



Clinicopathological Profile of Hepatic Involvement in Type-2 Diabetes Mellitus and Its Significance

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Abstract

Aims and Objectives : Involvement of liver as an organ complication in Type 2 Diabetes Mellitus (T2DM) is known. Studies are few about their baseline parameters from our country. Study the disease burden and clinicopathological profile of hepatic involvement in T2DM, find the associations with known risk factors and thus try to identify simple markers of advanced disease.

Material and Methods : A screened group of randomly selected 47 patients of T2DM without other liver diseases (viral, alcoholic, drug, autoimmune, etc.) was selected. Their clinical (age, sex, body mass index, family history, blood pressure), biochemical (transaminases, lipid profile), and hepatic ultrasonographic (USG) and histopathological (HPE) profiles were studied. Segregation was done according to the histological severity and duration of diabetes (<5yrs, 5-10yrs, >10yrs).

Results : On histology, normalcy was maintained in 17%, only fatty change was present in 43%, nonalcoholic steatohepatitis (NASH) could be identified in 40% with more advanced disease in 23%. Prevalence of cirrhosis was low. Positive family history, hypertension, longer duration, female sex and increased body mass index were significantly associated with NASH; more advanced disease was associated with male sex only. Incident lipid profile and transaminases levels were non-contributory. In the early stage, USG detected abnormality correlated poorly with HPE.

Conclusion : The burden of hepatopathy in T2DM is high; with improving cardiovascular mortality, a higher burden awaits us in the next decade or so. Naturally, it becomes imperative to the treating clinician for targeting this aspect of diabetic complication from the very beginning of therapy. ©

INTRODUCTION

Diabetes Mellitus (DM) can alter hepatic morphology and physiology; DM itself can be precipitated by hepatic disorders.¹ Though there are ethnic variations, non-alcoholic fatty liver disease (NAFLD) is increasingly being recognized as an important and common condition associated with obesity, Type 2 diabetes (T2DM), hypertriglyceridemia and hypertension (HTN).¹ The spectrum can extend from clinical near normalcy or simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis (often accompanied by steatosis), and even hepatocellular carcinoma.² However, the natural history is not well delineated.² Progression to more severe stages is variable.³ NASH is supposed to be a more severe part of NAFLD that can lead to cirrhosis, terminal liver failure, and hepatocellular carcinoma.³ Most cases present in the 5th or 6th decade with a female preponderance, though recent data suggest a male predominance.¹⁻³ Iron overload

or hemochromatosis gene mutations are rarely associated with NAFLD in Asian Indians.⁴

Typically, liver biopsy is the ultimate test for diagnosis and delineation of the extent.² It is usually not done except for situations of conflicting diagnosis.⁵ Liver ultrasonography (USG), although not sensitive enough to differentiate simple steatosis from more advanced hepatic involvement, is widely used.⁶ Elevated alkaline phosphatase and decreased platelets have found to be indicators of advanced grades.⁷ Increase in GGT may be among the earliest biochemical markers.¹ Hyperbilirubinemia, prolongation of the prothrombin time and hypoalbuminemia are infrequent but it often has an AST/ALT ratio of < 1.^{7,5} High triglyceride levels have been found to be independently associated with advanced fibrosis.⁸

Schematically, a presumed diagnosis of NAFLD can be made in a patient of T2DM who has mild elevations of transaminases, insignificant alcohol consumption, no other liver disease, is not on potentially steatogenic medications and has signs of steatosis on a liver USG scan.⁷ Though some histological markers have been identified for worst outcomes, longitudinal studies are lacking.^{9,3,7} The minimum

