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) typically follows a benign non progressive clinical course, there is evidence that NAFLD even in the absence of liver 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 affect portal venous pressure in absence of fibrosis, and to correlate the severity of steatosis with the portal vein diameter. **Patients and Methods:** this study included eighty patients with NAFLD. All participants were subjected to detailed history taking, clinical examination, laboratory tests, abdominal ultrasound and portal vein duplex as well as liver biopsy to determine the histological degree of steatosis and to exclude 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% with mean 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 the degree 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 spleen in 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 A virus; HBsAg = hepatitis B surface antigen, HCC = hepatocellular carcinoma; HCV = hepatitis C virus; HDL = high density lipoprotein; HVP = 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 activator inhibitor; TNF = tumor necrosis factor.

Introduction

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

Although nonalcoholic fatty liver disease (NAFLD) typically follows a benign non 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. Patients with 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 second pathogenic stimulus causes oxidative stress, reactive oxygen species formation, and cytokine production⁽⁴⁾.

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

Several studies investigated the prevalence of portal hypertension and esophageal varices in

patients with well-characterized and liver biopsy-confirmed NAFLD, and found that portal hypertension and esophageal varices were not uncommon among patients with NAFLD⁽⁵⁾.

The purpose of this study was to assess the possibility of steatosis to induce portal hypertension in absence of fibrosis in patients with nonalcoholic fatty liver disease, and to correlate the severity of portal hypertension with the histological degree of steatosis. Diagnosis of portal hypertension using ultrasound techniques such as duplex ultrasonography and color 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 organ transplantation (ASCOT) - Ain Shams University Hospitals - as donors for living related liver transplantation (LDLT) were enrolled in this study. They were collected over a period of two years. The study was approved by the Ethics Committee of Ain Shams University Hospitals, Cairo, Egypt, in accordance with local research governance requirements.

Inclusion criteria:

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

- 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, viral hepatitis, 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 blood count (CBC), international normalized ratio (INR), liver and renal profiles including serum albumin, total proteins, total bilirubin, direct bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), serum creatinine, alpha-fetoprotein (AFP) levels, viral markers (HCV Ab, HBs Ag, HBc Ab, 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 scanner instrument with a 3.5 MHz convex transducer was performed to all participants. The patients were scanned in the supine position with comment on: liver size, echogenicity, splenic size according to its longest axis (normally it is up to 12-13 cm ⁽⁷⁾, and PV diameter and patency. Measurement of the portal vein diameter was taken in quiet respiration at the hilum of the liver just before bifurcation into right and left. For measurement of portal vein diameter, the central portion of the cursors was fixed at the echogenic outer wall of the vein. The wall of the portal vein was excluded from the measurement. Supervision was made during the data collection by the principal investigators to assure the data quality. Ultrasound features of

portal hypertension include: dilated portal vein > 13 mm, re-canalization of para-umbilical vein, portal systemic collateral pathways, splenomegaly or ascites ⁽⁸⁾

Portal vein duplex: using intercostal approach, color Doppler was used to evaluate the direction 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 supine position during suspended inspiration (figure 1). Usually, blood flow in the portal vein is hepatopetal (toward the liver) during the entire cardiac cycle. With a further increase in 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 system composed of a real-time electronic linear type B-mode scanner and a pulsed Doppler flow 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 using a 16-gauge Klatskin needle. The length of the histological specimens was no less than 2.5 cm. All biopsy specimens were placed in 10% neutral buffered formalin solution for fixation and embedded in paraffin blocks. Serial sections (sectioned at 4-µm intervals) 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 measures were expressed as mean $\pm$ SD, quantitative non-parametric measures as median and percentiles, and both numbers and percentages were used to express categorized data. Student t, Wilcoxon rank-sum, Spearman's ranked correlation, and Chi square tests were used to analyze data. Linear Correlation coefficient was

used for detection of correlation between two quantitative variables in one group. ANOVA test was used for comparison 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 50 men (62.5%) and 30 women (37.5%) with their age ranged from 18 to 44 years (mean 30.08 ± 11.70). None of the studied patient was hypertensive or diabetic and none of them was 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) with hepatopetal direction of the blood flow in all patients (table 1). Sixty patients had portal vein diameter ≥ 13 mm (75%), twenty of them (25%) had PV diameter > 13 mm. Spleen size ranged from 11-12 cm with mean of (11.4 ± 0.5). None of the participants had recanalization of para-umbilical vein, portal-

systemic collateral pathways, splenomegaly or ascites.

