Serum Visfatin in patients with chronic hepatitis C

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Abstract: Background: The role of visfatin in non alcoholic fatty liver diseases (NAFLD) is now well known accordingly, the aim of this work was to study the serum level of Visfatin in patients with chronic hepatitis C (HCV) and their relations to the nutritional state of patients as well as the biochemical markers of liver disease. Subjects: This study was carried out on 75 male subjects classified into five groups all of them were subjected to measurement of body mass index (BMI), lipid profile, liver function tests, PCR for HCV, serum visfatin level & liver biopsy when ever possible was done. Results: Mean serum Visfatin level was significantly elevated in group II (HCV & cirrhosis) and group IV (HCV & steatosis) than in group V. ($P < 0.05$). Significant positive correlation was found between serum visfatin & BMI, degree of inflammation & fibrosis. ($P<0.05$) On the other hand, significant negative correlation was noted between serum visfatin & apolipoprotein A1. ($P<0.05$). Conclusion: High levels of visfatin in patients with HCV and steatosis than other patients' groups suggest its involvement in the process of steatosis and its progression. Furthermore, high levels of visfatin in patients with HCV-induced cirrhosis and schistosomiasis suggest its role in liver fibrogenesis.

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1. Introduction:

The growing interest in the biology of adipose tissue derived from the understanding that fat is not only a passive energy depot, but functions as a hormonally active tissue, capable of producing numerous molecules, including cytokines, chemokines and adipokines. [(1)] The term "adipokines" comprises a group of polypeptide hormones which are expressed predominantly, although not exclusively, by adipose tissue in a regulated manner. [(3)] These molecules are secreted into the circulation and regulate the functions of different tissues through local, central and/or peripheral actions. [(2)]

Visfatin is a recently discovered adipokine that exerts insulin-mimicking effects, by activating the insulin receptor in a manner distinct from that of insulin. [(3, 4)] Visfatin is an insulin-mimetic adipokine that was originally discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors (whence its alternative name, pre-B-colony enhancing factor, or PBEF). [(5)] It is up-modulated in models of acute lung injury and sepsis. [(6)]

Dahl et al [(7)] have suggested that visfatin is an inflammatory mediator in cardiovascular pathologies based on its localization and actions in macrophages within atherosclerotic lesions. Moreover, it has been reported that visfatin up regulates endothelial matrix metalloproteinases (MMP-2/-9). [(8)]

Similarly to insulin, visfatin enhances glucose uptake by myocytes and adipocytes, and inhibited hepatocyte glucose release, resulting into insulin resistance in hepatocytes which distorts directly glucose metabolism, especially the control over glucose output into the circulation and interferes with cell survival and proliferation, while hepatic fatty acid synthesis remains stimulated by compensatory hyperinsulinaemia, resulting in steatosis . [(9)] Visfatin is secreted by activated lymphocytes, monocytes, and neutrophils. It induces the cellular expression of inflammatory cytokines such as TNF-α, IL-1and IL-6; visfatin-induced IL-6 expression might be involved in the pathogenesis of IR (insulin resistance) associated with visceral obesity. IL-6 has been demonstrated to promote IR via induction of SOCS (suppressor of cytokine signaling) proteins. [(10)] Since visceral adipose tissue is strongly associated to insulin resistance and non-alcoholic fatty liver disease (NAFLD), visfatin represent a possible link between visceral fat, insulin resistance and the development of NAFLD. [(11,12)]

Although circulating visfatin was found to be higher in NAFLD than in normal individuals, its lower expression in adipose tissue of NAFLD patients suggests that such difference is not due to adipose tissue overproduction. Taken together these

findings suggest a possible role for visfatin in the pathophysiology of NAFLD. [12] Fibrosis and cirrhosis are the final outcomes of all chronic liver disease; however, some morphological and biological differences distinguish fibrosis due to NASH from the one secondary to other causes of liver damage. The main cell type responsible for extracellular matrix deposition is represented by HSCs, which undergo activation in conditions of liver injury, acquiring a phenotype that enables them to participate in the liver wound healing process. [13]

The profibrogenic mechanisms operating in NASH are partly in common with those observed in other chronic liver diseases. However, the increase in circulating adipokines, oxidative stress generated by accumulation of fat in hepatocytes and the hormonal profile associated with the metabolic syndrome might have a specific role for the induction of fibrogenesis in this condition. [13] Taken together, these data identify a role for visfatin in obesity-related diseases, including insulin resistance and metabolic syndrome, and suggest possible implications in liver pathophysiology, especially in the context of NAFLD. [14]

Aim of the work

The aim of this work was to study the serum level of Visfatin in patients with chronic hepatitis C (HCV) and their relations to BMI of patients as well as the biochemical markers of liver disease.

