

Assessment of Hepatotoxicity of Obesity in Female Patients' through Histopathological, Biochemical and Immunohistochemical Study

Ragia M. Hegazy^{1,2,*}, Waleed H. Almalki¹, Eman Farouk^{2,3}, Hala F. M. Kamel^{3,4}

¹College of Pharmacy, Umm Al-Qura University (UQU), Makkah, Saudi Arabia

²College of medicine, Benha University, Egypt

³College of medicine, Umm Al-Qura University (UQU), Makkah, Saudi Arabia

⁴College of medicine, Ain Shams University, Egypt

Abstract Background: Obesity is a multifactorial condition that involve complex interactions of metabolic, physiologic, social and behavioral factors. Obese individual has an increased incidence of non alcoholic fatty liver disease (NAFLD) which is one of the most common causes of chronic liver disease. The complications of NAFLD remain undescribed. Aim of the work: was to determine the progression of complications of NAFLD. Material and Methods: 20 liver biopsies from female patients' were evaluated and categorized, according to the NAS(Non Alcoholic ActivityScore) system. Biopsies were processed for histopathological and immunohistochemical study beside liver function tests. Results:Liver biopsies from obese person showed single or multiple fat droplets inside the cytoplasm of the most of liver cell, ballooning of hepatocytes with positive immunohistochemical reaction for Mollary bodies occurred. Moreover inflammatory cells were detected in hepatic lobules together with hepatic cell necrosis. These changes were associated with pericellular ad peiportal fibrosis. There were significant increases in liver functions ($P < 0.05$). Conclusions:It is likely that regular assessment of liver functions and histopathological changes in obese will continue to play an important role in the diagnosis and management of people with NAFLD. Recommendations:Further studies are still required to determine the role of liver biopsy and liver function tests in monitoring therapeutic responses. The grading and staging NAFLD also requires further evaluation.

Keywords Obesity, Liver Functions, Immunohistochemistry, NAFLD, Liver Biopsy

1. Introduction

Obesity has reached epidemic proportion with a prevalence that continues to increase; obesity is due to combined effects of multiple gene, lifestyle and environmental factors. There is significant paralleling rise in incidence of obesity and diabetes[1, 2]. Non alcoholic fatty liver diseases (NAFLD) emerging as one of the most common cause of chronic liver disease[2, 3].

NAFLD occurs in the setting of metabolic syndrome in which insulin resistance plays key role. Metabolic syndrome is a metabolic disorder related to obesity[4]. Obesity, increases delivery of free fatty acid to the liver, fatty acid metabolism is impaired with accumulation of triglyceride in liver of obese persons suggesting a defect in mitochondrial lipid oxidative accumulation of tissue triglyceride and acylCoAenzyme derivative may activate serine kinase pathway that induce insulin resistance by blocking the

insulin receptors leading to type 2 diabetes mellitus[4, 5].

Biochemical and Histopathological assessment will continue to play an important role in the diagnosis and management of people with NAFLD[6-8]. Liver biopsy is appropriate methods to determine the severity of liver damage accurately and may reveal an additional cause of liver damage[3, 9, and 10].

Causes of NAFLD remain un-described so the **aim of the present study is to** determine the complications of NAFLD through analysis of the changes in liver histopathology and its relation with liver function tests deterioration.

2. Materials and Methods

Subjects

Twenty females were involved in the study had been subdivided into two groups. Group (1): Five female patients with normal body mass index ≤ 25 were used as control. Group (2): fifteen female obese patients [body mass index (BMI) ≥ 35 were enrolled prospectively and consecutively in the study. All patients underwent bariatric surgery at the Benha University Hospital.

* Corresponding author:

r.m.hejazy@hotmail.com (Ragia M. Hegazy)

Published online at <http://journal.sapub.org/ajmms>

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Exclusion Criteria: were patients being treated with hepatotoxic or antineoplastic drugs, or with primary liver disorders other than fatty liver that could account for HcS.

Ethical approval: An ethical approval for this study from Ethics Committee in Benha University Faculty of Medicine Hospitals and all subjects provided written informed consent about the aim of the study had obtained before conduction of the study.

Biochemical assay

The serum was extracted from each blood sample for spectrophotometric determination of the levels of γ -glutamyltransferase (GGT), aspartate transaminase (AST) and alanine transaminase (ALT) [11], alkaline phosphatase (ALP) [12], total protein [13], and albumin [14]. Also, the globulin values and albumin/globulin ratios were calculated.