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histological features of NASH include steatosis (at least > 5 percent of observed hepatocytes)-macro and micro vesicular fat (either or mixed) especially in zone 1/3, lobular and or portal infiltration with either or both acute and chronic inflammatory cells with or without fibrosis.¹⁰ Ballooning degeneration, Mallory hyaline and cirrhotic changes are considered advanced features.^{2,9} Various grades and stages of inflammation and fibrosis have been suggested but none universally accepted.⁵ Presence of nuclear glycogen has been a constant feature.^{3,7} Little is known about their baseline clinical, biochemical and histological profile in the Indian perspective.^{11,4}

The aim of study was the apparent prevalence and profile of hepatic involvement as assessed by incident clinical parameters, biochemical markers, USG and histopathology, in a clinic based cross sectional Indian population of Type-2 DM. The patients will be initially segregated according to their histological severity and then by the duration of DM, and their clinicobiochemical parameters will be compared. An attempt will be made to find simple clinicobiochemical markers of advanced hepatic involvement, if possible.

MATERIAL AND METHODS

The population prevalence and its standard deviation is not known for hepatopathy of diabetic origin. So for an adequate study strength, a prevalence of 50% was considered (the population prevalence of fatty liver in general is around 24%)¹² and for a precision of 85% the calculated patient population was 44. A screened population of 100 consecutive patients having the inclusion criteria was planned and from them, numbers generated by simple random selection without replacement would select 50 patients (considering dropouts). Their incident clinicopathological profiles will be studied after necessary consent.

Consecutive patients of T2DM were screened for the initial cohort from the Diabetes Clinic of NRS Medical College, Kolkata over a period of six months and the specific numbers identified earlier were included. Patients with history of alcoholism, positive viral markers (HBV, HCV), positive ANA, certain drug intake (steroids, amiodarone, tamoxifene, estrogens, methotrexate, statins, glitazones, etc.), bariatric surgery, recent parenteral nutrition and BMI > 35 and < 17 were excluded.¹³ Patients with overt cardiac, significant renal and or hepatic dysfunction, recent stroke and pregnancy diabetes were also excluded for relevant reasons.^{13,7}

Parameters studied included (1) Clinical- age, sex, body mass index, blood pressure, family history of T2DM and hypertension (HTN; SBP- systolic blood pressure, DBP- diastolic blood pressure) in a first degree relative; (2) Biochemical-serum bilirubin (Bilirn.), ALT(alanine aminotransferase), AST(aspartate aminotransferase), alkaline phosphatase(ALKP), albumin, creatinine (creat.) and lipid profile(TCHL-total cholesterol, TGL-triglyceride, LDL- low density lipoprotein cholesterol, HDL- high density

cholesterol, VLDL); (3) Sonologic – USG detected fatty liver, regenerating nodules or cirrhosis and (4) Histopathological picture of liver biopsy (HPE) .

All patients underwent a complete clinical, anthropometric, and laboratory investigations, including USG and liver biopsy.⁷ Body weight was measured in light clothing and without shoes to the nearest half kilogram and height to the nearest half centimeter; three blood pressure readings were obtained at 2-minute intervals and averaged, and used in the analyses.⁸ Serum analysis was done in a semi autoanalyser. Brightness and posterior attenuation were considered indices of the extent of fatty infiltration and fibrosis.⁶ The patients selected were initially segregated according to their predefined histologic types, and then according to their duration from initial diagnosis: group-A < 5 yrs., group- B 5-15 yrs. and group- C > 15 yrs. (this division was arbitrary).

A biopsy specimen with minimal fragmentation and having a length of ≥ 1 cm or ≥ 7 portal tracts was considered adequate.⁷ NASH was defined as steatosis in more than 5% of observed hepatocytes and evidence of inflammation, lobular or pericentral venular, and or fibrosis (zone 3/zone 1) with or without Mallory hyaline, ballooning degeneration and cirrhosis.⁹ Cases with steatosis only or normal histology were considered as non-NASH (N-NASH). NASH was divided into two subgroups. One with the minimum criteria of steatosis (>5%) and inflammatory infiltrate and or fibrosis termed nonadvanced NASH (NA-NASH); and the other with ballooning degeneration and or Mallory hyaline or cirrhotic change along with the minimal changes, termed advanced NASH (A-NASH).^{7,9,10} Staining was done with, hematoxylin and eosin, and reticulin. No control population was taken, as liver biopsy in normal subjects was not permitted by the ethical committee. Statistical analysis was done by calculation of difference of the standard error of two means or two proportions with a 2 tailed.

