The relationship between serum dipeptidyl peptidase-4 enzyme and nonalcoholic fatty liver disease in diabetic and nondiabetic patients

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Background
Dipeptidyl peptidase-4 (DPP4) is a membrane-associated peptidase. It has widespread organ distribution throughout the body and exerts pleiotropic effects. The liver expresses DPP4 to a high degree.
Nonalcoholic fatty liver disease (NAFLD) is more prevalent in patients with type 2 diabetes mellitus (T2DM), and is associated with increased mortality rates. Currently, there is no approved pharmacologic agent for the management of NAFLD. We need to discover more agents in the pathogenesis of NAFLD to attack it. Therefore, the aim of this work is to study the relationship between serum DPP4 enzyme and NAFLD in diabetic and nondiabetic patients.
Patients and methods
This study was conducted on 160 patients divided equally into four groups: the control group included healthy participants; the T2DM group included type 2 diabetic patients without NAFLD; the NAFLD group included nondiabetic NAFLD patients; and the T2DM-NAFLD group included T2DM patients with NAFLD. Laboratory investigation included glycosylated hemoglobin, liver enzymes, lipid profile, and serum DPP4 enzyme.
Results
DPP4 was significantly higher in the T2DM-NAFLD group compared with the other three groups, and in the NAFLD group and T2DM group compared with the control group. There was a significant direct correlation between serum DPP4 and BMI, glycosylated hemoglobin, serum cholesterol, triglycerides, and low-density lipoprotein (LDL). There was a significant inverse correlation between serum DPP4 and high-density lipoprotein (HDL).
Conclusion
DPP4 is significantly higher in diabetic patients compared with nondiabetic patients and in NAFLD patients compared with non-NAFLD patients. DPP4 can be proposed as a novel candidate in NAFLD pathogenesis.

Keywords:
diabetes mellitus, dipeptidyl peptidase-4 enzyme, nonalcoholic fatty liver disease

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological disease encompassing a spectrum of diseases ranging from simple hepatic steatosis to inflammatory nonalcoholic steatohepatitis (NASH) with increasing levels of fibrosis and eventually hepatic cirrhosis. The increase in the incidence of type 2 diabetes mellitus (T2DM) and obesity leads to an increase in the incidence of NAFLD [1]. The pathogenesis of NAFLD has been associated with insulin resistance, diabetes, oxidative stress, and lipotoxicity [2]. Until now, there is no approved treatment for the management of NAFLD [3–6]. Therefore, we need to determine more agents in the pathogenesis of this disease to attack it.

Dipeptidyl peptidase-4 (DPP4) enzyme is a ubiquitous glycoprotein and occurs as a cell-membrane-bound protein found on the surface of epithelial and acinar cells and in endothelial cells, fibroblasts, and lymphocytes [7,8]. It is also present in a soluble form known as cell surface antigen CD26 (CD26/DPP4). This soluble form is found in many biological fluids [9]. DPP4 is widely expressed in numerous tissues including endothelial cells in multiple vascular beds, rendering the enzyme highly accessible to peptide substrates circulating through the gut, liver, lung, and kidney. The liver expresses DPP4 in many places as it presents in bile canaliculi, hepatocytes and hepatic stellate cells. This widespread distribution indicates that DPP4 has pleiotropic biological actions [10]. Therefore, the aim of

this work is to study the relationship between serum DPP4 enzyme and NAFLD in diabetic and nondiabetic patients.

Patients and methods

This study was conducted on 160 patients divided into four groups: the control group included 40 healthy individuals; the T2DM group included 40 type 2 diabetic patients without NAFLD; the NAFLD group included 40 nondiabetic NAFLD patients; and last, the T2DM-NAFLD group included 40 T2DM patients with NAFLD. These patients were collected from the outpatient clinic of the Internal Medicine Department. Members of the study groups were subjected to thorough history taking and clinical examination. Laboratory investigation was performed for all patients and included glycosylated hemoglobin (HbA1c), serum aspartate aminotransferase, alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), and lipid profile. Serum DPP4 enzyme level was measured by a quantitative sandwich enzyme immunoassay technique using a commercially available kit (Quantikin Human Clusterin Immunoassay; R&D Systems Inc., Minneapolis, Minnesota, USA) according to the manufacturer's instructions.

