Research Article

Soluble CD٣٦ and Pon-١ As Novel Markers of Fatty Liver in Type ٢ DM

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Abstract
The aim of the present study is to predicate early fatty change in the liver in type ٢ diabetes by measuring sCD٣٦, paroxanase ١(PON-١) and nitric oxide level and estimating the degree of insulin resistance (by calculation of HOMA/IR). The study was carried out on ٨٥ subjects divided into ٢٥ patients with type ٢ DM of less than one year duration with normal liver enzymes, ٢٥ patients with type ٢ DM of more than one year duration with normal liver enzymes, ٢٥ patients with type ٢ DM of more than one year duration with elevated liver enzymes, in addition to ١٠ apparently healthy subjects (as control group) of matched age and sex. Each individual was subjected to general examination, abdominal examination, abdominal ultrasonography, laboratory investigations in the form of routine investigations; CBC, fasting blood glucose, two hour post prandial, renal function test, liver function tests, lipograme, Prothrombin time, concentration and special investigation in the form of; serum fasting insulin and calculation of HOMA/IR, SolubleCD٣٦, PON-١ by enzyme immunoassay (EIA) and nitric oxide. There was a statistically significant elevation of fasting insulin level, HOMA/IR, sCD٣٦ and PON-١ when comparing group I with group II and III; and group II to group III. While serum nitric oxide was significantly lower in group II and III when compared to group I. In patients groups there were positive correlations of sCD٣٦ with fasting glucose (r= ٠.٤, p=٠.٠١), fasting insulin (r= ٠.٨, p=٠.٠٠١), HOMA/IR (r= ٠.٨١, p=٠.٠٠٠١), TGs (r = ٠.٤١, p=< ٠.٠٥) and PON-١ (r= ٠.٨١, p= ٠.٠٠٣). While there was significant negative correlation of sCD٣٦ with nitric oxide (r= -٠.٧٦, p= ٠.٠١). Concerning PON-١ there were significant positive correlations with fasting insulin (r= ٠.٧٩, p=٠.٠٠٠١), HOMA/IR (r= ٠.٧٤, p=٠.٠٠٠١) and nitric oxide (r= ٠.٧٥, p=<٠.٠٥). While there was significant negative correlation with HDL-c (r= -٠.٤٢, p= ٠.٠٠٨). In patient group III there was significant positive correlation of sCD٣٦ with ALT (r= ٠.٦٢, p=٠.٠٠١) and GGT (r= ٠.٦, p= ٠.٠٣)
Conclusion: Plasma sCD٣٦ is a significant predictor for early degree of fatty liver in patients with type ٢ DM. Also there were strong correlations between fasting insulin and HOMA/IR with sCD٣٦, PON-١ and nitric oxide.
Key words: Soluble CD٣٦, PON-١, Nitric oxide, Type ٢ diabetes and Fatty liver.

Introduction
Diabetes mellitus **(**DM) is "a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both."[١] Type ٢ DM is due to insulin resistance or reduced insulin sensitivity[٢]. This form of diabetes accounts for ~٩٠–٩٥٪[٣]. Type ٢ fatty liver is not a medical term, but this condition is linked to type ٢ DM. In many cases, a fatty liver will not cause symptoms. However, the accumulated fat can lead to inflammation and scarring of the liver tissue, which can result in liver failure[٤].

Soluble CD٣٦ and parao-xanase١ were discovered as indicators of liver injury in diabetic patients. CD٣٦, also known as fatty acid translocase (FAT)[٥] and platelet glycol-protein IV or IIIb, is a multispecific, integral, ٨٨-kDa membrane glycolprotein expressed on the surface of a wide variety of cell types including adipocytes, skeletal muscle cells, platelets, endothelial cells and monocytes/macrophages[٦]. A soluble form of CD٣٦ (sCD٣٦), a marker of altered tissue CD٣٦ expression, was identified inhuman plasma, and elevated levels were found in obesity and type ٢ DM[٧].

Paraoxonase-١ (PON-١), is a high-density lipoprotein (HDL-c), synthesized in liver, associated enzyme that protects low-density lipoprotein cholesterol (LDL-c) and HDL-C against oxidative damage .This enzyme is thought to degrade oxidized phospholipids and play an important role in the organism's antioxidant and anti-inflammatory system. Liver diseases are associated with increased oxidative stress and decrease serum PON-١ activity[٨].

