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Clinico-Pathological Profile of NAFLD in Type 2 Diabetes Mellitus

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Abstract
Non Alcoholic Fatty Liver Disease (NAFLD) is a spectrum of disorders seen in people without consumption of alcohol in amounts known to be toxic to the liver and characterized by pathological appearance of predominant macro-vesicular hepatic steatosis. Non-Alcoholic Steato-Hepatitis (NASH) is a metabolic liver disease in which fatty change i.e steatosis is accompanied with lobular inflammation, hepatocyte injury and/or hepatic fibrosis. NASH can be defined pathologically as significant steato-hepatitis not resulting from alcohol, drugs, toxins, infectious agents or other identifiable exogenous causes. Abnormal liver function tests secondary to NASH/NAFLD has become the most common liver disease worldwide.
This study was planned to evaluate the clinical and biochemical profile of 85 diabetic -out-patients and in-patients attending, the Department of Medicine, Government T.D.Medical College, Alappuzha. The study concluded that NASH prevalence is strongly associated with Female gender, hypertension, dyslipidemia and family history. Further, the occurrence of NASH was higher in those with longer duration of Type 2 Diabetes, uncontrolled Diabetes, higher waist-hip ratio and higher BMI. Presence of combination of low serum albumin levels, low-normal platelet counts, acanthosis nigricans and non-tender hepatomegaly was found to be a strongly predictive of NASH in diabetics.

MATERIALS AND METHODS

The study was conducted on 85 Type 2 Diabetic patients from OPD and general IP wards of Department of General Medicine, Govt TD Medical College, Alappuzha between 2011-2012, after getting approval from the Institutional Research Committee and Ethical committee with the following objectives:

1) Study the prevalence of NASH in type 2 DM
2) Study the risk factors for NASH in patient with type 2 DM

It was a descriptive study on 85 patients above 40 years of age, meeting the ADA 2011 criteria of Type 2 Diabetes and having elevated serum transaminases.

NAFLD was considered in patients with less than 20gm of ethanol consumption per day, Elevated liver transaminases (ALT /AST ratio) and presence of abnormal fat accumulation in the liver on ultrasound imaging. In very rare instances, liver biopsy was performed.

The ADA 2011 Criteria for the diagnosis of Diabetes mellitus was used to select patients.

These were Symptoms of diabetes plus RBS ≥ 200 mg /dl, Fasting plasma glucose ≥126 mg/dl, HBA1c ≥ 6.5% or 2 hour plasma glucose ≥200 mg/dl during an OGTT.

Individuals with consumption of ethanol >20g/ day, usage of drugs known to cause steatosis like amiodarone, valproic acid, isoniazid, phenytoin, carbamazpine, corticosteroids, tamoxifen, methotrexate or high dose estrogen in the previous six months, those with prior history of liver disease, jejuno-ileal bypass or small bowel resection and diseases like malignancy, presence of any liver disease that can cause fatty liver such as chronic hepatitis C, autoimmune liver disease and Wilson's disease were excluded.

All Type 2 Diabetes Mellitus patients who met the inclusion criteria during the study period underwent routine blood examination, RBS, Lipid profile, LFT, urine routine, viral markers (HBs Ag/ Anti HCV) and USG abdomen.

In relevant cases, auto-immune screening (ANA), serum Ferritin and S Cerruloplasmin/ examination for KF ring were done. EDTA vacutainers and plain vacutainers without anticoagulant were used to collect blood for estimation of biochemical parameters.

GOD-POD method was used to estimate blood sugar. Triglyceride, Total Cholesterol and HDL was estimated using ERBA Mannheim diagnostics kit and LDL calculated by Friedwald formula. BMI was calculated from Weight/Height in M2.

All prospective participants were subjected to detailed past, personal and family history taking and physical examination. Standardized questionnaires with these details, current medical treatments and relevant life-style behaviours were completed.