Histopathological study: Liver biopsies were obtained at the moment of bariatric surgery, using a Tru-cut of 18-G. Only samples longer than 14mm on histological examination were included in the study [20,21]. Liver biopsies were obtained from obese patients. Liver biopsies were immediately fixed in buffered formalin and processed for paraffin section; Sections were obtained and stained with hematoxylin and eosin, to assess cumulative hepatocyte changes and necroinflammatory, fibrotic or architectural modifications and Masson trichrome stain to detect fibrosis. The intensity, distribution (centrizonal, panzonal and azonal) and quality (macrovesicular, multivesicular or mixed) of the HcS were analyzed. The term multivesicular HcS is used to refer to hepatocytes showing multiple small-medium sized vesicles without nuclear displacement to the cell periphery. This was assessed as present or absent. In addition, we noted the presence or absence of lobular inflammation, pericellular and portal fibrosis and of any other hepatocellular injury. So features of steatohepatitis include hepatocellular injury (beyond simple fatty change), inflammation and fibrosis (Table 1). Hepatocyte ballooning is the most characteristic feature of steatohepatitis [16].

Table (1). Typical lobular changes occurring in steatohepatitis [16]

Hepatocellular injury	Ballooning
	Apoptosis/necrosis
	Mallory's hyaline
Inflammation	Neutrophil polymorphs
	Other cells (e.g. T lymphocytes, macrophages)
Fibrosis	Perisinusoidal
Pericellular	

Immunohistochemical and quantitative study: To identify "surgical hepatitis" and thus to obtain correct scores for lobular inflammation, immunoperoxidase techniques were applied, using an automatized system (Lab Vision Autostainer 720), introducing primary antibodies (Ab) into myeloperoxidase (MPO) (polyclonal Ab, prediluted, Master Diagnostica -MD-, Spain) and CD68 (monoclonal Ab 512H12, prediluted, MD). Anti-p62 Ab (Monoclonal Ab 3P62LCK, 1/500 dilution, BD Transduction) was also

applied to locate MDB.

Statistical analysis: All quantitative data were presented as mean (X) \pm Standard Deviation (SD) and were compared using the unpaired Student's test. Data entered and analyzed using SPSS v 17.0. P value <0.05 was considered statistically significant. *In Histopathological and immuno his to chemistry:* Fields were estimated using the Olympus BX40, DOT med,s Shipping Quote Service, image analyzer computer system at the Pathology Department, Faculty of Medicine, Ain Shams University. Measurements were performed within 10 non overlapping fields for each animal at $\times 400$ magnification.

3. Results

3.1. Biochemical Results

Serum activities of transaminases, SGPT, SGOT and ALP, and serum albumin are given in Table 1 where the single dose of APAP significantly elevated SGPT, SGOT activities when compared to normal animals (Table 1).

Subjects involved in this study were twenty five female patients who subdivided into two groups according to their body mass index; where Group 1: BMI ≤ 25 ; while Group 2 BMI ≥ 35 .

Table (2). Biochemical effect of obesity on liver functions

Groups	BMI	AST U/L	ALT U/L	ALK.PH U/L	ALB G/L
GI (N=5)	≤ 25	57 \pm 2.9	26.1 \pm 4.3	20.0 \pm 1.5	45.6 \pm 5.5
GII (N=20)	≥ 35	120 \pm 2.4 P <0.05 *	124.1 \pm 3.2 P <0.05 *	27.2 \pm 3.4 P >0.5	39.8 \pm 2.5 P >0.5

Values are expressed as mean \pm SD, N=number per each group.

* P < 0.5 : Significant statistically difference; while N.S. P > 0.5 : non significant value

Table (3). Showing the mean area % of anti-CD68 immunostaining, SD and P value in normal liver cells vs. fatty liver cells

	anti-CD68 in normal liver cells	anti-CD68 in fatty liver cells
Mean \pm SD	3.988 \pm 0.669	11.184 \pm 1.779
P value	0.2438	0.026
Significance		*

Values are expressed as mean \pm SD, n=number per each group.

* P < 0.5 : Significant statistically difference; while N.S. P > 0.5 : non significant value

Table (4). Showing the mean area % of anti p62 Ab immunostaining SD and P value in normal liver cells vs. fatty liver cells

	anti p62 Ab in normal liver cells	anti p62 Ab in fatty liver cells
Mean \pm SD	0.4388 \pm 0.132	6.587 \pm 0.913
P value	0.2512	0.00212
Significance		*

Values are expressed as mean \pm SD, n=number per each group.

