



To Study the Prevalence of Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes and to Correlate it with Lipid profile and Glycated Hemoglobin in a Hospital

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Abstract

As incidence of diabetes is continuously increasing in india and diabetic dyslipidemia is a leading cause of non Alcoholic fatty liver disease which may progress to cirrhosis and hepatocellular carcinoma. No proven treatment is available to treat NAFLD. It can be prevented or its progression may be attenuated by early treatment of dyslipidemia in diabetes.

Material and Methods: - A hospital-based prospective study was carried out in 200 diabetic patients, 100 of them found to be on statin therapy for diabetic dyslipidemia for at least 3 months from history, above 35 years age including both sexes irrespective of community or background which were present in inpatient department of medicine, Mata Chanan Devi hospital, New Delhi, during the time period of 2014-2016. It is a 210 bedded, tertiary care hospital in west Delhi, where the patients travel from all northern India.

Results: - 91 of the 200 were found to have NAFLD by ultrasonography. The demographic and lipid profile such as total cholesterol, serum triglycerides, serum HDL, serum LDL and serum VLDL were recorded. The mean age of the patients was male 66.46 ± 11.83 and female 62.96 ± 11.16 years. Mean BMI in non-NAFLD statin group 26.48, non-NAFLD non-statin group 26.77, NAFLD statin group 29.14 and NAFLD non-statin group 30.08 (not significant). 54% of patients with metabolic syndrome and only 6% of non metabolic syndrome patients had NAFLD. Prevalence of various grades of NAFLD in statin vs. non statin groups, total (33 vs. 58%), grade 1 (21 vs. 49%), grade 2 (7 vs. 8%), grade 3 (1 vs. 5%). Mean of various serum lipid parameters in statin vs. non statin groups is total cholesterol (117.9 vs. 171.53), TG (120.13 vs. 184.49), LDL (60.27 vs. 99.26), VLDL (24.04 vs. 36.21), non-HDL (84.3 vs. 136.16) with p value < 0.05 (significant), HDL (37.89 vs. 39.7). The total prevalence of NAFLD in diabetics was found to be lower than was found in various other studies, this was found to be associated to the statin therapy that 100 of the total group was already on at time of study for > 3 months. Diabetics on statin therapy are found to have a significantly lowered lipid parameters and also lower prevalence of NAFLD than non statin group. No significant difference was noted in the liver enzymes and ECG changes.

Conclusion: - Thus our study has found that statins not only improve the diabetic dyslipidemia but also reduces the prevalence of NAFLD and is also safe.

Recommendation: - Large randomized double blind trails are needed to prove the drug effects.

Keywords: NAFLD, diabetes, statin, metabolic syndrome, lipid parameters.

Introduction

Presently, the world is in a pandemic of type 2 diabetes with exponential growth of diabetics. Diabetic dyslipidemia is a leading cause of

atherosclerotic disease process like coronary artery disease. If we diagnosed and treat dyslipidemia in early stage, we can decrease the risk of atherosclerotic diseases^[1]. Studies have

also noted an increase in the prevalence of non-alcoholic fatty liver disease especially in diabetes [2]. Majority of these diabetics are found to have metabolic syndrome. Some authors even consider NAFLD as one of the manifestations of metabolic syndrome and indirect marker of CVD risk. Nonalcoholic fatty liver disease (NAFLD) is a term for a broad continuum of liver illnesses extending from the rather benign steatosis to Non alcoholic steatohepatitis (NASH) and can progress to severe cryptogenic cirrhosis and also a risk factor for hepatocellular carcinoma [3-7].

The most significant risk factors for NAFLD include the components of metabolic syndrome: obesity, glucose intolerance or diabetes, hypertension, and dyslipidemia, particularly elevated triglycerides and low levels of HDL cholesterol [8]. Metabolic syndrome was diagnosed as per IDF 2005 criteria. Though liver biopsy is the gold standard method for diagnosis of NAFLD [9,10], Ultrasonography which is non-invasive, simple tool, can be used for the early detection of NAFLD in asymptomatic patients. Liver Ultrasonography for detecting liver steatosis has sensitivity of 60-94% and specificity of 88-95%. This study will be conducted to estimate the prevalence of NAFLD as diagnosed by ultrasound examination of the liver [11, 12].