Special emphasis was given on risk factors: (1)Central obesity (>90cm in males;>80cm in females Asians) (2)Signs of Insulin resistance(Acanthosis nigricans /Skin tags / PCOD) (3) Duration of diabetes in years (4) BMI (Kg /m2) Normal 19- 24.99, Overweight: 25-29.99, Obesity >30 (5) Waist hip ratio : > or equal 1.0 male and >

0.9 female (6) Hypertension (BP>130 mm systolic or > 85 mm diastolic or on medication)

Biochemical evaluation was done to estimate lipid profile and detect Dyslipidemia with Hypertrig-lyceridemia: >150 mg/dl; or on medication); Low HDL <40 mg/dl in female or < 50 mg/dl in male or on medication) high LDL Value (>100 mg/dl) and Hypercholesterolemia (> 200 mg/dl).

Uncontrolled diabetes was diagnosed with RBS > 200 mg/dl with or without medications.

Patients were classified into two groups- the first one (group A) consists of patients with Type 2 Diabetes who had normal liver function tests and the second group (group B) consisted of patients suffering from NAFLD in conjunction with Type 2 Diabetes.

Statistical Analysis was done using Statistical Package for Social Sciences (SPSS) version 10. Chi square (χ^2) test was used as nonparametric test for comparisons and associations between different parameters of the data. Sensitivity and specificity of significant variables to detect NASH and to compare proportions within each group were also found out. The risk to develop NASH for various parameters was done with Logistic regression analysis. For all statistical evaluations, a two-tailed probability of value, < 0.05 was considered significant.

RESULT

85 patients with type 2 Diabetes were included in the study after evaluation with the inclusion and exclusion criteria. 6 patients were detected to have NASH, and only 1 gave consent for liver biopsy. Further evaluation excluded 2 patients as they had Hemochromatosis and Wilson's disease.

Table 1: NASH among Diabetic

NASH	Frequency	Percent
Normal	79	92.9
NASH	6	7.1
Total	85	100

In our study, 7.1% patients with type 2 Diabetes had NASH. (6 out of 85)

Table 2: Association between NASH and Gender

| Gender | Group | | Total |
	Normal	NASH	
Male	31	2	33
	39.20%	33.30%	38.80%
Female	48	4	52
	60.80%	66.70%	61.20%
Total	79	6	85
Chi Square: 0.089; P > 0.05			

The occurrence of NASH among diabetics in our study was found to be higher in female (60.70%).

Table 3: Association between NASH and Family History of Diabetes

| Family History | Group | | Total |
	Normal	NASH	
Nil	77		77
	97.50%		90.60%
Diabetes Mellitus	2	6	8
	2.50%	100.00%	9.40%
Total	79	6	85
Chi Square: 62.136; P < 0.001			

In our study, all patients with NASH had a strong family history of diabetes and a duration of diabetes more than 10 years. (P value <0.001).

Table 4: Association between NASH and Insulin Resistance

| Type of Insulin Resistance | Group | | Total |
	Normal	NASH	
Nil	62		62
	78.50%		72.90%
Acanthosis nigricans	14	6	20
	17.70%	100.00%	23.50%
Skin Tags	3		3
	3.80%		3.50%
Total	79	6	85
Chi Square: 20.981; P < 0.001			

All the 6 NASH patients in our study showed signs of insulin resistance. All the 6 had acanthosis nigricans. However, both skin tags and acanthosis nigricans were seen in about 20% of non-NASH diabetic patients.

Table 5: Association between Body Mass Index and Diabetes

| BMI | Group | | Total |
	Normal	NASH	
Normal	43		43
	54.40%		50.60%
Over Weight	36	6	42
	45.60%	100.00%	49.40%
Total	79	6	85
Chi Square: 6.609; P < 0.005			

Risk Factor analysis revealed BMI to be in over-weight category in all the cases detected to have NASH. (P value was significant)

Table 6: Association between NASH and Serum Triglyceride Levels

| Triglycerides | Group | | Total |
	Normal	NASH	
Normal	44		44
	55.70%		51.80%
Hypertriglyceridemia	35	6	41
	44.30%	100.00%	48.20%
Total	79	6	85
Chi Square: 6.926; P < 0.01			

