

Role of Serum Dipeptidyle Peptidase IV (DDP IV) Activity in Development of Non – Alcoholic Steatohepatitis in Patients with Non-Alcoholic Fatty Liver Disease

Khaled Abd Elhamed Mohammed¹, Inas El Khedr Mohammed¹, Hany Haroun Kaiser¹,
Khaled Rafik Elbaz¹, Hesham Drwesh^{2*}

¹Department of Internal Medicine, Ain Shams University, Cairo Governorate, Egypt.

²Critical Care Department, Theodor Bilharz Research Institute (TBRI), Giza Governorate, Egypt.

ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is defined as the abnormal accumulation of lipids, primarily in the form of triglycerides in individuals who do not consume significant amounts of alcohol (≤ 20 g ethanol/d). It is characterized by a spectrum of disease varying from simple steatosis through to steatohepatitis with fibrosis and scarring, which can lead to cirrhosis. Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26) is a protein that, in humans, is encoded by the DPP4 gene.

Aim of the work: To study the role of serum dipeptidyle peptidase IV activity in development and progression of simple steatosis to non-alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease and its role in follow up the progression to chronic liver disease.

Methods: This study was conducted as a case-control study in Internal Medicine Department of Ain Shams University Hospital and included 30 patients with non-alcoholic fatty liver (group I) and 30 patients with non-alcoholic steatohepatitis (group II) , 30 healthy individuals were taken as a control group (III). All cases are subjected to full history clinical examination full Lab., abdominal sonar and assessment of (DDP IV, CD26).

Results: It is suggested that circulating DPP4 may play a role in the progression of non – alcoholic fatty liver disease (NAFLD) to non – alcoholic steatohepatitis because of its ability to differentiate simple steatosis from steatohepatitis.

Conclusion: When NAFLD is induced by nutritional overload, hepatic inflammation enhances hepatic DPP4 expression. Accelerated degradation of GLP-1 by DPP4 inhibits insulin secretion and causes hyperglycemia. Hyperglycemia further enhances DPP4 expression, with further worsening in glucose metabolism. The increased hepatic expression of DPP4 in NAFLD patients suggests that DPP4 may be involved in the onset and/or progression of NAFLD. Hepatic inflammation may induce this phenomenon, although DPP4 causes deteriorations in systemic glucose metabolism.

Keywords: Non-Alcoholic Fatty Liver, Steatohepatitis, (DDP IV, CD26).

*Correspondence to:

Dr Hesham Drwesh,
Critical Care Department,
Theodor Bilharz Research Institute, Giza, Egypt.

Article History:

Received: 11-08-2018, **Revised:** 09-09-2018, **Accepted:** 27-09-2018

Access this article online

Website: www.ijmrp.com	Quick Response code 
DOI: 10.21276/ijmrp.2018.4.5.056	

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as the abnormal accumulation of lipids, primarily in the form of triglycerides in individuals who do not consume significant amounts of alcohol (≤ 20 g ethanol/d). It is characterized by a spectrum of disease varying from simple steatosis through to steatohepatitis with fibrosis and scarring, which can lead to cirrhosis.¹

Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26) is a protein that, in humans, is encoded by the DPP4 gene.² Although various factors are responsible for the development of

NAFLD, a high glucose load is known to induce DPP-4 expression in HepG2 cells and hepatic DPP-4 mRNA expression level in the livers is significantly higher in patients with NAFLD, compared to healthy subjects.³

Also experienced a case of refractory NAFLD that was successfully treated with sitagliptin, a DPP-4 inhibitor. Moreover, it is reported that sitagliptin ameliorates liver enzymes and hepatocyte ballooning in patients with nonalcoholic steatohepatitis.

So, DPP-4 inhibitors ameliorate hepatic injury and glucose impairment in patients with NAFLD.⁴

AIM OF THE WORK

The aim of this work is to study the role of serum dipeptidyle peptidase IV activity in development and progression of simple steatosis to non-alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease and its role in follow up the progression to chronic liver disease.

PATIENTS AND METHODS

This study was conducted as a case-control study in Internal Medicine Department of Ain Shams University Hospital and included 30 patients with non-alcoholic fatty liver (group I) and 30 patients with non-alcoholic steatohepatitis (group II), 30 healthy individuals were taken as a control group (III)

Exclusion Criteria

1. Diabetes mellitus.
2. Alcoholic patient.
3. Viral hepatitis B and C.
4. Drug-induced liver disease e.g. amiodarone and methotrexate.
5. Metabolic liver diseases including Wilson disease and hemochromatosis.
6. Hepatocellular carcinoma.

Study Design

All subjects participating in the study were asked to sign a consent before inclusion. Then, they were subjected to:

1. Medical history and clinical examination.
2. Calculation of body mass index [body weight in kg divided height square in meters (kg/m²)].
3. Routine laboratory hematology and chemistry (complete blood picture, prothrombin time and INR, total and direct serum bilirubin, AST, ALT, serum albumin, serum urea and serum creatinine).
4. Lipid profile include:
 - A. Triglyceride.
 - B. HDL cholesterol.
 - C. LDL cholesterol.
 - D. Total cholesterol.
5. Serum dipeptidylpeptidase-4 level.
6. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR).
7. Imaging including abdominal ultrasound for diagnosis of non-alcoholic fatty liver and exclusion of other etiology.
8. Fibroscan to quantify liver fibrosis.
9. Non-alcoholic fatty liver disease fibrosis score.

