



ESTIMATION OF SERUM AND ASCITIC FLUID LEPTIN LEVELS IN CHRONIC LIVER DISEASE

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ABSTRACT

Background: 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, Recent attention has been focused on the liver profibrogenic role of leptin in human and animal models so, we aimed to estimate the serum and the ascitic fluid leptin levels in chronic liver disease.

Subjects & Method: Serum and ascitic leptin levels were measured using ELIZA test in patients with liver cirrhosis (GI), hepatocellular carcinoma (GII), fatty liver (GIII) and healthy control (GIV).

Results: Serum leptin levels were significantly elevated in the three patients 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 GI and GII were more than the serum levels suggesting intra-abdominal production of leptin.

Conclusion: The elevated leptin level in group GI and GII, patients may play a role in the pathogenesis and progression of the disease. The elevation of ascitic fluid leptin levels more than serum levels in patients with hepatocellular carcinoma may suggest an intra-abdominal production of leptin.

Keywords: *Leptin, Cirrhosis, hepatocellular carcinoma, fatty liver.*

INTRODUCTION

Chronic liver diseases are defined by continuity of clinical or biochemical evidence of hepatic dysfunction for longer than six months (Suchy FJ)^[1]. Most forms of late-stage chronic liver disease often-manifest cirrhosis (Liou Cao)^[2]. Compensated cirrhosis; enough healthy liver tissue remains to carry out the important functions of the liver (UMMC, 2013; BLT, 2011)^[3]. As the disease progresses to the point where there is not enough functional liver tissue remaining to support metabolic demands, "decompensated cirrhosis" emerges. A cirrhotic patient is deemed "decompensated" upon the emergence of symptoms such as ascites, variceal bleeding, hepatic encephalopathy or jaundice (Garcia-Tsao)^[4].

Leptin, the product of the obese gene, is mainly produced by adipose tissues and, to a lesser extent, by tissues such as the stomach, skeletal tissue and placenta (Zhang Y)^[5]. The leptin receptor (OBR), belongs to the class I

cytokine receptor family (which includes receptors for IL-6, IL-12 and prolactin), and exists in at least six alternatively spliced forms with cytoplasmic domains of different length, known as OBRa, OBRb, OBRc, OBRd, OBR e and OBRf. (Tartaglia. LA.)^[6]. Leptin is known to regulate energy homeostasis. Mounting evidence has advocated leptin to have a regulatory function in immunity similar to the function of a pro-inflammatory cytokine. Several studies have found that circulating leptin levels increase during infection and inflammation, suggesting that leptin is part of the immune response and host defense mechanisms. Leptin levels are acutely increased by many acute phase factors, such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6, and during bacterial infection, or lipopolysaccharide (LPS) challenge. Leptin acts on monocytes/macrophages by inducing eicosanoid synthesis, nitric oxide (Faggioni R)^[7], and several pro-inflammatory

cytokines. Moreover, leptin induces chemotaxis of neutrophils and the release of oxygen radicals. The role of leptin in the innate and adaptive immune responses has also been reviewed (**La Cava A**)^[8]. Leptin is secreted into the blood stream in a circadian rhythm and proportional to body fat mass. Although leptin serves as a regulatory mediator between the brain and the periphery through modulating the hypothalamo-pituitary-adrenal (HPA) axis, its circulating level is also regulated by hormones secreted by the HPA system, including corticosteroids, prolactin, and insulin.

Furthermore, leptin expression can be negatively regulated by fasting, beta adrenergic agonists and thiazolidinediones. In addition, leptin secretion is higher in females than in males for any given age and body fat mass, i.e. it is sexually dimorphic (**Balci H**)^[9].

Clinical data, which investigated serum leptin concentrations in patients with chronic viral hepatitis, indicated that cirrhotic patients due to HBV infection had significantly higher leptin levels compared to the controls, and serum leptin levels were associated with the stage of liver fibrosis. In addition, it was suggested that increased serum leptin levels might represent a negative prognostic factor for response to lamivudine mono-therapy in patients with chronic hepatitis B. (**Lee, A. T.**)^[10]. Higher leptin levels were shown to be associated with cirrhosis development due to chronic HCV infection, but not with their histological features. These controversial results regarding the association of leptin with chronic HCV hepatitis needs further investigation (**Manolakopoulos S**)^[11]. Fatigue is a frequent and disabling symptom reported by patients with CHCV. Its mechanism is poorly understood. Recent attention has focused on the role of leptin and energy expenditure in CHCV

(**Ferrua B**)^[12]. There is evidence suggesting that leptin facilitates T-lymphocyte-mediated hepatotoxicity by facilitating the activation of tumour necrosis factor alpha (TNF- α) and interleukin 18 (IL-18), actions that may contribute to steatosis associated inflammation (**Faggioni R**)^[13].

