European Journal of Academic Essays 1(10): 17-22, 2014
ISSN (online): 2183-1904
ISSN (print): 2183-3818
www.euroessays.org

Oxidative Stress, Antioxidant and Leptin Status in Patients with Nonalcoholic Steatohepatitis

< Mohamed Ahmed Hassanien >[1], < Saad-El-Din Abd-El-Fattah Abou-El-Noeman >[2] < Ehab Aboueladab >[3]

[1]Clinical Biochemistry and Medical Education Departments, College of Medicine,
King Abdul-Aziz University, Jeddah, Saudi Arabia;
Medical Biochemistry, College of Medicine,
Tanta University, Egypt
mohammedhassanien700@yahoo.com

[2]Clinical Biochemistry and Medical Education Departments, College of Medicine,
King Abdul-Aziz University, Jeddah, Saudi Arabia
nooman1234@hotmail.com

[3]Damietta University, Faculty of Specific Education, New Damietta City, Egypt;
Alfarabi College for Dentistry and Nursing, Basic Science department,
Jeddah, Saudi Arabia
ehab10f@msn.com

Abstract: Non-alcoholic steatohepatitis (NASH) is characterized by morphological features indistinguishable from alcoholic hepatitis in individuals who do not consume excess alcohol. NASH can progress to cirrhosis, and liver failure is now the second leading cause of death in these patients. Although the prevalence of NASH appears to be increasing, the etiopathogenesis remains poorly understood. The present study aimed to evaluate the mechanisms involved in developing NASH through biochemical and laboratory parameters in Egyptian patients. This study was conducted on twenty five patients with NASH and twenty normal subjects of matched age and sex as control. All the patients were evaluated for several biochemical parameters through liver function tests, lipid profile, fasting and postprandial blood glucose, malondialdhyde, glutathione peroxidase activity, advanced glycated end products, and serum leptin. Data obtained from these tests were statistically evaluated. Obesity, hyperlipidaemia, and diabetes mellitus were prevalent among patients with NASH. There was significant increase in malondialdhyde and glutathione peroxidase activity in NASH patients. In addition, there were significant increases in leptin and basic fibroblast growth factor in NASH patients compared to control. Despite examining in different demography, we reestablished obesity, non insulin dependent diabetes mellitus and dyslipidemia as the important risk factors for NASH. We also found that enhanced oxidative stress was responsible for the second hits to liver in our patients, leading to NASH apart from simple steatosis. We reaffirmed the role of leptin in the pathogenesis of NASH.

Keywords: Non-alcoholic steatohepatitis (NASH), Leptin, Risk factors

1. Introduction

The pathophysiological characteristics, such as hepatic fat accumulation (steatosis); hepatocyte injury, manifested by swollen or "ballooned cells"; lobular inflammation with inflammatory infiltrated cells, predominantly neutrophils with or without; fibrosis of the liver, typically periventicular/ pericellular in distribution, have been found to be associated with non-alcoholic steatohepatitis (NASH) [1]. NASH has the potential to progress to liver fibrosis, cirrhosis, and even hepatocellular carcinoma [2]. The prevalence of NASH is higher in patients with obesity, insulin resistance, type 2 diabetes mellitus, and hyperlipidaemia [3-5].

The pathogenesis of NASH is yet to be understood. Fatty liver (hepatic steatosis) might eventually progress to NASH, however, little is known about the factors responsible for the transition from fatty liver to NASH. According to the "two-hits" hypothesis [6, 7], fatty liver is considered as the first hit, while enhanced oxidative stress is the second hit, leading to NASH. Fatty liver occurs due to accumulation of lipid in the liver, mainly triglycerides. The mechanisms leading to lipid accumulation are also not completely understood, but it could potentially result from insulin resistance [8, 9] and decreased disposal of fatty acids from impaired mitochondrial β-oxidation or deficient production of very low density lipoprotein [10]. Enhanced oxidative stress arises as an

Corresponding Author: Mohammed Hassanien, Clinical Biochemistry Department, College of Medicine, King Abdul-Aziz University, Email: mohammedhassanien700@yahoo.com, Tel: 00966564576503

imbalance between oxidants, namely free radicals, and antioxidants in favor of the former, leading to autopropagative process lipid peroxidation [11]. The liver normally responds to the chronic presence of oxidants by increasing the synthesis of protective anti-oxidant pathways such as those based on reduced glutathione (GSH). If these are inadequate, the products of lipid peroxidation create and amplify oxidative stress developing an inflammatory process in the liver [12].