Assay of Human Dipeptidyl Peptidase 4

The kit uses a double-antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to assay the level of human Dipeptidyl Peptidase4 (DPP4) in samples. Add Dipeptidyl Peptidase4 (DPP4) to monoclonal antibody enzyme well which is pre-coated with human DPP4 monoclonal antibody, incubation; then, add DPP4 antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then, carry out incubation and washing again to remove the uncombined enzyme. Then, add chromogen solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human substance DPP4 of sample were positively correlated.^{1,2,10}

Storage Conditions

1. The kit shall be stored at 2-8°C, and the coated microwell plate shall be stored at a dry place.
2. The reagents shall be kept stable in the period of validity; and substrate shall be colorless. The substrate shall be changed in time in case of development or blueing.

Washing Method

Manually Washing Method: Shake away the remain liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35 ml after-dilution washing solution into the well, and marinate 1-2 minutes. Repeat this proc washing method.

Manually Washing Method: Shake away the remain liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35 ml after-dilution washing solution into the well, and marinate 1-2 minutes. Repeat this process according to requirements.

Automatic Washing Method: If there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance.

ASSAY PROCEDURE

1. Standard Dilution

2. The quantity of the plates depends on the quantities of to-be-tested samples and the standards. It is suggested to duplicate each standard and blank well. Every sample shall be made according to required quantity, and try to use the duplicated well as possible.

3. Inject Samples:

- 1) **Blank Well:** Do not add samples and DPP4-antibody labeled with biotin, streptavidin-HRP, only chromogen solution A and B, and stop solution are allowed; other operations are the same.
- 2) **Standard Wells:** Add standard 50 µl, streptavidin-HRP 50 µl (since the standard already has combined biotin antibody, it is not necessary to add the antibody).
- 3) **To Be Test Wells:** Add sample 40 µl, and then add both DPP4-antibody 10 µl and streptavidin-HRP 50 µl. Then, seal the sealing membrane, and gently shaking, incubated 60 minutes at 37°C.
4. **Confection:** Dilute 30 times the 30° washing concentrate with distilled water as standby.
5. **Washing:** Remove the membrane carefully, and drain the liquid, shake away the remaining water.
6. Add chromogen solution A 50 µl, then chromogen solution B 50 µl to each well. Gently mixed, incubate for 10 minutes at 37°C away from light.
7. **Stop:** Add stop solution 50 µl into each well to stop the reaction (the blue changes into yellow immediately).
8. **Final Measurement:** Take blank well as zero, measure the Optical Density (OD) under 450 nm wavelength which should be carried out within 10 minutes after adding the stop solution.
9. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use kinds of software to make calculations.

Standard Dilution		
480 pg/ml	Standard No.5	120 µl originalstandard + 120 µl standard diluents
240 pg/ml	Standard No.4	120 µl standard No.5 + 120 µl standard diluents
120 pg/ml	Standard No.3	120 µl standard No.4 + 120 µl standard diluent
60 pg/ml	Standard No.2	120 µl standard No.3 + 120 µl standard diluent
30 pg/ml	Standard No.1	120 µl standard No.2 + 120 µl standard diluent

Homeostasis Model Assessment of Insulin Resistance

The HOMA model was originally designed as a special case of a more general model called HOMA-CIGMA. The approximating equation for insulin resistance, in the early model, used a fasting plasma sample, and was derived by used of the insulin-glucose product, divided by a constant: (assuming normal- weight, normal subjects < 40 years, having 100% β-cell function an insulin resistance of 1)

$$\frac{\text{Glucose} \times \text{Insulin}}{405}$$

HOMA-IR =

Glucose in mass units mg/dL. IR is insulin resistance. Insulin is given in mU/L. Glucose and insulin are both during fasting.

A sample of 5ml of venous blood sample was drawn from the ante cubital vein for measurement of fasting blood glucose, fasting blood insulin and detection of insulin resistance (by HOMA-IR). Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as insulin (pmol/L) x glucose (umol/L) / 22.5. Lower index indicates greater insulin sensitivity (Bruno et al., 2009).

Non-Alcoholic Fatty Liver Disease Fibrosis Score (NFS)

NFS was calculated as per the following formula: -1.675+0.037 X age (years) + 0.094 X body mass index (BMI, kg/m2) + 1.13 X impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 X AST/ALT ratio - 0.013 X platelet (X109 /L) - 0.66 X Albumin (g/dL) (Pathik et al., 2015).

Statistical Analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as

mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- A one-way analysis of variance (ANOVA) when comparing between more than two means.
- Post Hoc test: Least Significant Difference (LSD) was used for multiple comparisons between different variables.
- Chi-square (X²) test of significance was used in order to compare proportions between two qualitative parameters.
- Pearson's correlation coefficient (r) test was used for correlating data.
- Receiver operating characteristic (ROC curve) analysis was used to find out the overall predictivity of parameter in and to find out the best cut-off value with detection of sensitivity and specificity at this cut-off value.
- Probability (P-value)
 - P-value ≤0.05 was considered significant.
 - P-value ≤0.001 was considered as highly significant.
 - P-value >0.05 was considered insignificant.