Students T-test with equal or unequal variance (as indicated by Fishers Test). Non parametric tests were considered if the values would not have a normal distribution. A 'p' value of less than 0.05 was considered significant.

OBSERVATION

A total of 47 patients consented for the entire procedures. The mean fasting plasma glucose was 184 ± 80.17 mg/dl (N=100), 2 hr. post prandial plasma glucose was 266 ± 97.8 mg./dl (N=140). Total cholesterol (TCHL) was 172.3 ± 43.1 mg./dl (N=135) was raised in 54% (2% > 2 times normal), alkaline phosphatase (N=190 I.U./L; 164.5 ± 73.9 I.U./L) was raised in 17% (>2 times in 6%). Most of the patients presented with raised triglycerides (TGL- 207.9 ± 81.5 mg/dl; N=150 mg/dl) and low HDL cholesterol (HDL- 36.2 ± 9.8 mg/dl; N=40 mg/dl); LDL cholesterol (LDL) was 95.04 ± 37.8 mg/dl (N=100 mg/dl).

The diagnosis of fatty liver was initially done by USG, using standardized criteria, by a single operator who was

unaware of the other data.¹⁰ It was abnormal in 66% of the cases. The HPE examination was also by a single person who was blinded for the rest of the parameters. In HPE, normalcy was maintained in 17%, glycogen deposition was very common (80.8%-74.4%), steatosis was found in 63.8%, inflammation in 36.2%, fibrosis in 31.9%, ballooning degeneration in 27.7% and Mallory hyaline in 4.25%. NASH was evident in 40%, A-NASH in 23% and cirrhosis in 2.12%.

Table 1 enumerates the sex, family history (F/H-DM& HTN) and USG finding distributions of the histologically differentiated groups. Table 2 and 4 shows the clinicobiochemical parameter distribution these groups; Table 3 and 5 elaborates their p-values. The Tables 6 and 8 compares the clinicobiochemical parameters of the patients segregated by duration, while Tables 7 and 9 depicts their p-values. Table 10 outlines the USG findings according to duration and Table 11 elaborates the histologic status of all the patients according to duration. (N=upper limit of normal value, ns=statistically not significant, s=statistically

significant, n=number of patients).

The USG showed fatty change in ~ 50% cases of N-NASH while it was ~ 100% in cases of NASH (100% in A-NASH, p<0.05). Strong association of family history (both DM and or HTN) was found in NASH compared to N-NASH (50% Vs 11-21%, p<0.05), however the relation did not become stronger in cases of A-NASH (p >0.05). Male sex was significantly higher compared to females (78% Vs 22%, p<0.05) in case of A-NASH, while females were similar (52% Vs 47%, p>0.05) in total NASH. However, in total number of cases studied (n=47, F=16, NASH in F=9), prevalence of NASH in females was higher compared to males (56% Vs 32%, p< 0.05).

High BMI was strongly associated with NASH, but for the more advanced variety (A-NASH) the effect was not more (p=0.57). Increasing duration also had a very significant association with NASH, which, however did not contribute to A-NASH (p=0.98). Both increased SBP and DBP were significantly associated with NASH, more so with DBP; again they were not more significantly associated with A-NASH (p=0.58 and 0.71).The incident lipid parameters did not differ amongst the various histological groups.

