.31%)	10 (10.52%)	20 (21.05%)
60-69	1 (2%)	1 (2%)	6 (12%)	1 (1.05%)	2 (2.10%)	7 (7.36%)
Total	7 (14%)	6 (12%)	37 (74%)	10 (10.52%)	19 (20%)	66 (69.47%)
Overall % of NAFLD	34.48			65.51		

The Mean TG level among cases is 240.24 ± 100.52 as compared to 108.5 ± 21.12 among controls whereas the values 250.24 ± 134.52, 118.5 ± 22.4 were discretely in female cases and controls. As observed in table 1 mean LDL/HDL values were 4.93, 2.20 respectively in male cases and controls in comparison with values of 3.03, 2.13 in female individuals. The mean values of TC/HDL ratio were observed as 8.26 and 3.41 in male cases and controls whereas in female cases and controls the values were 7.49 and 3.76 respectively. [Table 1]

Table 3: Correlation of NAFLD with risk factors

			Central obesity	Hypertension	Dyslipidemia	Type 2 DM	Hypothyroidism
Male n=50	Control	Yes	21 (42%)	23 (46%%)	15 (30%)	32 (64%)	12 (24%)
		No	29 (58%)	27 (54%)	35 (70%)	18 (36%)	38 (76%)
	Case	Yes	32 (64%)	29 (58%)	30 (60%)	36 (72%)	18 (36%)
		No	18 (36%)	21 (42%)	20 (40%)	14 (28%)	32 (64%)
	Relative risk		1.36 (p=0.005)	1.37 (p=0.001)	1.55 (p=0.001)	1.26 (p=0.001)	1.56 (p=0.005)
Female n=95	Control	Yes	35 (36.84%)	21 (22.10%)	26 (27.36%)	24 (25.26%)	15 (15.78%)
		No	60 (63.15%)	74 (77.89%)	69 (72.63%)	71 (74.73%)	80 (84.21%)
	Case	Yes	76 (80%)	69 (72.63%)	70 (73.68%)	71 (74.73%)	61 (64.21%)
		No	19 (20%)	26 (27.36%)	25 (26.31%)	24 (25.26%)	34 (35.78%)

	Relative risk		2.18 (p=0.001)	1.89 (p=0.001)	2.21 (p=0.001)	1.57 (p=0.001)	1.69 (p=0.001)

The Mean AST levels among male cases are 26.08 ± 12.14 as compared to 18.01 ± 2.16 among controls. And the assessment in female cases and controls were 29.18 ± 10.34, 23. 18.12 ± 5.16 individually. The Mean ALT level among cases were 33.69 ± 12.44 as compared to18.55 ± 6.02 among controls and the mean ALT in female cases and controls were 34.58 ± 13.34 and 18.46 ± 5.02 respectively. Mean ALP levels out of males were 198.98±26.75 and 51.25±9.32 in cases and controls and in females ALP values were 211.95±59.27 and 49.02±6.25 respectively. The average GGT level in male cases were 34.11 ± 10.54 as compared to12.99 ± 3.18 amongcontrols and the mean levels in female cases and controls were 35.22 ±9.94 13, .52 ± 3.21 respectively. The mean Deritis ratio (AST/ALT ratio) among cases were 0.77 ± 1.34 as compared to 0.99 ± 0.19 among controls and the values in female cases and controls were 0.84 ± 1.39, 0.98 ± 0.26 respectively. Deritis Ratio is < 1 in cases and >1 in controls. Mean BMI among male cases was 26.14 ± 4.59 and 24.17 ± 3.34 among controls and among females the values were 29.13 ± 3.59 and 23.61 ± 1.34 in cases and controls respectively. As observed, mean values of albumin in male cases and controls were 4.3±1.2, 4±0.5 as compared to values 7.64±4.58, 5.26±3.29 in female cases and controls respectively. It was shown that mean values of uric acid in male and female cases and controls were 6.99±3.59, 5.1±2.36 and 7.64±4.58, 5.26±3.29 respectively. The other hepatic amylase, creatine kinase, choline esterase and 5'NT enzymes were also studied and the results were tabulated in table 1. It was shown (table 2) the prevalence of NAFLD was in females (65.15%) than in males (34.48%) and in 40 - 49 age group in both males (34.48%) and females (43.15) followed by 24%, 37.89% under age group of 50-59 in males and females respectively and 16%, 10.52% were under age group of 60-70 in males and females individually. The dominant risk factor observed in majority of male cases was type 2 DM (72%) followed by central obesity (64%), dyslipidemia (60%), hypertension (58%), and least was hypothyroidism (36%).the predominant risk factor in females was central obesity (80%), succeeded by type 2 DM (74.73%), dyslipidemia (73.68%), hypertension (72.63%), and least was hypothyroidism (64.21%). It was observed that the prevalence of all undertaken risk factors was shown higher in females than males. [Table 3]