RESULTS

Table 3 shows highly statistically significant difference between groups according laboratory data as in AST, ALT,, PT, INR, direct bilirubin triglycerides, total cholesterol and fasting insulin, where they have higher value in group II in comparison to group I and III, but there are no statistically significant in serum urea, serum creatinine and CBC

Table 1: Comparison between groups according demographic data.

Demographic Data	Group I	Group II	Group III	x2/F*	p-value
Gender					
Male	17 (56.7%)	14 (46.7%)	20 (66.7%)	2.443	0.295
Female	13 (43.3%)	16 (53.3%)	10 (33.3%)		
Age (years)					
Mean±SD	44.80±9.08	52.23±8.23	48.63±12.01	4.222	0.058
Range	22-62	35-65	29-69		

Table 2: Comparison between groups according anthropometric measurements.

Anthropometric measurements	Group I	Group II	Group III	F	p-value
Body wt (kg)					
Mean±SD	100.17±13.53	103.93±6.87	97.83±8.64	1.483	0.096
Range	80-145	88-112	65-111		
Ht (m)					
Mean±SD	1.76±0.05	1.71±0.14	1.76±0.05	2.812	0.066
Range	1.67-1.85	1.23-1.85	1.67-1.85		
BMI (wt/(ht)²)					
Mean±SD	32.18±3.81	34.01±3.47	31.30±3.73	1.921	0.176
Range	24.69-43.3	26.56-38.75	19.28-36.06		

Table 3: Comparison between groups according laboratory data and complete blood count.

Laboratory data		Group I	Group II	Group III	F	p-value
AST	Mean±SD	31.83±5.75	49.87±8.24	20.37±7.19	130.456	<0.001
	Range	22-42	36-64	11-42		
ALT	Mean±SD	37.00±4.46	58.63±10.41	22.10±4.58	203.514	<0.001
	Range	24-45	35-69	14-32		
S Alb	Mean±SD	4.41±0.35	3.78±0.62	4.61±0.29	28.345	<0.001
	Range	4-4.9	3-4.9	4-4.9		
Pt	Mean±SD	13.07±0.71	13.76±1.31	13.10±0.74	4.847	<0.001
	Range	11.8-14	11.8-17.2	11.8-14.3		
INR	Mean±SD	1.02±0.02	1.14±0.18	1.02±0.02	13.031	<0.001
	Range	1-1.08	1-1.6	1-1.06		
Serum Urea	Mean±SD	37.37±8.41	41.33±9.32	28.13±7.96	18.675	<0.001
	Range	28-54	28-58	13-49		
S. Creatinine	Mean±SD	1.11±0.21	1.14±0.26	0.79±0.22	20.156	<0.001
	Range	0.7-1.5	0.6-1.6	0.5-1.4		
Triglyceride	Mean±SD	232.00±113.58	259.27±104.69	134.17±32.84	15.617	<0.001
	Range	100-490	105-480	85-195		
Total cholesterol	Mean±SD	222.40±48.80	240.93±49.76	167.83±30.41	22.479	<0.001
	Range	150-330	130-339	120-239		
Fasting blood glucose	Mean±SD	99.93±22.79	106.73±11.52	93.00±12.08	5.317	0.007
	Range	79-115	79-124	75-114		
F.insulin	Mean±SD	12.13±2.74	17.76±4.04	8.75±0.81	75.894	<0.001
	Range	8.5-17	11-24.4	7.1-10		
TLC	Mean±SD	7.91±1.94	6.98±2.02	8.05±1.63	2.894	0.061
	Range	4.5-11	3-11	5.19-11		
Hb	Mean±SD	14.36±0.73	13.83±1.34	14.36±0.68	2.989	0.056
	Range	13-15.6	10-15.6	13.3-15.6		
Plt	Mean±SD	264.93±135.15	300.53±573.93	283.10±50.44	0.081	0.922
	Range	160-880	125-3320	207-360		

Table 4: Comparison between groups according bilirubin.

Bilirubin		Group I	Group II	Group III	F	p-value
Total	Mean±SD	1.05±0.24	1.10±0.36	0.88±0.33	4.032	0.021
	Range	0.3-1.3	0.3-1.9	0.3-1.3		
Direct	Mean±SD	0.18±0.10	0.30±0.31	0.13±0.09	6.109	0.003
	Range	0.04-0.5	0.04-1.2	0.04-0.3		

Table 5: Comparison between groups according HOMA IR.

HOMA IR	Group I	Group II	Group III	F	p-value
Mean±SD	3.18±0.89	4.81±1.46	2.00±0.28	59.913	<0.001
Range	1.84-4.79	2.53-7.35	1.4-2.49		

Table 6: Comparison between groups according NAFLD fibrosis score.

NAFLD fibrosis score	Group I	Group II	Group III	F	p-value
Mean±SD	-2.18±1.44	-0.75±1.49	-3.31±1.00	28.09	<0.001
Range	-4.479-0.706	-3.341-1.066	-5.313--1.578		

Table 7: Comparison between groups according serum DPP-IV level.