NAFLD was diagnosed by ultrasound. All the other possible etiologies of hepatitis were excluded. None of these patients had alcohol-related disease in the history and none of them had alcohol intake.

The study was conducted from March 2016 to December 2016. The protocol of this study was approved by ethical committee of Faculty of Medicine. Written consent was taken from all studied individuals before enrolling in the study.

Exclusion criteria were as follows: viral hepatitis B or C, storage diseases including Wilson's disease, hemochromatosis, and α-1 antitrypsin deficiency, autoimmune hepatitis, drug-induced liver disease, type 1 DM, history of malignancies, chronic or acute diseases of the liver, heart, or kidney. Patients treated with metformin, thiazolidinediones, incretin-based therapy, and statin drugs were excluded.

Statistical methodology

Data were analyzed using Statistical Package for Social Science SPSS software computer program, version 15 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented in mean and SD. Qualitative data were presented in frequency and percentage. To compare between groups, we used χ^2-test, analysis of variance, and least significant difference. Correlation between two parameters was done using correlation coefficient. Significance level value was P less than or equal to 0.05.

Results

There were no significant differences between the four groups as regards age and sex. BMI was significantly higher in the three patient groups compared with the control group.

Liver enzymes were significantly higher in both NAFLD groups compared with T2DM and control groups. Serum cholesterol, LDL, and triglycerides were significantly higher in the three patient groups compared with the control group and HDL was significantly lower in the three patient groups compared with the control groups. HbA1c was significantly higher in both diabetic groups compared with the NAFLD group and the control group (Table 1).

DPPIV was significantly higher in the T2DM-NAFLD group compared with the other three groups and in the NAFLD group and T2DM group compared with control group, whereas there was no significant difference between T2DM and NAFLD groups (Table 1).

There was a significant direct correlation between serum DPP4 enzyme and BMI, HbA1c, liver enzymes, serum cholesterol, triglycerides, and LDL. There was a significant inverse correlation between serum DPP4 enzyme and HDL. There was no significant correlation between serum DPP4 enzyme and age (Table 2).

To adjust the confounding factors, the multivariate logistic regression analysis was done, and the results showed that the following factors had a significant association with NAFLD: serum DPP4 enzyme, HbA1c, BMI, serum total cholesterol, and HDL (Table 3).

Discussion

In the current study, serum DPP4 levels in T2DM patients and T2DM-NAFLD were significantly higher than in normal control and NAFLD group, respectively. In addition, there was a significant direct correlation between serum DPP4 and HbA1c. These results are in agreement with findings by other studies [11,12]. In a study using HepG2 cells, DPP4 expression was enhanced by high glucose, but not by high insulin. Therefore, it is

Table 1 Comparison between the four groups as regards different parameters (*n*=40)