The high circulating leptin levels associated with obesity may contribute to NASH in two ways, either by promoting insulin resistance and elevated circulating insulin level or by altering insulin signaling in hepatocytes to promote increased intracellular fatty acids [13]. Leptin may also influence the progression from hepatic steatosis to NASH as leptin could regulate inflammatory response and selectively enhance the secretion of certain inflammatory cytokines. [14, 15] Moreover, Bouloumie et al. recently claimed that leptin could enhance generation of reactive oxygen species (ROS) by increasing oxidation of fatty acids and oxidation of resultant acetyl-CoA through the tricarboxylic acid (TCA) cycle.[16]

Reports are scarce in the medical literature with regard to the role of the biochemical factors, such as oxidant and antioxidants levels, circulating leptin, in developing steatohepatitis in non-alcoholic patients. This study attempts to reestablish correlations between those important biochemical factors to unveil the pathogenic mechanism leading to NASH in an Egyptian cohort.

2. Materials and Methods

In this study, 25 patients with NASH and 20 healthy persons as controls were enrolled. All the participants were selected from the outpatient clinic of Tropical Medicine Department at Tanta University Hospital in Egypt. Informed written consent was obtained from each participant. The patients with NASH underwent a detailed clinical and laboratory evaluations, such as including liver function tests, hepatitis markers, to rule out other possible liver diseases. All the patients in the study group also underwent an upper abdominal ultrasonography. Liver biopsy was done in two selected cases. Indirect haemagglutination was carried out to rule out bilharziasis. None of the patients had renal dysfunction, drug toxicity, active infection, or recent gastrointestinal bleeding. Neither they received any antibiotic nor any vaso active drug at the time of study. Menopausal female, alcoholic, or cardiac patients were excluded.

Medical history of all the participants including controls was noted, and they were all clinically examined. BMI was calculated as weight (in kilograms) divided by the square of height (in meters) for all patients and controls (overweight: BMI between 25 – 29.9 kg/m2, obesity: BMI 29.9 kg/m2). Obesity was further classified as class-I obesity (BMI between 30 – 34.9 kg/m2), class-II obesity (BMI between 35 – 39.9 kg/m2), and extreme obesity (BMI equal to or more than 40 kg/m2).

All participants received the same dietary protein intake at least one day before the study. On the day of study, all of them were fasting for at least 12 h and rested in bed. Blood samples were taken from patients of study and controls groups. Laboratory investigations including liver function tests, lipid profile, fasting and postprandial blood glucose, lipid peroxidation end products (malondialdhyde or MAD), advanced glycated end products (AGEP), glutathione peroxidase (GPx) enzyme activity, and serum leptin were carried out. Serum leptin level was measured by a commercially available ELISA kit (The DSL- 10-23100 Human Leptin ELISA Kit; Diagnostic System laboratories, Webster, Texas). It is a direct Sandwich ELISA based assay, where human leptin molecules from samples is captured to the wells of a microtiter plate coated by pre-titered amount of polyclonal rabbit anti-human leptin antibodies, and unbound materials are washed away. Next step involves binding of a biotinylated monoclonal antibody to the captured human leptin and conjugation of alkaline phosphatase to biotinylated antibodies, followed by washing of free antibody-enzyme conjugates and quantification of immobilized antibody-enzyme conjugates by monitoring alkaline phosphatase activities in the presence of the substrate p-nitrophenyl phosphate. The enzyme activity is measured spectrophotometrically monitoring absorbance at 405 nm (due to production of the yellow colored product p-nitrophenol). The increase in absorbance is directly proportional to the amount of captured human leptin; hence, in the unknown sample, leptin can be quantitated from a reference curve generated with reference standards of known concentrations of human leptin. [17, 18]

The data were analyzed using SPSS for windows 8.0, 1977, SPSS, Chicago, IL. The results are expressed as mean ± SD. Independent sample t-student and Levene's tests were performed to compare the mean BMI and other serum parameters between control and study groups. All analyses were two tailed, and a p value of less than 0.05 was accepted as statistically significant.