S.DPP-IV level	Group I	Group II	Group III	I vs. II	I vs. III	II vs. III
Mean±SD	2.06±0.57	3.48±0.51	0.37±0.10	<0.001	<0.001	<0.001
Range	1.194-3.29	2.483-4.353	0.129-0.58			

Table 8: Comparison between groups according pelvic abdominal U/S.

Pelvic-abdominal US	Group I	Group II	Group III	x2	p-value
Average	11 (36.7%)	3 (10%)	0 (0%)	107.703	<0.001
Coarse	0 (0%)	6 (20%)	0 (0%)		
Enlarged	17 (60%)	19 (63.3%)	0 (0%)		
Fine parenchymatous	1 (3.3%)	2 (6.7%)	0 (0%)		
Normal	0 (0%)	0 (0%)	30 (100%)		
Total	30 (100%)	30 (100%)	30 (100%)		

Table 9: Comparison between groups according fibro-scan.

Fibro-scan	Group I	Group II	Group III	x2	p-value
F0	12 (40%)	0 (0%)	30 (100%)	69.946	<0.001
F1	9 (30%)	7 (23.3%)	0 (0%)		
F2	7 (23.3%)	11 (36.7%)	0 (0%)		
F3	2 (6.7%)	7 (23.3%)	0 (0%)		
F4	0 (0%)	5 (16.7%)	0 (0%)		
Total	30 (100%)	30 (100%)	30 (100%)		

Table 10: Correlation between serum DPP-IV and other parameters, using Pearson correlation Coefficient in group I, II.

Parameters	S.DPP-IV level	
	r	p-value
Age (years)	-0.238	0.205
Body wt (kg)	-0.488	0.006
Ht (m)	0.082	0.666
BMI (wt/(ht) ²)	-0.597	<0.001
TLC	0.209	0.268
Hb	0.131	0.490
Pit	0.344	0.063
Total	0.036	0.848
Direct	0.488	0.011
Ast	-0.124	0.514
Alt	-0.108	0.572
S.alb.	0.393	0.032
Pt	-0.196	0.300
INR	0.163	0.391
Serum urea	0.047	0.804
Serum creat	0.292	0.117
Triglyceride	-0.400	0.028
Total cholesterol	0.450	0.013
Fasting blood glucose	-0.452	0.012
F.insulin	-0.882	<0.001
HOMA IR	-0.913	<0.001
NAFLD fibrosis score	0.492	0.006

Table 11: Correlation between serum HOMA IR and other parameters, using Pearson correlation Coefficient in group I, II.

Parameters	HOMA IR	
	r	p-value
Age (years)	.314*	0.015
Body wt (kg)	.444**	<0.001
Ht (m)	-.368**	0.004
BMI (wt/(ht) ²)	.592**	<0.001
TLC	-0.190	0.145
Hb	-.270*	0.037
Pit	-0.009	0.946
Total	0.130	0.322
Direct	0.221	0.090
Ast	.605**	<0.001
Alt	.508**	<0.001
S.alb.	-.358**	0.005
Pt	0.178	0.175
INR	.340**	0.008
Serum urea	0.170	0.194
Serum creat	-0.021	0.871
Triglyceride	.591**	<0.001
Total cholesterol	0.084	0.521
Fasting blood glucose	.572**	<0.001
F.insulin	.968**	<0.001
NAFLD fibrosis score	.427**	<0.001

Table 12: Correlation between serum NAFLD fibrosis score and other parameters, using Pearson correlation Coefficient in group I, II.

	NAFLD fibrosis score	
	r	p-value
Age (years)	0.591	<0.001
Body wt (kg)	0.533	<0.001
Ht (m)	-0.085	0.521
BMI (wt/(ht)2)	0.629	<0.001
TLC	-.278*	0.032
Hb	-0.394	0.002
Plt	-.309*	0.016
Total	.257*	0.048
Direct	0.467	<0.001
Ast	0.635	<0.001
Alt	0.541	<0.001
S.alb.	-0.859	<0.001
Pt	.309*	0.016
INR	0.526	<0.001
Serum urea	0.243	0.061
Serum creat	0.157	0.231
Triglyceride	0.236	0.069
Total cholesterol	0.184	0.159
Fasting blood glucose	0.203	0.120
F.insulin	0.426	0.002

Table 13: Diagnostic Performance of groups in Discrimination of serum DPP-IV level (ug/ml).

Groups	Cut-off.	Sen.	Spe.	PPV	NPV	Accuracy
I vs. II	≤2.9	90%	86.7%	87.9%	96.3%	97.3%
I vs. III	≥0.58	100%	100%	100%	100%	100%
II vs. III	≥0.58	100%	100%	100%	100%	100%

Receiver operating characteristics (ROC) curve was used to define the best cut off value of serum

DPP-IV level. Cut off value of DPP-IV was used to different between fatty liver with steatohepatitis and fatty liver only.

DPP-IV level > 2.9ug/ml indicates fatty liver with steatohepatitis and DPP-IV level <2.9ug/ml indicates presence of fatty liver only.

DPP-IV level >0.58 ug/ml indicates presence of fatty liver with or without steatohepatitis while DPP-IV <0.58ug/ml considered healthy person.