DISCUSSION

In our study, 145 subjects were ultrasound diagnosednon-alcoholic fatty liver disease (NAFLD) among type 2 DM and 145 age and sex matched controls. The mean age in the study group was 54.80±1.96 in males and 57.48±1.87 in females. Patients in the study group were found predominantly clustered in the 40-49 age categories. This may suggest a higher incidence of NAFLD in females in the Indian sub-continent. Elevated levels of Total Cholesterol, LDL, VLDL, TG, AST, ALT, GGT and various hepatic enzymes and decreased levels of HDL, AST/ALT ratio was found in cases compared to healthy controls. In our study, it was found that females were more prone to NAFLD than males and also showed considerable hike in all biochemical parameters and risk factors in comparison to male cases and controls. Our results were consistent with the previous works. [Table 4]

Table 4: Correction of Results with Previous Literature

Author name	Year	Sample size	Conclusion
Tatjana Novakovic etal,[8]	2014	170	Patients with non-alcoholic fatty liver are excessively obese, have greater waist line extent, consequently insulin resistance and impaired glucose metabolism, insulin resistance, dyslipidemia, risk factors known to be associated with the development of cardiovascular disease.
Qazi Najeeb etal,[9]	2015	430	Patients with NAFLD, there are considerable changes in biochemical markers. Thus, it seems essential that in clinical settings in cases in which biochemical and lipid changes are observed, sonography should be performed to examine individuals with NAFLD, since early diagnosis prevents further complications and delays them.
Santhoshakumari TMJ etal,[10]	2017	480	Central obesity and dyslipidemia was significantly higher in the NAFLD group compared to the control group.
Lucky R. Cuenza, etal,[11]	2017	100	Ultrasound-based grading of the severity of NAFLD is associated with abnormalities in the metabolic profile of patients. The FRS is correlated with increasing severity of NAFLD based on ultrasound. These findings suggest that the presence of NAFLD may be a marker for the presence of increased cardiovascular risk and may help identify patients who may benefit from more aggressive therapies to prevent development of adverse cardiovascular events.
B.D. Pardhe etal,[12]	2018	219	The result of this study suggests that there is an increased prevalence and significant changes in biochemical markers in cases of NAFLD. Timely diagnosis would help in delaying its complications and co-morbidities.
Anahita Zakeri etal,[13]	2018	80	The results showed that non-alcoholic fatty liver is more prevalent in females and older ages. Doing multi-center studies was recommended in Ardabil province or other

			provinces in Iran in future.
Roya mansour-ghanaei etal,[14]	2019	950	Biochemical markers and lipid profile are associated with NAFLD. Thus, it is recommended to investigate NAFLD in clinical settings in cases in which their changes are observed in patients through ultrasonography.
Kiran Namoos etal,[15]	2021	650	Mild elevations of biochemical markers like liver enzymes and lipid profile are associated with Non-alcoholic Fatty Liver Disease.

CONCLUSION

The present study has evaluated the utility of Serum lipid profile and liver enzymes as useful early biomarkers of Non-alcoholic fatty liver disease (NAFLD) in type 2 DM with good results. Elevated levels of Total Cholesterol, LDL, VLDL, TG, AST, ALT, GGT, albumin, uric acid, 5'NT, choline esterase, creatine kinase and decreased levels of HDL, AST/ALT ratio was found in cases compared to healthy controls. Our study supports the role of dyslipidemia and elevated liver enzymes which help explain the proposed etio- pathogenesis of insulin resistance and oxidative stress. We conclude from our study that obesity, dyslipidemia, hypertension, hypothyroidism and Type 2 DM elevated liver enzymes and lipid profile could be biomarkers for the early diagnosis of NAFLD with Type 2 DM and other comorbidities.

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