Table 7 Association between NASH and HDL Levels

| HDL | Group | | Total |
	Normal	NASH	
Low	32	6	38
	40.50%	100.00%	44.70%
Normal	47		47
	59.50%		55.30%
Total	79	6	85
Chi Square: 7.985; P < 0.01			

Table 8: Association between NASH and Total Cholestrol

| Total Cholesterol | Group | | Total |
	Normal	NASH	
Normal	60	4	64
	75.90%	66.70%	75.30%
High	19	2	21
	24.10%	33.30%	24.70%
Total	79	6	85
Chi Square: 0.258; P > 0.05			

Elevated Triglyceride and low HDL was uniformly found in all the diabetics people with NASH, and this was statistically significant (P<0.001). However, Serum Cholesterol was not significant in association.

Table 9: Association between NASH and Waist Hip Ratio

| Waist Hip Ratio | Group | | Total |
	Normal	NASH	
Normal	74	2	76
	93.70%	33.30%	89.40%
High	5	4	9
	6.30%	66.70%	10.60%
Total	79	6	85
Chi Square: 21.445; P < 0.001			

There was a statistically significant association between high waist- hip ratio with prevalence of 66.7% in the NASH population.

Table 10: Association between NASH and Uncontrolled Diabetes

| RBS | Group | | Total |
	Normal	NASH	
Normal	77	2	79
	97.50%	33.30%	92.90%
Uncontrolled DM	2	4	6
	2.50%	66.70%	7.10%
Total	79	6	85
Chi Square: 34.963; P < 0.001			

Of the patients with NASH, 66.7 % had uncontrolled diabetes and this was a statistically significant relationship.

Table 11: Association between NASH and Hypertension

| Hypertension | Group | | Total |
	Normal	NASH	
Nil	77	1	78
	97.50%	16.70%	91.80%
Yes	2	5	7
	2.50%	83.30%	8.20%
Total	79	6	85
Chi Square: 48.178; P < 0.001			

In our study, 83.30% of diabetic NASH individuals had hypertension (Statistically significant).

Table 12: Association between NASH and Smoking

| Personal History | Group | | Total |
	Normal	NASH	
Nil	76	5	81
	96.20%	83.30%	95.30%
Smoking	3	1	4
	3.80%	16.70%	4.70%
Total	79	6	85
Chi Square: 2.059; P > 0.05			

Smoking was not a statistically significant factor in occurrence of NASH.

Table 13: Association between NASH and Non Tender Hepatomegaly

| Hepatomegaly | Group | | Total |
	Normal	NASH	
Nil	78		78
	98.70%		91.80%
Yes	1	6	7
	1.30%	100.00%	8.20%
Total	79	6	85
Chi Square: 71.935; P < 0.001			

All the NASH diabetic patients had non tender hepatomegaly.

Table 14: Association between NASH and USG Abdomen (Fatty Liver)

| USG | Group | | Total |
	Normal	NASH	
Normal	74		74
	93.70%		87.10%
Fatty Liver	5	6	11
	6.30%	100.00%	12.90%
Total	79	6	85
Chi Square: 43.429; P < 0.001			

Similarly, USG abdomen revealed a Fatty Liver in all the NASH Diabetics. (Statistically significant, p<0.001)

Table 15: Association between NASH and Low Normal Platelet Count

| Platelet Count | Group | | Total |
	Normal	NASH	
Low Normal (1.5 - 2)	7	4	11
	8.90%	66.70%	12.90%
Normal (2 - 4.5)	72	2	74
	91.10%	33.30%	87.10%
Total	79	6	85
Chi Square: 16.539; P < 0.001			

Further, all diabetic patients with NASH had platelet count on the low normal side (between 1.5 -2 lakh / mm3).

Table 16: Association between NASH and Low Serum Albumin

| S. Albumin | Group | | Total |
	Normal	NASH	
Hypoalbuminemia	16	4	20
	20.30%	66.70%	23.50%
Normal	63	2	65
	79.70%	33.30%	76.50%
Total	79	6	85
Chi Square: 6.676; P < 0.005			

Another statistically significant association was between Serum Albumin level and NASH, with 66.7 % of the diabetic NASH having had low serum albumin.