Table 14: Diagnostic Performance of groups in Discrimination of HOMA IR.

Groups	Cut-off.	Sen.	Spe.	PPV	NPV	Accuracy
I vs. II	>3.36	80%	70%	72.7%	77.8%	82.2%
I vs. III	>2.49	73.3%	100%	100%	78.9%	90.7%
II vs. III	>2.49	100%	100%	100%	100%	100%

Receiver operating characteristics (ROC) curve was used to define the best cut off value of HOMA IR:

Cut off value of HOMA IR was used to different between fatty liver with steatohepatitis and fatty liver only.

HOMA IR > 3.36 ug/ml indicates fatty liver with steatohepatitis while HOMA IR level <3.36ug/ml indicates presence of fatty liver only.

HOMA IR >2.49 indicates presence of fatty liver with or without steatohepatitis while HOMA IR <2.49 considered healthy person.

Table 15: Diagnostic Performance of groups in Discrimination of NAFLD fibrosis score.

Groups	Cut-off.	Sen.	Spe.	PPV	NPV	Accuracy
I vs. II	> (-1.38)	73.3%	73.3%	73.3%	73.3%	75.4%
I vs. III	> (-2.44)	60%	86.7%	81.8%	68.4%	72.9%
II vs. III	> (-1.58)	76.7%	100%	100%	81.1%	91.5%

Receiver operating characteristics (ROC) curve was used to define the best cut off value of NAFLD fibrosis score:

Receiver operating characteristics (ROC) curve was used to define the best cut off value of HOMA IR:

Cut off value of NAFLD fibrosis score was used to different between fatty liver with steatohepatitis and fatty liver only.

NAFLD fibrosis score > -1.83ug/ml indicates fatty liver with steatohepatitis while NAFLD fibrosis score <-1.83ug/ml indicates presence of fatty liver only.

NAFLD fibrosis score >-2.44 indicates presence of fatty liver only while NAFLD fibrosis score <-2.44 indicates healthy person.

NAFLD fibrosis score >-1.58 indicates presence of fatty liver with steatohepatitis while NAFLD fibrosis score < -1.85 indicates healthy person.

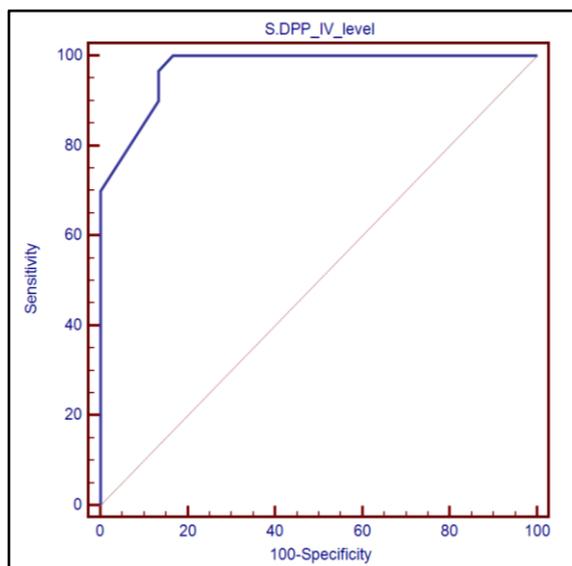


Fig. 1: Sensitivity and specificity between group I and group II according S.DPP IV level

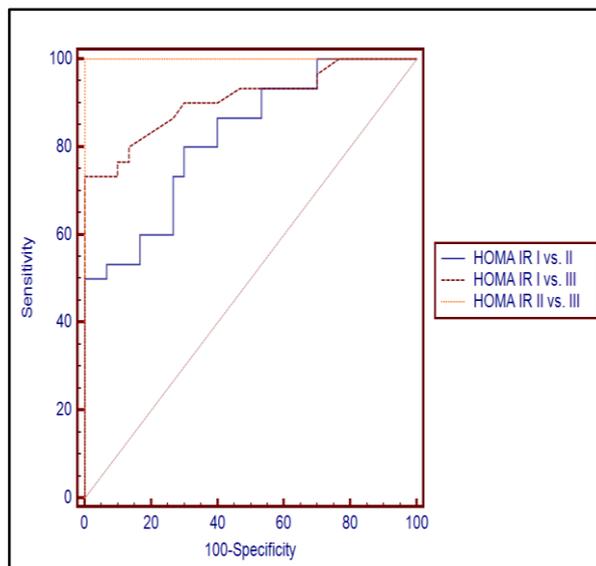


Fig. 4: Sensitivity and specificity between different groups according to HOMA IR