The association of levels of Lipid profile and Glycated hemoglobin will be compared between type 2 diabetics with NAFLD and without NAFLD at our institution, Mata Chanan Devi Hospital.

Objectives

To study the prevalence of Non alcoholic fatty liver disease (NAFLD) in Indian Type 2 diabetic patients. To compare Lipid profile and Glycated hemoglobin in between groups of type 2 diabetes with NAFLD and without NAFLD. To compare prevalence and lipid profiles in between groups of type 2 diabetes on statin therapy and those not on statin.

Material and Methods

A prospective study was carried out in 200 diabetic patients in above 35 years age including both sexes irrespective of community or background which were present in inpatient department of medicine, Mata Chanan Devi hospital, New Delhi, during the time period of 2014-2016. Known liver disease, HBsAg or HCV positivity, ingestion of hepatotoxic drug(s), Known alcoholics with daily consumption >20gm/day were not included in the study. Diabetes is a self reported disease. The diagnosis of diabetes is based on American diabetic association criteria. Before starting the study, permission was taken from institutional ethical committee. A written informed consent was taken. A detailed history was taken and careful physical examination was done. Based on history, 100 of the group were found to be on statin therapy for more than 3 months.

Lipid profile was done from each subject after 8-12 hr fasting. Other baseline investigation like haematological profile, blood urea, serum creatinine, serum aminotransferase, serum uric acid, serum electrolytes, ECG was also carried out at the time of admission. Blood sugar was analysed using hexokinase method. All the data and various findings including the past history, present diagnosis, blood sugar, HbA1c, nonfasting and fasting lipid profile of all subjects were tabulated and evaluated using Microsoft Excel. Non HDL cholesterol was calculated by total cholesterol – HDL cholesterol.

Statistical Analysis

Statistical package for social sciences (SPSS) 20.0 software is used for data analysis. Pearson Chi-Square test is done for Sex distribution in different age group and in different parameters. Null Hypothesis: There is no significant difference in the mean value of different parameters between two groups i.e. $\eta_1 = \eta_2$. Alternate Hypothesis: There is a significant difference in the mean value of different parameters between two groups i.e. $\eta_1 \neq \eta_2$. Level of Significance: $\alpha = 0.05$. Statistical test used is Mann-Whitney test. Decision

Criterion: We compared the P-Value with the level of significance. If $P < 0.05$, we reject the null hypothesis and accept the alternate hypothesis. If $P \geq 0.05$, we accept the null hypothesis.

Results

Out of 200 patients, there were 106 females and 94 males subjects. Minimum age is 36 years and maximum age is 96 years. Mean age in males is 66.46 ± 11.83 and in females 62.96 ± 11.16 .

Table - 1 Sample distribution according to group.

GROUP	N	%
Non NAFLD	109	54.5%
NAFLD	91	45.5%
TOTAL	200	100%

Of the 200 type 2 diabetic patients included in our study, 91 are found to have various grades of NAFLD and 109 had grade 0 NAFLD (normal liver) on ultrasonography. Prevalence of NAFLD in our study group is found to be 45.5%.

Table - 2 Gender distributions in the groups.

GROUP	MALE		FEMALE	
	N	%	N	%
Non NAFLD	55	59%	54	51%
NAFLD	39	41%	52	49%
TOTAL	94	100%	106	100%

Above table shows that 41% of men have NAFLD and 49% of women have NAFLD.

Table - 3 Comparison of various parameters between the groups.