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

For statistical purpose the patients of the current study were sub-divided into three subgroups 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 $\pm$ 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 $\pm$ 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 $\pm$ SD of their ages is 32.93 ± 8.65

The results showed statistical significant difference between the 3 subgroups regarding the age ($p=0.05$), spleen size ($p=0.00$), TLC ($p=0.02$), FBS ($p=0.00$), serum sodium ($p=0.027$), and cholesterol ($p=0.018$). The higher values are in higher degrees of steatosis (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 (/mm ³)	150.00	354.00	250.38	57.65
TLC(/mm ³)	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)	<i>P</i> <i>value</i>
Hemoglobin (g/dl)	13.7±0.9	14.01±1.3	13.5±1.09	0.48
Platelets (/mm ³)	251.2±50.2	265.7±65.7	236.3±54.3	0.38
TLC(/mm ³)	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-term longitudinal 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-related morbidity and mortality ⁽¹²⁾.

Portal hypertension (PHT) is a severe complication of liver cirrhosis leading to a higher risk of development of gastro-esophageal varices, ascites, hepatorenal syndrome, and hepatic

encephalopathy. Among patients with NASH, the portal hypertensive syndrome appears only in those with advanced cirrhosis ⁽¹³⁾. The mechanisms by which steatosis could induce PHT are not fully understood. Leptin seems to play a role in the pro-fibrogenic responses in the liver ⁽¹²⁾. To date it is not clear whether the architectural and functional alterations seen in patients with steatosis in absence of cirrhosis might induce a significant increase of portal pressure. Steatosis,

without fibrosis, may lead to changes in liver blood flow, which are poorly understood ⁽¹⁴⁾.

In a study published in 2014 on ten patients presenting with metabolic disorder without any other known cause of liver disorders, 2 died during perioperative course of major liver resection most probably due to hepatorenal syndrome on top of acute portal hypertension related to the surgical procedure and chronic portal hypertension which was undetermined. The authors concluded as fibrosis was not present or marginal in liver specimens, and the real cause of chronic portal hypertension in patients with metabolic disorders 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 from 10-14 mm. Sixty patients (75%) showed either high upper limit of normal PV diameter (13mm) or higher diameters (>13mm). Although patients of the current study didn't have PV diameters exceeding 14mm, there was significant positive correlation between the degree of steatosis and portal vein diameter.

This is in some similarity with *Franque et al.*, who found elevated hepatic venous portal gradient (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 patients with steatosis had portal hypertension ⁽⁵⁾.

It is of notice that changes in liver blood flow in steatosis partly resemble those seen in cirrhosis, in which the intra-hepatic portal flow is usually reduced attributed to an increased intrahepatic vascular resistance resulting in increase in the portal pressure ⁽¹⁶⁾.

In the current study a statistically highly significant positive correlation existed between the degree of steatosis and size of the spleen. This was in agreement with *Suzuki et 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

steatosis compress 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 levels were higher in NASH patients with higher degrees of steatosis ⁽¹⁸⁾. Elevated ALT levels are the most common liver abnormality in NAFLD and NASH, whereas alkaline phosphatase and γ -glutamyltransferase are less frequently elevated. NAFLD is a common explanation for abnormal liver test results and accounts for asymptomatic elevation of aminotransferase levels in up to 90% of cases ⁽¹⁹⁾. Also, there was statistically significant positive correlation between the degree of steatosis 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 levels in NASH patient, whereas the level of HDL was markedly lower ⁽²⁰⁾.