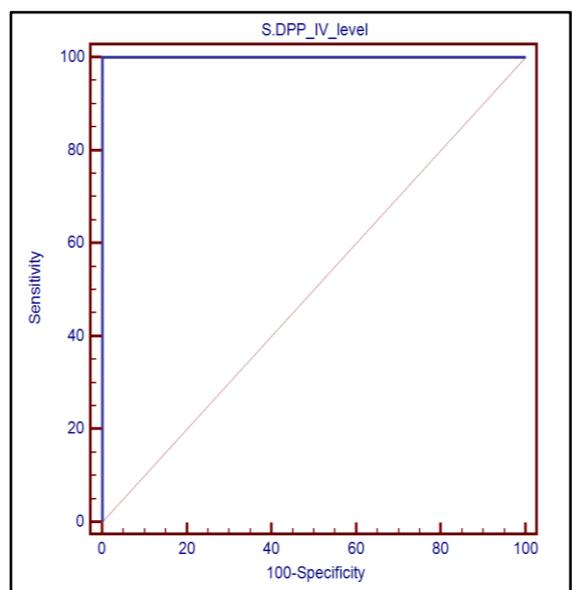


Fig. 2: Sensitivity and specificity between group I and group III according S.DPP IV level

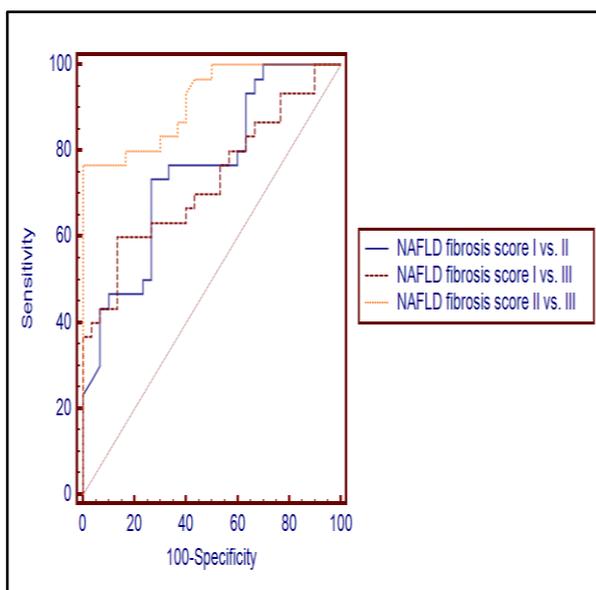


Fig. 5: Sensitivity and specificity between different groups according to NAFLD fibrosis score

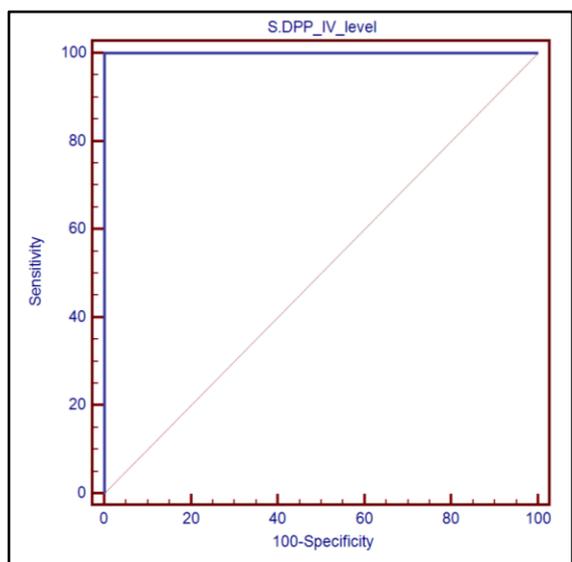


Fig. 3: Sensitivity and specificity between group II and group III according S.DPP IV level.

DISCUSSION

Samples obtained tissue from 60 NAFLD patients (31 males and 29 females) and 30 control subjects (20 males and 10 females). Our study showed no statistical significant differences between all studied groups as regard gender and age.⁵

Obtained tissue samples from 17 NAFLD patients (9 males and 8 females) and 10 control subjects (5 males and 5 females). The control group was younger than the NAFLD group.

Our study showed no statistical significant difference between all studied groups as regard complete blood count, BMI. While there were statistically significant differences between groups according AST, ALT, total cholesterol and direct bilirubin and highly statistically significant difference between groups according laboratory data, HOMA IR and NAFLD fibrosis score.

BMI, ALT and LDH were significantly higher in the NAFLD patients than in the control group. Nutritional parameters, including total cholesterol, triglyceride and fasting plasma glucose levels, were higher in the NAFLD patients than in the control group, although these differences were not statistically significant.⁵

Our study showed also highly statistically significant difference between groups according S. DPP-IV level, pelvic-abdominal U/S and fibro-scan.

Increased serum activity and/or hepatic expression of DPP4 have been reported in various hepatic diseases. Serum DPP4 activity was significantly higher in patients with chronic hepatitis C virus (HCV) infection and primary biliary cirrhosis than in healthy controls. Increased DPP4 protein was also detected in the ileum and liver in HCV-infected patients.⁶

In animal models, elevated serum DPP4 activity was observed in rat cirrhosis induced by diethyl-nitrosamine, phenobarbital and carbon tetrachloride, and was positively correlated with serum transaminase levels. So, destruction of liver cells may increase the serum activity and hepatic expression of DPP4.⁷

DPP4 also appears to be involved in liver diseases originating from hepatic steatosis. Indeed, DPP4 activity was greater in NAFLD patients than in control subjects and patients with T2DM, and DPP4 activity was correlated with HOMA2-IR.⁸