* P < 0.5 : Significant statistically difference; while N.S. P > 0.5 : non significant value

Liver function tests in obese female patients (Group 2, N=20) showed significant escalation ($P < 0.5$) of serum transaminases (AST, and ALT) in comparison with control (Group 1, N=5), while alkaline phosphatase level and serum albumin value in obese female patients (Group 2) showed non significance changes ($P > 0.5$) in comparison with control (Group 1) as shown in Table (2).

3.2. Morphometric Results

The area % of positive anti-CD68 immunostaining, anti-p62Ab immunostaining and anti-MPO – myeloperoxidase - Ab immunostaining cells in liver significantly increased

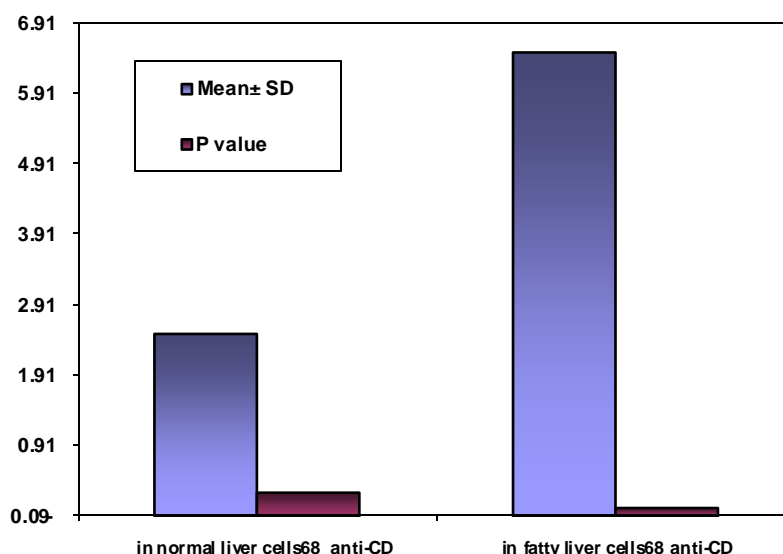
in obese persons as compared to control group (table 1,2,3, histogram. 1,2,3).

Table (5). Showing the mean area % of a anti-MPO -myeloperoxidase-Ab immunostaining SD and P value in normal liver cells vs. fatty liver cells

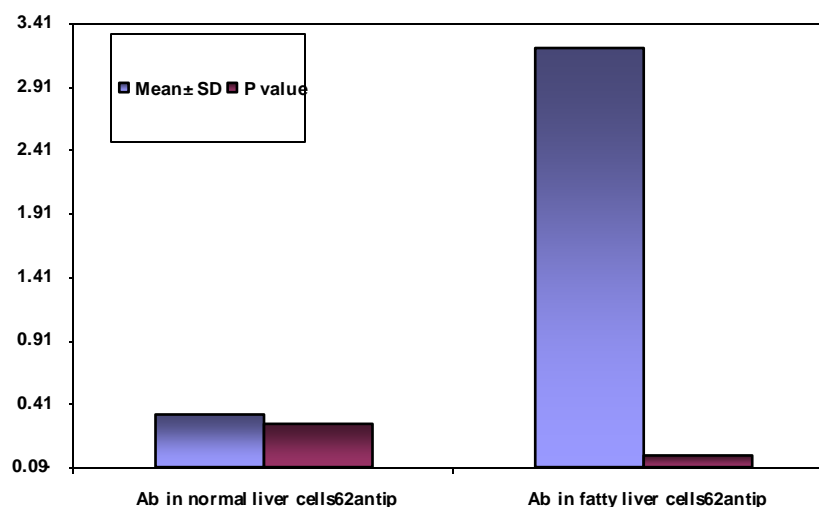
	anti-MPO in normal liver cells	anti-MPO in fatty liver cells
Mean \pm SD	0.978 \pm 0.346	8.998 \pm 0.965
P value	0.132	0.0121
Significance		*

Values are expressed as mean \pm SD, n=number per each group.