Serum creatinine and transaminases did not show any

Table 1 : Sex, family history and USG abnormality distribution

	Sex-M:F	F/H-DM	F/H-HTN	Both	USG Abn.
TOTAL n=47	31:16	15	13	9	31
N-NASH n=28	21:07	6	4	3	13
NASH n=19	10:09	9	9	6	18
A-NASH n=9	7:02	5	3	2	9
NA-NASH n=10	3:07	4	6	4	9

Table 5 : P value of the parameters of Table-4

Group	HDL	VLDL	Creat	ALT	AST	ALK P	ALB
NASH vs. N-NASH	0.38	0.76	0.79	0.42	0.56	0.02	0.72
A-NASH vs. N-NASH	0.55	0.47	0.59	0.63	0.62	0.27	0.68
NASH vs. A-NASH	0.91	0.49	0.61	0.31	0.41	0.5	0.95
NA-NASH vs. A- NASH	0.87	0.31	0.44	0.12	0.16	0.32	0.93

Table 2 : Clinical parameter distribution of histological groups.

Group	Age-years	Duration-years	BMI-kg/m ²	SBP-mmHg	DBP-mmHg	TCHL-mg/dl	TGL-mg/dl	LDL-mg/dl
NASH n=19	55.3±8.7	9.75±3.1	30.9±2.8	147.1±18.1	91±7.8	181.5±42.02	203.6±76.97	100.8±42.1
A-NASH n=9	57.6±8.7	9.72±2.4	30.1±3.9	142.8±16.7	89.6±7.1	186.1±50.2	192.9±65.6	110.5±50.7
N-NASH n=28	51.1±8.3	4.2±4.5	23.1±2.3	129.5±21.04	81.9±9.2	168.9±41.8	212±85	93.7±32.9
NA-NASH n=10	53.5±8.2	9.78±3.6	31.6±1.3	150.6±18.5	92±8.1	177.8±33.6	212.2±84.02	93.1±31.6

Table 3 : P value of the parameters of Table-2

Group	Age	Duration	BMI	SBP	DBP	TCHL	TGL	LDL
NASH vs. N-NASH	0.12	0.000005	<0.0000007	0.006	0.001	0.34	0.74	0.53
A-NASH vs.N-NASH	0.07	0.003	0.0000005	0.12	0.04	0.35	0.57	0.28
NASH vs.A-NASH	0.56	0.98	0.57	0.58	0.71	0.81	0.74	0.63
NA-NASHvsA- NASH	0.35	0.96	0.31	0.39	0.56	0.69	0.62	0.41

Table 4 : Clinical parameter distribution of the histological groups

Group	HDL-mg/dl	VLDL-mg/dl	Creat-mg/dl	ALT-mg/dl	AST-mg/dl	ALK P-IU/L	ALB-mg/dl
NASHn=19	37.7±10.4	36.4±19.9	1.03±0.6	45.1±20.9	41.6±19.02	155.3±75.1	3.9±0.51
A-NASHn=9	37.3±7.7	38.9±14.1	0.91±0.5	53.9±16.2	48.9±21.4	134.5±54.1	3.9±0.36
N-NASHn=28	35.03±9.3	34.9±12.9	1.1±0.9	50.1±19.4	44.9±18.4	104.5±67.4	3.9±0.34
NA-NASHn=10	38.1±12.2	40.9±22.5	1.13±0.6	38±21.5	35.7±14.4	171.9±84.7	3.91±0.6

Table 6 : Clinical parameter distribution according to duration

Group	Age years	BMI kg/m ²	SBP mmHg	DBP mmHg	TCHL- mg/dl	TGL- mg/dl	LDL- mg/dl	HDL- mg/dl	VLDL- mg/dl
An=21	49.6±7.97	23.4±2.5	125.5±19.2	80.4±9.02	160±36.5	199.9±82.2	85.7±28.1	37.3±9.3	35.3±12.9
Bn=13	52.7±8.1	28.1±4.1	134.5±19.4	84.8±6.3	183.1±45.2	242.8±80.5	97.9±41.3	36.7±11	44.4±20.4
Cn=13	57.8±7.8	28.9±4.7	157.7±9.7	94.9±5.9	181.5±45.6	185.8±69.1	107.2±43.6	34.2±8.8	26.7±7.97