Serum DPP4 activity was significantly higher in NASH patients than in control subjects and was correlated with the histopathological grade of liver disease. Furthermore, the intensity of hepatic DPP4 immunostaining was correlated with the extent of hepatic steatosis. So, IR, which is thought to promote the progression of NAFLD and NASH, is associated with the serum activity and hepatic expression of DPP4.⁹

Ryskjaer reported that the plasma DPP4 activity was significantly elevated in patients with T2DM, and was correlated with fasting glucose and HbA1c levels. In one study, serum DPP4 activity was reduced in patients with T2DM, and DPP4 activity was negatively correlated with glucose and HbA1c levels.¹⁰ These discrepancies in diabetic patients may be due to factors such as disease duration, patient age and glycemic control.¹¹

Miyazaki stated that DPP4 expression is higher in NAFLD liver than in healthy liver. Hepatic mRNA levels of DPP4 were evaluated by RT-PCR in NAFLD patients and in the control group. DPP4 expression was 15-fold higher in the NAFLD liver than in the control liver.⁵ To determine possible associations with IR in NAFLD, DPP4 expression levels were compared among groups of patients stratified by Hemeostasis Model Assessment-Insulin Resistance (HOMA-IR) < 2.5 and ≥ 2.5 . DPP4 expression levels were significantly lower in patients with HOMA-IR ≥ 2.5 than in patients with HOMA-IR < 2.5 .

DPPIV may act by several possible mechanisms in NASH pathogenesis: First, it might be regulating the insulin resistance of liver which determines the steatosis in liver. Second, DPPIV might direct the immune response towards proinflammatory Th1 type rather than anti-inflammatory Th2 type which subsequently may initiate hepatic inflammation. Third, DPPIV might control the fibrogenesis in the liver by mediating the interaction of extracellular matrix proteins with cells of immune system and hepatocytes.

The insulin resistance is believed to be main pathology leading to NASH; therefore the treatment of NASH has been targeted to regulate insulin sensitivity by either lifestyle modification or drugs. Increatin; namely Glucagon-Like Peptide 1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP) are hormones stimulating glucose-dependent insulin secretion.¹²

Correlations revealed positive correlation and significant between S.DPP-IV and NAFLD fibrosis score direct bilirubin, total

cholesterol and negative correlation and significant between serum DPP-IV and BMI, Fasting blood glucose, F. insulin, HOMA IR.

Finreis showed a positive correlation between serum DPP4 activity and HOMA2-IR in NAFLD patients. They also showed that DPP4 activity in NAFLD patients with glucose intolerance was lower than that in NAFLD patients with normal glucose tolerance, complicating the role of IR in DPP4 activity in NAFLD.⁸

Miyazaki analyzed correlations between hepatic DPP4 expression levels and metabolic factors. Hepatic DPP4 expression levels were negatively correlated with HOMA-IR and BMI.⁵ Among biochemical parameters, DPP4 expression levels were positively correlated with total cholesterol levels, but not with triglyceride levels, or with other parameters, such as ALT, LDH, γ -glutamyl transpeptidase and platelet number.

In a study done by Balaban, serum DDPIV activity was higher in NASH patients when compared to controls and the activity was correlated with BMI. However, age or sex of patients did not have any effect on DDPIV activity.⁹ Similar to the intensity of CD26 staining in liver, serum DPPIV activity was correlated with hepatosteatosis and grade but not with stage. Serum DPPIV level is strongly correlated with serum direct bilirubin level.

Miyazaki stated that serum DPPIV activity was significantly higher in patients with NASH than in controls.⁵ Serum DPPIV activity correlated with grade and steatosis. But, there was no association with zonal DPPIV staining, stage or class. Serum DPPIV activity was not associated with clinical (sex, age, anthropometric measurements) or laboratory (liver enzymes, lipid levels, fasting glucose, OGTT, HOMA, CRP) parameters, except for body mass index.

Receiver operating characteristics (ROC) curve was used to define the best cut off value of serum DPP-IV level:

- **Group I vs. II:** ≤ 2.9 DPP-IV, sensitivity 90%, specificity 86.7%, PPV 87.9%, NPV 96.3% and accuracy 97.3%.
- **Group I vs. III:** ≥ 0.58 DPP-IV, sensitivity 100, specificity 100%, PPV 100%, NPV 100% and accuracy 100%.
- **Group II vs. III:** ≥ 0.58 DPP-IV, sensitivity 100, specificity 100%, PPV 100%, NPV 100% and accuracy 100%.

Balaban investigated the changes related to DPPIV in NASH patients.⁹ They concluded that NASH is a disease affecting significant proportion of the populations and has an unknown pathogenesis. The diagnosis of NASH is based on clinical exclusion of other liver diseases and demonstrating the characteristic histopathological findings. If the alterations related to DPPIV are a consequence of liver injury specific to NASH, the serum DPPIV activity could be used to differentiate simple steatosis from steatohepatitis and DDPIV inhibitors could be a novel candidate in NASH treatment.