	Control group (mean±SD)	T2DM group (mean±SD)	NAFLD group (mean±SD)	T2DM-NAFLD group (mean±SD)	ANOVA test	*P* value	LSD (Significance)
Age (years)	48.0±8.9	51.3±8.7	49.8±9.1	54.1±9.4	1.165	>0.05	–
BMI (kg/m^2)	24.1±2.5	27.9±3.6	28.3±4.4	28.1±5.1	5.108	<0.05	Control vs. the other three groups
ALT (U/l)	25.81±5.3	24.6±4.9	52.4±12.1	56.1±11.6	5.718	<0.05	Both NAFLD groups vs. control and T2DM groups
AST (U/l)	26.3±6.7	26.2±5.8	35.2±7.6	38.2±7.4	6.178	<0.05	Both NAFLD groups vs. control and T2DM groups
GGT (U/l)	23.1±2.3	28.5±3.4	88.1±7.6	95.4±10.1	6.82	<0.05	Both NAFLD groups vs. control and T2DM groups
HbA1c (%)	4.6 ±0.9	7.4±1.2	4.8±0.7	7.5±1.1	15.7	<0.05	Both T2DM vs. control and NAFLD groups
Serum DPP4 (ng/ml)	1675.3±244.8	2908.8±436.7	2825.1±399.2	3431.8 ±492.3	48.2	<0.05	T2DM-NAFLD group vs. the other three groups T2DM and NAFLD groups vs. control group
Total cholesterol (mg/dl)	168.5±29.7	192.3±37.4	202.0±28.7	205.6±31.1	9.182	<0.05	The three patient groups vs. the control group
Serum TG (mg/dl)	135.1±23.7	157.6±31.5	148.1±31.2	171.1±36.4	12.21	<0.05	The three patient groups vs. the control group
Serum LDL (mg/dl)	99.2±24.8	111.7±32.2	107.7±19.1	121.2±31.4	9.56	<0.05	The three patient groups vs. the control group
Serum HDL (mg/dl)	46.4±7.9	42.0±10.4	40.7±8.7	39.2±12.6	8.19	<0.05	The three patient groups vs. the control group
Sex [*n* (%)]							
Males	7 (17.5)	9 (22.5)	8 (20)	9 (22.5)	χ^2=0.132	>0.05	–
Females	33 (82.5)	31 (77.5)	32 (80)	31 (77.5)			–

ALT, alanine aminotransferase; AST, aspartate aminotransferase; DPP4, dipeptidyl peptidase-4; GGT, γ-glutamyl transferase; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalchoholic fatty liver disease; T2DM, type 2 diabetes mellitus; TG, triglycerides.

Table 2 Correlation between serum dipeptidyl peptidase-4 and other parameters in the diabetic patients

	Serum DPP4	
	r	*P*
Age	0.218	>0.05
BMI	0.784	<0.05
HbA1c	0.709	<0.05
Total cholesterol	0.655	<0.05
Triglyceride	0.648	<0.05
LDL	0.642	<0.05
HDL	–0.601	<0.05
ALT	0.722	<0.05
AST	0.745	<0.05
GGT	0.812	<0.05

ALT, alanine aminotransferase; AST, aspartate aminotransferase; DPP4, dipeptidyl peptidase-4; GGT, γ-glutamyl transferase; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3 Multivariate logistic regression analysis of independent variables associated with nonalchoholic fatty liver disease

Factors	Odds ratio (95% CI)	*P* value
Serum DPP4 enzyme	2.52 (1.57–4.05)	<0.001
BMI	1.79 (1.19–2.69)	<0.001
HbA1c	3.05 (1.46–6.38)	<0.001
Total cholesterol	1.04 (1.02–1.06)	<0.001
HDL	1.63 (1.08–2.47)	<0.001

CI, confidence interval; DPP4, dipeptidyl peptidase-4; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein.

speculated that hyperglycemia may induce the hepatic DPP4 expression, but insulin may not affect the transcription of DPP4 [13]. The in-vitro assays reported that glucose decreases DNA methylation of *DPP4* gene. Hypomethylation of this gene enhances glucose-mediated DNA expression and thus increases the release of DPP4 by the liver, which leads to an increase in soluble DPP4. This might lead to metabolic deteriorations including steatosis [14].

These results are in contrast to findings by other studies that reported decreased DPP4 levels in diabetic patients. Researchers speculated that these discrepancies in diabetic patients may be because of factors such as diabetes duration, patient age, and blood glucose control. Exposure of the body to high concentration of glucose for long duration promotes biosynthesis of DPP4 as described before [15,16].

In this study, there was a significant direct correlation between serum DPP4 enzymes, BMI, total cholesterol, triglycerides, and LDL, and a significant inverse correlation between serum DPP4 enzyme and HDL. Similarly, other studies have reported that circulating DPP4 correlated with BMI and waist circumference [15,17]. It was demonstrated that enlargement of adipocytes enhances the release of DPP4 from fat cells into the circulation [17]. Kanazawa *et al.* [18] provides evidence that CD26/DPP4 may be useful as a biomarker for increased risk of obesity.