2. Subjects and Methods

This study was carried out on seventy-five male subjects classified into five groups:

Group I: Fifteen patients with Chronic Hepatitis C (CHC).

Group II: Fifteen patients with CHC and liver cirrhosis. (Diagnosis of liver cirrhosis was based on clinical, laboratory and ultrasonographic findings).

Group III: Fifteen patients with mixed CHC and Schistosomal hepatic fibrosis.

Group IV: Fifteen patients with mixed CHC and steatosis.

Group V: Fifteen control healthy subjects

Male subjects were selected to exclude gender differences in visfatin hormone levels. Patients with history of the following were excluded from our study: Hepatitis B, autoimmune hepatitis, any alcohol intake, patients receiving hepatotoxic drugs. Other conditions associated with high visfatin values also were excluded including; type 2 diabetes, rheumatoid arthritis, and cardiovascular disease, the use of antiepileptic drugs, inflammatory bowel disease, renal failure, and renal transplant recipients. Patients gave consent to participate in the study. The protocol was approved by the committee of ethics, faculty of medicine, Alexandria University.

All groups were subjected to the following: Thorough history taking, clinical examination, calculation of BMI. Investigations which include: routine laboratory investigations; urine and stool examination, CBC, blood urea and serum creatinine, fasting and 2hr-postprandial blood sugar and lipid profile (triglycerides, cholesterol, VLDL, LDL, HDL and Apolipoprotein). Liver function tests; ALT, AST, prothrombin activity, serum albumin, serum bilirubin (total and direct), serum alkaline phosphatase, and gamma glutamyl transferase (GGT) were measured.Viral markers: serum HCV-antibodies using third generation ELISA and HCV-RNA using PCR-RNA quantitative were performed. Ultrasound examination of the abdomen and liver biopsies whenever possible (all cases in group I, 4 cases in group II, 4 cases in group III and 10 cases in group IV). In addition, sigmoidoscopy was performed to diagnose cases with mixed schistosomal affection.

Visfatin plasma concentrations was conducted by using enzyme immunosorbent assay, Visfatin, C-terminal, (Human) EIA Kit, catalog No.: EK-003-80, Phoenix Pharmaceuticals, Belmont, USA.

Statistical methods:

All analyses were performed using ANOVA test. All values are expressed as the mean ±standard deviation. Variables significantly deviating from normal distribution were logarithmically transformed. (F = ANOVA test, P = probability)

3. Results:

Significant differences were found between the studied groups and controls regarding hemoglobin level, red blood cells, white blood cells and platelets count (Table I). In addition, there were also a statistically significant difference between all groups and group IV as regards; serum cholesterol level, triglycerides, HDL, LDL, VLDL and apolipoprotein A1 levels (Table II).

Statistical difference between the studied groups regarding AST, ALT, total bilirubin, serum albumin and serum visfatin levels was summarized in (table III, IV and V).

Table (1): Comparison between the different studied groups regarding blood picture

		Mean	Standard Deviation	F	Sig.
Hb	**I**	11.40	2.051	9.488	.0001*
	II	12.23	2.210		(groups II,III,IV)
	III	11.22	2.234		
	IV	14.33	1.764		
	V	14.53	1.324		
RBCs	**I**	3.78	.702	4.489	.003*
	II	3.84	.774		
	III	3.71	.981		(groups II,III,IV)
	IV	4.34	.748		
	V	4.69	.637		
Platelets	**I**	201.07	160.298		.002*
	II	156.13	71.592	4.485	(groups II,III,IV)
	III	154.07	86.315		
	IV	179.07	67.646		
	V	212.67	55.053		
WBCs	**I**	8.01	2.611	3.677	.009*
	II	4.52	2.077		(groups II, III,IV)
	III	7.93	5.077		
	IV	7.51	1.899		
	V	7.31	1.820		

Table (II): Comparison between the different studied groups regarding lipid profile

