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Nonalcoholic fatty liver disease (NAFLD) and Portal Hypertension in Egyptian Patients

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Abstract

Background and Study aims: *Although nonalcoholic fatty liver disease (NAFLD) typicallyfollows a benign non progressive clinical course, there is evidence that NAFLD even in the absence ofliver fibrosis can induce portal hypertension which has serious sequelae such as splenomegaly,esophageal varices and ascites. The aim of this work is to assess the possibility of NAFLD to affectportal venous pressure in absence of fibrosis, and to correlate the severity of steatosis with the portalvein diameter. Patients and Methods: this study included eighty patients with NAFLD. All participantswere subjected to detailed history taking, clinical examination, laboratory tests, abdominal ultrasoundand portal vein duplex as well as liver biopsy to determine the histological degree of steatosis and toexclude liver fibrosis. Results: the patients were 50 males (62.5%) and 30 females (37.5%), their*

meanage was 30.08±11.70. The degree of steatosis according to liver biopsies ranged from 3%-20% withmean of (14.57 ± 5.74) and the portal vein diameter measured by portal vein duplex ranged from 10-14mm with mean of (11.5 ± 1.24). There was statistically significant positive correlation between thedegree of steatosis and portal vein diameter, spleen size, fasting blood sugar, total cholesterol, LDL,HDL and ALT. Conclusion: hepatic steatosis correlates with portal vein diameter and size of the spleenin the absence of fibrosis and can lead to portal hypertension.

Keywords: *NAFLD; steatosis; portal hypertension; liver biopsy; spleen*

Abbreviations: AFP = alpha-fetoprotein; ALT = alanine aminotransferase;ANA=antinuclear antibody, anti-HBc Ab= antibodies against hepatitis B core antigen;ASMA=anti smooth muscle antibody, CBC = complete blood count; HAV = hepatitis Avirus; HBsAg = hepatitis B surface antigen, HCC= hepatocellular carcinoma; HCV =hepatitis C virus; HDL= high density lipoprotein; HVPG= hepatic venous portal gradient,INR = international normalized ratio; LDL= low density lipoprotein; LDLT= Living donor liver transplantation; NAFLD =nonalcoholic fatty liver disease; NASH=nonalcoholic steatohepatitis; PHT=portal hypertension; PAI= plasminogen activatorinhibitor; TNF= tumor necrosis factor.

Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a wide spectrum ofconditions associated with over accumulation of fat in the liver ranging from NAFLD orsimple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis [(1)].

Although nonalcoholic fatty liver disease (NAFLD) typically follows a benignnon progressive clinical course, NASH is a potentially serious condition; as many as 25%of patients may progress to cirrhosis and experience complications of portal hypertension,liver failure, and hepatocellular carcinoma [(2)].

Portal hypertension (PHT) is a severe complication of liver cirrhosis. Patientswith PHT are at risk to develop gastro-esophageal varices and massive gastrointestinal bleeding, ascites, hepatorenal syndrome, and hepatic encephalopathy [(3)].

Current understanding of the progression of NAFLD and NASH involves a "2-hit" hypothesis in which the initial metabolic disturbance causes steatosis and a secondpathogenic stimulus causes oxidative stress, reactive oxygen species formation, andcytokine production [(4)]

To date, it is not clear whether liver steatosis per se (i.e., in the absence of severefibrosis or liver cirrhosis) might increase portal pressure and trigger the portalhypertensive syndrome.

Several studies investigated the prevalence of portal hypertension and esophagealvarices in patients with well-characterized and liver biopsy-confirmed NAFLD, andfound that portal hypertension and esophageal varices were not uncommon amongpatients with NAFLD [(5)].

The purpose of this study was to assess the possibility of steatosis to induce portalhypertension in absence of fibrosis in patients with nonalcoholic fatty liver disease, and tocorrelate the severity of portal hypertension with the histological degree of steatosis. Diagnosisof portal hypertension using ultrasound techniques such as duplex ultrasonography andcolor Doppler imaging are the modalities of choice, because they are noninvasive, rapid,and highly sensitive and specific [(6)].

Patients and Methods

Eighty adult patients with NAFLD presented to Ain Shams center of organtransplantation (ASCOT) -Ain Shams University Hospitals- as donors for living relatedliver transplantation (LDLT) were enrolled in this study. They were collected over aperiod of two years .The study was approved by the Ethics Committee of Ain ShamsUniversity Hospitals, Cairo, Egypt, in accordance with local research governancerequirements.