PARAMETERS	ULTRASOUND NAFLD	N	Mean	Std. Deviation	t-value	p-value
WT in KG	non-NAFLD	109	68.87	6.33	6.84	<0.001
	NAFLD	91	76.03	8.45		
HT in Meters	non-NAFLD	109	1.61	0.07	1.20	0.23
	NAFLD	91	1.60	0.06		
BMI	non-NAFLD	109	26.59	2.60	7.55	<0.001
	NAFLD	91	29.74	3.29		
WAIST in cm	non-NAFLD	109	89.20	5.88	7.12	<0.001
	NAFLD	91	95.27	6.15		
HIP in cm	non-NAFLD	109	94.69	3.55	2.72	0.01
	NAFLD	91	96.23	4.46		
WHR	non-NAFLD	109	0.94	0.07	4.91	<0.001
	NAFLD	91	0.99	0.07		
SBP in mm of Hg	non-NAFLD	109	122.66	14.25	2.84	0.01
	NAFLD	91	128.24	13.38		
DBP in mm of Hg	non-NAFLD	109	74.68	9.19	2.38	0.02
	NAFLD	91	77.80	9.29		
DURATION OF DIABETIS in years	non-NAFLD	109	9.57	6.46	1.20	0.23
	NAFLD	91	10.62	5.77		
HTN in years	non-NAFLD	109	7.72	6.97	0.31	0.76
	NAFLD	91	7.41	7.08		
CHOL in mg%	non-NAFLD	109	132.97	44.11	3.75	<0.001
	NAFLD	91	158.78	53.13		
TG in mg%	non-NAFLD	109	124.97	63.47	4.51	<0.001
	NAFLD	91	185.05	120.64		
HDL in mg%	non-NAFLD	109	40.61	14.08	1.96	0.05
	NAFLD	91	36.62	14.67		
LDL in mg%	non-NAFLD	109	71.20	30.95	3.64	<0.001
	NAFLD	91	90.02	41.99		
VLDL in mg%	non-NAFLD	109	25.10	13.17	4.21	<0.001
	NAFLD	91	36.14	23.28		
CH	non-NAFLD	109	3.47	1.15	6.04	<0.001
	NAFLD	91	4.87	2.07		
NON HDL	non-NAFLD	109	96.20	35.49	4.98	<0.001

	NAFLD	91	127.03	51.70		
FBS in mg/dl	non-NAFLD	109	150.83	75.33	3.43	<0.001
	NAFLD	91	188.97	81.82		
PPBS in mg/dl	non-NAFLD	109	223.72	87.61	2.84	0.01
	NAFLD	91	260.46	95.08		
HbA1c in%	non-NAFLD	109	7.60	2.02	3.20	<0.001
	NAFLD	91	8.61	2.44		

Above table compares the clinicopathological parameters between NAFLD and non NAFLD groups. It shows that weight, BMI, waist circumference (WC), WHR, blood pressure, duration of diabetes, total cholesterol, TG, LDL,

VLDL, CH, NON-HDL, FBS, HbA1c are significantly elevated (p<0.005) and HDL is significantly lesser in NAFLD group compared to non NAFLD group.

Table - 4 Comparing prevalence of metabolic syndrome (as per IDF 2005 CRITERIA), obesity and morbid obesity among various grade of NAFLD.

NAFLD grades VS METABOLIC SYNDROME	IDF 2005 CRITERIA (2 OR MORE)					Obesity >25	Morbid obesity >30
	GRADE 0	GRADE 1	GRADE 2	GRADE 3	TOTAL		
METABOLIC SYNDROME	77 (46%)	68 (41%)	15 (9%)	6(4%)	166 (83%)	132 (79%)	39 (23%)
NON METABOLIC SYNDROME	32 (94%)	2(6%)	0	0	34 (17%)	34 100%	13 (38%)
SUB TOTAL	109	70	15	6	200	166	52

Above table shows an 83% prevalence of metabolic syndrome as per IDF 2005 CRITERIA with 79% of them being obese and 23% being morbidly obese. 54% of subjects with metabolic syndrome as per these criteria have NAFLD with

41% having grade 1. Among subjects without metabolic syndrome 100% were obese and 38% were morbid obese. About 6% of them have NAFLD and all were grade 1.

Table – 5 Grades of NAFLD verses components of lipid profile.

Grades	Number	Total Chol	TG	HDL	LDL	VLDL	Non HDL	CH	% on statins
0	109	133	125	41	71	25	96	3.5	61
1	70	167	185	39	95	36	132	4.8	30
2	15	140	218	29	80	43	124	5.7	47
3	6	104	100	31	54	20	74	4	83

Above table shows the averages of lipid parameters among various grades of NAFLD. The parameters did not correlate with grades in ascending fashion as expected, especially in higher grades.