Table 17: Association between NASH and Type of Oral Hypoglycemic Drug (OHA)

| OHA | Group | | Total |
	Normal	NASH	
Nil	1	1	2
	1.30%	16.70%	2.40%
Sulphonylurea	65	3	68
	82.30%	50.00%	80.00%
Biguanides	9	2	11
	11.40%	33.30%	12.90%
Both	4		4
	5.10%		4.70%
Total	79	6	85
Chi Square: 8.726; P < 0.05			

Sulphonylureas were the most common OHA in the NASH diabetic (50%), followed by biguanides (12.9%).

In a nutshell, our findings were surmised as:

Parameters	B (NASH)	± S.E.	Odds Ratio
Age	3.979	9.460	5.349
Duration of DM	16.968	3.413	2.339
Past History	0.197	4.701	1.218
Family History	11.248	22.674	7.676
BMI	1.841	11.563	6.301
Waist Hip Ratio	0.022	2.382	0.978
Insulin Resistance	0.570	1.513	1.769
Hypertension	0.781	3.705	2.185
Hepatomegaly	7.139	4.273	1.260
AST	0.253	8.086	1.288
ALT	0.424	5.586	1.528
Total Cholesterol	1.298	1.270	3.663
HDL	11.568	9.621	6.587

Discussion

In recent times, abnormal liver tests attributable to hepatic steatosis or NASH have become the most common liver disease in the community. Depending on the criteria set for defining abnormal aminotransferase value in studies such as the Third National Health and Nutritional Examination Survey (NHANES III), the incidence of NAFLD/NASH is between 3 and 23% of the adult population. [1,2,4,10-13].

More recent studies depict a dramatic increase in both the prevalence and severity of NASH in patients with type 2 diabetes [3,6,9,15-19]. Extrapolation of the current trends on the continuing epidemic of obesity and type 2 diabetes predict a very high rise in the prevalence of NASH during the coming years.

Review of literature in earlier studies on NAFLD/NASH emphasized the good overall prognosis [2,7]. The error in this view was subsequently cleared by more recent and detailed studies.

Mounting evidence points to progression to cirrhosis in 20–25% of cases with NASH. [3,4,9-

[12,22] and some cases of 'cryptogenic cirrrhosis' may be attributable to NASH, in which the histological features of steatohepatitis have resolved [8,9,14-18,27]. Rare cases of sub-acute hepatic failure have also been attributed to possible NASH [28]. Recent studies based on case definitions according to fibrotic severity indicate progression to liver failure in those with significant fibrosis. [8,12,29].

Thus, it is of paramount importance to identify population vulnerable to developing NASH from amongst the large pool of diabetic and obese patients, as well as in identifying the set point at which treatment is to be initiated to prevent irreversible liver damage.

Hepatic imaging based studies have detected Hepatic steatosis and/or raised alanine transferase levels in approximately 70% of obese people.[5,9,11,14-18]and NASH in around 20% [1,11].

Factors like obesity, type 2 diabetes and hepatic steatosis predispose to both NASH as well as fibrotic progression in hepatitis C [30,31]. Obesity is recognised now as an independent risk factor for development of alcoholic cirrhosis [32]. Thus, 'NASH determinants' promote cirrhosis, hepatic complication of obesity, insulin resistance and diabetes, and cirrhosis in background of chronic viral hepatitis or alcoholism.

With this background, this study was conducted as an attempt to ascertain clinic-pathological factors that are strongly associated to development of NASH in type 2 Diabetes.

Of the 85 type 2 diabetic patients enrolled in our study, 6 were diagnosed to have NASH. The prevalence of NASH in type 2 diabetes was found to be 7.1% (6 out of 85). Females in our study had a higher occurrence of NASH (60.70%).These findings were comparable to other studies. (Omagari KH et al , Zimmet P et al) [24-26].