Table 7 : P value – Duration parameters of Table-6

Group	Age	BMI	SBP	DBP	TCHL	TGL	LDL	HDL	VLDL
A vs. B	0.285	0.002	0.2163	0.115	0.149	0.159	0.376	0.837	0.184
B vs. C	0.135	0.67	0.0017	0.0004	0.934	0.075	0.596	0.563	0.013
A vs. C	0.002	0.002	0.0000005	0.000005	0.179	0.609	0.143	0.342	0.027

Table 8 : Clinical parameter distribution according to duration

Group	Creat-mg/dl	Bilirn-mg/dl	ALT-mg/dl	AST-mg/dl	ALKP- IU/L	ALB-mg/dl
An=21	1.03±0.81	0.719±0.198	47.6±19.3	46.9±19.7	120.8±71.2	3.9±0.37
Bn=13	0.88±0.45	0.776±0.384	48.3±17.8	33.3±12.1	118.6±94.3	3.99±0.33
Cn=13	1.3±0.81	0.859±0.384	49.1±22.7	49.6±17.8	136.2±49.8	3.97±0.58

significant variation, though the mean ALT/AST ratio was more than 1; serum albumin was also unaltered. The ALKP was significantly higher in cases of NASH (p=0.02) but did not increase in A-NASH. Highest ALT (53.9 ± 16.2 mg/dl) was associated with A-NASH and highest ALKP (171.9 ± 84.7 mg/dl) with NA-NASH but none were in the clinically striking levels.

The BMI and blood pressure were significantly higher in the longer duration age groups (B and C) as was the age, but the lipid measures did not vary, except for VLDL-C that was significantly lower in group B and C.

Serum creatinine did not differ; group B had the lowest mean AST while group C had the highest ALKP; only the AST alteration was statistically significant (p=0.016).

Normalcy was highest in patients with shorter duration (61.9%); fatty changes became significantly evident with longer duration (groups B and C), though group-A had a higher prevalence compared to population data (33.3% Vs 24%). Cirrhosis was found only in group C but was not a statistically significant finding.

Table 9 : P value – Duration parameters of Table-8

Group	Creat	Bilirn	ALT	AST	ALKP	ALB
A vs. B	0.504	0.813	0.914	0.022	0.952	0.495
B vs. C	0.132	0.324	0.927	0.016	0.579	0.906
A vs. C	0.387	0.915	0.849	0.692	0.476	0.739

None of the patients of highest duration (group-C) had a normal USG study or liver biopsy. All the histological abnormalities expressed highest prevalence in group-C, with the exception of nuclear glycogen deposition- it was uniformly high in all the groups (71.4%, 84.6%, 93.3% p>0.05). Mallory hyaline (marker of irreversible damage) was the least observed pathology and found only in group-C (15.4%). In group-A, 2 patients having ballooning degeneration (reversible damage) did not have any other abnormality on histology (including steatosis) – these patients were not segregated as NASH. The group of A-NASH patients increased uniformly from group-A to group-C (4.76%, 27.1%, 53.8% p<0.05). The single case of cirrhosis was included in the category of NASH and A-NASH patients. His liver biopsy had patchy areas of steatosis and he could be identified in the USG scan as a possible case of cirrhosis (but fatty change was not identified).