		Mean	Standard Deviation	F	Sig.
cholesterol	**I**	124.07	42.516	8.039	.0001*
	II	123.67	62.314		
	III	138.40	64.293	IV # other groups.	
	IV	231.13	76.800		
	V	139.40	58.058		
TGs	**I**	75.93	22.327	12.113	.0001*
	II	187.20	47.644		
	III	87.53	36.424	IV # other groups.	
	IV	97.93	84.218		
	V	93.73	35.101		
HDL	**I**	72.20	15.907	11.638	.0001*
	II	72.73	13.609		
	III	43.47	24.118	IV # other groups.	
	IV	44.07	14.733		
	V	71.53	17.960		
LDL	**I**	127.13	8.861	7.224	.0001*
	II	126.13	12.609		
	III	107.27	8.216		
	IV	147.67	18.027	IV # other groups.	
	V	124.33	38.814		
VLDL	**I**	22.47	7.060	13.057	.0001*
	II	28.00	3.891		
	III	24.33	7.880		
	IV	44.67	16.762	IV # other groups	
	V	27.13	6.534		
Apolipoprotein A1	**I**	115.87	4.794	6.840	.0001*
	II	114.80	3.098	IV # other groups.	
	III	115.67	7.277		
	IV	104.67	12.344		
	V	115.13	3.889		

Table (III): comparison between the different studied groups regarding liver function

		Mean	Standard Deviation	F	Sig.
AST	I	40.53	14.372	3.979	.006*
	II	53.40	35.874		(group II,III)
	III	45.27	23.423		
	IV	67.93	57.528		
	V	22.00	5.477		
ALT	I	66.40	19.145	17.881	.0001*
	II	43.07	8.972		
	III	36.93	13.074		(group IV,I)
	IV	123.40	67.232		
	V	46.07	11.361	11.361	
Total Bilirubin	I	1.65	1.65	8.65	0.001*
	II	3.21	2.69		(In group II,III,IV)
	III	2.95	1.41		
	IV	1.75	1.23		
	V	0.90	1.42		

Table (IV): Comparison between the different studied groups regarding serum albumin

	Mean	Standard Deviation	F	Sig.
I	3.80	.705	15.949	.000
II	2.09	.623		
III	2.96	.593		
IV	3.00	.615	II, III, IV # other groups.	
V	3.85	.341		

Table (V): Comparison between the different studied groups regarding plasma Visfatin

		Mean	Standard Deviation	P	Sig.
S Visfatin(ng/ml)	**I**	612.00	13.638		
	II	631.87	15.811	.001*	(group II, group IV)
	III	608.20	16.476		
	IV	638.80	15.086	.001*	(group IV, group V)
	V	602.33	7.480		

Mean serum Visfatin level ranged from 590 ng /ml to 665 ng/ml among different studied groups with highest mean level of Visfatin found in group IV (638.80 ng /ml) followed by group II (631.87 ng/ml) showing the highest significant values.

A significant positive correlation was noted between BMI and serum visfatin. Also significant positive correlations were noted between serum levels of Visfatin and postprandial and fasting blood sugar. Significant positive correlations were found between levels of Visfatin and serum cholesterol and serum triglycerides and VLDL. Furthermore, significant positive correlation was also noted between serum levels of Visfatin and each of AST and ALT level. Moreover, a significant positive correlation was noted between the degree of inflammation, fibrosis and serum visfatin.

On the other hand, significant negative correlation was noted between serum levels of Visfatin and HDL, serum albumin and apoA1. In addition, there was a significant negative correlation

between prothrombin activity and serum visfatin. (TableVI)(Figures 1-4)

Table (VI): Correlation between S. visfatin and some other variables.

S Visfatin	R	P
Cholesterol	.347(**)	0.002
TGs	.264(*)	0.022
AST	.278(*)	0.016
ALT	.375(**)	0.001
BMI	.279(*)	0.015
HDL	-.259(*)	0.026
LDL	0.16	0.17
VLDL	.399(**)	0
Apolipoprotein	-.258(*)	0.025
Serum albumin	-0.406(*)	0.0001
HCV PCR	0.134	0.260
Degree of portal inflammation.	0.36(*)	0.0004
Degree of fibrosis	0.58(*)	0.0002