3. Results

Tables 1 and 2 present the results obtained by analyzing the main biochemical parameters. The main risk factors for NASH include overweight and obesity, hyperlipidaemia, and diabetes mellitus. We found significantly higher BMI in patients with NASH (32.1 ± 3.3 Kg/m2) as compared to control (23.6 ± 1.56 Kg/m2) (p = 0.008). Based on BMI, we categorized the participants as obese (80 %) and overweight (20 %). The obese patients were further categorized as class I (52 %) and class II (28 %) obese patients.

Table 1: The results of demographic variables in control and NASH patient

Variable	Control group	NASH
Number	20	25
Gender (M/F)	9/11	14/11
Age/Years	28.15 ± 10.05	38.72 ± 5.83

NASH patients showed significantly higher cholesterol, triglyceride (p = 0.021) and LDL (p = 0.000) compared to control. Compared to the control group, 68 % of patients of the study group had cholesterol over 5.2 mmol/L, while triglyceride level was equal or over 1.7 mmol/L in 56% NASH patients. Our study included 40 % patients suffering from non insulin dependent diabetes mellitus (NIDD).

Table 2: The results of main biochemical tests in control and NASH patients

Laboratory Test	Control group		NASH		p-value
	Mean	S.D.	Mean	S.D.	
Gender (M/F)	9/11		14/11		
Number	20		25		
Age/Years	28.15	10.05	38.72	5.83	
Body mass index (Kg/m$^{2)}$	23.6	1.56	32.1	3.3	0.008*
ALT (U/L)	17.65	4.54	34.24	15.6	0.000*
AST (U/L.)	14.85	4.36	29.2.4	12.69	0.000*
Albumin (g/dL)	4.43	0.22	4.12	0.18	0.665
Total Protein (g/dL)	8.18	0.27	7.01	0.20	0.760
Total Bilirubin (umol/L)	9.28	2.28	13.3	2.1	0.937
Direct Bilirubin (umol/L)	2.625	0.87	3.15	1.0	0.742
Prothrombin time (Second)	12.63	0.2	12.98	0.31	0.666
Fasting Blood Sugar (mmol/L)	4.5	0.3	8.3	5.1	0.000*
Post prandial blood sugar (mmol/L)	4.7	0.32	11.4	7.8	0.000*
Cholesterol (mmol/L)	4.5	0.25	5.3	0.32	0.250*
Triglyceride (mmol/L)	0.97	0.14	1.62	0.42	0. 001*
HDL (mmol/L)	1.09	0.1	1.13	0.07	0.021*
LDL (mmol/L)	2.4	0.7	3.4	0.28	0.000*
MAD (nmol/ml)	2.44	0.17	3.13	0.33	0.012*
Advanced glycated end products (A.U.)	2.78	0.27	2.86	0.48	0.000*
GPx (U/L)	554.25	91.17	772.36	132.33	0.012*
S.Leptin (ng/ml)	7.9	0.57	20.15	2.78	0. 000*

*** Significant P < 0.05**

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, HDL = High density lipoprotein, LDL = Low density lipoprotein.

In liver function tests, patients with NASH showed significantly higher elevation in liver enzymes, AST and ALT, compared to the control group (p = 0.000 for both AST and ALT), and the AST/ALT ratio was less than one in both groups. However, serum total bilirubin (13.3 ± 2.1 µmol/L) and direct bilirubin (3.15 ± 1.0 umol/L), serum albumin (4.12 ± 0.18 g/dL), total protein (7.01 ± 0.20 g/dL), and prothrombin time (12.98 ± 0.31 s) were within the normal expected range in patients with NASH.

In addition, we also found significantly higher lipid peroxidation end products (MAD) and GPx activity in patients with NASH when compared to control (p = 0.012 for both MAD and GPx). There were significant increases in both AGEP as well as in serum leptin in patients with NASH compared to control group (p = 0.000 for both AGEP and serum leptin); increased AGEP was mainly observed in patients with NIDD.

4. Discussion

Obesity, diabetes mellitus, and dyslipidemia have been recognized as the major risk factors associated with NASH [19-21]. Our findings are also in agreement with this fact, and Table 2 clearly shows that obesity, dyslipidaemia, and diabetes mellitus are indeed prevalent among patients with hepatic steatosis and NASH. These metabolic disorders indicate the presence of insulin resistance that may offer some explanation with regard to the pathophysiological mechanisms of NASH. Such metabolic disorders are developed from the imbalance between storage of triglyceride in the liver and adipose tissue due to the net transfer of triglyceride from adipose tissue to liver, leading to hepatic steatosis (fatty liver) and it is considered as the first hit to liver [8, 9]. Risk factors of NASH increase the

circulating levels of free unsaturated fatty acids with enhanced concentration of those in liver. Oxidation of free fatty acids results in the autopropagative process of lipid peroxidation.