DISCUSSION

No genomic abnormality has been observed in cholesterol and bile acid synthesis in NAFLD, but hepatic cholesterol have been found to be rich in PUFA than SFA suggesting abnormal cholesterol metabolism.¹⁴ This phenomenon may establish the relation with insulin resistance syndrome and gallstones. Mitochondrial dysfunction leads to increased hepatic oxidative stress and lipid peroxidation. This leads

Table 10 : Distribution of USG findings:

Total = 47 (Patients)	Group A n=21	Group B n=13	Group C n=13	Significance
Normal n=15	13 (61.9%)	2 (15.4%)	0	A>B p<0.025 B vs. c = ns A>C p<0.001
Fatty change n=31	8 (38.1%)	11 (84.6%)	12 (92.3%)	A<B p<0.005 B vs. c = ns A<C p<0.001
Cirrhosis n=1	0	0	1 (6.3%)	A vs. B vs. C=ns

Table 11 : Distribution of liver histopathology

Item	Group A	Group B	Group C	Significance
Parameters	n=21	n=13	n=13	P<0.05
Normal biopsy	6 (28.57%)	2 (15.38%)	0	A>C A vs. B = ns B vs. C = ns
Nuclear glycogen deposition	15 (71.4%)	11 (84.6%)	12 (92.3%)	A>C A vs. B = ns B vs. C = ns
Cytoplasmic glycogen deposition	12 (57.1%)	11 (84.6%)	12 (92.3%)	A > C A vs. B = ns B vs. C = ns
Fatty changes(micro & macro)	8 (38%)	9 (62.2%)	13 (100%)	A<B<C
Mallory hyaline	0	0	2 (15.38%)	A vs. B = ns C >B & A
Ballooning degeneration	3 (14.3%)	4 (30.76%)	6 (46.15%)	A< C A vs. B = n B vs. C = ns
Inflammation	2 (9.5%)	7 (53.8%)	8 (61.53%)	B vs. C = ns B & C >A
Fibrosis	2 (9.5%)	6 (46.2%)	7 (53.8%)	B vs. C = ns B & C >A
NASH	2 (9.5%)	7 (53.8%)	10 (76.9%)	B vs. C = ns B & C >A
A-NASH	1(4.76%)	3(27.1%)	7(53.8%)	C>B>A
USG fatty change	8(38.1%)	11(84.6%)	12(92.3%)	C=B>A
Cirrhosis	0	0	1 (7.69%)	A vs. B vs. C = ns

to direct hepatic injury by damaging cellular organelles and DNA by depleting mitochondrial DNA and stimulating the nuclear factor $\kappa\beta$ pathway.¹⁴ Free cholesterol, lower levels of cellular ATP, and calcium depletion inhibits protein folding in the endoplasmic reticulum, which results in release of inflammatory mediators. Worsened inflammation causes migration of macrophages into adipocytes leading to release of cytokines, more inflammation and fibrosis in the liver. Stem cell based reparative process which restores normal structure in liver is also abnormal in NAFLD.¹⁴

The 'gold standard' for diagnosing NAFLD is clinicopathological correlation, with confirmation of steatosis by liver biopsy and exclusion of other causes (e.g., alcohol) clinically.⁹ Steatosis is generally macro vesicular, although it may be mixed with micro vesicular droplets, which usually indicate mitochondrial injury.¹⁵ Ultrasonography detects fatty changes in the liver in 12.9%-16.4% of individuals in the population at large.¹⁶ Specific symptoms related to the hepatobiliary system were infrequent (dyspepsia and ill-defined right upper quadrant discomfort -19.1%) as was clinical hepatomegaly. Serum bilirubin and albumin were normal. Raised enzyme levels (ALT, AST, and ALKP) were hardly more than twice the normal and this level of difference was of no use for practical diagnostic purposes, however their levels are known to fluctuate.^{17,16} The association of NAFLD with HTN is well documented; systolic being more commonly associated.¹⁷ In our study, HTN was associated with NASH but diastolic HTN had a stronger relation.¹⁷ Incident lipid parameters did not predict severity status probably because dyslipidemia is a common association of T2DM of any stage.¹⁷