There is experimental evidence indicating that leptin acts as a tumor promoter in NASH-related hepatocellular carcinoma (HCC). Liver hyperplasia was evident at an earlier stage of NAFLD and HCC was observed in higher rates in ob/ob mice; metabolic aberrations, rather than cirrhosis, initiated the hepatic neoplastic process (**Yang S**)^[14].

SUBJECT AND METHODS

This study was carried out on 100 individual selected from in patient clinic of Menouf Fever hospital, Tropical med. Dep. Faculty of medicine. They were 56 female and 44 male there age ranged between (28 – 66) years with mean age 46.9 ± 6.33 . the study is carried out from Sep. 2016 to April 2017

They were divided into four groups:

Group I: include (30 patients) with liver cirrhosis, they were 18 (60%) male and 12 (40%) female with mean age 45.5 years and they were divided into two subgroup: **Group Ia:** Include (15Patients) with ascites, **Group Ib:** Include (15 patients) without ascites, **Group II:** Include (30patients) with hepatocellular carcinoma. They were 20 (66.66) male and 10 (33.34%) female with mean age 44.46 years they were divided into two subgroups: **Group IIa:** Include(15Patients) with ascites, **Group IIb:** Include (15 patients) without ascites, **Group III:** Include (20 patients) with fatty liver they were 5 (25 %) male and 15 (75%) female with mean age 45.35 years, **Group IV:** Include (20 persons) healthy individuals, they were 13 (65%) male and 7 (35%) female there age ranged 47.2 years.

Exclusion criteria: chronic liver disease with diabetes melites, renal insuffancy obesity, pregnancy, coronery or endocrinal diseases.

N.B: The study did not exclude diabetic melites and obesity from group III.

All patients and control subjected to the following:

1- Full history taking thorough clinical examination and laboratory investigation in the form of:

Complete blood picture, Blood urea and serum creatinine, Fasting and post-prandial blood sugar. Serum cholesterol and triglycerides Liver

function tests (total serum bilirubin, ALT, AST, LDH, Alkaline phosphatase, serum albumin and prothrombin time and activity), Alpha feto protein, Ascitic fluid examination (chemical and bacteriological) Estimation of serum and ascetic fluid leptin levels, Abdominal Ultrasonography.

2- Ascitic fluid samples for chemical cytological and bacteriological investigation:

- Ascitic fluid samples were collected in the same sitting of serum sample under complete aseptic conditions.

3- Estimation of serum and ascetic leptin levels:

- This was done using direct ESA kit for quantitative determination of leptin level by Enzyme assay manufactured by diagnostic Biochem Canada.

Statistical analysis: Data was analyzed using Microsoft Excel 2007. Parametric data was expressed as means and non-parametric data was expressed as number and percentage. Student's test was done to compare between groups. Pearson Correlation Coefficient was done to correlate between different parameters among groups. Analysis of variance (ANOVA) test was used to estimate the difference between the means of more than two groups. P value of < 0.05 considered non-significant, P value of ≤ 0.05 considered significant, P value of < 0.01 was considered highly significant.

RESULTS

Table (1): Laboratory finding of the studied groups

	Group I	Group II	Group III	Group IV	ANOVA	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD		
	N= 30	N= 30	N= 20	N= 20	F	P-value
(ALT) (U/L)	109.533 ± 40.941	71.900 ± 29.139	48.250 ± 5.447	27.500 ± 6.833	39.661	<0.001*
(AST) (U/L)	109.433 ± 36.719	93.600 ± 19.877	33.500 ± 9.822	21.800 ± 9.088	80.702	<0.001*
Total serum bilirubin (mg %)	3.813 ± 1.611	5.767 ± 2.351	0.620 ± 0.317	0.630 ± 0.298	63.381	<0.001*
(ALP) (U/L)	165.733 ± 67.513	205.400 ± 92.437	127.550 ± 34.327	115.100 ± 35.389	9.326	<0.001*
Serum Albumin (g/dl)	2.423 ± 0.447	2.397 ± 0.506	3.943 ± 0.348	4.290 ± 0.165	141.888	<0.001*
Prothrombin activity (%)	43.733 ± 21.141	51.267 ± 15.065	92.500 ± 3.706	89.050 ± 6.143	71.598	<0.001*
(LDH) (U/L)	132.467 ± 21.299	270.933 ± 69.976	103.700 ± 10.878	77.000 ± 15.079	117.194	<0.001*
Alpha fetoprotein (mg/ml)	82.633 ± 44.562	616.433 ± 245.606	4.395 ± 1.634	6.220 ± 1.850	126.048	<0.001*
lood urea	42.833 ± 6.330	44.733 ± 4.748	41.050 ± 4.236	42.050 ± 5.165	2.209	0.092
Serum Creatinine	0.950 ± 0.136	0.953 ± 0.125	0.875 ± 0.189	0.875 ± 0.174	2.013	0.117
Serum Cholestrol (mg/dl)	121.333 ± 20.129	98.200 ± 4.985	227.100 ± 23.479	117.150 ± 9.560	288.138	<0.001*
Serum Triglycerides (mg/dl)	103.033 ± 18.261	104.967 ± 21.745	167.150 ± 9.938	106.800 ± 15.168	67.575	<0.001*

