

Evaluation of Serum and Ascitic Fluid Leptin in Chronic Liver Diseases

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ABSTRACT:

Background/Aim: Chronic liver diseases are considered a major health problem in the world. Leptin is a member of cytokine family secreted by the adipocytes and known to reduce food intake. It was first linked to obesity but latter, it was found to be implicated in the pathogenesis of a wide variety of diseases like inflammation and inflammatory bowel diseases. Recent attention has been focused on the liver profibrogenic role of leptin in human and animal models. This study aiming to evaluate the serum and ascitic fluid leptin levels in patients with chronic liver diseases and its relation to the severity of the disease.

Patients & Methods: Serum and ascitic fluid leptin levels were measured using ELIZA test in patients with liver cirrhosis (GI), hepatocellular carcinoma. (G II), fatty liver (G III) and healthy control (GIV).

Results: serum leptin levels were significantly elevated in the three patient groups when compared to the control group with significantly higher levels in females than males in all groups. This suggests a role of leptin in the pathogenesis and progression of chronic liver diseases. The ascitic fluid leptin levels in the GI and GII were more than the serum levels suggesting intra abdominal production of leptin.

Conclusion: Leptin was significantly elevated in the serum and ascitic fluid in patients of spontaneous bacterial peritonitis than those of sterile ascites. This suggests a role of infection in this elevation.

Key words: Ascitic Fluid Leptin, Chronic Liver Diseases.

INTRODUCTION

Chronic liver diseases considered a major health problem in the world. Its major causes include Schistosomiasis, chronic hepatitis B, chronic hepatitis C, alcohol, fatty infiltration, autoimmune hepatitis and haemochromatosis. It is very important because it may end in liver cirrhosis which is complicated by liver cell failure, ascites, splenomegaly, hepatic encephalopathy, bleeding esophageal varices and malignant transformation⁽¹⁾. Chronic liver

diseases, which include cirrhosis and hepatocellular carcinoma (HCC), are responsible for an estimated 1.5 million deaths annually⁽²⁾. Egypt has a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma⁽³⁾. A community based study in Egypt stated that there is an increased number of patients suffering from cirrhosis which was estimated to be about 300,000 cases in the Nile Delta⁽⁴⁾. In fact, patients with

chronic liver diseases show many symptoms like fatigue, anorexia, malnutrition and loss of weight. These disabling symptoms impair the quality of life of these patients ⁽⁶⁾. The exact pathogenesis of these symptoms is poorly evaluated. Recent attention has been focused on the role of the leptin in chronic liver diseases. In cirrhosis, leptin levels have been found to be increased by many investigators ⁽⁶⁾.

Leptin is a member of cytokine family, primarily secreted from the adipocytes of white fat which plays an important role in the regulation of body weight in mammals ⁽⁷⁾. Minor levels of regulated leptin expression also occur at other sites such as placenta, skeletal muscle, the stomach fundus, and culture-activated hepatic stellate cells (HSCs) ⁽⁸⁾. Leptin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight, as well as in T lymphocytes and vascular endothelial cells ⁽⁹⁾. Human leptin is a 16-kDa (kilodalton), 146 amino acid residues nonglycosylated protein. It was first linked to obesity but later on it was found to be implicated in the pathogenesis of a wide variety of diseases like inflammation ⁽¹⁰⁾ and inflammatory bowel diseases ⁽¹¹⁾. Hyperleptinemia was also reported in bacterial peritonitis induced in healthy mice ⁽¹²⁾. In humans, leptin is thought to act as a satiety hormone as an afferent signal in a negative-feedback loop that maintains constancy of adipose mass and regulates the adaptive neuroendocrine and metabolic response to alterations in nutritional state ⁽¹³⁾.

Although serum leptin concentrations vary considerably among individuals, several findings indicate that leptin secretion is mainly correlated with body fat content and sex, being higher in obese than lean subjects and in females than in males ⁽¹⁴⁾. Leptin plasma level has been found to be reduced in malnourished patients ⁽¹⁵⁾ and low ⁽¹⁶⁾ or in the normal range ⁽¹⁷⁾ in patients suffering from chronic illness. The amount of leptin expressed by adipocyte correlates well with the lipid content of the cells. Once synthesized, leptin is secreted and not stored in the cell ⁽¹⁸⁾. Leptin acts on the brain to regulate food intake, energy expenditure, and

neuroendocrine function. It conveys to the brain information about the size of energy stores and activates hypothalamic centers that regulate energy intake and expenditure ⁽¹⁹⁾, leptin levels have been correlated with basal insulin levels. Prolonged insulin infusion or supra physiologic insulin levels markedly increase circulating leptin levels ⁽²⁰⁾. Leptin is distributed in several organs and cleared mainly by the kidney ⁽²¹⁾. The importance of the kidney as a major organ for leptin clearance is suggested by higher plasma leptin concentrations being observed in patients with renal impairment and end-stage renal disease when matched by sex, age, or adiposity ⁽²²⁾. High leptin levels might contribute to cachexia in patients with end-stage renal disease. It has been suggested that leptin is filtered by the glomeruli and degraded by renal epithelial cells ⁽²³⁾. Leptin's effects on body weight are mediated through effects on hypothalamic centers that control feeding behavior, hunger, body temperature and energy expenditure ⁽²⁴⁾. Leptin was found to decrease food intake, increase motor activity, and increase energy production and body temperature ⁽²⁵⁾. In culture, the administration of leptin stimulates proliferation and differentiation of haematopoietic stem cells ⁽²⁶⁾ and leptin increases myelopoiesis, erythropoiesis and lymphopoiesis ⁽²⁷⁾. In cirrhosis, which is often associated with hypermetabolism ⁽²⁸⁾; serum leptin level was found to be significantly elevated and it may play a role in the pathogenesis of symptoms like fatigue ⁽²⁹⁾. So, liver fibrosis should be added to the list of conditions in which leptin play a role and its significance in chronic liver diseases must be widely evaluated ⁽³⁰⁾. The high circulating leptin levels associated with obesity in humans may thus contribute to hepatic steatosis in two ways: (a) by promoting insulin resistance and elevated circulating insulin levels and (b) by altering insulin signaling in hepatocytes so as to promote increased concentrations of intracellular fatty acids and their conversion to triglycerides ⁽³¹⁾. Elevated leptin levels may also contribute to fatty liver disease even in the absence of obesity, ⁽³²⁾ observed increased leptin levels in Japanese students with fatty liver, independent of body mass index

(BMI).