About 100 patients in the study group were found to be on statin therapy for more than 3 months.

Table – 13 Statin therapy verses NAFLD prevalence in the study group.

	Statin group	Non statin group	Total
NAFLD	33	58	91
Non NAFLD	67	42	109
Total	100	100	200

Above table shows the prevalence of NAFLD in the 2 subgroup based on statin therapy.

Table – 7 Comparison of various parameters between groups in patients on statin therapy for >3 months and not on statins

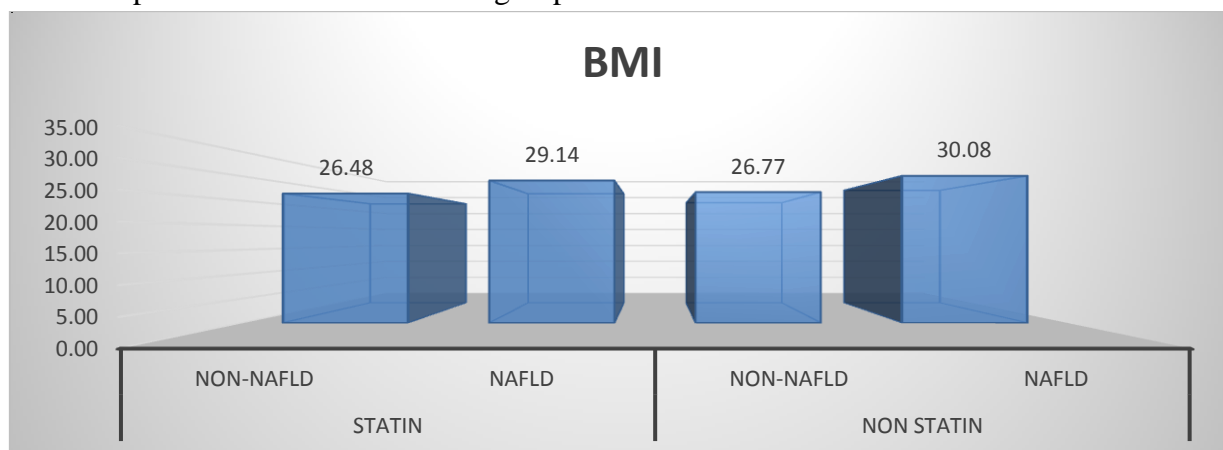
PARAMETERS	ULTRASOUND NAFLD GRADE	Statin group		Non statin group	
		N	Mean	N	Mean
AGE	non-NAFLD	67	69.448	42	62.90
	NAFLD	33	65.818	58	59.55
WT in KG	non-NAFLD	67	68.642	42	69.24
	NAFLD	33	76.455	58	75.79
HT in Meters	non-NAFLD	67	1.612	42	1.61
	NAFLD	33	1.621	58	1.59
BMI	non-NAFLD	67	26.480	42	26.77
	NAFLD	33	29.142	58	30.08
WAIST in cm	non-NAFLD	67	90.030	42	87.88
	NAFLD	33	97.636	58	93.93
HIP in cm	non-NAFLD	67	94.403	42	95.14
	NAFLD	33	95.212	58	96.81
WHR	non-NAFLD	67	0.955	42	0.92
	NAFLD	33	1.026	58	0.97
SBP in mm of Hg	non-NAFLD	67	122.390	42	123.10
	NAFLD	33	129.390	58	127.59
DBP in mm of Hg	non-NAFLD	67	74.776	42	74.52
	NAFLD	33	77.576	58	77.93
DURATION OF DIABETIS in years	non-NAFLD	67	11.313	42	6.79
	NAFLD	33	14.303	58	8.52
HTN in years	non-NAFLD	67	10.105	42	3.90
	NAFLD	33	10.091	58	5.88
CHOL in mg%	non-NAFLD	67	116.630	42	159.05
	NAFLD	33	120.480	58	180.57
TG in mg%	non-NAFLD	67	111.510	42	146.45
	NAFLD	33	137.640	58	212.03
HDL in mg%	non-NAFLD	67	39.075	42	43.07
	NAFLD	33	35.485	58	37.26
LDL in mg%	non-NAFLD	67	60.105	42	88.90
	NAFLD	33	60.606	58	106.76
VLDL in mg%	non-NAFLD	67	22.313	42	29.55
	NAFLD	33	27.546	58	41.03
CH	non-NAFLD	67	3.191	42	3.91
	NAFLD	33	4.161	58	5.28
NON HDL	non-NAFLD	67	82.406	42	118.20
	NAFLD	33	88.133	58	149.17
FBS in mg/dl	non-NAFLD	67	145.280	42	159.67
	NAFLD	33	170.030	58	199.74
PPBS in mg/dl	non-NAFLD	67	219.240	42	230.86
	NAFLD	33	243.240	58	270.26
HBA1c in%	non-NAFLD	67	7.334	42	8.03
	NAFLD	33	7.746	58	9.11