Table (3): Serum level of leptin in the studied groups

	Group I	Group II	Group III	Group IV	ANOVA	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD		
	N= 30	N= 30	N= 20	N= 20	F	P-value
Serum level of leptin	32.07 ± 15.625	21.192 ± 7.305	15.912 ± 5.827	12.719 ± 3.449	18.595	<0.001*

Table (4): Serum level of leptin in the studied groups

	Group Ia	Group Ib	Group IIa	Group IIb	Group III	Group IV	ANOVA	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD		
	N= 15	N= 15	N= 15	N= 15	N= 20	N= 20	F	P-value
Serum level of leptin	27.957 ±12.719	36.191 ±17.539	19.344 ±7.749	23.039 ± 6.571	15.912 ±5.827	12.719 ±3.449	12.968	<0.001*

Table (5): Serum leptin and ascitic leptin in-group Ia and IIa

		Group Ia			Group IIa			T-Test	
		Mean ±SD			Mean ±SD			t	P-value
Serum level of leptin	Mean ±SD	27.957	±	12.719	19.344	±	7.749	2.240	0.033*
Ascitic leptin	Mean ±SD	28.459	±	8.694	36.626	±	11.143	-2.238	0.033*

Table (6): Serum level of leptin in relation to sex

Groups	Sex						T-Test	
	Male			Female			t	P-value
	Mean	±	SD	Mean	±	SD		
Group I	29.519	±	14.824	40.917	±	4.562	-2.570	0.016*
Group II	20.748	±	11.490	35.500	±	4.197	-3.903	0.001*
Group III	15.636	±	3.067	16.060	±	6.999	-0.151	0.881
Group IV	13.258	±	3.634	11.719	±	3.076	0.949	0.355

Table (7): Relation between sex and serum leptin & ascetic leptin

Groups		Sex						T-Test	
		Male			Female			t	P-value
		Mean	±	SD	Mean	±	SD		
Group I	Serum level of leptin	29.519	±	14.824	40.917	±	4.562	-2.570	0.016*
	Ascetic leptin	32.549	±	12.457	47.250	±	6.384	-3.757	0.001*
Group II	Serum level of leptin	20.748	±	11.490	35.500	±	4.197	-3.903	0.001*
	Ascetic leptin	35.220	±	9.930	43.600	±	7.321	-2.359	0.026*

DISCUSSION

Chronic liver diseases may progress to fibrosis, accumulation of fibrillar extracellular matrix (ECM). Fibrosis is a dynamic process, considered as the result of a chronic "wound healing" response (Friedman SL) [15].

Regarding hepatic cancer, there are approximately 626.000 new cases of primary

liver cancer worldwide, almost all represented by hepatocellular carcinoma (HCC) with a high mortality ratio (0.93), 598.000 of these patients die from this cancer each year, making HCC the third most common cause of cancer death (Parkin DM) [16].

Leptin has proven to be important in the progression of hepatic steatosis (Magkos F1)

[17]. As a result of synergistic action of other adipocytokines such as tumor necrosis factor (TNF- α), fatty liver may progress to steatohepatitis by lipoapoptosis induction, which leads to the recruitment of inflammatory cells, contributing to its progression and subsequently causing hepatocellular damage, such as cell swelling and appearance of Mallory bodies (Magkos F) [18]. Leptin has proven to be important in the progression of hepatic steatosis (Magkos F) [19].

Our study showed significantly higher ascitic fluid leptin level compared to serum in our patient in both group I, II.

This is in agreement with (Giannini E) [20] who reported similar results and suggested intra-abdominal production of the leptin to explain this higher level reported that is due to hyper expression in leptin mRNA on activated hepatic stellate cells and found it is correlated with fibrosis in cirrhotic patients to be source of increased the leptin in tissue and serum of patients with the chronic liver disease.