No imaging method (USG, CT scan, MRI) is probably able to distinguish between simple steatosis and NASH or indicate accurately the stage of fibrosis.^{3,16} The sensitivity of each imaging method increases with the degree of fatty infiltration, with at least 33% steatosis being optimal.¹⁷ They often fail to identify alterations at a threshold of below 30%.¹⁷ A clinical diagnosis of NAFLD before biopsy based on serologic and imaging studies has been found to be correct in 53%-83% cases.¹⁶ The negative predictive value of a normal USG scan has been estimated to be around 55%-87%.¹⁶ Though there are fallacies, ultrasonography seems to be useful for the diagnosis of presumed NAFLD, on an average up to 75% can reveal steatosis. On USG, fatty change was found in 63.8% cases, and cirrhosis in 2% cases. On statistical analysis, fatty change became significantly evident between group A and group C. The discordance between abnormal USG and abnormal histology was highest in group A – nearly 30% patients expressing fatty liver in USG did not have any histological abnormality; this discordance eased out with increasing duration. The higher prevalence with increasing age may be easily explained by the higher prevalence of obesity and the cumulative glucotoxicity.^{16,17}

Histologic features used to distinguish NASH from simple steatosis are controversial and vary in the literature.^{16,10} NASH has been defined as zone 3 predominate macro vesicular steatosis in combination with hepatocyte ballooning and a mixed inflammatory infiltrate, often with characteristic perisinusoidal and pericellular fibrosis.⁹ Alcoholic liver disease and NAFLD have similar histologic features; they cannot be distinguished by means of liver

biopsy, meticulous history and certain biomarkers are helpful.^{17,9} The prevalence of NAFLD tends to be higher among males and almost universal among morbidly obese diabetics.⁹ NASH had been found to be present in 18.5% of obese subjects (compared to 2.7% of lean subjects) and in nearly 50% of severely obese people with T2DM.^{17,9}

In the histological findings, there was high prevalence of nuclear and cytoplasmic glycogen deposition, which is probably indicative of the uncontrolled glycaemic status of the patients. No fatty change was found in 17 patients (36.2%) and a total normal biopsy was seen in 6 patients (17%). Isolated ballooning degeneration was evident in 2 patients of group-A, their significance is difficult to state but subjects with ballooning degeneration only are also known to progress.¹⁷ Steatosis had a high prevalence and in all patients, it was both macro and micro vesicular, though international studies have shown a very high prevalence of macro vesicular fat with a low prevalence of micro vesicular fat, which is supposed to be associated with a more sinister prognosis.^{3,7,9} The reason for this discrepancy is probably related to either the high prevalence of insulin resistance in our population (leading to more mitochondrial injury) or they are merely macro vesicular fat in their early formative stages.¹⁵ Overall, the prevalence of micro and macro vesicular fatty changes uniformly increased with duration.

Mallory hyaline was evident only group C. For evidence of inflammation and fibrosis, the increase from group A to group B and group C was significant, but between group B and group C, it was not significant. The prevalence of NASH was high (40.4%), but A-NASH was 23%. The relative proportions of NASH to NAFLD in various studies have been around 1:10.¹⁷ The histologic criteria to define NASH remaining tentative, selection biases might have operated differently in the various series.^{17,9} A selection bias might also have been the reason in our study.

Prevalence of cirrhosis did not differ amongst the three groups. Prevalence of NASH increased with the duration of T2DM (9.5% in group A to 76.9% in group C). The cases of NASH predominantly occurred in the 5th and 6th decade (mean age 55.3 yrs), with a female preponderance (56% Vs 32%).¹⁸ Most (73.33%) of the patients of NASH were obese with a mean BMI 30.9 kg/m², and the most common clinical finding being asymptomatic hepatomegaly (27%). The mean diastolic blood pressure was 91 mmHg, TGL 203.6 mg/dl, TCHL 181.5 mg/dl and LDL 100.8 mg/dl. Highest LDL was found in A-NASH (110.5 ± 50.7 mg/dl); lowest HDL was associated with N-NASH (35.03 ± 9 mg/dl). The findings on liver biopsy of the NASH patients were – glycogen deposition (100%), fatty changes (100%), inflammatory infiltrates (90%), fibrosis (78.9%), ballooning degeneration (58%) and Mallory hyaline bodies (10.5%).¹⁸ The prevalence of cirrhosis was very low compared to the prevalence of NASH (2% Vs 40%).⁸ This is probably related to the still prevalent high cardiovascular mortalities in these patients, which kills them before they can reach the stage of cirrhosis.¹⁹ The pattern of liver-related death is not uniform across the spectrum