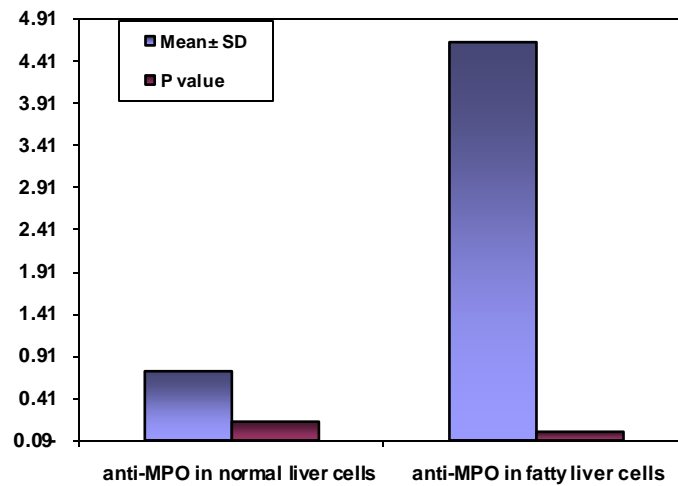
* $P < 0.5$: Significant statistically difference; while N.S. $P > 0.5$: non significant value



Histogram (1). Showing the mean area % of anti-CD68 immunostaining, SD and P value in normal liver cells vs. fatty liver cells



Histogram (2). Showing the mean area % of anti-p62Ab immunostaining SD and P value in normal liver cells vs. fatty liver cells



Histogram (3). Showing the mean area % of anti-MPO -myeloperoxidase- Abimmunostaining SD and P value in normal liver cells vs. fatty liver cells

3.3. Histological Results

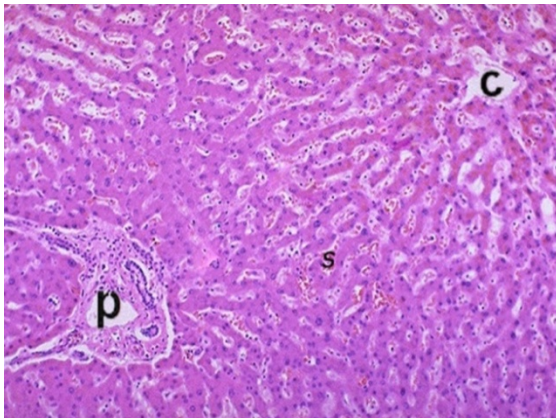


Figure (1). A photomicrograph of a section of control liver, showing central vein (c). Radiating cords of liver cells surrounded by sinusoids and portal area (p). (H&Ex200)

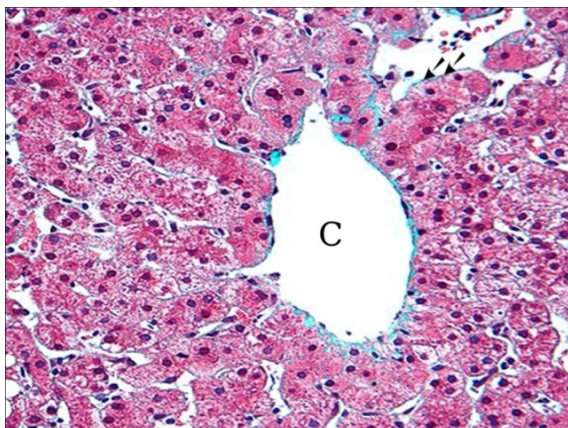


Figure (2). A photomicrograph of a section of control liver, showing minimal amount of fibrotic tissue around central vein (c). (Masson trichrome x400)

The liver of the control group showed hepatic lobules. The center of the lobule was the central vein (Fig. 1). hepatic

cords separated by sinusoids and showed minimal amount of collagen fibers around central vein (Fig. 2).

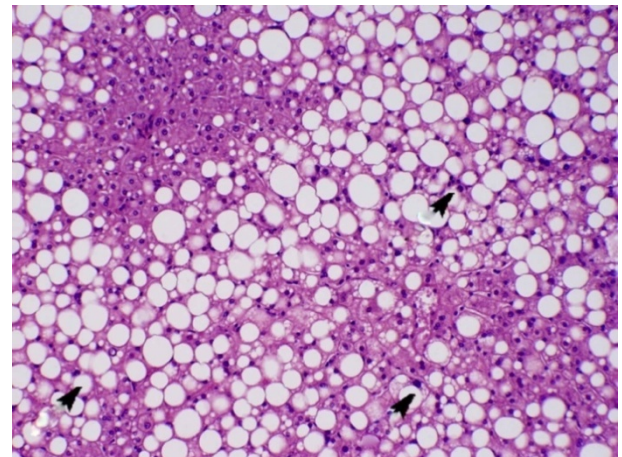


Figure (3). A photomicrograph of a section of liver from obese person, showing liver cells loaded with large single fat droplet (arrowheads). Some hepatocytes contain multiple fat droplets. (H&Ex200)