Above table compares various parameters between statin and non statin groups. Statin therapy is the single variable differentiating the 2 groups and no confounding factors have been found. Statin induced lipid lowering is equally seen in both the NAFLD and non NAFLD groups.

Thus, to compare the effects of statin in various parameters the study group is divided into 4 subgroups as

- Non statin with NAFLD
- Non statin without NAFLD
- Statin with NAFLD
- Statin without NAFLD

Graph – 1 Comparison of BMI in the 4 sub groups.



Above graph shows that BMI is higher in NAFLD non statin group than in non NAFLD group.

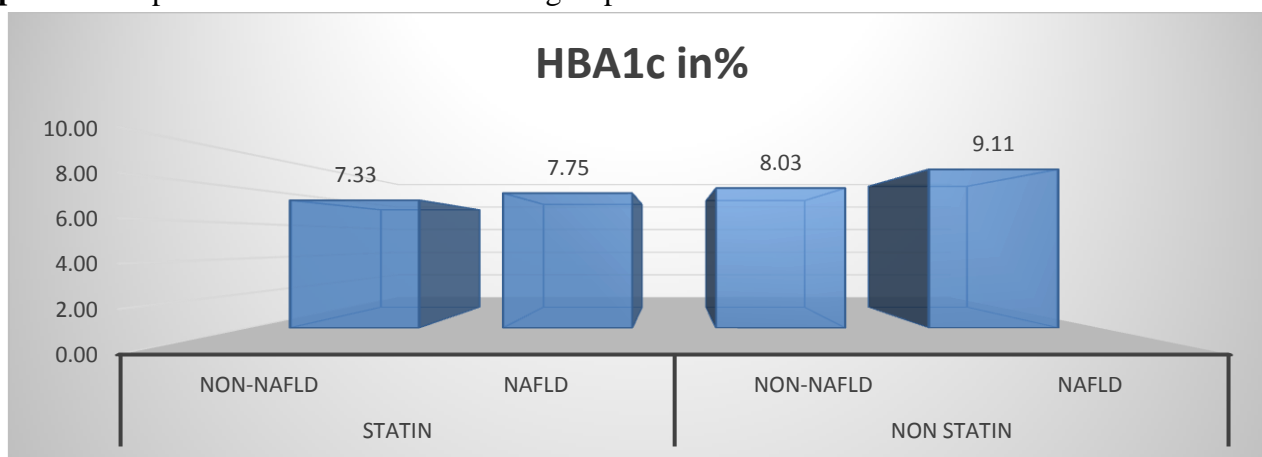
Table – 6 Grades of NAFLD verses grades of HbA1c.

HbA1c Grades VS NAFLD GRADE				
	<7 (%)	7-8.9	>9	TOTAL
GRADE 0	53 (49%)	32 (29%)	24 (22%)	109
GRADE 1	20 (29%)	26 (37%)	24 (34%)	70
GRADE 2	5 (33%)	5 (33%)	5 (33%)	15
GRADE 3	4 (67%)	1 (17%)	1 (17%)	6
TOTAL	82	64	54	200

Above table compares various grades of NAFLD among level of HbA1c. Uncontrolled DM as seen by HbA1c >7 is in 118 subjects, 62 (68%) of them