The aim of this work is to determine the utility of soluble CD٣٦ and PON-١ as indicators of insulin resistance and fatty liver change in type ٢ DM.

Subjects and methods

The present study was carried out at the Clinical Pathology Department, Faculty of Medicine, Minia University. It was conducted on ٧٥ consecutive patients with type ٢ DM who were selected from the attendants of the Diabetes Outpatient Clinic of Minia University Hospital, through the period from October, ٢٠١١ to April ٢٠١٢. They were ٣٧ males, and ٣٨ females, their ages ranged from ٣٠ to ٥٦ years. The study also has ١٠ apparently healthy volunteers who served as a control group.

They were classified into: Group (I): ٢٥ diabetic patients (١٢ males and ١٣ females) of less than one year duration with normal liver enzyme; **Group (II):** ٢٥ diabetic patients (١٢ males and ١٣ females) of more than one year duration with normal liver enzymes; **Group (III)**: ٢٥ diabetic patients (١٣ males and ١٢ females) of more than one year duration with elevated liver enzymes and **Group (IV)** :includes ١٠ apparently healthy subjects

Patients with body mass index more than ٣٠ kg/m² or positive serological markers for viral hepatitis or any systemic disease (e.g. Renal impairment, hypertension..etc) and history of hepatotoxic drugs were excluded. All the study subjects were submitted to: history**,** clinical examination and abdominal ultrasonography

Sampling protocol:

About ٧ ml of venous blood were withdrawn from each subject by sterile veinpuncture after ١٢ hours fasting. One ml in EDTA containing tube: for CBC. Another ١ ml in EDTA containing tube was centrifuged and the expressed plasma was kept frozen at -٢٠°C for detection of soluble CD٣٦. The remaining ٥ ml divided into two plain tubes were left to be clotted then centrifuged. Separated serum was used for determination of fasting blood glucose, kidney function tests, liver function tests and lipid profile. The remaining serum was kept frozen at -٢٠°C for determination of fasting insulin, nitric oxide and paraoxanease-١.

Laboratory investigations:

- **Routine:**

Complete blood count (CBC): It was determined by automated cell counter, Sysmex KX-٢١N (TAO Medical Incorporation, Japan).

Biochemical tests:

- Fasting serum glucose, renal function tests, liver function tests, total cholesterol and triglycerides (TGs) were determined using fully automated clinical chemistry auto-analyzer system Konelab ٢٠i (Thermo Electron Incorporation, Finland)**.**
- Serum high density lipoprotein- cholesterol (HDL-c) was determined using Microlab ٢٠٠ (Vital Scientific W-Holland) using kits supplied by Human Gesellschaft Fur Biochemical and Diagnostic mbh, Germany[١٢]**.** Serum low density lipoprotein- cholesterol (LDL-c) was calculated according to Friedewald equation.

- **Special investigations:**

Estimation of fasting insulin: By an enzyme immunoassay**,** kit was supplied by (DRG International, Inc. USA)[٩].

Estimation of sCD٣٦: By quantitative sandwich enzyme immunoassay, kit was supplied by (ADIPO BIO-SCIENCE, INC Walsh Ave. Suite C Santa Clara, USA)[١٠].

Estimation of PON-١: enzyme immunoassay method for quantitative determination of PON-١ concentration, kit was supplied by (Wuhan ElAab Science Co., Ltd Wuhan "٤٣٠٠٧٩" China)[١١].

Estimation of Nitric oxide: for quantitative determination of nitric oxide concentration, kit was supplied by (R&D System, Inc. USA)[١٢].

All analyses were performed with version ١٩ of Statistical Package of Social Science (SPSS).

Results

The present prospective hospital based study included seventy five patients with type ٢ DM

in addition to ten apparently healthy volunteers who served as a control group.

Demographic data for the studied groups are shown in table (I).
Fasting insulin was significantly increased in group I, group II and group III when compared with group IV (p=٠.٠٠٠١, p=٠.٠٠٠١ and p=٠.٠٠٠١ respectively). On com-paring group I with group II and group III there was significant increase values in group II and group III (p=٠.٠٠٠١ and p=٠.٠٠٠١ respectively). Also there was significant increase in fasting insulin in group III when compared with group II (p= ٠.٠٠٠١). HOMA/IR was significantly increased in group I, group II and group III than group IV (p= ٠.٠٠٠١, p=٠.٠٠٠١ and p=٠.٠٠٠١ respectively). On comparing group I with group II and III the HOMA/IR ratio was significantly increased in group II and Group III (p=٠.٠٠٠١ and p=٠.٠٠٠١ respectively). When comparing group II with group III there was significantly increased ratio in group III (p=٠.٠٠٠١) as shown in tables (II).