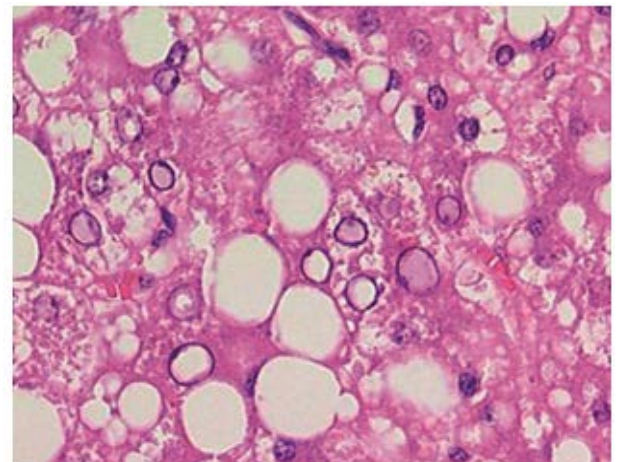


Figure (4). A photomicrograph of a section of liver from obese person, showing some hepatocytes with glycogenated nucleus (arrow) (H&Ex400)

Examination of a section of the liver from obese person showed liver loaded with large single fat droplets (Fig,3), some hepatocytes showed glycogenated nucleus(Fig,4), Presence of balloon cells with intracytoplasmic Mallory's hyaline (Fig, 5) and others showed fatty liver with cellular infiltration (Fig,6). Examination of the liver by Masson trichrome showed pericellular and perivascular fibrosis (Fig, 7), other hepatocytes showed hepatic cell necrosis , cellular infiltration and fibrosis(Fig,8),

Examination of a section of the liver from obese person showed macrophages aggregation in necrotic areas of liver(Fig,9).

Degenerated hepatocytes showed positive immune reactive Mallory bodies around the nucleus of ballooned hepatocytes (Fig,10), while other ballooned hepatocytes showed aggregations of polymorphonuclear leukocytes (Fig,11).

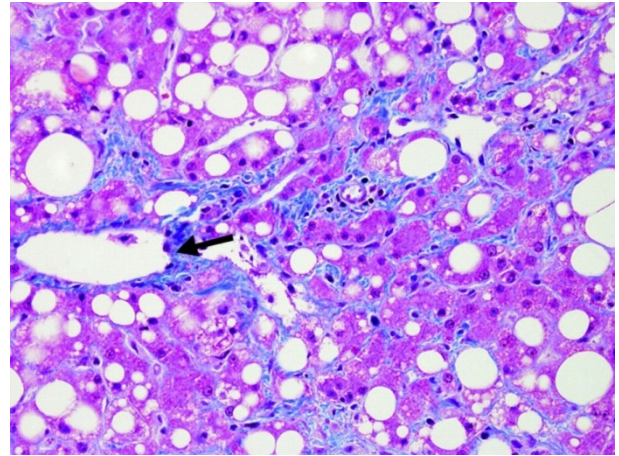


Figure (7). A photomicrograph of a section of liver from obese person, showing fatty liver with perivascular fibrosis (arrow). (Masson trichrome x400)

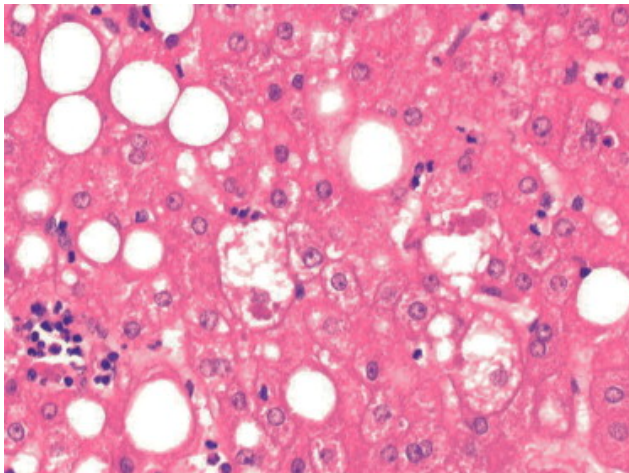


Figure (5). A photomicrograph of a section of liver from obese person, showing Presence of balloon cells with intracytoplasmic Mallory's hyaline (arrow). Few inflammatory infiltrates are present (arrow heads) (H&Ex400)