In our study, all patients with NASH had a strong family history of diabetes and duration of diabetes more than 10 year (P value <0.001). The studies by Struben V et al had also proven impact of past medical and family history to metabolic disorders that underlie NASH particularly type 2 diabetes,

and other features and complications of insulin resistance such as arterial hypertension and coronary heart disease.[33]

Further, in our study group, acanthosis nigricans as markers of insulin resistance was found in all the 6 patients. Among the non Nash diabetic insulin resistance was found to be in form of both acanthosis and skin tags. Almost similar findings were reported by Marchesini G et al. [6]

Similarly in the laboratory tests, 100 percent diabetics people with NASH in our group were found to have elevated levels of triglyceride , LDL and low HDL levels, and could further indicate insulin resistance. These were comparable to studies by Fan J-G et al who had established the strong association between impaired adipose function and abnormal hepatic lipid partitioning leading to spectrum of NAFLD.[36]

In our study group, risk factor assessment revealed that 66.7% of the NASH population had an elevated waist to hip ratio and 100 % of them fell in the category of overweight, when BMI was calculated. Similar findings have been reported in studies by Angulo P et al. [34] .

Re-analysis of data derived from a population-based surveys by Manchanayake MM et al and Koonen DP et al identified a significant association between dietary cholesterol and cirrhosis, irrespective of cause. Similar statistically significant association was also seen in our study group, with Hypertension and dyslipidemia seen in 83.30% of diabetic NASH individuals, and all having elevated levels of triglyceride and low HDL levels [37,38] . Regarding glycemic control, we established that 66.7 % of our study group had uncontrolled diabetes and a significant relationship were made among the diabetic NASH. In our study, Serum cholesterol and smoking in diabetic NASH as a risk factor didn't yield a statistical significant results .

Non tender hepatomegaly was present in all patient with NASH diabetic. We also found that the sensitivity (100%) and specificity (98.73%) for this clinical sign .Similar picture held true for USG abdomen , in the aspect that all NASH had a

detectable fatty liver (Sensitivity 100% and specificity 93%) .

In addition, we found that diabetic patients with NASH had platelet count on the low normal side . Another strong association was with respect to the serum albumin value. In our group, 66.7 % of the diabetic NASH had low serum albumin which had a significant p value and a specificity of nearly 80%. These were comparable to other studies.(Palekar et al) [39]

Thus the need of the hour is to identify handy and easily accessible clinical models that might help in early recognition and management of this epidemic. Palkar et al created a model that sumarized 5 risks factors (age > 50, female sex, AST >45, BMI > 30, AST/ALT ratio > 0.80, and hyaluronic acid level>55. Yet another larger European study by Angulo P et al assessed the utility of the NASH Test (Biopredictive), a complex test including 13 clinical and laboratory parameters. Yet many more scoring systems to better identify NAFLD patients with NASH are under development and awaiting validation, especially in South Asian population. The adaptability, reliability and ease of such tests in a resource constrained nation like ours is a matter under speculation. [40].

From our study, we conclude that amongst patients with diabetes, those with longer duration of diabetes, poor glycemic control, and a strong family history of hypertension, diabetes and dyslipidemia were more prone to develop NASH.

This study also suggests asides from the recognised indicators like obesity, non tender hepatomegaly, acanthosis nigricans, hypertrig-lyceridemia and low HDL levels, other risk factors like duration of diabetes, low normal platelet levels and low serum albumin level might also be used to identify predisposition to develop NASH and initiation of appropriate life-style and pharmaceutical interventions to attempt disease progression.

Conclusion

1) The prevalence of NASH amongst Diabetics was higher in female gender. There was a strong association between hypertension, dyslipidemia and a family history of diabetes.

2) The incidence of NASH amongst Diabetics was parallel to duration of diabetes and was invariably linked to poor glycemic control, insulin resistance (especially acanthosis nigricans), elevated Triglyceride levels and low HDL levels.

3) Non tender hepatomegaly was identified as a clinical sign with high sensitivity and specificity to predict NASH.A high waist hip ratio and a high BMI was seen frequently.

4) Low serum albumin and low normal platelet were other laboratory findings strongly associated with incidence of NASH amongst diabetics.

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