Soluble CD٣٦ level was significantly increased in group I, group II and group III as compared with group IV (p=٠.٠٠١, p=٠.٠٠١ and p=٠.٠٠١ respectively). On comparing values in group I with that of group II and group III there was significant increase levels in group II and group III (p=٠.٠٠١ and p=٠.٠٠١ respectively). Also there was significant increase in sCD٣٦ level in group III when compared with group II (p=٠.٠٠٠١). Concerning nitric oxide values, there was significantly decreased levels in group I, group II and group III as compared with those in group IV (p=٠.٠٠١, p=٠.٠٠١ and p= ٠.٠٠١ respectively). On comparing group I with group II and group III there was significantly decreased values of nitric oxide in group II and group III (p=٠.٠٠١ and p=٠.٠٠١ respectively).
Also, there was significant decrease in nitric oxide values in group III when compared with group II (p=٠.٠٠١). PON-١ was significantly increased in group I, group II and group III as compared with group IV (p=٠.٠٠١, p=٠.٠٠١ and p= ٠.٠٠١ respectively). Also, on comparing group I with group II and III the PON-١ levels were significantly increased in group II and group III (p=٠.٠٠١and p=٠.٠٠١ respectively). On comparing group II with group III there was significantly increased levels in group III (p= ٠.٠٠١) as shown in table (III).

There were significant positive correlations of sCD٣٦ with fasting glucose (r=٠.٤; p=٠.٠١), fasting insulin (r= ٠.٨; p=٠.٠٠١), HOMA/IR (r=٠.٨١; p=٠.٠٠٠١), TGs (r =٠.٤١, p=< ٠.٠٥) and PON-١ (r=٠.٨١, p=٠.٠٠٣) in patients groups. While there was significant negative correlation of sCD٣٦ with nitric oxide (r=-٠.٧٦, p=٠.٠١). Concerning nitric oxide there were significant negative correlations with fasting insulin (r=-٠.٨٠; p=٠.٠٠٠١) and HOMA/IR (r=-٠.٧٨; p= ٠.٠٠٠١). While there was significant positive correlation of nitric oxide with PON-١ (r=٠.٧٥, p=<٠.٠٥). PON-١ were significantly positively correlated with fasting insulin (r=٠.٧٩; p=٠.٠٠٠٠١) and HOMA/IR (r= ٠.٧٤; p= ٠.٠٠٠٠١). While there was significant negative correlation with HDL-c (r= -٠.٤٢, p=٠.٠٠٨) as shown in table (IV) .

In patient group III there were significant positive correlations of sCD٣٦ with ALT (r= ٠.٦٢; p=٠.٠٠١) and GGT (r= ٠.٦, p=٠.٠٣) as shown in table (V).

Table (I): Demographic Characteristics of the studied groups

Variable	Group I	Group II	Group III	Group IV	P – value		
					$G_I$vs G_{II}	$G_I$vs G_{III}	G_{II}vs G_{III}
Age (year)					٠.٧	٠.٥٣	٠.٨
Range	٣١-٤٨ y	٣٣ – ٤٩y	٣٠ -٥٠y	٣٨-٥٦y			
Mean±SD	٣٩.٩±٨.٦	٤١.٤±٨.٦	٤٠.٩±١٠.٣	٤٧±٩.٣			
p-value	p =٠.٢	p =٠.٦	p=٠.٧				
Sex(female/male)					٠.٥	٠.٨	٠.٧
No.	١٣/١٢	١٣/١٢	١٢/١٣	٥/٥			
%	٥٢/٤٨٪	٥٢/٤٨٪	٤٨/٥٢٪	٥٠/٥٠٪			
p-value	p =٠.٥	p =٠.٧	p =٠.٩				
BMI(Kg/m٢)					٠.٥	٠.٩	٠.٤
Range	٢١-٢٤.٧	٢١.٤-٢٤.٦	٢١.٩-٢٣	١٨.٤-١٩.٦			
mean±SD	٢٣±١.٧	٢٣±١.٦	٢٢.٩±١	١٩±٠.٦			
p-value	p=٠.٠٠١*	p =٠.٠٠٠١*	p=٠.٠٠١*				