In the current study there was significant correlation between the degree of steatosis and fasting blood glucose which is in agreement with *Lankarani et al.*, who found elevated levels of fasting blood sugar in patients with NAFLD ⁽²¹⁾. NAFLD is closely related to metabolic syndrome in which there is increase in the visceral fat with increased 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 production of inflammatory cytokines, but also possibly to trigger cell signaling by interaction with a TNF- 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 the degree of steatosis and Triglycerides, which is different than results of *Khurram and Ashraf*, who found hypertriglyceridemia in studied population of NASH ⁽²³⁾.

Dyslipidemia in patients with NAFLD is atherogenic in nature and it is characterized by increased levels of serum triglycerides and decreased levels of HDL cholesterol. The mechanisms for these profound alterations in lipid and lipoprotein profiles in NAFLD are not well understood, but they have generally been attributed to hepatic overproduction of the very low density lipoprotein (VLDL) particles and deregulated clearance of various lipoproteins from the circulation⁽²⁴⁾.

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

In this study there was no statistically significant correlation between the degree of 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, fasting blood sugar, total leukocytic count, serum sodium and cholesterol being higher with increased degree of steatosis. The prevalence of NAFLD and NAFLD-related fibrosis was found to increase with age⁽²⁷⁾. Older patients have more NAFLD risk factors, such as hypertension, obesity, diabetes and hyperlipidaemia⁽²⁷⁾.

In our study there were 50 (62.5%) males and 30 (37.5%) females, and this is in agreement with the study of *Lankarani et al.* who found higher incidence of NAFLD in male patients⁽²¹⁾. NAFLD was initially thought to be more common in women, but this opinion lacks empirical support⁽²⁷⁾. Another Asian study showed higher prevalence of NAFLD in men than in women⁽²⁸⁾. Male gender was also associated with elevated aminotransferase levels, the presence of

histological NASH, hepatic fibrosis and overall mortality in patients with NAFLD⁽²⁹⁾. This may be attributed to the fact that female hormones are protective against the development of NAFLD for their role/interplay with lipid metabolism in liver. Vice versa, androsterone and androgens may have unfavorable effects 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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References

1. Bacon BR, Farahvash MJ, Janney CG and Neuschwander-Tetri BA. "Nonalcoholic steatohepatitis: an expanded clinical entity". *Gastroenterol.* 1994; 107:1103-9.
2. Chalasani N, Younossi Z, Lavine J, Diehl A, Brunt E, Cusi K, et al. "The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association". *Hepatology*. 2012; 55: 2005-23.
3. Browning JD, Kumar KS, Saboorian MH and Thiele DL. "Ethnic differences in the prevalence of cryptogenic cirrhosis". *Am J Gastroenterol.* 2004; 99: 292-8.
4. Tilg H and Diehl AM. "Cytokines in alcoholic and nonalcoholic steatohepatitis". *N Engl J Med.* 2000; 343:1467-76.