Inclusion criteria:

- Adult patients
- Proved hepatic steatosis by clinical, laboratory, ultrasound and histologicalfeatures.

- Written informed consent was obtained from each participant.

Exclusion criteria:

- Patients with established liver fibrosis or cirrhosis diagnosed by liver biopsy
- Other causes of hepatic diseases/ affection such as alcoholic liver disease, viralhepatitis, autoimmune hepatitis, hemochromatosis, Wilson disease, thrombophilia,metabolic liver diseases.
- Other diseases that lead to portal hypertension e.g. Schistosomiasis.
- Other causes of hepatic pathology such as HCC.

Patient Assessment: All patients were subjected to:

Full history taking, clinical examination and laboratory tests including complete bloodcount (CBC), international normalized ratio (INR), liver and renal profiles includingserum albumin, total proteins, total bilirubin, direct bilirubin, alanine transaminase(ALT), aspartate transaminase (AST), serum creatinine, alpha-fetoprotein (AFP)levels, viral markers (HCVAb, HBsAg, HBcAb, HAV Ab), autoimmune markers(ANA, ASMA), serum iron, serum ferritin, blood sugar and lipid profile (LDL, HDL,total cholesterol and triglycerides).

Abdominal ultrasound imaging using Toshiba "Just Vision" real-time scannerinstrument with a 3.5 MHz convex transducer was performed to all participants. Thepatients were scanned in the supine position with comment on: liver size, echogenicity,splenic size according to its longest axis (normally it is up to 12-13cm [(7)], and PVdiameter and patency. Measurement of the portal vein diameter was taken in quietrespiration at the hilum of the liver just before bifurcation into right and left. Formeasurement of portal vein diameter, the central portion of the cursors was fixed at theechogenic outer wall of the vein. The wall of the portal vein was excluded from themeasurement. Supervision was made during the data collection by the principalinvestigators to assure the data quality. Ultrasound features of portal hypertensioninclude: dilated portal vein > 13mm, re-canalization of para-umblical vein, portalsystemiccollateral pathways, splenomegaly or ascites [(8)]

Portal vein duplex: using intercostal approach, color Doppler was used to evaluate thedirection of the flow in the main portal vein i.e. hepatopetal/non-hepatopetal(hepatofugal or bidirectional) and diameter of the portal vein, with subjects in the supineposition during suspended inspiration (figure 1). Usually, blood flow in the portalvein is hepatopetal (toward the liver) during the entire cardiac cycle. With a further increasein portal venous pressure, the blood flow direction becomes to-and-fro biphasic), and finally,direction is reversed (hepatofugal) *(9).* The device used was an ultrasonic duplex systemcomposed of a real-time electronic linear type B-mode scanner and a pulsed Dopplerflow meter using Fast Fourier transform (Toshiba SAL 50A/SDL-01A, Toshiba Corp,Tokyo, Japan).

Percutaneous liver biopsy: to assess the degree of steatosis and to exclude fibrosis.

Ultrasonography-guided liver biopsies were performed under conscious sedation usinga 16-gauge Klatskin needle. The length of the histological specimens was no less than2.5 cm. All biopsy specimens were placed in 10% neutral buffered formalin solutionfor fixation and embedded in paraffin blocks. Serial sections (sectioned at 4-µmintervals) were concurrently stained with Hematoxylin-Eosin and Masson's trichrome.Degree of steatosis was identified according to the NAFLD activity score (NAS)scoring system *(10)*

***Statistical Analysis*:** IBM SPSS Statistics for Windows, version 19.0 (IBM Corp.,Armonk, NY, USA) was used for data analysis. Quantitative parametric measureswere expressed as mean ±SD, quantitative non-parametric measures as median andpercentiles, and both numbers and percentages were used to express categorized data.Student t, Wilcoxon rank-sum, Spearman's ranked correlation, and Chi square testswere used to analyze data. Linear Correlation coefficient was

used for detection ofcorrelation between two quantitative variables in one group. ANOVA test was used forcomparison among different times in the same group in quantitative data. If positive,Tukey's test was used to compare between different groups. Probability of error (P)values <0.05 were considered statistically significant.