of NAFLD;¹⁸ poor outcomes have been more frequent in patients in whom biopsies show ballooning degeneration and Mallory hyaline or fibrosis.^{9,17}

Multiple associations of NASH could be identified in our study by the univariate analysis (due to small sample size multivariate analysis was not feasible). These included strong family history of either DM or HTN or both, female sex, both systolic and diastolic BP, higher BMI, longer duration of DM and to some extent raised ALKP.^{17,8,7} However, for more advanced disease (A-NASH), a significant association could be established with male sex only.²⁰ Estradiol is a potent endogenous antioxidant and is supposed to be protective for females.²⁰ Variant estrogen receptors have been found to be expressed largely in male patients and this might contribute to their higher risk.²⁰ Patients with lesser degrees of hepatic involvement (N-NASH) compared to NASH had a shorter duration of diagnosis of DM, lower BMI and lower alkaline phosphatase, less strong family history and fewer USG abnormalities. The lipid parameters, transaminases, albumin and bilirubin levels did not differ.

Majority of patients do not develop significant complications, but around 28% of the patients may develop serious liver sequel.⁵ Liver biopsy, though the recommended diagnostic procedure, entails a recorded death risk of 0.01%.⁵ With a projected high population burden of T2DM and NAFLD, liver biopsy will not be a viable option for disease staging in those at risk of advanced fibrosis. More simple noninvasive markers are the need of the day.⁵

CONCLUSION

Mechanisms responsible for the progression of fatty liver to NASH and beyond are still not clear and there is disagreement on the exact histological definitions as well as the elements that indicate progression.^{14,7} Serum enzyme levels cannot be used to predict the histologic severity, USG detected fatty change may be a sensitive indicator of NAFLD, especially in longer duration patients.^{9,17} On histology, steatosis is very common; micro and macro vesicular fatty changes have an equal prevalence. Mallory hyaline, ballooning degeneration were infrequent while nuclear and cytoplasmic glycogen deposition were too abundant.⁹ Though there is significant progression of NASH over the years, there is hardly any significant prevalence of cirrhosis.⁸ The combination of history, physical examination, non-invasive tests and imaging studies are useful for excluding other disease.^{9,6} Liver biopsy with its inherent risks is the most sensitive and specific means to diagnose the hepatic status in T2DM.⁷

NASH is associated with strong family history of T2DM or HTN, higher BMI and longer duration; significantly raised alkaline phosphatase may be a non-invasive indicator. Male sex probably predisposes to advanced hepatic involvement. However, in a primary care setting where NAFLD is common, a positive ultrasound scan in association with metabolic risk factors (T2DM) and other simple associations in the absence positive serologic evidence of chronic liver disease

is likely to be adequate for diagnosis NASH or advanced NASH. Involvement of the liver as organ complication in DM was so long not given due care. Significant prevalence of hepatic involvement in recent onset T2DM and absence of non-involvement in advanced cases as seen in this study compels us to divert our vision. Liver as an organ for glucose metabolism provides the platform for achieving metabolic stability.¹⁴ Our analyses underline the existence of an exceedingly large population at risk of forth coming liver failure in the next decades, provided they survive the burden of cardiovascular disease mortality.^{17,18} Early hepatic intervention to improve the metabolic status should be one important component of diabetes care.

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