Leptin may also influence the progression from hepatic steatosis to steatohepatitis. Given the similar intracellular signaling pathways stimulated by leptin and several inflammatory cytokines, it is perhaps not surprising that leptin could regulate inflammatory responses⁽⁶³⁾. There is evidence suggesting that leptin facilitates T-lymphocyte-mediated hepatotoxicity by facilitating the activation of tumor necrosis factor alpha (TNF- α) and, interleukin 18 (IL-18), actions that may contribute to steatosis-associated inflammation⁽⁶⁴⁾. Within the hepatocytes, leptin appears to regulate the expression and activity of several proteins that mediate oxidative stress⁽⁶⁵⁾. In a surprising and provocative study,⁽⁶⁶⁾ detected leptin expression and protein synthesis in activated hepatic stellate cells. Moreover, it has been demonstrated that serum leptin levels correlate with hepatic steatosis in chronic hepatitis C and the levels of leptin increase according to the progression of the stage of fibrosis in chronic HCV-infected patients. Moreover, the severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C⁽⁶⁷⁾.

AIM OF THE WORK

The aim of this work is to evaluate the serum and ascitic fluid leptin levels in patients with chronic liver diseases and its relation to the severity of the disease.

PATIENTS AND METHODS

This study was carried out on 50 individuals selected from the inpatient and outpatient clinic of the Tropical Medicine Department, Tanta University hospital, they were 25 males and 25 females their ages ranged between 33-53 years. They were classified as follows:

Group I: Included 20 patients with liver cirrhosis and ascites. They were 10 males and 10 females. Their ages ranged between 38 -52 years.

Group I was subdivided into 2 subgroups:

Group IA: Included 10 patients with liver cirrhosis and sterile ascites. They were 5 males and 5 females.

Group IB: Included 10 patients with liver cirrhosis and spontaneous bacterial peritonitis. They were 5 males and 5 females.

Group II: Included 10 patients with hepatocellular carcinoma. They were 5 males and 5 females. Their ages ranged between 43 -53 years.

Group III: Included 10 individuals with fatty liver. They were 5 males and 5 females. Their ages ranged between 35 -51 years.

Group IV (control group): Included 10 healthy individuals. They were 5 males and 5 females. Their ages ranged between 33 -53 years.

Exclusion criteria:

Patients with diabetes mellitus, renal insufficiency, and obesity, pregnancy, coronary or endocrinal diseases were excluded from the study except in group III some patients were diabetic.

All patient and control groups were subjected to the following

1-Thorough history taking with stress on (Symptoms of liver cell failure& gastrointestinal symptoms)

2-Full clinical examination including

3-Calculation of body mass index (BMI):

Measurement of height in meters and weight in kilograms and calculation of body mass index (BMI) using the following equation:

$$\text{BMI} = \frac{\text{Weight}}{\text{Height}^2}$$

3-Laboratory investigations

- Fasting and post-prandial blood sugar.
- Serum cholesterol and triglycerides
- Hepatitis B surface antigen.
- Hepatitis C virus antibody estimated by 3rd generation ELISA
- Liver function tests
- Alpha fetoprotein .

- Ascitic fluid examination (chemical and bacteriological).
- Estimation of the serum and ascitic fluid leptin levels (8).

4-Abdominal ultrasonography

Ascitic fluid samples:

Ascitic fluid samples were collected in the same sitting of serum sample under complete aseptic conditions. Ascitic fluid sample was stored in sterile tubes in - 70°C till the time of examination

Estimation of serum and ascitic leptin levels:

This was done using direct ELISA Kit for quantitative determination of leptin level by Enzyme Immunoassay manufactured by Diagnostic Biochem Canada (8).

Principle of the test:

Competition occurs between an unlabelled antigen (presents in standard, control and patient samples) and an enzyme- labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtitre plate reader. The intensity of the color formed is inversely proportional to the concentration of leptin in the sample. A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and control can be directly read

Assay procedure:

- Specimen pretreatment no preparation is needed for the samples before the procedure.
- All reagents must reach the room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption

Steps:

*Prepare working solution of leptin-HRP (Leptin-Horseradish Peroxidase) conjugate and wash buffer.

*Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.

*Pipette 50 µl of each calibrator, control and specimen samples into corresponding labelled wells in duplicate.

*Pipette 100 µl of the conjugate working solution into each well.

*Incubate into a plate shaker (approximately 200-rpm) for 2 hours at room temperature.

*Wash the wells 3 times with 300µl of diluted wash buffer per well and tap plate firmly against absorbent paper to ensure that it is dry.

*Pipette 150 µl of the substrate solution into each well at timed intervals.

Incubate on a plate shaker for 15-20 minutes at room temperature.

*Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 7.

*Read the plate on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution.

Sensitivity:

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD (optical density) of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Diagnostic Biochem Canada direct leptin ELISA kit is 0.5 ng/ml.

RESULTS

Group I included 10 males (50 %) and 10 females (50 %). Group II included 5 males (50 %) and 5 females (50 %). Group III included 5 males (50 %) and 5 females (50 %), and group IV included 5 males (50%) and 5 females (50%).