Results

The demographic features of the studied population showed that there were 50men (62.5%) and 30 women (37.5%) with their age ranged from 18 to 44 years (mean30.08±11.70). None of the studied patient was hypertensive or diabetic and none of themwas alcoholic. The mean of the body mass index BMI of the patients was (27.25±2.70).Liver biopsy showed macro-steatosis ranging from 3% to 20% with mean of(14.575± 5.746) in absence of liver fibrosis (F0) in all patients.

Portal vein diameter ranged from 10-14 mm with mean of (11.5±1.24) withhepatopetal direction of the blood flow in all patients (table1). Sixty patients had portalvein diameter ≥ 13mm (75%), twenty of them (25%) had PV diameter > 13mm. Spleen sizeranged from 11-12 cm with mean of (11.4±0.5). None of the participants had recanalizationof para-umbilical vein, portal-systemic collateral pathways, splenomegaly orascites.

There was statistically significant correlation between the degree of steatosis andportal vein diameter (figure 2), spleen size (figure 3), fasting blood sugar, cholesterol level, LDL, HDL, ALTand serum sodium, but there were no statistically significant correlation between thedegree of steatosis and complete blood picture parameters, INR, total proteins, albuminand triglycerides (table 2).

For statistical purpose the patients of the current study were sub-divided into threesubgroups according to the degree of steatosis by liver biopsy:

Group I: steatosis (3%-10%); (n=20); included 12 males (60%) and 8 females(40%). The mean±SD of their ages is 26.30±5.12.

Group II: steatosis (10%-15%); (n=28); included 18 males (64.3%) and 10 females(35.7%). The mean±SD of their ages is 29.50 ±4.50

Group III: steatosis (15-20%); (n=32); included 20 males (62.5%) and 12 females(37.5%). The mean±SD of their ages is 32.93±8.65

The results showed statistical significant difference between the 3 subgroupsregarding the age (*p=0.05*), spleen size *(p=0.00)*, TLC *(p=0.02)*, FBS *(p=0.00)*, serumsodium *(p=0.027)*, and cholesterol *(p=0.018)*. The higher values are in higher degrees ofsteatosis (table 3).

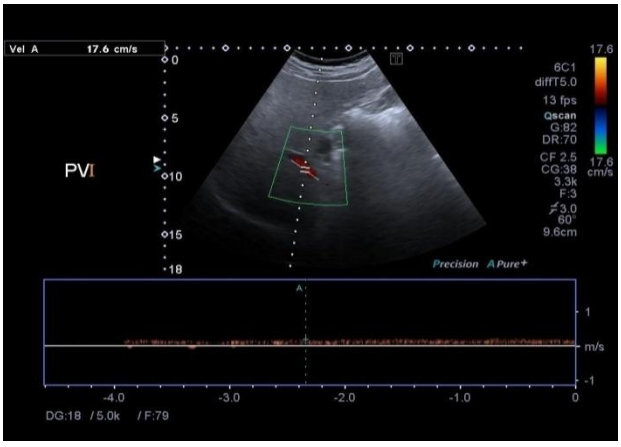

Figure 1: Portal vein flow

Table (1): Descriptive data of the studied patients

	Minimum	Maximum	Mean	SD
Hemoglobin (g/dl)	11.70	16.50	13.75	1.15
Platelets (/mm³)	150.00	354.00	250.38	57.65
TLC(/mm³)	3.00	12.20	6.41	1.90
Total proteins(gm/l)	6.60	8.50	7.57	0.45
Serum albumin(gm/l)	3.30	5.30	4.38	0.42
Total bilirubin(mg/dl)	0.23	1.50	0.63	0.25
Dir. bilirubin (mg/dl)	0.10	0.30	0.15	0.06
ALT (U/L)	5.00	43.00	22.98	8.13
AST (U/L)	12.00	39.00	26.55	7.22
FBG (mg/dl)	73.00	100.00	87.90	6.21
Na(meq/l)	133.00	147.00	139.30	3.20
K(meq/l)	3.60	5.10	4.38	0.33
Creatinine(mg/dl)	0.50	1.30	0.82	0.20
BUN (mg/dl)	6.00	19.00	12.00	3.43
INR	0.87	1.20	0.97	0.07
Cholesterol(mg/dl)	70.00	195.00	122.23	32.62
TG (mg/dl)	36.00	183.00	82.33	31.34
HDL (mg/dl)	35.00	72.00	50.45	7.20
LDL (mg/dl)	42.00	128.00	75.28	19.67
PV diameter(mm)	10.00	14.00	11.50	1.24
Spleen size (cm)	11.00	12.00	11.53	0.51
Degree of steatosis (%)	3	20	14.575	5.746