To assess whether there are relationships between oxidative stress and the development of NASH, we measured MAD as one of the end products of lipid peroxidation and GPx activity as an index to the antioxidant status. We found a significant increase in both MAD and GPx activity in patients with NASH as compared to control ($p = 0.012$ for both). This is in accordance with the previous reports suggesting a significant increase in oxidative stress parameters in patients with NASH. [12, 20, 22] In these studies, although the authors found significant increase in malondialdhyde levels [20, 22] in NASH patients, they did not find any significant difference in GPx activity[22] in those patients while comparing to control group. The enhanced GPx activity in our study could be explained in terms of disease progress. At early stages of the disease with normal liver function, the liver responds to pro-oxidant by increasing synthesis of protective antioxidants enzymes as GPx; however, liver capacity to synthesize antioxidants enzymes may be seriously affected with disease progression. The NASH patients enrolled in our study could still be in the earlier stages of disease progression.

As a result of enhanced lipid peroxidation in NASH patients, oxidative stress builds up and gradually amplifies, resulting in an imbalance between antioxidants and pro-oxidants in favor of the latter; this, in turn, can cause liver injury and is considered as the second hit to the liver leading to the development of NASH. This suggests that oxidative stress and lipid peroxidation may in part play critical role in the pathogenesis and probably in progression of NASH. [11, 12].

As mentioned above that obesity in prevalent among NASH patients (Table 2). To assess the influence of obesity in developing NASH, we examined serum leptin of patients in the study group as well as in control group. Leptin is closely involved in the regulation of food intake, body composition, and energy expenditure; hence, leptin is involved in the pathogenesis of obesity.[23] Leptin has been known to promote insulin resistance [24], elevate circulating insulin levels, and augment inflammatory and profibrogenic response in the murine liver exposed to hepatotoxic chemicals. [13, 25-27] Moreover, Bouloumie et al [16] demonstrated that leptin can induce oxidative stress in human endothelial cells. These findings, in conjunction with the observation that leptin is expressed and synthesized by the activated hepatic stellate cells, [27] support the notion that leptin might play a role in the pathogenesis of NASH. In the present study, we also found significantly high serum leptin in NASH patient when compared to the control group ($p = 0.000$), correlating with the previous reports those suggest association of higher levels of leptin with NASH. [19, 28] In contrast, Chalasani et al. [28] failed to detect statistically significant difference in serum leptin levels between NASH patients and controls. This could be due to the similarity in percent body fat and subcutaneous fat in NASH patients and their controls. Contrary to the study by Chalasani et al., [29] we had 80% obese among NASH patients, indicating that those patients might have had higher body fat or higher subcutaneous fat than controls to account for the higher levels of serum leptin in our study. It has already been shown that the amount of total body and subcutaneous fat are critical in determining the serum levels of leptin in obese patients [30].

Based on the above findings, we can explain the association between the increase of serum leptin and oxidative stress parameters in NASH patients through the effects of leptin on generating reactive oxygen species ROS. Leptin enhances ROS production by increasing beta oxidation of fatty acids, and oxidation of the resultant acetyl-CoA through TCA cycle in turn enhances oxidative stress responsible for the second hit inducing NASH. Increased fatty acid oxidation is mediated through stimulating the activity of carnitinepalimitoyltransferase-1 (CPT-1) and inhibiting that of acetyl-CoA caroxylase (ACC), pace-setting enzymes for fatty acid oxidation and synthesis, respectively. This whole process is mediated via protein Kinas A (PKA). PKA may stimulate CPT-1 (a key enzyme of beta oxidation of fatty acids) via two mechanisms; either by decreasing malonyl-CoA levels through phosphorylation, followed by inactivation of ACC or by modulating the interactions between CPT-1 and the cytoskelton through phosphorylation of intermediate filaments in malonyl- Co-A independent manner [31, 32].

5. Conclusions

The current study was performed in Egypt. Despite the change in demographical characteristics of the study group, we reestablished the fact that obesity, non insulin dependent diabetes mellitus and dyslipidemia are indeed important risk factors for the development of hepatic steatosis and NASH. In accordance with the previous studies, we also found enhanced oxidative stress as responsible parameter for the second hit to liver, resulting in NASH apart from simple steatosis. We reaffirmed the role of leptin in the pathogenesis of hepatic steatosis and development of steatohepatitis through its metabolic effects and increasing oxidative stress.