In group I, the age was ranged between 38 - 53 years with a mean of 47 ± 5 years. In group II, the range was 43 - 53 years with a mean of 47.4

± 3 years. In group III, the range was 35 - 51 years with a mean of 44.6 ± 5.6 years. In group IV, the range was 33 - 52 years with a mean of 43.9 ± 7 years. There was no statistically significant difference between all studied groups as regards age ($P > 0.05$).

The collected data of the general examination of all studied groups including the weight, the height and body mass index (Table 1) revealed that; The weight showed a statistically significant increase in group III when compared to the other groups ($P < 0.05$).

As regard the height; there was no statistically significant difference between all studied groups as regards height ($P > 0.05$).

Body mass index showed a statistically significant increase in group III when compared to other groups ($P < 0.05$).

Viral markers in the group I showed that, 80% of cases showed anti HCV positive and 20 % showed positive HBs Ag in the serum. In group II, 60% of cases showed positive anti HCV, 20 % showed positive HBs Ag and 20 % showed positive both anti HCV and HBs Ag in the serum.

Fasting and Postprandial blood sugar levels (Table 1): showed significant elevation in the group III of fatty liver when compared to the other groups ($P < 0.05$).

Serum cholesterol and triglyceride level (ng/dl) in the studied groups (Table 1): showed a statistically significant decrease in the group I and II when compared to the other groups. On the other hand, they were found to show statistically significant increase in group III when compared to the other three groups ($P < 0.05$).

Table (2) shows liver function tests of the studied groups:

Serum Bilirubin: There was a statistically significant elevation of the serum bilirubin in group I of liver cirrhosis and group II of hepatocellular carcinoma in relation to group III fatty liver and control group IV ($P < 0.05$).

Serum Alanine amino transferase (ALT) and Serum Aspartate amino transferase (AST):

There was a statistically significant elevation of ALT in group I of liver cirrhosis and group II of hepatocellular carcinoma in relation to group III fatty liver and control group IV ($P < 0.05$). Also, there was elevation of the mean value of serum ALT in group III in comparison to group IV but the difference was non significant ($P > 0.05$).

Serum albumin and Prothrombin activity: There was statistically significant decrease of serum albumin in both group I and group II when compared to group III and control group IV ($P < 0.05$).

Serum Alkaline phosphatase (ALP):

There was a statistically significant elevation of serum ALP (alkaline phosphatase) in group I and II in relation to group III and control group IV ($P < 0.05$) with a non-statistically significant difference between group I and II and between group III and IV ($P > 0.05$).

Serum Lactate dehydrogenase (LDH):

Serum lactate dehydrogenase was significantly elevated in both group I and group II in relation to the other groups ($P < 0.05$) without significant difference between group I and II or between group III and IV ($P > 0.05$).

Results of alpha fetoprotein:

There was significant elevation of alpha fetoprotein in the group II of hepatocellular carcinoma in relation to the other groups ($P < 0.05$). Also, there was a statistically significant elevation of alpha fetoprotein in the group I of liver cirrhosis when compared to the group III (fatty liver) and group IV (control).

Results of ascitic fluid examination:

Ascitic fluid albumin: In group I, its level ranged between 0.5 - 1.9 gm/dl with a mean of 1.09 ± 0.379 gm/dl. In group II, the range was 1 - 1.9 gm/dl with a mean of 1.36 ± 0.34 gm/dl.

Serum ascitic albumin gradient (SAAG): In group I, its level ranged between 1.1 - 1.5 gm/dl with a mean of 1.27 ± 0.13 gm/dl. In group II,

the range was 0.9 - 1.4 gm/dl with a mean of 1.1 ± 0.18 gm/dl.

Total leucocytic count of ascitic fluid: In group I, its level ranged between 35-650 cell/cc with a mean of 275 ± 196 cell/cc. In group II, the range was 65 - 150 cell/ cc with a mean of 106 ± 297 cell/ cc

Serum leptin level (ng/ml) in all studied groups Table (3): There was a statistically significant increase in the serum leptin level in patient groups (G I, G II and G III) in comparison to the control group ($P < 0.05$), with a non significant difference between the three patient groups I, II and III ($P > 0.05$).

Ascitic fluid leptin level (ng/ml) in the group I and II Table (4): There was non-significant difference of ascitic fluid leptin levels between the two groups ($P > 0.05$).

Comparison between serum and ascitic fluid leptin in each group I and II (table5):

There was a significant increase of leptin levels in the ascitic fluid when compared to the serum levels in both groups ($P < 0.05$).

Comparison between serum leptin in subgroup Ia and Ib (Figure 1): The group I of liver cirrhosis was subdivided into 2 subgroups: There was a statistically significant increase of serum leptin levels in the group Ib than in group Ia ($P < 0.05$).

Comparison between ascitic fluid leptin in subgroup Ia and Ib (Figure 1): There was a statistically significant increase of ascitic fluid leptin levels in the group Ib than in group Ia ($P < 0.05$).

Statistical comparison of serum leptin in all studied groups in relation to sex (Figure 2): there was statistically significant increase of serum leptin levels in the females when compared to the leptin levels in males in the same group ($P < 0.05$).