***: Statistically significant**

Table (II): Results of fasting glucose, fasting insulin and HOMA/IR of the studied groups

Variable	Group I	Group II	Group III	Group IV	P – value		
					G_I vs G_{II}	$G_I$vs G_{III}	G_{II} vs G_{III}
Fasting glucose(mg/dl)					٠.٠٤	٠.٠٠٣*	٠.١١
Range	١٦٧.٩-٢٠٤	١٦٩-٢٣٧	١٨٢-٢٥٦.٥	٧٧.٧-٨٧.٧			
Mean±SD	١٨٦.٤±١٨.٥	٢٠٣.٢±٣٤	٢١٩.٣±٣٧.٥	٨٢.٧±٥			
p- value	p =٠.٠٠٠١*	p =٠.٠٠١*	p=٠.٠٠٠١*				
Fasting Insulin(µIU/ml)					٠.٠٠٠١*	٠.٠٠٠١*	٠.٠٠٠١*
Range	١٣.٥-١٩.٥	٢٦.٥-٣٧.٧	٤٠.٧-٦٣.١	٩.٨-١٣			
Mean±SD	١٦.٥±٣	٣٢.١±٥.٦	٥١.٩±١١.٢	١١.٤±١.٦			
p- value	p =٠.٠٠٠١*	p =٠.٠٠٠١*	p =٠.٠٠٠١*				
HOMA/IR					٠.٠٠٠١*	٠.٠٠٠١*	٠.٠٠٠١*
Range	٥.٣-١٠.١	١٢.٢-٢٠	٢٠.٧-٣٥	٢-٢.٦			
mean±SD	٧.٧±٢.٤	١٦.١±٣.٩	٢٧.٩±٧.١	٢.٣±٠.٣			
p- value	p =٠.٠٠٠١*	p =٠.٠٠٠١*	p =٠.٠٠٠١*				

***: Statistically significant**

Table (III): Results of sCD٣٦, nitric oxide and PON-١ of the studied groups

Variable	Group I	Group II	Group III	Group IV	P – value		
					G_I vs G_{II}	G_I vs G_{III}	G_{II} vs G_{III}
sCD٣٦(ng/ml)					٠.٠٠١*	٠.٠٠١*	٠.٠٠٠١*
Range	٣٩.٤-٤٧.٨	١٨٣.٩-١٩٦.٩	٥٩٢-٩١٤	١٨.٦-٢٣.٤			
Mean±SD	٤٣.٦±٤.٢	١٩٠.٨±٦.٩	٧٥٣.١±١٦١	٢١±٢.٤			
p-value	p =٠.٠٠١*	p =٠.٠٠١*	p=٠.٠٠١*				
Nitric oxide(µmol/L)					٠.٠٠١*	٠.٠٠١*	٠.٠٠١*
Range	٦٢-٨٢	٤٧.٨-٥٩.٩	٢٩-٥١	٧٧-٨٩.٦			
mean±SD	٧٢.٣±١٠.١	٥٣.٩±٦.١	٤٠.٢±١١.٢	٨٣.٣±٦.٣			
p- value	p =٠.٠٠١*	p =٠.٠٠١*	p =٠.٠٠١*				
PON-١(ng/ml)					٠.٠٠١*	٠.٠٠١*	٠.٠٠١*
Range	٥.٢-١٢.٣	٢٦.٧-٧٠.٧	٧٥.٢-١٢٤.٢	٤.٤-٥			
mean±SD	٨.٨±٣.٥٦	٤٨.٧±٢٢	٩٩.٧±٢٤.٥	٤.٧٥±٠.٣			
p-value	p =٠.٠٠١*	p =٠.٠٠١*	p =٠.٠٠١*				

***: Statistically significant**

Table (IV): Correlations of sCD٣٦, PON-١ and nitric oxide with laboratory finding in patients groups.