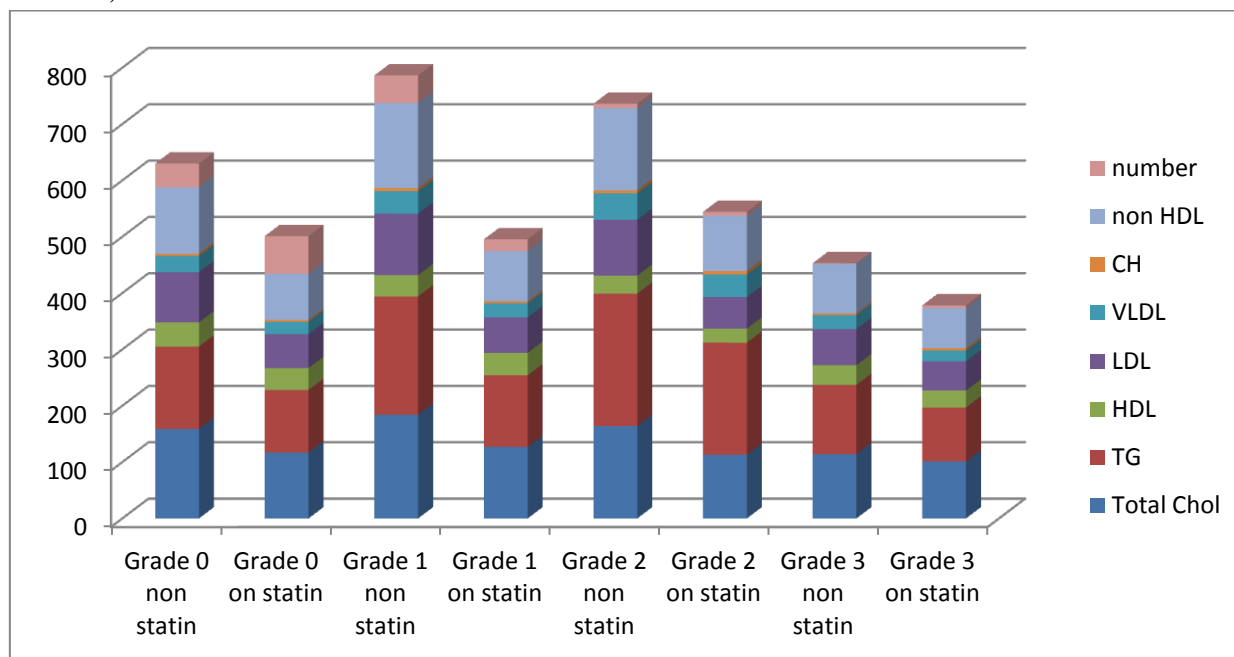
have NAFLD. 53 (49%) with HbA1c <7 have normal liver.

Graph – 2 Comparison of HbA1c in the 4 sub groups.



Above graph shows higher average HbA1c in non statin NAFLD group than other groups.

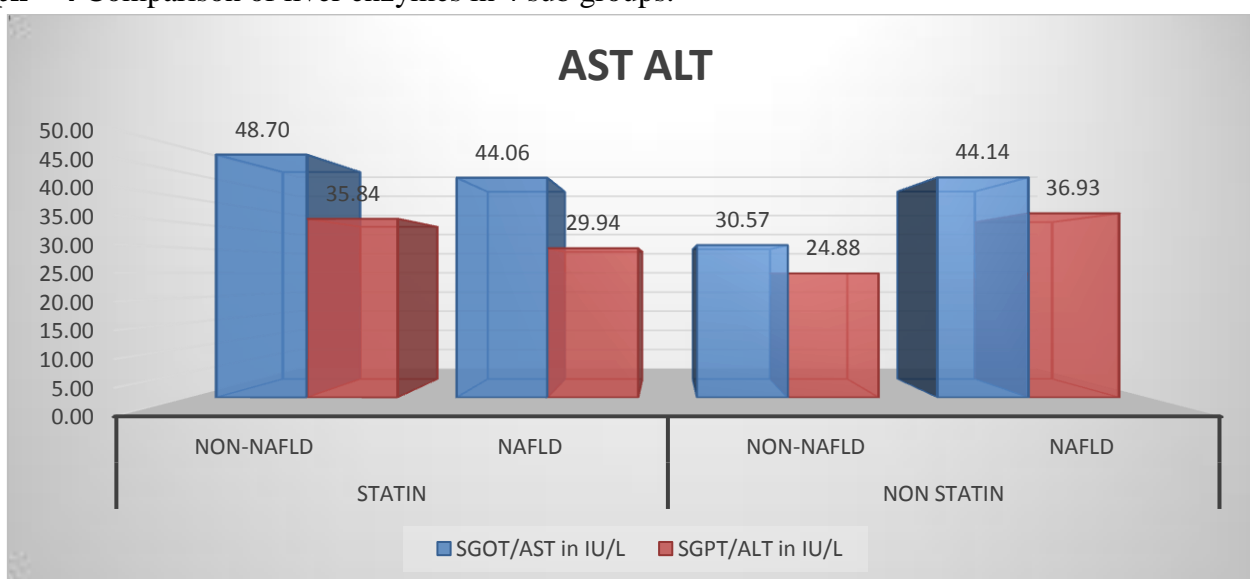
Graph - 3 Comparison of various lipid parameters between various grades of NAFLD in 2 groups (statin and non statin)