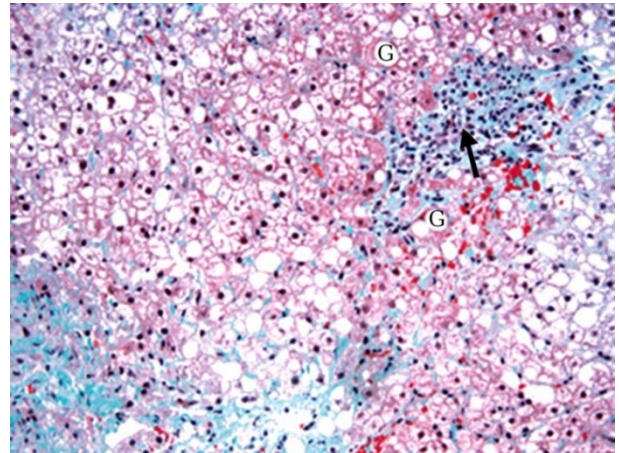


Figure (8). A photomicrograph of a section of liver from obese person, showing hepatic cell necrosis (G),cellular infiltration and fibrosis (arrow).(Masson trichrome x400)

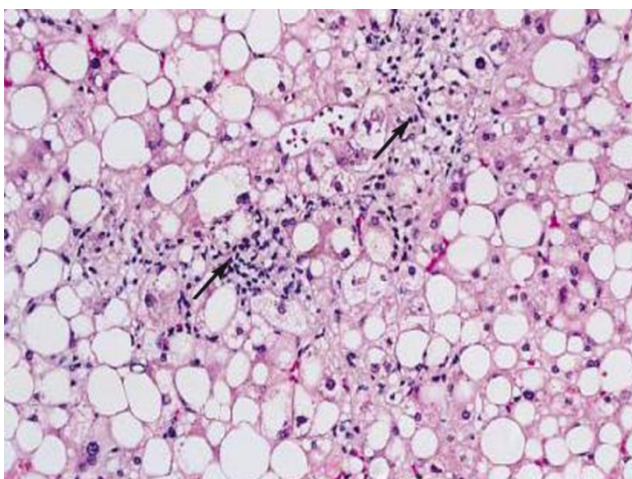


Figure (6). A photomicrograph of a section of liver from obese person, showing fatty liver with cellular infiltration (arrow) (H&Ex400)

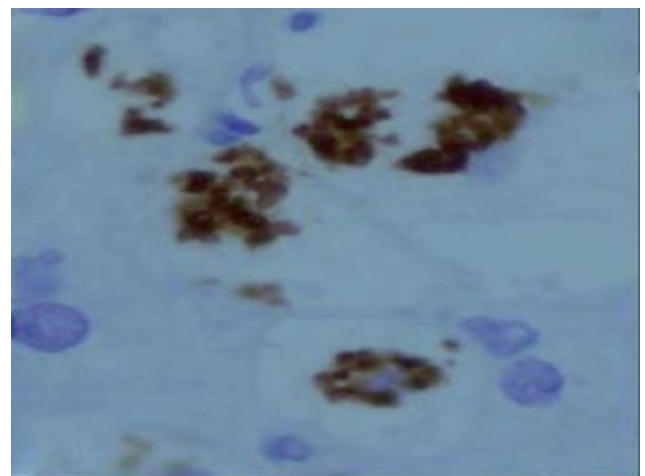


Figure (9). A photomicrograph of a section of liver from obese person, showing macrophages aggregation in necrotic areas. (anti-CD68 Abimmunostaining x 400)