Dependent variable	sCD٣٦	Nitric oxide	Paraoxanase١
Fasting glucose	r = ٠.٤* p =٠.٠١	r = -٠.٣١ p =٠.١٢	r = ٠.٣٧ p =<٠.٠٥
Fasting insulin	r =٠.٨* p =٠.٠٠١	r = - ٠.٨٠* p =٠.٠٠٠١	r = ٠.٧٩* p ٠.٠٠٠٠١
HOMA/IR	r =٠.٨١* p =٠.٠٠٠١	r = - ٠.٧٨* p= ٠.٠٠٠١	r = ٠.٧٤* p = ٠.٠٠٠٠١
Total cholesterol	r =٠.١٤ p =٠.٩	r = -٠.٢٤ p=٠.٨	r = ٠.٢ p = ٠.٢
Triglyceride	r =٠.٤٣* p =<٠.٠٥*	r = -٠.٣٢ p = <٠.٠٥	r = ٠.٥* p =٠.٠٤*
HDL	r =٠.٢ p =٠.٦	r = ٠.١٨ p = ٠.٥٦	r = - ٠.٤٢* p = ٠.٠٠٨
LDL	r =٠.١٣ p = ٠.٧٦	r = -٠.١٤ p=<٠.٥	r = ٠.٢٤ p = ٠.٤٦
PON-١	r = ٠.٨١* p =٠.٠٠٣	r = ٠.٧٥* p = <٠.٠٥	_____
Nitric oxide	r = - ٠.٧٦* p =٠.٠١	_____	_____

Grades of r: ٠.٠٠-٠.٢٤ = weak or no association; ٠.٢٥-٠.٤٩ = fair association; ٠.٥٠-٠.٧٤ = moderate association; ≥ ٠.٧٥ = strong association.

***= statistically significant**

Table (V): Correlations of sCD٣٦, Nitric oxide and PON-١ with liver enzymes (ALT, AST and GGT) in group III

Variables	sCD٣٦	Nitric oxide	Paraoxanease ١
ALT	r =٠.٦٢*	r =٠.٠٦	r =٠.١٢
	p =٠.٠٠١	p =٠.٣٧	p =٠.٥
AST	r =٠.٢	r =-٠.٠٦	r =٠.١٤
	p =٠.٠٣	p =٠.٢	p =٠.٧
GGT	r =٠.٦*	r =٠.٣	r =٠.٠٨
	p =٠.٠٣	p =٠.١٩	p =٠.٤

Discussion

The WHO describes DM as the most common endocrine disease in the world(١٣). Type٢ DM is a risk factor for progressive liver disease and mortality in patients with fatty liver. So the diagnosis and evaluation of fatty liver is an important part of management of diabetes(١٤). The definitive diagnosis of fatty liver is based on the histological examination of liver biopsy samples. However, it is an invasive and costly procedure and is associated with many complications(١٥).

Aminotransferase levels are directly associated with liver dysfunction, but that association is affected by insulin action and inflammation(١٦). Aminotransferase levels, even at normal levels, correlate positively with proinflammatory cytokines and negatively with anti-inflammatory cytokines(١٧).

The results of the recurrent study revealed significant increase levels of fasting glucose, fasting insulin and HOMA/IR in patient group than control group. This was in agreement with Handberg et al.,(١٨) and Renuka et al.,(١٩). Another investigators Piyali et al.,(٢٠) explained that difference is due to, prolonged state of hyperglycemia leads to excess lipid supply. When FFA (Free Fatty Acids) are elevated for a prolonged period, they have a direct effect on insulin action in skeletal muscle tissue and liver, reducing the normal responses to insulin to promote glucose uptake and to suppress hepatic glucose output, respectively.

Concerning sCD٣٦, our study found that there was a significantly increased sCD٣٦ level in patients groups than in control group. This was in agreement with Handberg et al.,(١٨). The increased levels of sCD٣٦ are more likely to be explained as follow; type ٢ diabetes is associated with increased levels of fasting blood glucose. This increase leads to accumulation of fat in the liver, lead to increase FFA in the liver. Monocyte and macrophage will uptake the FFA and this lead to increase the expression of sCD٣٦.

Liani et al.,(٢١) found that sCD٣٦ levels among diabetic patient with duration more than one year are significantly higher than those of less than one year duration. This is in consistent with the present study as sCD٣٦ levels were significantly higher in group II and III when compared with group I.

PON-١ concentration in the current study showed significant increase in patients group than control group. This was in agreement with Renuka et al.,(١٩). The authors state that reason of this difference till present is unknown. However, several explanations are suggested, a larger proportion of the paraoxonase protein could be inactive in diabetes either because of the presence of an endogenous circulating inhibitor or perhaps because of increased glycosylation of paraoxonase.