Acknowledgment

The authors acknowledge the participation of patients and the assistance of technical staffs at Tropical Medicine Department at Tanta University Hospital in Egypt for this study.

References

[1] Lawrence, S., B. Emmet and M. Willisc, 1998. Steatosis and Steatohepatitis in liver disease. Churcholl Livingstone, London.

[2] Shimada, M. , E Hashimoto, M Taniai, K Hasegawa, H Okuda, N Hayashi, K Takasaki and J. Ludwig, 2002. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol., 37 (1): 154-160.

[3] Wanless, I.R. and J.S. Lentz, 1990. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology, 12 (5): 1106-1110.

[4] Chitturi, S., S. Abeygunasekera, G.C. Farrell, J. Holmes-Walker, J.M. Hui, C. Fung, R. Karim, R. Lin, D. Samarasinghe, C. Liddle, M. Weltman and J. George, NASH and insulin resistance: Insulin hypersecretion and

specific association with the insulin resistance syndrome. Hepatology, 35 (2): 373-379.
[5] James, O.F. and C.P. Day, 1998. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol., 29 (3): 495-501.
[6] Day C.P. and O.F. James, 1998. Steatohepatitis: a tale of two "hits"? Gastroenterology, 114 (4): 842-845.
[7] Chitturi, S. and G.C. Farrell, 2001. Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis., 21 (1): 27-41.
[8] Poretsky, L. and B. Piper, 1994. Insulin resistance, hypersecretion of LH, and a dual-defect hypothesis for the pathogenesis of polycystic ovary syndrome. Obstet Gynecol., 84 (4): 613-621.
[9] Pagano, G., G. Pacini, G. Musso, R. Gambino, F. Mecca, N. Depetris, M. Cassader, E. David, P. Cavallo-Perin and M. Rizzetto, 2002. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology, 35 (2): 367-372.
[10] Esteban M, 1999. Fatty liver in sciff's diseases of the liver. 8th ed. Lipincot-Raven Publishers, Philadelphia.
[11] Robertson G., I. Leclercq and G.C. Farrell, 2001. Nonalcoholic steatosis and steatohepatitis. II. Cytochrome P-450 enzymes and oxidative stress. Am J Physiol Gastrointest Liver Physiol., 281 (5): G1135-G1139.
[12] Stärkel, P., C. Sempoux, I. Leclercq, M. Herin, C. Deby, J.P. Desager and Y. Horsmans, 2003. Oxidative stress, KLF6 and transforming growth factor-beta up-regulation differentiate non-alcoholic steatohepatitis progressing to fibrosis from uncomplicated steatosis in rats. J Hepatol., 39 (4): 538-546.
[13] Cohen, B., D. Novick and M. Rubinstein, 1996. Modulation of insulin activities by leptin. Science, 274 (5290): 1185-1188.
[14] Loffreda, S., S.Q. Yang, H.Z. Lin, C.L. Karp, M.L. Brengman, D.J. Wang, A.S. Klein, G.B. Bulkley, C. Bao, P.W. Noble, M.D. Lane and A.M. Diehl, 1998. Leptin regulates proinflammatory immune responses. Faseb J., 12 (1): 57-65.
[15] Bemelmans, M.H., L.J. van Tits and W.A. Buurman, 1996. Tumor necrosis factor: function, release and clearance. Crit Rev Immunol., 16 (1): 1-11.
[16] Bouloumie, A., T. Marumo, M. Lafontan and R. Busse, 1999. Leptin induces oxidative stress in human endothelial cells. Faseb J., 13 (10): 1231-1238.
[17] Imagawa, K., Y. Matsumoto, Y. Numata, A. Morita, S. Kikuoka, M. Tamaki, C. Higashikubo, T. Tsuji, K. Sasakura, H. Teraoka, H. Masuzaki, K. Hosoda, Y. Ogawa and K. Nakao, 1998. Development of a sensitive ELISA for human leptin, using monoclonal antibodies. Clin Chem., 44 (10): 2165-2171.
[18] Shibata, H., N. Sasaki, T. Honjoh, I. Ohishi, M. Takiguchi, K. Ishioka, M. Ahmed, M. Soliman, K. Kimura and M. Saito, 2003. Feline leptin: immunogenic and biological activities of the recombinant protein, and its measurement by ELISA. J Vet Med Sci., 65 (11): 1207-1211.