Miyazaki compared the mRNA expression levels of DPP4 in liver biopsy samples from NAFLD patients to those of control livers. In NAFLD patients, we also examined correlations between DPP4 expression levels and metabolic factors, including homeostasis model assessment-insulin resistance (HOMA-IR), body mass index (BMI), and serum cholesterol and triglyceride levels.⁵ To examine the potential effects of nutritional factors, DPP4 expression levels were analyzed in HepG2 cells subjected to various culture conditions. Hepatic DPP4 mRNA expression was significantly greater in NAFLD patients than in control subjects. DPP4 expression levels were negatively correlated with HOMA-IR

and positively correlated with serum cholesterol levels. In HepG2 cells, high glucose significantly enhanced DPP4 expression, whereas insulin, fatty acids and cholesterol did not. Increased hepatic expression of DPP4 in NAFLD may be associated with metabolic factors, including insulin resistance, and may adversely affect glucose metabolism in this liver disease.

Itou also experienced a case of refractory NAFLD that was successfully treated with sitagliptin, a DDP-4 inhibitor. Moreover, it is reported that sitagliptin ameliorates liver enzymes and hepatocyte ballooning in patients with nonalcoholic steatohepatitis.⁴ So, DPP-4 inhibitors ameliorate hepatic injury and glucose impairment in patients with NAFLD.

CONCLUSION

Hepatic DPP4 is involved in the progression of NAFLD in the following ways:

- i) When NAFLD is induced by nutritional overload, hepatic inflammation enhances hepatic DPP4 expression.
- ii) Accelerated degradation of GLP-1 by DPP4 inhibits insulin secretion and causes hyperglycemia.
- iii) Hyperglycemia further enhances DPP4 expression, with further worsening in glucose metabolism.

The increased hepatic expression of DPP4 in NAFLD patients suggests that DPP4 may be involved in the onset and/or progression of NAFLD. Hepatic inflammation may induce this phenomenon, although DPP4 causes deteriorations in systemic glucose.

ACKNOWLEDGEMENT

We would like to express great thanks to:

Khaled Abd Elhamed Mohammed , Inas El Khedr Mohammed, Hany Haroun Kaiser, Khaled Rafik Elbaz & Hesham Drwesh.

REFERENCES

1. Hazlehurst JM and Tomlinson JW (2013): Non-alcoholic fatty liver disease in common endocrine disorders. *Eur J Endocrinol*; 169(2): R27-R37.
2. Kameoka J, Tanaka T, Nojima Y, et al. (1993): Direct association of adenosine deaminase with a T cell activation antigen, CD26. *Science*; 261 (5120): 466–9.
3. Eguchi Y, Hyogo H, Ono M, et al. (2012): Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol*; 47:586–595.

4. Itou M, Kawaguchi T, Taniguchi E, et al. (2012): Dipeptidyl Peptidase IV Inhibitor Improves Insulin Resistance and Steatosis in a Refractory Nonalcoholic Fatty Liver Disease Patient: A Case Report. *Case Rep Gastroenterol*; 6:538–544.

5. Miyazaki M, Kato M, Tanaka K, et al. (2012): Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. *Molecular Medicine Reports*; 5: 729-733.

6. Itou M, Kawaguchi T, Taniguchi E, et al. (2008): Altered expression of glucagon-like peptide-1 and dipeptidyl peptidase IV in patients with HCV-related glucose intolerance. *J Gastroenterol Hepatol*; 23: 244-251.

7. Lakatos PL, Firneisz G, Borcsiczky D, et al. (2000): Elevated serum dipeptidyl peptidase IV (CD26, EC 3.4.14.5) activity in experimental liver cirrhosis. *Eur J Clin Invest*; 30: 793-797.

8. Firneisz G, Varga T, Lengyel G, et al. (2010): Serum dipeptidyl peptidase-4 activity in insulin resistant patients with non-alcoholic fatty liver disease: a novel liver disease biomarker. *PLoS One*; 5:e12226.

9. Balaban YH, Korkusuz P, Simsek H, et al. (2007): Dipeptidyl peptidase IV (DDP IV) in NASH patients. *Ann Hepatol*; 6: 242-250.

10. Ryskjaer J, Deacon CF, Carr RD, et al. (2006): Plasma dipeptidyl peptidase-IV activity in patients with type-2 diabetes mellitus correlates positively with HbA1c levels, but is not acutely affected by food intake. *Eur J Endocrinol*; 155: 485-493.

11. McKillop AM, Duffy NA, Lindsay JR, et al. (2008): Decreased dipeptidyl peptidase-IV activity and glucagon-like peptide-1(7-36)amide degradation in type 2 diabetic subjects. *Diabetes Res Clin Pract*; 79: 79-85.

Source of Support: Nil. **Conflict of Interest:** None Declared.

Copyright: © the author(s) and publisher. IJM RP is an official publication of Ibn Sina Academy of Medieval Medicine & Sciences, registered in 2001 under Indian Trusts Act, 1882. This is an open access article distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cite this article as: Khaled Abd Elhamed Mohammed, Inas El Khedr Mohammed, Hany Haroun Kaiser, Khaled Rafik Elbaz, Hesham Drwesh. Role of Serum Dipeptidyle Peptidase IV (DDP IV) Activity in Development of Non – Alcoholic Steatohepatitis in Patients with Non-Alcoholic Fatty Liver Disease. *Int J Med Res Prof*. 2018 Sept; 4(5):244-52. DOI:10.21276/ijmrp.2018.4.5.056