Table (2): Correlation between the degree of steatosis and other parameters:

Correlations		
	Degree of steatosis	
	r	P-value
Hemoglobin	-0.099	0.543
Platelets	-0.202	0.212
TLC	0.096	0.556
Total proteins	-0.269	0.094
Serum albumin	-0.174	0.284
Total bilirubin	-0.158	0.329
Direct bilirubin	0.184	0.255
ALT	0.363	0.021*
AST	0.236	0.142
Fasting blood glucose	0.550	<0.001*
Na	-0.322	0.042*
K	0.060	0.711
creatinine	-0.074	0.650
BUN	-0.044	0.786
INR	-0.026	0.875
Cholesterol	0.509	0.001*
TG	0.048	0.770
HDL	-0.382	0.015*
LDL	0.369	0.019*
PV diameter	0.336	0.034*
Spleen size	0.573	<0.001*

*Statistically significant difference.

Table (3): Comparison between the 3 sub-groups regarding differentLaboratory and ultrasound parameters:

Parameters	Steatosis (3-10%) (n=20)	Steatosis (10-15%) (n=28)	Steatosis (15-20) (n=32)	*P value*
Hemoglobin (g/dl)	13.7±0.9	14.01±1.3	13.5±1.09	0.48
Platelets (/mm³)	251.2±50.2	265.7±65.7	236.3±54.3	0.38
TLC(/mm³)	5.65±1.46	7.48±1.92	5.95±1.79	0.02*
INR	0.97±0.06	0.96±0.06	0.96±0.08	0.92
Total proteins (gm/l)	7.67±0.4	7.66±0.3	7.41 ±0.5	0.24
Serum albumin (gm/l)	4.44±0.34	4.42±0.46	4.31±0.44	0.70
Total Bilirubin (mg/dl)	0.70±0.20	0.62±0.33	0.58±0.19	0.496
Direct bilirubin (mg/dl)	0.14±0.05	0.15±0.06	0.16±0.06	0.715
FBS(mg/dl)	82.6±3.27	87.571±6.63	91.5±4.80	0.00*
ALT(U/L)	19.1±6.11	22.35±6.27	25.93±9.77	0.105
Sodium (meq/L)	141.60±2.98	138.50±3.05	138.58±2.89	0.02*
Cholesterol (mg/dl)	103±26.65	117±31.83	138±30.32	0.01*
Triglycerides (mg/dl)	81.10±29.79	86.35±25.43	79.56±37.88	0.838
HDL (mg/dl)	53.20±9.79	52.00±5.65	47.37±5.76	0.078
LDL(mg/dl)	65.70±14.46	76.14±17.65	80.0±84	0.173
PV diameter (mm)	10.80± 1.13	11.71±1.26	11.75±1.18	0.11
Spleen size(cm)	11.10±0.31	11.42±0.51	11.87±0.34	<0.0*

*Statistically significant difference.
INR, international normalized ratio; TLC: total leucocytic count; FBG: FASTING BLOOD GLUCOSE; ALT, Alanine transaminase;HDL, high density lipoprotein;LDL, low density lipoprotein.