[19] Uygun, A., A. Kadayifci, Z. Yesilova, A. Erdil, H. Yaman, M. Saka, M.S. Deveci, S. Bagci, M. Gulsen, N. Karaeren and K. Dagalp, 2000. Serum leptin levels in patients with nonalcoholic steatohepatitis. Am J Gastroenterol., 95 (12): 3584-3589.
[20] Loguercio, C., V. De Girolamo, I. de Sio, C. Tuccillo, A. Ascione, F. Baldi, G. Budillon, L. Cimino, A. Di Carlo, M.P. Di Marino, F. Morisco, F. Picciotto, L. Terracciano, R. Vecchione, V. Verde and C. Del Vecchio Blanco, 2001. Non-alcoholic fatty liver disease in an area of southern Italy: main clinical, histological, and pathophysiological aspects. J Hepatol., 35 (5): 568-574.
[21] Crespo, J., P. Fernández-Gil, M. Hernández-Guerra, A. Cayón, M. Mayorga, A. Domínguez-Diez, J.C. Fernández-Escalante and F. Pons-Romero, 2001. Are there predictive factors of severe liver fibrosis in morbidly obese patients with non-alcoholic steatohepatitis? Obes Surg., 11 (3): 254-257.
[22] Koruk, M., S. Taysi, M.C. Savas, O. Yilmaz, F. Akcay and M. Karakok, 2004. Oxidative stress and enzymatic antioxidant status in patients with nonalcoholic steatohepatitis. Ann Clin Lab Sci., 34 (1): 57-62.
[23] Kaplan, L.M., 1998. Leptin, obesity, and liver disease. Gastroenterology, 115 (4): 997-1001.
[24] Müller G, J. Ertl, M. Gerl and G. Preibisch, 1997. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J Biol Chem., 272 (16): 10585-10593.
[25] Sivitz, W.I., S.A. Walsh, D.A. Morgan, M.J. Thomas and W.G. Haynes, 1997. Effects of leptin on insulin sensitivity in normal rats. Endocrinology, 138 (8): 3395-3401.
[26] Ikejima, K., H. Honda, M. Yoshikawa, M. Hirose, T. Kitamura, Y. Takei and N. Sato, 2001. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. Hepatology, 34 (2): 288-297.
[27] Potter, J.J., L. Womack, E. Mezey and F.A. Anania, 1998. Transdifferentiation of rat hepatic stellate cells results in leptin expression. Biochem Biophys Res Commun., 244 (1): 178-182.
[28] Chitturi, S., G. Farrell, L. Frost, A. Kriketos, R. Lin, C. Fung, C. Liddle, D. Samarasinghe and J. George, 2002. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology, 36 (2): 403-409.
[29] Chalasani, N., D.W. Crabb, O.W. Cummings, P.Y. Kwo, A. Asghar, P.K. Pandya and R.V. Considine, 2003. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? Am J Gastroenterol., 98 (12): 2771-2776.
[30] Van Harmelen, V., S. Reynisdottir, P. Eriksson, A. Thörne, J. Hoffstedt, F. Lönnqvist and P. Arner, 1998. Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes, 47 (6): 913-917.
[31] Velasco, G., M.J. Geelen, T. Gómez del Pulgar and M. Guzmán, 1998. Malonyl-CoA-independent acute control of hepatic carnitinepalmitoyltransferase I activity. Role of Ca2+/calmodulin-dependent protein kinase II and cytoskeletal components. J Biol Chem., 273 (34): 21497-21504.
[32] Guzmán, M., G. Velasco, M.J. Geelen, 2000. Do cytoskeletal components control fatty acid translocation into liver mitochondria? Trends Endocrinol Metab., 11 (2): 49-53.

Author Profile

Mohamed Ahmed Hassanien developed the original idea and the protocol, abstracted and analyzed data, critical revision of the manuscript, and is guarantor, Administrative, technical, and material support

Saad-El-Din Abd-El-Fattah Abou-El-Noeman contributed in Acquisition of data, Analysis and interpretation of data, Drafting of the manuscript, Statistical analysis.

Ehab Aboueladab developed the original idea and the protocol, collected data, analyzed data, statistical analysis, wrote the manuscript, and is guarantor.