It is suggested that the weight gain early in life is closely linked to changes in hepatic DPP4 expression, which are in turn associated with variation in DPP4 methylation around exon 2. Methylation in exon 2 of human DPP4 has been previously shown to correlate negatively with its expression in visceral adipose tissue of obese women [19]. DPP4 methylation in adipose tissue correlated positively with HDL cholesterol levels in the blood, indicating a role of adipose tissue DPP4 in lipidemia [20].

DPP4 affects lipid metabolism by the inactivation of peptides such as glucagon-like peptide 1 (GLP-1) and neuropeptide Y. DPP4 also affects lipid metabolism directly. Depression of the gene encoding DPP4 directly increases the activity of the peroxisome proliferator-activated receptor-α pathway, which leads to increased lipid oxidation and reduced lipogenesis. These result in prevention of hepatic steatosis [21].

The results of this study showed a good relation between NAFLD and serum DPP4 level. Serum DPP4 level was significantly higher in patients in the T2DM-NAFLD group compared with those in the T2DM group, and in patients in the NAFLD groups compared with those in the control group. It was presumed that in patients with NAFLD the serum DPP4 activity would be increased and, by the dysfunctional enetro-insular axis, it might contribute to the impairment of glucose tolerance and the speedup of metabolic deterioration observed in NAFLD [22].

Another study showed that hepatic DPP4 mRNA expression level in the livers is significantly increased in NAFLD patients compared with healthy individuals. It also showed that serum DPP4 activity and hepatic expression of DPP4 are correlated with hepatic steatosis and grades of NAFLD [23]. Moreover, DPP4-deficient rats show lower levels of hepatic proinflammatory and profibrotic cytokines and reduced hepatic steatosis compared with wild-type rats. These favorable changes in lipid metabolism are independent of glucose metabolism [24].

In the current study, a positive correlation was found among GGT, ALT, and serum DPP4 levels, which supports the finding that an important part of excess serum DPP4 is of hepatic origin. When we analyzed serum DPP4 levels in both NAFLD groups separately (diabetic and nondiabetic) and compared them with the serum DPP4 level of T2DM group and control group, respectively, we concluded that it is the presence of steatosis that has an important impact on the serum DPP4 level and not the hyperglycemia alone. Similarly in other studies, DPP4 activity in serum and liver specimens of NAFLD patients correlates with serum GGT and ALT levels [23,24]. It was found that serum DPP4 level was significantly higher in the NAFLD group than in the control individuals and was correlated with the histopathological grade of liver disease [25]. Furthermore, the intensity of hepatic DPP4 immunostaining was correlated with the level of liver steatosis [23].

Several clinical studies have shown that GLP-1 receptor agonists reduced fat deposition in the liver and improved liver function independently of body weight reduction in type 2 diabetic patients [26,27]. Another study showed that administration of GLP-1 analog directly reduced triglyceride stores compared with control-treated cells in the absence of insulin [28]. These findings suggest that activation of GLP-1 signaling in the liver has beneficial effects on NAFLD and that DPP4 inhibitors may also affect liver function. However, the impact of improving liver function by DPP4 inhibitors still needs further studies.

From the previous, we hypothesize that hepatic DPP4 is involved in the initiation and progression of NAFLD in the following ways: obesity and/or hyperglycemia induce adipose tissue and hepatic DPP4 expressions. An increase in DPP4 levels leads to initiation or progression of steatosis by direct effect and to an increase in degradation of GLP-1, which also causes more hyperglycemia that leads to further enhancement

of DPP4 expression with further progression of NAFLD.

Therefore, we can conclude that DPP4 is significantly higher in diabetic patients compared with nondiabetic patients and in NAFLD patients compared with non-NAFLD patients, and there is a further increase in its level if both T2DM and NAFLD are present in the same patient. There was a significant direct correlation between DPP4 and BMI, total cholesterol, TG, LDL, and liver enzymes and an inverse correlation with HDL. DPPIV can be proposed as a novel candidate with several potential functions in NAFLD pathogenesis. Further studies are required to examine the physiological role of DPP4 in the NAFLD liver and to determine whether DPP4 may offer a new treatment target to suppress the progression of NAFLD even in nondiabetic patients.

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Conflicts of interest

There are no conflicts of interest.

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