Table (1): Weight, height, BMI and some Biochemical Parameters

	Group I No = 20	Group II No = 10	Group III No = 10	Group IV No = 10	All Pairwise statistical Comparison
Weight Mean±SD	69±3.9	713±4.5	85.3±7.4	69.7±3.36	Q1=1.49 Q2=11.9* Q3=0.3 Q4=90.8* Q5=1.03 Q6=10.1*
Height Mean±SD	1.66±0.062	1.67±0.053	1.66±0.058	1.7±0.029	No significant difference ($P > 0.05$)
BMI Mean±SD	25.07±2.18	25.59±1.08	30.68±1.9	23.8±0.55	Q1=0.85 Q2=4.11* Q3=1.86 Q4=28.15* Q5=2.362 Q6=5.177*
Blood glucose , Cholesterol, Triglycerides					
F BG (mg/dl) Mean ±SD	848±8.4	825 ±7.9	127±30.5	86.6±2.9	Q1=0.8 Q2=41* Q3=0.7 Q4=429* Q5=1.4 Q6=28*
PPBG (mg/dl) Mean±SD	118±10	107±13	180.5±7	121±6.2	Q1=1.4 Q2=3.8* Q3=0.6 Q4=4.5* Q5=1.8 Q6=2.7*
Cholesterol (mg/dl) Mean ±SD	1094±11	113±11	209±15	140±11	Q1=1.2 Q2=29.6* Q3=9.2* Q4=24.6* Q5=6.9* Q6=17.7*
Triglycerides (mg/dl) Mean ±SD	99.5 ± 224	1042 ± 17.4	162 ± 9.9	112 ± 16	Q1=1 Q2=12.4* Q3=2.5 Q4=9.8* Q5=1.3 Q6=8.5*

Table (1) cont.: Weight, height, BMI and some Biochemical Parameters

	Group I No = 20	Group II No = 10	Group III No = 10	Group IV No = 10	All Pairwise statistical Comparison
Blood picture					
Hb					
Mean ±SD	10±1.4	9.8±1.6	13.4±0.8	13.1±0.9	
RBCs	3.46 ±7.9	3.68±5.69	5.1 ±5.6	5.2±6.4	
Mean±SD					
WBCs	4980±1070	4109±1418	5630±1252	5180±967	
Mean ±SD					
Platelets	162 ±53	102 ±53	306 ±49	348±5	
Mean ±SD					

FBG = Fasting bl. glucose PPBG = Post prandial bl. glucose

Table (2): shows liver function tests of the studied groups

	Group I No = 20	Group II No = 10	Group III No = 10	Group III No = 10	All Pairwise statistical Comparison
T. Bilirubin					Q1 = 0.07 Q2 = 4.09*
Range	1 - 6	1 - 9.2	0.4 - 0.9	0.5 - 0.9	Q3 = 3.2* Q4 = 3.4*
Mean ±SD	2.63 ±1.4	3.69 ± 2.9	0.65 ± 0.1	0.67 ± 0.13	Q5 = 2.7* Q6 = 0.752
ALT (U/L)					Q1 = 0.017 Q2 = 3.1*
Range	22 - 149	22 - 113	22 - 55	16 - 37	Q3 = 4.26* Q4 = 2.7
Mean ±SD	62 ±30	61.5 ± 25.9	34.3 ± 9.2	26 ± 6.7	Q5 = 3.7* Q6 = 1.01
AST (U/L)					Q1 = 0.3 Q2 = 3.9*
Range	35 - 160	60 - 120	24 - 50	17 - 30	Q3 = 4.6* Q4 = 3.6*
Mean ±SD	75 ± 29	79.5 ± 18	30.5 ± 7.5	25.2 ± 4.4	Q5 = 4.3* Q6 = 0.6
ALP (U/L)					Q1 = 0.17 Q2 = 6.47*
Range	95 - 300	130 - 320	85 - 180	55 - 180	Q3 = 6.54* Q4 = 5.4*
Mean ±SD	213 ±56.4	211 ±64	120 ± 34.6	119 ± 45.5	Q5 = 5.51* Q6 = 0.05
LDH (U/L)					Q1 = 0.16 Q2 = 4.73*
Range	170 - 330	175 - 355	130 - 200	135 - 190	Q3 = 3.9* Q4 = 4.50*
Mean ±SD	263.9 ± 45	263 ± 51	160 ± 18.7	166 ± 16	Q5 = 3.7* Q6 = 0.32
S. Albumin					Q1 = 2.08 Q2 = 1.9*
Range (gm/dl)	1.7 - 3	1.9 - 3	4 - 4.5	4 - 4.5	Q3 = 17.6* Q4 = 15*
Mean ±SD	2.32 ± 0.38	2.5 ± 0.3	4.3 ± 0.2	4.1 ± 0.23	Q5 = 13.4* Q6 = 2.02
Prothrombin					Q1 = 0.8 Q2 = 4.9*
Range	28 - 70%	34 - 70 %	85 - 100%	85 - 100%	Q3 = 4.4* Q4 = 3.5*
Mean ±SD	53.3 ± 16	58.9 ± 11	97.4 ± 5.8	94.8 ± 5.4	Q5 = 3.1* Q6 = 0.38
Q1: G I versus G II	Q2: G I versus G III		Q3: G I versus G IV		Q4: G II versus G III
Q5: G II versus G IV	Q6: G III versus G IV		*: Significant (P < 0.05)		

Table (3): shows serum leptin in all studied groups

	GI No20	GII No10	GIII No10	GIV No10	All Pairwise statistical Comparison	
Serum leptin					Q1=0.59	Q2=0.968
Range (ng/ml)	35.2 -62.7	36.3 -59.3	35 - 55.8	7.2 - 28	Q3=15.3 *	Q4=0.32
Mean	47.86	46.6	45.8	15.9	Q5=12.7 *	Q6=12.4*
±	±	±	±	±		
SD	7.6	6.7	7.3	8.56		
Q 1: G I versus GII		Q 2: G I versus G III	Q3: G I versus G IV		Q4: GII versus GIII	
Q5: GII versus GIV		Q6: GIII versus GIV	*: Significant (P < 0.05)			

Table (4): shows ascitic fluid leptin in all studied groups

	GI (No20)	GII (No10)	t- test
Ascitic leptin			
Range (ng/ml)	44.7 - 74.9	47.1 -68.8	t=0.174
Mean	59.9	60.48	
±	±	±	
SD	8.79	8.39	

Table (5): shows comparison between serum and ascitic fluid leptin in each group I and II