The present study demonstrated positive significant correlations between sCD٣٦ with fasting glucose, fasting insulin and HOMA/IR in patients groups. This was in agreement with Jose-Mamuel et al.,(٢٢).

In the present study there was positive correlation of sCD٣٦ and triglycerides, this was in agreement with Handberg et al.,(٢٣) and Liani

et al.,[٢١]. This correlation is more likely due to; diabetic state associated with insulin resistance condition leads to increase expression of CD٣٦ in a number of tissues. This increase leads to more expression of CD٣٦ on macrophage, more fat accumulation and initiation of atherosclerotic lesions.

The current study shows significant positive correlation between sCD٣٦ and PON-١ in patients groups.

Latisha et al.,[٢٤] agreed with our finding. They explained this relation as follows; there is inverse association between CD٣٦ expression and total HDL, as well as, with HDL subfractions. CD٣٦ functions in the uptake of fatty acids and oxidized lipoproteins. Consequently, CD٣٦ has been identified to influence free FA and high-density lipoprotein (HDL) levels. This alternation of HDL-c will be reflected on PON-١ concentration level.

Concerning nitric oxide, our study stated that nitric oxide was negatively correlated with fasting insulin and HOMA/IR. This was in agreement with Paolo et al.,[٢٦] and Tessari et al.,[٢٧]. Also, our study showed significant positive correlation of nitric oxide with PON-١. This was in agreement with Acar et al.,[٢٨].

Another investigator Deepthi et al.,[٢٩] found positive correlation between PON-١ and nitric oxide. They explained this finding by; diabetes is a condition of hyperglycemia, hyper-glycemic condition has dual effect: First, high blood glucose depletes natural antioxidants and facilitates the production of reactive oxygen species which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA etc. The second effect, type٢ DM is associated with increased glycation of various plasma enzymes due to increased glucose concentration which further reduce the capacity of PON١ to prevent lipid peroxidation leading to increased tendency for lipid peroxidation.

In the present study, PON-١ was significantly positively correlated with fasting insulin and HOMA/IR.This was in agreement with Judit et al.,[٣٠] and Acar et al.,[٢٨].

Concerning the relation to HDL-c there was significant negative corre-lation with PON-١, Renuka et al.,[١٩] share with present study this finding. Another author Murakami et al.,[٣١] concluded that PON-١ activity is diminished in type ٢ diabetes mainly due to alternation in HDL-c com-position.

Soluble CD ٣٦ showed significant positive correlations with ALT and GGT in patients group III. This was in agreement with Jose-Manuel et al.,[٢٢] and Handberg et al.,[٢٣]. They mentioned that sCD٣٦ may be released from degrading cells just like ALT, thus increase with liver cell destruction associated with insulin resistance. With insulin resistance and obesity, plasma lipids are disturbed and accumulate in the liver, potentiating the "low-grade inflamemation." This may induce release/secretion of CD٣٦ by monocytes or macrophages (Kupffer cells) and may even release CD٣٦ from liver cells.

In agreement with our results another investigators Koonen et al.,[٣٢] andLaini et al.,[٢١] observed that sCD٣٦ was strongly related to liver enzymes and degree of fatty liver in diabetic patients. They explained that by; patients of diabetic fatty liver have dyslipidemia and insulin resistance leading to lipid deposition in hepatocyte. The lipid deposition causes induction of mitochondrial swelling, increased lysosomal fragility and impaired membrane integrity resulting in the release of hepatic enzymes from injured hepatocyte.

Conculusion

It is concluded that plasma sCD٣٦ is a significant predictor for early degree of fatty liver in patients with type ٢ DM, strong correlations between fasting insulin and HOMA/IR with sCD٣٦, PON-١ and nitric oxide. This may help in estimating the degree of insulin resistance in type ٢ diabetes. Also, the present study revealed many potential risk factors for development of diabetic fatty liver including: duration of DM, obesity and dyslipidemia.

It is recommend for further studies on a larger scale of patients to provide better understanding of the role of sCD٣٦, PON-١ and nitric oxide in early detection of fatty liver in type ٢ diabetes.

The method used for nitric oxide assessment needs to be standardized across different countries in order to obtain real idea about nitric oxide levels in diabetes. Estimation of Apo-A١ together with HDL is recommended to obtain better idea about PON-١ in diabetes. Finally more studies needed at molecular level for estimation of CD٣٦ and PON-١ genes expression.

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