Also, ascitic fluid leptin level are remarkably higher than the serum level and ascitic fluid leptin level in average twice as high as the serum level in the patient with hepatocellular carcinoma this is due to intra-abdominal production of the leptin (Van Hannelen V) [21].

In current study, we have found highly significant correlation between serum leptin and liver function tests in all studied groups.

This result is in agreement with (Green R. M.) [22] who explain alternation due to defect synthesis of coagulation factors. In addition this result is in agreement with (Ben-Ari Z) [23] who found that the leptin level increase when the liver function worsen likely reflecting a loss in the capacity to down regulate energy expenditure. In current study the lipid profile is highly significant in all studied groups with non-significant relation between to the leptin level. This result is in agreement with the study of (Cortez-pino) [24] who demonstrated that, obesity and dyslipidaemia were observed in 80% of the patients with non-alcoholic fatty liver disease, confirming that obesity and dyslipidaemia are risk factors of non-

alcoholic fatty liver disease while decrease the leptin in relation to lipid profile due to defective hepatic synthesis indicating poor prognosis (Riggio O) [25].

The current study showed statistically significant increase of the leptin level among patients groups in comparison to control group ($p < 0.05$) while there is a non-significant difference between the three patient groups (I, II, III). The results in cirrhotic patient group were agreement with (Trayhurn P) [26] who reported that patient's with cirrhosis had significantly higher serum leptin concentration compared with non-cirrhotic patients.

This due to stimulated production of some factors able to stimulate a dipocyte leptin synthesis and/or secretion of such as cytokines sexual steroids, insulin and cortisol, Moreover its possible due to development of portosystemic shunting in cirrhosis causes many of above mentioned secretogenous of leptin to increase leading to a multifactorial stimulation of leptin secretion (Testa R) [27]

This result agreed with (LeclereqlA) [28] who reported that chronic liver injury is dependent on leptin. This result also demonstrated that leptin is required for collagen I expression and production in the context of chronic liver injury and hepatic inflammation, possibly by modulatory transforming growth factor beta I (TGFB₁), bio-activation. These findings establish the essential physiological dependence of leptin for hepatic fibrogenesis in response to chronic liver injury.

In the current study serum leptin level in patient with HCC were significantly increased when compared to that of control group (21.192+ 7.305)

This result is in agreement with (Wang SN & Lee KT) [29] who reported that leptin receptor (ObR) is stimulator to class I cytokine receptors and signal transduction components of IL-6 family of cytokine the biological action of leptin will be effective when high amounts of IL-6 receptor activate to signaling pathways.

1) Janus Kinase (JAR) signal transducers and its activators to transcription (STAT16).

2) Phosphatidylinositol 3 kinase (PI3k) both promoting cell growth migration and invasion.

This study shows that, the serum leptin levels showed a statically significant increase in group III of fatty liver (Mean 15.912 + 5.827) in comparison to the control group.

This result is in agreement with (Uygun A) [30] reported that mean serum leptin level were significantly higher in patient with non-alcoholic fatty liver disease (NAFLD) when compared to control group, He stated that elevated serum leptin level might promote hepatic steatosis and steatohepatitis. This prospective control study, leptin level was significantly elevated in females than males in all studied groups.

This is in agreement with (Saber A) [31] and (McCullough A J) [32] who reported that women have significantly greater subcutaneous adipose tissue mass relative to omental adipose mass and estrogen increase serum leptin levels while testosterone decrease it. Thus, patient's sex needs to be considered when investigating the leptin level.

In current study, we found that BMI is highly significant in all studied groups with non-significant relation is found between leptin level and BMI in all studied groups.

This is in agreement with (Tungtrongchitr R) [33]. Who found no significant correlation between the leptin level and BMI in patient that have post hepatitis liver cirrhosis are wasted and may be over dehydrated thus, the excess in extracellular fluid that occur in all stages of liver disease causes weight parameters such as BMI to be in accurate.

CONCLUSIONS

From this study, we concluded that:

Estimation of the leptin level being a good option for clinical outcomes with chronic liver disease to be predicted in clinical practice for easy clinical application, Estimation of the serum and the ascitic fluid leptin is an excellent score for prediction of chronic liver disease which may progress to liver cell failure or hepatocellular carcinoma, Ascitic fluid leptin is significantly higher than the serum leptin. It may be due to intra

abdominal production of leptin, Patient with ascitic fluid leptin level twice the serum leptin level predicting hepatocellular carcinoma.

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