	Serum leptin	Ascitic leptin	t- test
Group I			
Range (ng/ml)	35.2 -62.7	44.7-74.9	t = - 4.6*
Mean	47.86	59.9	
±	±	±	
SD	7.6	8.79	
Group II			
Range (ng/ml)	36.3 -59.3	47.1 -68.8	t = - 4.06*
Mean	46.6	60.48	
±	±	±	
SD	6.7	8.39	

*: Significant (P < 0.05)

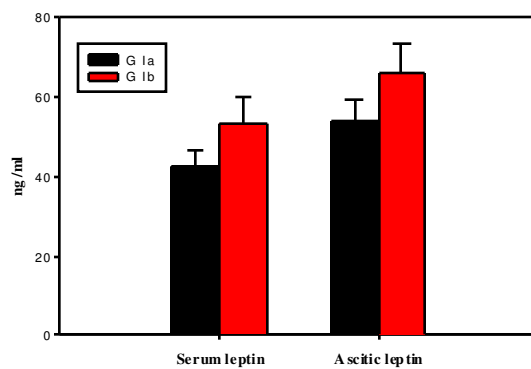


Fig. 1: shows comparison between serum and ascitic fluid leptin in each group Ia and b

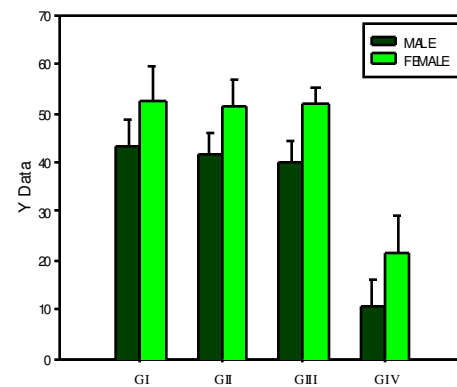


Fig. 2: shows statistical comparison of serum leptin in all studied groups according to sex

DISCUSSION

Most of acute and chronic liver diseases are characterized by inflammatory processes with enhanced expression of various pro- and anti-inflammatory cytokines in the liver. These cytokines are the driving force of many inflammatory liver disorders often resulting in fibrosis and cirrhosis⁽⁹⁾. Viral hepatitis is considered as one of the most important causes of chronic liver diseases. Hepatitis C virus (HCV) infection is a worldwide public health problem⁽⁴⁰⁾. HCV infection frequently leads to chronic hepatitis, which may progress to cirrhosis and even to hepatocellular carcinoma⁽⁴¹⁾. The mechanisms responsible for the pathogenesis of chronic HCV infection are not well known. One of the common and prominent histopathologic features in patients with chronic HCV infection is the presence of hepatic steatosis, which is believed to be an important co-factor in accelerating the development of liver fibrosis⁽⁴²⁾. The relationship between body mass index (BMI), steatosis, and fibrosis in chronic hepatitis C virus (HCV) infected patients suggests a role of steatosis in the progression of hepatitis C⁽⁴³⁾. Recent attention has suggested a critical role of leptin in hepatic inflammation and fibrogenesis in human and animal studies however, the

precise mechanisms underlying the profibrogenic action of leptin in the liver has not been well elucidated⁽⁴⁴⁾. As leptin plays a role in the modulation of immune response and inflammation⁽⁴⁵⁾, the increase in leptin production that occurs during infection and inflammation strongly suggests that leptin is a part of the cytokine cascade⁽⁴⁶⁾.

Ganong, (1999)⁽⁴⁷⁾ considered leptin as a member of the cytokine family secreted by the adipocytes and known to reduce food intake. It was first linked to obesity but later on it was found to be implicated in the pathogenesis of a wide variety of diseases like inflammation⁽¹⁰⁾, and inflammatory bowel diseases⁽¹¹⁾. It has been suggested that leptin might play a role in hepatic fibrosis. Isolated hepatic stellate cells have been shown to produce leptin during the in vitro transactivation process as reported by Potter et al., (1998)⁽⁶⁹⁾ and more recently, leptin has been shown to augment the inflammatory and profibrogenic response in the murine liver upon exposure to hepatotoxic chemicals⁽⁴⁸⁾.

The results of the present study showed that, the body mass index which is the index for obesity was significantly increased in the group III of fatty liver ($P < 0.05$) indicating that most of

them were obese when compared to other groups which have lower body mass index. Rocha et al, (2005)⁽⁴⁹⁾ revealed that body mass index and waist circumference are frequently associated with histological findings of steatosis and steatohepatitis and fibrosis in patients with non-alcoholic fatty liver diseases (NAFLD).

Concerning the lipid profile of all studied groups, our study showed a significantly high serum cholesterol and triglycerides in patients with fatty liver (the group III) when compared to their corresponding values in the other groups which showed a significantly lower serum cholesterol and triglycerides in patients with liver cirrhosis and those with hepatocellular carcinoma when compared with control ($P < 0.05$).

Our results were in agreement with the study of Cortez et al, (2003)⁽⁶⁰⁾ who demonstrated that, obesity and dyslipidaemia were observed in 80% of the patients with non-alcoholic fatty liver, confirming that obesity and dyslipidaemia are risk factors of non-alcoholic fatty liver disease. Decreased serum levels of cholesterol and triglycerides in patients of liver cirrhosis may be related to defective hepatic synthesis and malnutrition which is a well known and frequently reported complication in patients with liver cirrhosis. The decreased serum levels of cholesterol and triglycerides in cirrhotic patients may indicate a poor prognosis⁽⁶¹⁾. Liver function tests including serum transaminases are often used as screening tests among both low and high-risk populations. However these tests lack sensitivity and specificity for liver diseases and cirrhosis⁽⁶²⁾.