Above graph shows the means of various lipids in grades of NAFLD and in 2 groups based on statin therapy. TG and LDL are clearly high in non

statin group, but the same trend is not seen in grade 3. This could be because majority of grade 3 patients were on statin therapy.

Graph – 4 Comparison of liver enzymes in 4 sub groups.



Above graph shows a slight but insignificant raise of AST>ALT in statin group.

Discussion

A total of 200 patients (94 men and 106 women) with type 2 diabetes were included. The prevalence of NAFLD was 45.5%, with men having a lesser prevalence (41%) as compared to women (49%). Among the patients with NAFLD 43% were men and 57% were women. NAFLD

showed a bimodal peak with a female predominance. Average age was lower in NAFLD group (61yrs) compared to non NAFLD group (68yrs). More than half of the study population was obese and dyslipidemic, as almost 83% and 26%% had BMI >25 kg/m² and BMI >30kg/m², respectively. Prevalence of the metabolic syndrome (as per IDF

2005 criteria) was significantly higher in the NAFLD subgroup, as compared to those who did not have NAFLD (54% vs. 6%)^[13-17].

Mean HbA1c was also higher in NAFLD group (8.6%) compared to non NAFLD group (7.6%). Thus, uncontrolled DM also increases the prevalence of NAFLD. Duration of diabetes does not directly correlate with the prevalence or grade of NAFLD. Statin group consisted patients with longer duration of diabetes than non statin group. Using a cut off level of HbA1c>7% as a measure for poor control, 41% in the non-NAFLD subgroup and 59% in the NAFLD group had poor glycaemic control^[1, 2].

In our study, the prevalence of NAFLD, as detected by ultrasound, was 45.5% which is lesser compared with the prevalence found in other studies [Sanjay Kalra et al. (56.5%)^[18], Agarwal AK et al. (57.2%)^[19], T Targher G et al. (69.5%)^[20], Prashanth M et al. (87%)^[21], Somalwar M (56.6%)^[22]. Dyslipidemia and uncontrolled diabetes can increase the risk of development of higher grade of NAFLD, but by mere reduction of cholesterol or TG levels or by reducing HbA1c in a patient we cannot completely reverse the NAFLD grade. Thus, prevention is better than cure. Low HDL (36.5 mg/dL) and higher TG (185 mg/dL) are found in NAFLD group compare to non NAFLD group 40 and 125 mg/dL respectively^[23].

On reviewing the study proforma, 50% of patients were found to be on statin therapy for >3 months, which has reduced their total cholesterol and LDL levels significantly. It is also found that NAFLD prevalence is lower in statin group. Among statin group, prevalence of NAFLD is reduced (33%) compared to 58% in non statin group^[24-26]. Our study also showed that there is no significant elevation of liver enzymes in statin group as feared due to the rare and dose related hepatotoxicity of statin. Some studies also showed that statins can cause diabetes mellitus and glucose intolerance, but in our study group all were DM-2 and they did not show elevated HbA1c levels as compared to non statin group^[27-30].