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

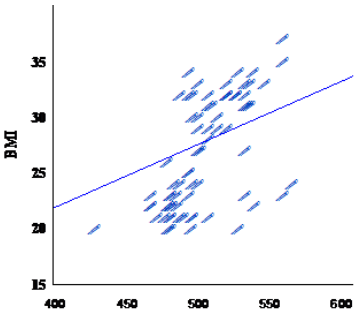

Figure (1): Correlation between visfatin & BMI

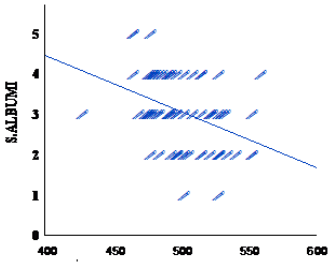

Figure (2): Correlation between visfatin & albumin

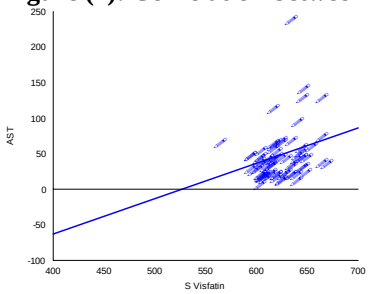

Figure (3): Correlation between visfatin & AST

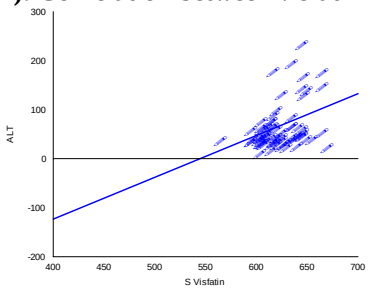

Figure (4): Correlation between visfatin & ALT

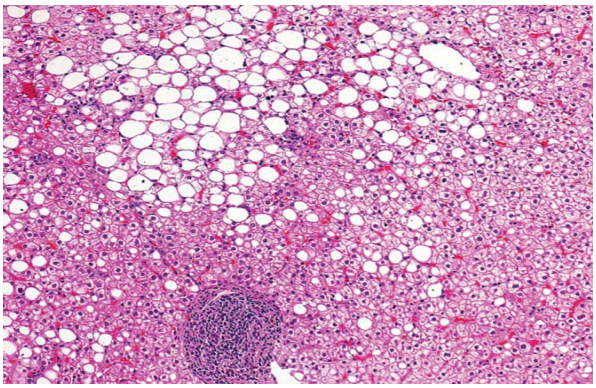

Fig 5: Histopathological image showing chronic hepatitis C with steatosis. The fat replaces less than one third of the liver tissue. A portal area with chronic inflammation is at the bottom of the field.

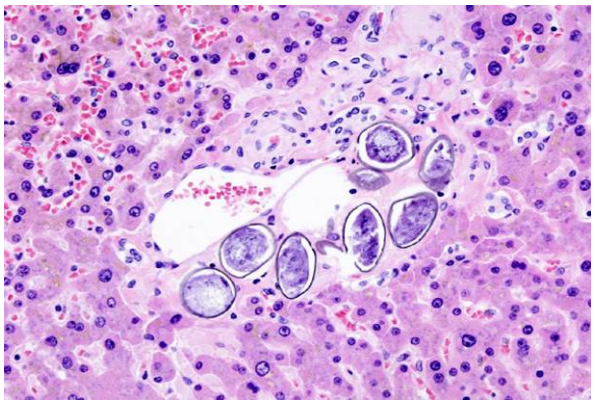

Fig.6: Histopathological image of schistosomiasis with deposition of calcified eggs in the hepatic portal tract.

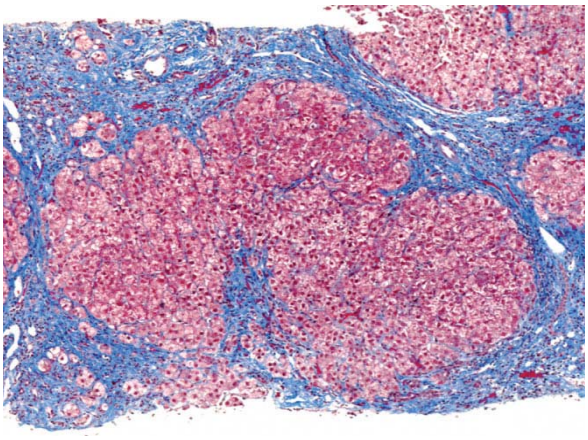

Fig7: Histopathological image showing Cirrhosis with chronic hepatitis

4. Discussion:

In view of the possible association of PBEF with adipose tissue, steatosis and insulin resistance, a question is being raised: if the circulating level of PBEF could be related to several pathological processes including HCV, post hepatitis cirrhosis, steatosis in humans. Accordingly, the aim of the present work was to study the serum level of visfatin in patients with some chronic liver diseases (CLD) and their relations to the biochemical markers of liver disease. The mean age of subjects was around 47 years. The mean BMI among groups in the present study was around 27.2 kg/m^2. In the present study no significant difference was found regarding BMI among the studied groups but there was a significant positive correlation between BMI and serum visfatin. In obesity-associated insulin resistance, circulating visfatin levels increases during the development of obesity, apparently due solely to secretion by abdominal white adipose tissue (WAT). [(15)]