The results of our study also showed altered liver function tests. Serum bilirubin, Alanin amino transferase (ALT) and Aspartate amino transferase (AST) were significantly higher in the patients of liver cirrhosis (G I) and hepatocellular carcinoma (GII). Our study also showed a statistically significant decrease of both serum albumin level and prothrombin activity in both groups of liver cirrhosis (G I) and hepatocellular carcinoma (G II) when compared to the other two groups.

These results were in-agreement with Green and Flamm, (2002)⁽⁶³⁾ who explained this alteration to be mainly due to a defect in the synthesis of coagulation proteins and albumin by hepatocytes in these conditions. On the other hand, prothrombin time is not useful for assessing liver function in patients with mild aminotransferase alteration, since prothrombin time can remain within normal limits for long periods unless a marked decrease in liver function occurs and patients with compensated liver cirrhosis may have normal prothrombin time. Giannini et al., (2005)⁽⁶⁴⁾ explained that the change in serum albumin level or prothrombin time may be associated with a decrease in functioning liver mass, although neither is specific for liver disease.

Serum leptin levels in our study showed statistically significant increase among patient groups in comparison to the control group ($P < 0.05$) with a non significant difference between the three patient groups (I, II and III) ($P > 0.05$).

Our results in cirrhotic patient group were in agreement with Trayhurn et al. (1999)⁽⁶⁵⁾ who reported that patients with cirrhosis had significantly higher serum leptin concentration compared to non-cirrhotic patients. The increase in serum leptin concentrations in cirrhotic patients may reflect the stimulated production of some factors able to stimulate adipocyte leptin synthesis and/or secretion, such as cytokines, sexual steroids, insulin and cortisol. Moreover, it is quite possible that the development of portosystemic shunting in cirrhosis causes many of the above mentioned secretagogues of leptin to increase, leading to a multifactorial stimulation of leptin secretion⁽⁶⁶⁾. Our results also were in agreement with Lin et al, (2002)⁽⁶⁷⁾ who stated that, serum levels of leptin and tumor necrosis factor alpha (TNF- α) were elevated in cirrhotic patients. The severity of liver cirrhosis was an important factor for the activation of tumor necrosis factor alpha (TNF- α) system and this activated tumor necrosis factor alpha (TNF- α) system conjointly with hyperleptinaemia might mediate malnutrition in patients with liver cirrhosis. Testa et al. (2000)⁽⁶⁸⁾ stated that, in the course of chronic viral liver

disease and serum leptin levels may reflect the extent of liver dysfunction. The tendency of circulating leptin concentration to increase may be interpreted as an early signal of the loss of the ability to down-regulate energy expenditure in response to anorexia as chronic viral hepatitis progresses toward liver cirrhosis. It has been demonstrated that serum leptin levels correlate with hepatic steatosis in chronic hepatitis C and the levels of leptin increase according to the progression of the stage of fibrosis in chronic HCV-infected patients. Moreover, the severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C⁽⁶⁷⁾.

Similar to our results, Faggioni et al., (2001)⁽⁶⁸⁾ and Liu et al., (2005)⁽⁶⁹⁾ found the serum leptin levels in patients with chronic hepatitis C was higher than that in controls. A major focus of recent studies is the role of leptin in the modulation of immune response and inflammation. The increase in leptin production that occurs during infection and inflammation strongly suggests that leptin is a part of the cytokine cascade, which regulates the innate immune response and host defense mechanisms. Leptin plays an important role in T-cell mediated immune responses and stimulates proliferation of CD4+ T cells and promotes Th1 responses⁽⁶⁴⁾. Our results also, were agreed by Wang and Lin, (2003)⁽⁶⁾ demonstrated that cirrhotic patients with or without hepatocellular carcinoma had increased serum leptin concentrations. However, they stated that, leptin did not appear to be associated with the development of hepatocellular carcinoma in cirrhotic patients. Lederq et al., (2002)⁽⁶¹⁾ reported that, hepatic fibrosis during chronic liver injury is dependent on leptin. Their results also demonstrated that leptin is required for collagen expression and production in the context of chronic liver injury and hepatic inflammation, possibly by modulating transforming growth factor beta 1 (TGFβ1) bioactivation. These findings establish the essential physiological dependence of leptin for hepatic fibrogenesis in response to chronic liver injury.

Moreover, Ikejima et al., (2005)⁽⁶³⁾ reported that, leptin and its functional receptors play a role in

hepatic fibrogenesis, most likely through up-regulation of transforming growth factor beta 1 (TGF-β) expression in the liver. They also demonstrated that leptin produced systemically from adipose tissue and locally from hepatic stellate cells (HSCs), acts as a profibrogenic cytokine and it is one of the key regulators of hepatic inflammation and contributes to the progression of hepatic fibrosis in various chronic liver diseases. Also, our results were in agreement with Piche et al., (2004)⁽⁶⁷⁾ who demonstrated that, serum leptin levels correlate with hepatic steatosis in chronic hepatitis C and the levels of leptin increase according to the progression of the stage of fibrosis in chronic HCV-infected patients. Moreover, the severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C. In our study serum leptin levels in patients with hepatocellular carcinoma were significantly increased when compared to that of control group ($P < 0.05$). The increased serum level of leptin in patients with hepatocellular carcinoma can be explained on the basis of many studies which revealed that, leptin has been demonstrated as a growth factor in colonic epithelial cells by stimulating the invasive capacity at early stages of neoplasia. Leptin has also been proved to be mitogenic in a number of tissues including colonic and breast tissues. Therefore, it is reasonable to speculate that the high levels of circulating leptin associated with obesity may lead to increased tumor cell growth⁽⁶²⁾.

Wang et al., (2004)⁽⁶³⁾ provided new insights into the carcinogenesis and progression of human hepatocellular carcinoma (HCC), suggesting that leptin could act in vivo as a growth factor towards hepatocytes, and may play a possible role in the process of initiation and progression of hepatocellular carcinoma (HCC), which could contribute to the explanation of the reasons why alcoholic and post-hepatitis liver cirrhosis patients with or without hepatocellular carcinoma have high levels of serum leptin and the reasons why obesity was a risk factor of developing hepatocellular carcinoma⁽⁶⁴⁾.