Conclusions

In our study prevalence of NAFLD in type 2 diabetes was found to be 45.5% by ultrasound, which is lower than the average of other similar studies. Weight, BMI, WHR, HbA1c, lipid parameters were found to be significantly higher in NAFLD group ($p<0.001$). No other parameter showed significance. But, these parameters did not follow a linear pattern as per the grades of NAFLD on ultrasound as expected. In view of the discrepancies, we found that a significant subgroup (100) of patients were already on statin therapy for >3 months for diabetic dyslipidemia, which has altered (lowered TG, total cholesterol, LDL, VLDL and slightly raised HDL levels) in the statin group. Upon comparison, non statin group showed a NAFLD prevalence of 58% whereas that of statin group was only 33%. Weight, BMI, WHR, HbA1c did not show significant difference between these groups. Grade of NAFLD did not comparably reduce, may be because irreversible changes have occurred. Thus, our study concluded beyond doubt that statins has reduced the prevalence of NAFLD due to their lipid lower effect. Our study also found that statin neither had any deterioration in liver enzymes or glycaemic control as believed, hence found to be safe.

Recommendations

- NAFLD is considered the hepatic manifestation of metabolic syndrome and also as an independent risk factor for CVD. Thus, clinicians should consider it as part of the management of the other components of this syndrome.
- So far, preliminary data suggest that weight loss can be beneficial and should be encouraged in overweight patients with NAFLD.
- No proven, effective treatment is currently available for NASH. "prevention is better than cure", early and aggressive management of dyslipidemia and insulin resistance by lipid lowering drugs like statins and by lowering HbA1c below 7,

we can prevent NAFLD development in diabetics.

- Statin may not reverse the irreversible damage that has already occurred in higher grades. It is recommended as prevention and not a cure to NAFLD in diabetic dyslipidemias.
- As insulin resistance has a key role in the development of NAFLD, treating insulin resistance in the NAFLD population is a promising strategy. A multimodal treatment plan that targets obesity, insulin resistance, hyperlipidemia and hypertension might be the best option.

Limitations

A limitation of our study is that the diagnosis of NAFLD was based on ultrasonography and was not confirmed by liver biopsy. Ultrasonography is by far the commonest method of diagnosing NAFLD in clinical practice and has very good sensitivity and specificity. The sensitivity and specificity of ultrasound for detecting hepatic steatosis varies from 60 to 94% and 88 to 95%, respectively. Studies suggest that liver biopsy is seldom necessary to diagnose NAFLD.

Our study is based on limited number of DM-2 patients of a region coming to a particular institute in north India, further larger randomized control studies are required to establish firmly the usefulness and safety of statins in NAFLD and DM-2.

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LIST OF ABBREVIATION USED

S no		
1	%	Percentage
2	<	Less than
3	>	More than
4	NAFLD	Non Alcoholic Fatty Liver Disease
5	Non NAFLD	Non Non Alcoholic Fatty Liver Disease
6	M	Male
7	F	Female
8	WT	Weight in Kgs
9	HT	Height in Meters
10	BMI	Body Mass Index
11	WHR	Waist-Hip Ratio
12	SBP	Systolic Blood Pressure
13	DBP	Diastolic Blood Pressure
14	HTN	Hypertension
15	CHOL	Cholesterol
16	TG	Triglycerides
17	HDL	High Density Lipoprotein
18	LDL	Low Density Lipoprotein
19	VLDL	Very Low Density Lipoprotein
20	CH	Chylomicrons
21	FBS	Fasting Blood Sugar
22	PPBS	Post Prandial Blood Sugar
23	LFT	Liver Funtion Test
24	SGOT/AST	Aspartate Amino Transferase
25	SGPT/ALT	Alanine Amino Transferase
26	ALP	Alkaline Phosphatase
27	GGT	Gamma Glutamate Transferase
28	ECG	Elesctrocardiography
29	DM 2	Diabetes mellitus type 2
30	IDF	International Diabetes Federation
31	WHO	World Health Organization
32	CHD	Coronary Heart Disease
33	HCC	Hepatocellular carcinoma