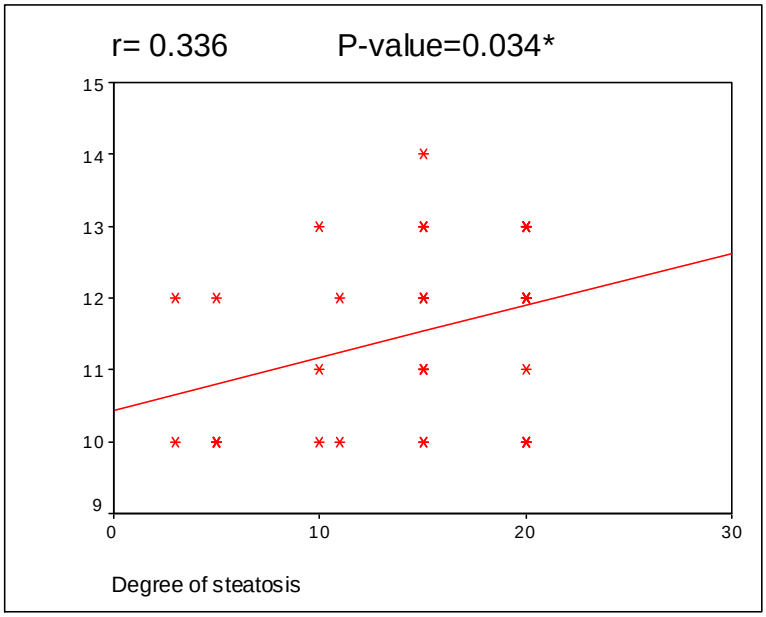

Figure 2: correlation between steatosis and portal vein diameter

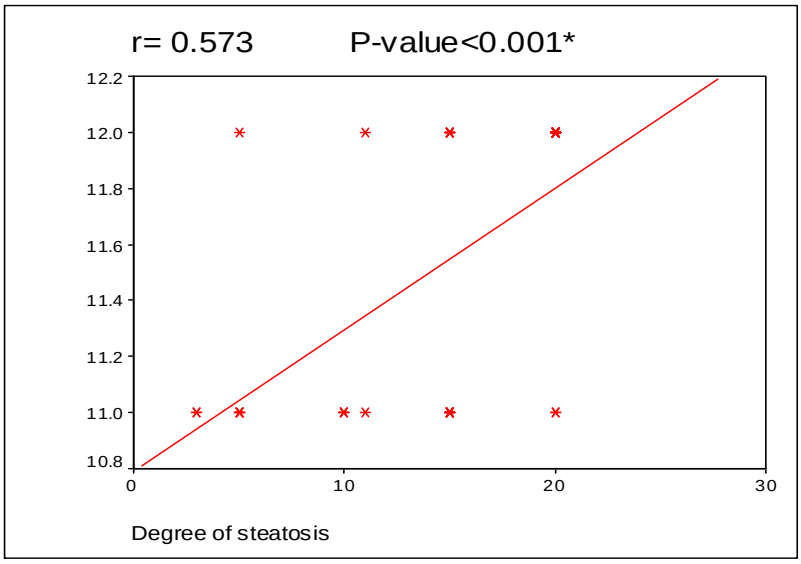

Figure 3: correlation between the steatosis and spleen size.

Discussion

NAFLD prevalence has grown to epidemic proportions [(11)]. Long-termlongitudinal studies suggest that NAFLD has a benign non progressive clinical course,whereas NASH is a serious condition with increased risk of both overall and liver-relatedmorbidity and mortality [(12)].

Portal hypertension (PHT) is a severe complication of liver cirrhosis leading to ahigher risk of development of gastro-esophageal varices, ascites, hepatorenal syndrome,and hepatic encephalopathy. Among patients with NASH, the portal hypertensivesyndrome appears only in those with advanced cirrhosis [(13)]. The mechanisms by whichsteatosis could induce PHT are not fully understood. Leptin seems to play a role in thepro-fibrogenic responses in the liver [(12)]. To date it is not clear whether the architectural and functional alterations seen inpatients with steatosis in absence of cirrhosis might induce a significant increase of portalpressure. Steatosis,

without fibrosis, may lead to changes in liver blood flow, which arepoorly understood [14].

In a study published in 2014 on ten patients presenting with metabolic disorderwithout any other known cause of liver disorders, 2 died during perioperative course ofmajor liver resection most probably due to hepatorenal syndrome on top of acute portalhypertension related to the surgical procedure and chronic portal hypertension which wasundetermined. The authors concluded as fibrosis was not present or marginal in liverspecimens, and the real cause of chronic portal hypertension in patients with metabolicdisorders should be investigated with further studies [15].

Patients of the current study showed degree of steatosis ranging from 3% to 20%,with portal vein diameter ranging from10-14 mm. Sixty patients (75%) showed eitherhigh upper limit of normal PV diameter (13mm) or higher diameters (>13mm). Althoughpatients of the current study didn't have PV diameters exceeding 14mm, there wassignificant positive correlation between the degree of steatosis and portal vein diameter.

This is in some similarity with *Francque et al.*, who found elevated hepatic venous portalgradient (HVPG) in overweight patients with NASH [14]. Similarly, *Mendes et al.*performed a study on patients of NASH with no fibrosis and found that (6%) of patientswith steatosis had portal hypertension [5].