In our study, the serum leptin levels showed a statistically significant increase in the group III

of fatty liver when compared to the control group IV ($P < 0.05$).

Giannini et al, (1999)⁽⁶⁵⁾ recorded statistically significant increase in serum leptin in patients with hepatic steatosis and those of chronic hepatitis with non significant difference between the two groups ($P > 0.05$) and this is similar to our results.

Our results also were in agreement with Uygun et al., (2000)⁽⁶⁶⁾ reported that mean serum leptin levels were significantly higher in patients with non-alcoholic fatty liver diseases (NAFLD) when compared to healthy controls. They stated that, elevated serum leptin levels might promote hepatic steatosis and steatohepatitis.

Our results also were in agreement with Chitturi et al., (2002)⁽⁶⁷⁾ who stated that, circulating leptin levels were increased in both men and women with non-alcoholic fatty liver diseases (NAFLD) and they stated that leptin together with serum c-peptide and age, proved to be a positive predictor of the severity of hepatic steatosis but not of liver inflammation and fibrosis. Diehl, (2004)⁽⁶⁸⁾ suggested that the increased basal expression of leptin in nonalcoholic steatohepatitis patients leads to overproduction of tumor necrosis factor alpha which may play a significant role in the pathogenesis of non-alcoholic steatohepatitis as a "second hit" following the development of simple steatosis as previously reported. Thus the overproduction of tumor necrosis factor alpha induced by leptin may lead non-alcoholic steatohepatitis to the progressive disease ultimately resulting in liver cirrhosis and hepatocellular carcinoma⁽⁶⁹⁾. It is suggested that leptin might contribute to hepatic steatosis induction by altering insulin signaling in hepatocytes and promoting increased intracellular fatty acid accumulation. Also, leptin may help to induce insulin resistance an almost universal finding in patients with non-alcoholic fatty liver diseases. Moreover, in a later stage, leptin might cause hepatic steatosis to turn into steatohepatitis by amplifying selected proinflammatory responses⁽⁶¹⁾. Our study showed a significantly higher ($P < 0.05$) ascitic fluid leptin levels compared to serum in our patients in both group I and II. This was in agreement with Giannini et al,

(2004)⁽⁷⁰⁾ who reported similar results and suggested intra abdominal production of leptin to explain this higher level. Ott et al., (2004)⁽⁷¹⁾ reported hyperexpression of leptin mRNA on activated hepatic stellate cells and found it to correlate with fibrosis in cirrhotic patients, therefore suggesting the cirrhotic liver to be a source of increased leptin in tissue and in serum of patients with chronic liver diseases which may be a more accurate explanation. The present study showed a statistically significant increase in both serum and ascitic fluid leptin levels in patients with liver cirrhosis and spontaneous bacterial peritonitis (group Ib) than their corresponding levels in those with sterile ascites (group Ia) despite absence of corresponding difference in liver functions in both groups. This suggests synergistic effect of infection and cirrhosis on leptin levels. Cirrhosis alone can elevate serum and ascitic fluid leptin as previously described, and infection can augment this response by stimulating the proinflammatory cytokines including tumor necrosis factor alpha (TNF- α) which in turn stimulates leptin production both locally and systemically as explained experimentally by Sarraf et al, (1997)⁽⁷²⁾. This finding was supported by the observation of Lin et al., (2002)⁽⁶⁷⁾ who reported that administration of tumor necrosis factor alpha (TNF-alpha) has been shown to increase the plasma leptin concentration, suggesting that, a cytokine-leptin link may play a role during chronic inflammation. Our explanation is also supported by the work of Moshlyedi et al (1998)⁽⁷³⁾ who induced peritonitis in healthy mice and recorded hyperleptinaemia in response to bacterial peritonitis. In our study, we found that serum leptin was significantly elevated in females than males in all studied groups and this was in agreement with McGullough et al., (1998)⁽⁷³⁾ who found the serum leptin to be elevated in patients with liver cirrhosis than control subjects and this elevation was gender dependant increasing in females more than males in patient and control groups. Also, Liu et al., (2005)⁽⁶⁹⁾ found their female subjects to have significantly higher serum leptin levels than the males. Havel et al., 1996⁽⁷⁴⁾ stated that, serum leptin levels are sexually differentiated,

with higher levels in women. One explanation for this finding is that women have significantly greater subcutaneous adipose tissue mass relative to omental adipose mass ⁽⁷⁾. In humans, leptin mRNA expression differs among various fat depots being greater in subcutaneous fat than omental fat ⁽⁸⁾. The amplitude of leptin pulses has been reported to be greater in women than men ⁽⁹⁾, though the physiological importance of this observation remains to be determined. One potential explanation for the sexual difference in leptin levels is the different effects of androgens and estrogens on leptin levels with estrogen reported to increase serum leptin levels ⁽⁷⁾ and testosterone observed to decrease them ⁽⁸⁾.

CONCLUSION

The elevated leptin levels in patients of chronic liver diseases suggests that leptin may play a role in the pathogenesis and progression of chronic liver diseases

RECOMMENDATIONS

A Leptin level is elevated in patients of chronic liver diseases and this suggests that leptin may play a role in the pathogenesis and progression of chronic liver diseases.

Further large studies are recommended to study the possible immunoregulatory role and potential role of leptin in chronic liver diseases in a large scale.