5. Mendes FV, Suzuki A, Sanderson SO, Lindor KD, Angulo P. "Prevalence and indicators of portal hypertension in patients with nonalcoholic fatty liver disease". *Clin Gastroenterol Hepatol*. 2012; 10: 1028-33.
6. Khan A.N. and Cho KJ. "Portal Hypertension Imaging". Medscape (2013).
7. Benter T, Klühs L and Teichgräber U. "Sonography of the Spleen". *J Ultrasound Med*. 2011; 30:1281-93.
8. Allan R. "Ultrasound assessment of portal hypertension". *Sound effects* 2006; 1:22-7.
9. Nićiforović D, Till V, Lovrenski J, Govorcín M, Lucić Z, Lovrenski J. "Duplex Doppler sonography in portal hypertension". *Med Pregl*. 2007; 60:161-7.
10. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;41:1313-21.
11. Angulo P, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. "Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease". *J Hepatol*. 2004; 41:943-9
12. Adams LA, Sanderson S, Lindor KD, and Angulo P. "The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies". *J Hepatol*. 2005; 30:145-53.
13. Cichoz-Lach H, Celinski K, Slomka M, and Kasztelan-Szczerbińska B. "Pathophysiology of portal hypertension". *J Physiol Pharmacol*. 2008; 59:231-5.
14. Francque S, Wamutu S, Chatterjee S, Van Marck E, Herman A, Ramon A. "Nonalcoholic steatohepatitis induces non-fibrosis-related portal hypertension associated with splanchnic vasodilation and signs of a hyperdynamic circulation". *Liver Int*. 2010; 30:365-75.
15. Le Bian Az, Costi R, Idrissi M and Smadja C. "Liver resection and metabolic disorders: An undescribed mechanism leading to postoperative mortality". *World J Gastroenterol*. 2014; 20(39): 14455-62.
16. Suzuki K, Kirikoshi H, Yoneda M, Mawatari H, Fujita K, Nozaki Y. "Measurement of spleen volume is useful for distinguishing between simple steatosis and early stage nonalcoholic steatohepatitis". *Hepatology Research* 2010; 40:693-700.
17. Colman JC, Britton RS, Orrego H, Saldivia V, Medline A, Israel Y. "Relation between osmotically induced hepatocyte enlargement and portal hypertension". *Am J Physiol* 1993; 245:G382-7.
18. Shi JP, Xun YH, HU CB, Zhang L, Liu H, Lou GQ. "Clinical and histological features of nonalcoholic fatty liver disease". *Chinese journal of hepatology* 2009; 11:812-6.
19. Clark JM, Brancati FL and Diehl AM. "The prevalence and etiology of elevated aminotransferase levels in the United States". *Am J Gastroenterol*. 2003; 98:960-7.
20. Wang Z, Xu M, Peng J, Jiang L, Hu Z, Wang H, et al. "Prevalence and associated metabolic factors of fatty liver disease in the elderly". *Exp Gerontol*. 2013; 48:705-9.
21. Lankarani KB, Ghaffarpasand K, Mahmoodi M, Lotfi M, Zamiri N, Heydari ST et al. "Nonalcoholic fatty liver disease in southern Iran: A population based study". *Hepat Mon*. 2013; 13:e9248.
22. Fukuchi S, Hamaguchi K, Seike M, Himeno K, Sakata T, Yoshimatsu H. "Role of Fatty Acid Composition in the Development of Metabolic Disorders in Sucrose-Induced Obese Rats". *Exp Biol Med*. 2004; 229:486-93.

23. Khurram M and Ashraf MM. A clinical and biochemical profile of biopsy-proven non-alcoholic Fatty liver disease subjects. *Journal of College of Physicians and Surgeons Pakistan* 2007; 17:531-4.
24. Taskinen MR, Adiels M, Westerbacka J, Söderlund S, Kahri J, Lundbom N et al. Dual metabolic defects are required to produce hyper triglyceridemia in obese subjects. *Arterioscler Thromb Vasc Biol.* 2011; 31:2144-50.
25. Kwak MS, Kim D, Chung GE, Kang SJ, Park MJ, Kim YJ, et al. Serum bilirubin levels are inversely associated with nonalcoholic fatty liver disease. *Clin Mol Hepatol.* 2012; 18: 383-90.
26. Yu J, Chu ES, Wang R, Wang S, Wu CW, Wong VW, et al. Heme-oxygenase protects against steatohepatitis in both cultured hepatocytes and mice. *Gastroenterol.* 2010; 138:694-704.
27. Amarapurkar D, Kamani P, Patel N, et al (2007). Prevalence of nonalcoholic fatty liver disease: population based study. *Ann Hepatol* 2007; 6: 161–3.
28. Fan JG, Zhu J, Li XJ, Gupte P, Kumar P, Agal S, et al. "Prevalence of and risk factors for fatty liver in a general population of Shanghai, China:.". *J Hepatol.* 2005; 43:508–14.
29. Chen ZW, Chen LY, Dai HL, Chen JH, Fang LZ. Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. *J Zhejiang Univ Sci B.* 2008; 9: 616–22.
30. Farrell GC, McCullough AJ, Christopher P. Non-Alcoholic Fatty Liver Disease: A Practical Guide. Day John Wiley & Sons 2013. 328 pages. *Online library. Wiley.com* /doi/10.1002/9781118556153.ins/summa.