It is of notice that changes in liver blood flow in steatosis partly resemble thoseseen in cirrhosis, in which the intra-hepatic portal flow is usually reduced attributed to anincreased intrahepatic vascular resistance resulting in increase in the portal pressure [16].

In the current study a statistically highly significant positive correlation existedbetween the degree of steatosis and size of the spleen. This was in agreement with *Suzukiet al.*, who found larger splenic size in patients with simple steatosis and NASH [16].

There are reports which had suggested that enlarged hepatocytes arising from steatosiscompress the sinusoids and lead to portal hypertension in NASH patients [17].

In this study there was significant positive correlation between the degree of steatosis and ALT, which is in agreement with *Shi et al.*, who found that ALT levelswere higher in NASH patients with higher degrees of steatosis [18]. Elevated ALT levelsare the most common liver abnormality in NAFLD and NASH, whereas alkaline phosphatase and γ-glutamyltransferase are less frequently elevated. NAFLD is a common explanation forabnormal liver tests results and accounts for asymptomatic elevation of aminotransferase levelsin up to 90% of cases [19].

Also, there was statistically significant positive correlation between the degree ofsteatosis and cholesterol and LDL level and significant negative correlation with HDL.

This is in agreement with *Wang et al.* who found higher total cholesterol and LDL levelsin NASH patient, whereas the level of HDL was markedly lower [20].

In the current study there was significant correlation between the degree ofsteatosis and fasting blood glucose which is in agreement with Lankarani et al., whofound elevated levels of fasting blood sugar in patients with NAFLD [21]. NAFLD isclosely related to metabolic syndrome in which there is increase in the visceral fat withincreased production of TNF-α from the adipocytes and alteration of levels of adiponectin, resistin, and PAI-1. TNF-α has been shown not only to cause the productionof inflammatory cytokines, but also possibly to trigger cell signaling by interaction with aTNF-α receptor that may lead to insulin resistance. This increases free fatty acid formation, cholesterol and LDL levels[22].

On the other hand, there was no statistically significant correlation between thedegree of steatosis and Triglycerides, which is different than results of *Khurram andAshraf*, who found hypertriglyceridemia in studied population of NASH [23].

Dyslipidemia in patients with NAFLD is atherogenic in nature and it is characterized byincreased levels of serum triglycerides and decreased levels of HDL cholesterol. Themechanisms for these profound alterations in lipid and lipoprotein profiles in NAFLD arenot well understood, but they have generally been attributed to hepatic overproduction ofthe very low density lipoprotein (VLDL) particles and deregulated clearance of variouslipoproteins from the circulation [24].

In this study there was no significant correlation between the degrees of steatosisand total bilirubin, unlike the study of *Min-Sun Kwak et al*., which showed that serumbilirubin level was inversely associated with the prevalence of NAFLD [25]. Serum bilirubin has anti-oxidant and cytoprotective effects. Previous experimental research alsosupports the role of bilirubin as a protective marker of NAFLD [26].

In this study there was no statistically significant correlation between the degreeof steatosis and hemoglobin, platelets and serum albumin, which is in agreement with*Lankarani et al*. [21].

On dividing the patients into three subgroups according to the degree of steatosis,there was significant difference between them regarding the age, spleen size, fastingblood sugar, total leukocytic count, serum sodium and cholesterol being higher withincreased degree of steatosis. The prevalence of NAFLD and NAFLD-related fibrosiswas found to increase with age [27]. Older patients have more NAFLD risk factors, suchas hypertension, obesity, diabetes and hyperlipidaemia[27].

In our study there were 50 (62.5%) males and 30 (37.5%) females, and this is inagreement with the study of *Lankarani et al*. who found higher incidence of NAFLD inmale patients [21]. NAFLD was initially thought to be more common in women, but thisopinion lacks empirical support [27]. Another Asian study showed higher prevalence of NAFLD in men than in women [28]. Male gender was also associated with elevatedaminotransferase levels, the presence of histological NASH, hepatic fibrosis and overall mortality in patients with NAFLD [29]. This may be attributed to the fact that femalehormones are protective against the development of NAFLD for their role/ interplay withlipid metabolism in liver. Vice versa, androsterone and androgens may have unfavorableeffects on liver function and hepatocytes [30].

Conclusion

Hepatic steatosis correlates with portal vein diameter and size of spleen in the absence of fibrosis and can lead to portal hypertension.

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Conflicts of Interest: There are no financial or other relations that could lead to aconflict of interest.

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