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تقييم مستوى اللبوتين في المصل والسائل البريتوني في أمراض الكبد المزمنة

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اقسام طب المنطق الحارة والحميات والكيمياء لحيوية الطبية*

المقدمة :

المضاعفات التي تسببها مرضي تشمع الكبد والاستسقاء وتضخم الطحال وفشل وظائف الكبد ونزف من دوالي المريء وأورام الكبد. و يتعرض المرضي لمشكلات الإرهاق وسوء التغذية ونقص الوزن. وقد تعتبر أمراض الكبد المزمنة من المشكلات الصحية الخطيرة في مصر والعالم وتكمن أهميتها في سجلات بعض الدراسات الحديثة ارتفاع نسبة اللبوتين في مصل الدم في حالات أمراض الكبد المزمنة وقد وجد أن معدل هرمون اللبوتين يرتفع في هؤلاء المرضي مما يقترح وجود علاقة بين اللبوتين وأمراض الكبد المزمنة. و اللبوتين هو عبارة عن هرمون تم فصله وتحديده حديثا ويرتبط بجين السمنة، ومن المعروف أن اللبوتين ينظم وزن الجسم عن طريق الإقلال من الطعام مع زيادة معدلات لطاقة المبولة، وهو يعمل غالبا مركزيا عن طريق ما تحت سرير المخ، وبالرغم من أننا لا نعلم الكثير عن هرمون اللبوتين إلا أنه من الواضح له يلعب دورا أساسيا في حدوث السمنة حيث وجد أن نسبة اللبوتين في مصل دم تتناسب ودرجة وثيقة مع نسبة الدهون في الجسم ومؤشر كتلة الجسم. سجلت بعض الدراسات الحديثة ارتفاع نسبة اللبوتين في مصل الدم عند مرضي تشمع الكبد، واقترحت أن اختلال وظيف الكبد يؤثر علي مستوي اللبوتين في مصل الدم، ولكن الطريقة لمسببة لذلك لم تدرس بشكل كافي. وهكذا نري أن العلاقة بين اللبوتين وأمراض الكبد المزمنة تعتبر غمضة في الوقت الحاضر.

الهدف من البحث:

قياس مستوي اللبوتين في المصل والسائل البريتوني في أمراض الكبد المزمن
مادة البحث:

تمت هذه الدراسة علي المجموعت التالية:

- 1- مرضي تشمع الكبد المصحوب بالاستسقاء البريتوني
- 2- مرضي سرطان الكبد المصحوب بالاستسقاء البريتوني
- 3- مرضي الكبد الدهني.
- 4- مجموعة الأصحاء.

طرق البحث:

جميع الأشخاص في لدراسة خضعوا للآتي:

- 1- تاريخ المرض كملًا.
- 2- فحص طبي كامل شامل الوزن والطول ومؤشر كتلة الجسم.
- 3- موجت صوتية علي البطن.
- 4- التحاليل المعملية: سكر صائم وبعد الأكل بساعتين في مصل الدم . نسبة كولسترول كلي وجزئي. نسبة دهون ثلاثية . وظائف كبد وتشمل: (أزيمت كبد نسبة الصفراء نسبة الألبومين نسبة لبروثرومبين.
- 5- دلالات التهاب الكبد الفيروسي المزمن.
- 6- نسبة اللبوتين في مصل الدم و لسائل البريتوني.

النتائج:

توصلت لدراسة للنتائج التالية:

في مجموعة المرضي المصلين بلعبد الدهني وجد أن مؤشر كتلة الجسم أعطي من مجموعة الأصحاء كما وجدت زيادة ذات دلالات إحصائية في معدلات الدهون الثلاثية وكولسترول في نفس المجموعة عن الأصحاء.

وجد زيادة ذات دلالات إحصائية في مستوي هرمون اللبوتين في مرضي تشمع الكبد المصحوب بالاستسقاء البريتوني و مرضي سرطان الكبد المصحوب بالاستسقاء البريتوني و مرضي الكبد الدهني بلمقارنة بمجموعة الأصحاء ولم توجد فروق ذات دلالة إحصائية بين مستوي اللبوتين في ذات الثلاث مجموعت . كما وجد أن مستوي هرمون اللبوتين في السائل البريتوني اعلي منها في المصل. وجد زيادة ذات دلالات إحصائية في مستوي هرمون اللبوتين في المصل و لسائل البريتوني لمرضي تشمع الكبد المصحوب بالاستسقاء البريتوني و الالتهاب البريتوني التلقائي مقارنة بمرضي تشمع الكبد المصحوب بالاستسقاء البريتوني.

الخلاصة والاستنتاج:

من خلال النتائج السابقة أمكن التوصل للآتي:

- 1- السمنة واضطرب نسبة الدهون في الدم تعتبر عوامل مؤدية ومصاحبة لمرض الكبد الدهني غير الكحولي، ولا بد من أخذ هذه العوامل في الاعتبار عند وضع خطة لعلاج هؤلاء لمرضي.
 - 2- ارتفاع مستوي اللبوتين في مصل الدم في حالات أمراض الكبد المزمنة يقترح أن اللبوتين يلعب دورا في حدوث التطورات في أمراض الكبد المزمنة مثل تشمع و مرض الكبد الدهني غير الكحولي.
 - 3- ارتفاع مستوي اللبوتين في مصل الدم في مرضي تشمع الكبد يقترح أن اللبوتين يلعب دورا في حدوث سوء التغذية عند هؤلاء المرضي.
 - 4- ارتفاع مستوي اللبوتين في المصل و السائل البريتوني لمرضي تشمع الكبد المصحوب بالاستسقاء البريتوني و الالتهاب البريتوني التلقائي يقترح ان للعدوى دورا في هذا الارتفاع فضلا عن دور كبد كذلك ارتفاع مستوي هرمون اللبوتين في السائل البريتوني اعلي منها في المصل يرجح وجود مصدر للإفراز بالبطن.
- دور اللبوتين في أمراض الكبد المزمنة لازال يحتاج للدراسة ويوصي بعمل دراسات مستقبلية واسعة لاستيضاح هذا الأمر.