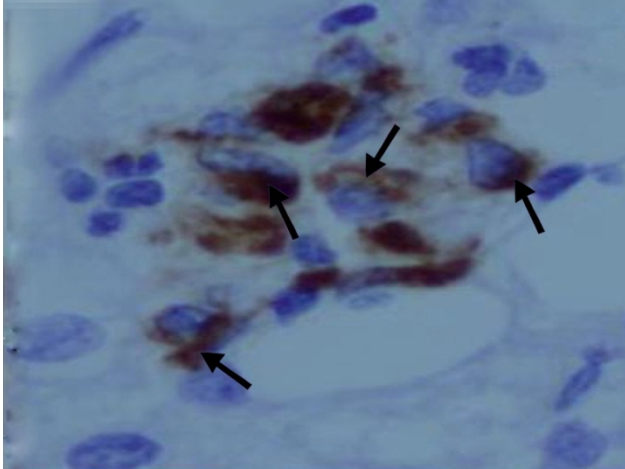
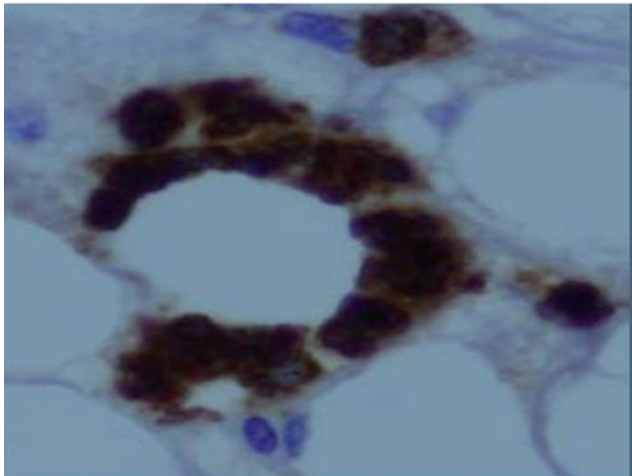


Figure (10). A photomicrograph of a section of liver from obese person, showing immune reactive Mallory-Denk bodies around the nucleus of ballooned degenerated hepatocytes (arrow). (anti p62Ab immunostaining x 400)



Figure(11). A photomicrograph of a section of liver biopsy in NAFLD in obese patients showing an aggregation of polymorphonuclear leukocytes around dying hepatocytes. (anti - MPO - myeloperoxidase - Ab immunostaining x 400)

4. Discussion

Fatty liver is a metabolic syndrome related to increased delivery of free fatty acid to the liver of obese individuals [5,6]. The present study showed that the cytoplasm of a great number of hepatocytes were distended with single large fat droplet that displaced the nucleus to the periphery (macrovesicular steatosis). Some hepatocytes showed microvesicular steatosis in the form of small multiple vesicles. These results were in accordance with other investigators [10, 17]. Moreover, hepatocellular injury in the form of ballooning and necrosis was recorded in the present work. Also, some authors declared that when the capacity of liver cells to store fat was decreased, fatty acid becomes toxic to the cells leading to cell death [13, 17]. Lipid mediators secreted by adipocytes like tumor necrosis factor and other metabolites could lead to liver cell death [10].

Fatty liver was associated with increased expression of the

membrane receptors rendering the cells susceptible to apoptosis [18-20]. In the present study, Mallory bodies were irregular acidophilic material composed of intermediate filaments aggregates. These bodies were associated with steatohepatitis. Inflammatory cells neutrophils and macrophages were detected in the liver parenchyma of the present work. Mitochondrial dysfunction associated with NAFLD might lead to release of reactive oxygen species. This leads to release of proinflammatory cytokines [21]. Moreover, inflammatory mediators were released from adipose tissue leading to inflammatory changes [21-23].

Hepatocytes with vacuolated (glycogenated nucleus) were seen in the present work. Some scientists declared that the presence of these nuclei denoted glycogen accumulation occurring in the setting of insulin resistance [4].

Pericellular and periportal fibrosis were detected in the present study. Also, some investigators declared that fibrosis was due to mitochondrial injury or due to mobilization of profibrogenic cytokines in response to hyperglycemia and inflammatory necrotic change [10, 20, and 21]. Progression of liver fibrosis was dependent on the presence of diabetes [4, 14, and 15]. Other work declared that fibrosis was recognized as an indicator of liver damage and was the best prognostic markers for morbidity and mortality [24-26]. The present study revealed aggregation of Kupfer cells and stimulation to phagocytic debris and release of interleukin I (IL I) and tumor necrosis factor (TNF).

Meanwhile, the present work showed extensive periportal fibrosis with apparent reduction in the severity of steatosis and steatohepatitis.

5. Recommendations

Sustained weight reduction in association with exercise can decrease fatty liver, fatty inflammation and fibrosis and reversed insulin resistance and not need for medical treatment. Regular assessment of liver function tests beside liver biopsies of obese adults, should be applied to define the existence of NAS or otherwise of NASH.

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