Regarding serum albumin level, chronic hepatitis C patients had a normal range of albumin level as liver functions were maintained as they were discovered early in the course of the diseases. There was a significant difference in group II (cirrhotics), III (mixed shistosomal hepatic fibrosis) and IV (mixed steatosis) regarding the serum albumin level, Also a significant negative correlation between serum visfatin and albumin level. This can be explained by high serum visfatin in patients with chronic liver disease in which hypoalbuminemia is already present as a result of decreased synthetic functions of the liver. [(16)]

In the present study, significant positive correlation was found between total serum cholesterol, serum triglycerides, LDL, VLDL and serum visfatin. This finding might be attributed to visceral adiposity that is present in conditions associated with high cholesterol, triglycerides, LDL and VLDL as a result of insulin resistance that causes elevation of serum visfatin. [(17)]

As regards serum HDL, apolipoprotien A1 in the present study there was a negative correlation between serum visfatin and HDL and apolipoprotien A1. The present study was conducted on patients with chronic liver diseases. Lipoproteins play an important role in the absorption of dietary cholesterol, long chain fatty acids and fat soluble vitamins. All patients suffered from hepatitis C virus infection in the present work, Hypolipidemia is more marked in cases suffering from hepatitis C virus and this abnormality is directly related to viral load and viral response.[(18)] In the present study a

significant positive correlation was found between serum ALT, AST and serum visfatin. The same correlation was also observed by Carlson [19] this could be explained by the fact that visfatin is a proinflammatory cytokine so naturally it correlates with the degree of hepatic inflammation.

Prothrombin was found to have a significant negative correlation with serum visfatin. High serum visfatin associated with a low prothrombin activity is due to associated chronic liver disease causing both high serum visfatin and low Prothrombin concentration. Also a significant positive correlation was found between serum visfatin and the degree of portal inflammation as well as fibrosis this is in consistent with Aller R. et al [20] who showed a relation of visfatin with portal inflammation in his study. This could be explained by the insulin mimetic effect of visfatin which may have a role in hepatic inflammation. Secondly, visfatin could play a direct inflammatory role. The inflammatory relations of visfatin have clear molecular explanations. For example, visfatin induces the productionof IL-6 in human monocytes whereas IL6 negatively regulates visfatin gene expression in adipocytes.

Regarding difference in the serum visfatin levels among the studied groups. Significant differences among groups were found, with the highest levels in group IV (mixed CHC and steatosis) followed by group II (CHC and liver cirrhosis). Visfatin could play a role such as an insulin mimetic molecule producing inflammation in the liver; this may explain why it was higher in cirrhotic patients as well. Inflammation in the liver triggers repair responses that involve activation of hepatic stellate cells to myofibroblasts, a process that ends in cirrhosis. [21] In obese patients, the primary abnormality may be genetically induced insulin resistance, with a secondary increase of serum triglyceride levels due to enhance of peripheral lipolysis. The resulting hepatic supply of fatty acids and insulin may increase triglyceride deposition in the liver. [22]

Moreover, visfatin levels could predict the presence of the portal inflammation; this molecule could involve a non-invasive technique to determine this pathological change. This question overlaps with the more general issue of how the various adipokines interact with each other because the net effect of the simultaneous release of several agents with diverse biological properties is not readily predictable. Addressing such problems will require the development of new pharmacological tools that target specific adipokine systems. As a consequence, we anticipate that new therapeutic targets will be identified to realize control of these systems.

5. Conclusions:

From the previous results we can conclude that high levels of visfatin in patients with HCV and steatosis than other patients' groups suggest its involvement in the process of steatosis and its progression. High levels of visfatin in patients with HCV-induced cirrhosis and schistosomiasis suggest its role in liver fibrogenesis.

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