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## Original Article

**Dipeptidyl peptidase IV (DDP IV) in NASH patients**Yasemin H Balaban;<sup>1</sup> Petek Korkusuz;<sup>2</sup> Halis Simsek;<sup>3</sup> Hale Gokcan;<sup>4</sup> Gokhan Gedikoglu;<sup>5</sup> Asli Pinar;<sup>6</sup>  
Gulsen Hascelik;<sup>7</sup> Esin Asan;<sup>2</sup> Erhan Hamaloglu;<sup>8</sup> Gonca Tatar<sup>3</sup>**ABSTRACT**

**Objective(s):** Non-alcoholic steatohepatitis (NASH) is a chronic liver disease with unknown etiology. The insulin resistance, immune mechanisms and oxidative stress are the main factors in its pathogenesis. Dipeptidyl peptidase IV (DPP IV) or CD26 is a protein with endocrine and immune functions. This study aimed to elucidate the changes related to DPP IV in NASH patients. **Methods:** Serum and urinary DPP IV activities were measured in 31 NASH patients and 17 healthy controls. The liver biopsies of 29 patients were immunolabeled for CD26. **Results:** The mean age of patients were 46 ±

11 years and 14 (45%) of them were female. The serum DPP IV activity was higher in patients ( $57.3 \pm 7.8$  U/L) than controls ( $43.6 \pm 10.6$  U/L) ( $p < 0.0001$ ), and correlated with the histopathological grade ( $p = 0.038$ ,  $r = 0.373$ ) and hepatosteatosis ( $p = 0.018$ ,  $r = 0.423$ ) but not with stage ( $p = 0.286$ ), class ( $p = 0.286$ ) or CD26 staining ( $p = 0.743$ ). The urinary DPP IV activity was similar in patients ( $1.52 \pm 0.94$  U/mmol creatinine) and controls ( $1.37 \pm 0.68$  U/mmol creatinine) ( $p = 0.861$ ). Three acinar zones of liver had equal CD26 expression ( $p = 0.076$ ). The intensity of CD26 immunostaining was correlated with histopathological grade ( $p = 0.001$ ) and hepatosteatosis ( $p = 0.003$ ) but no correlation with stage or class could be detected ( $p = 0.610$  and  $0.956$ , respectively). **In Conclusions:** The serum DPP IV activity and the staining intensity of CD26 in liver are correlated with histopathologic grade of NASH and hepatosteatosis. DPP IV can be proposed as a novel candidate with several potential functions in NASH pathogenesis.

**Key words:** CD26, dipeptidyl peptidase IV, non-alcoholic steatohepatitis, metabolic syndrome.

**Introduction**

Nonalcoholic steatohepatitis (NASH) is defined as hepatic steatosis associated with inflammation and fibrosis in the absence of significant alcohol consumption.<sup>1</sup> NASH is the most common cause of liver enzyme elevation in the populations which have been attacked by current epidemics of obesity, diabetes and so metabolic syndrome.<sup>2-4</sup> The prognosis of NASH has been debated but nearly half of the patients have progressive fibrosis and one in five of them develop cirrhosis.<sup>5</sup> The recurrence of NASH after liver transplantation and the development of nonalcoholic fatty liver disease (NAFLD) in a quarter of transplanted patients for cryptogenic cirrhosis support believe that NASH is a metabolic disease. Although high prevalence and relatively poor prognosis of NASH urgently necessitate effective treatments, there is currently no drug which can improve the prognosis of NASH.

Dipeptidyl peptidase IV (DPP IV; E.C 3.4.14.5) is synonym with CD26, adenosine deaminase binding protein (ADAbp), and T cell activation protein. DPP IV is a cell-

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surface dipeptidase that activates or inactivates the peptides by removing proline or alanine from second position of N-terminal. DPPIV inactivates incretins which improve insulin resistance and  $\beta$  cell function. Apart from this enzymatic activity, DPPIV has function as a receptor and interacts with several proteins such as adenosine deaminase, HIV gp 120 protein, fibronectin, collagen, chemokine receptor CXCR4, and CD45. DPPIV also serves as a co-stimulatory molecule to activate T cells. DPPIV is primarily expressed on T lymphocytes, endothelial cells and epithelial cells. Its soluble form can be detected in plasma and body fluids. The change in serum DPPIV level is associated with autoimmune diseases, infections, cancers, depression and also liver diseases.<sup>6-8</sup>

DPPIV inhibitors are new group of oral antidiabetic agents which have been shown to improve insulin resistance in prediabetic people and patients with type 2 diabetes.<sup>9-11</sup> Experimental animal studies and clinical studies have firmly established that DPPIV inhibition improve glucose tolerance and  $\beta$  cell function without any adverse effect on normal physiology, therefore, DPPIV is a drug target and DPPIV inhibitors have been approved by FDA for the combination or monotherapy of type 2 diabetes.<sup>9-16</sup> Since NASH is strongly associated with insulin resistance and DPPIV inhibitors improve both hepatic and peripheral insulin sensitivity, DPPIV inhibitors are also novel candidates for the treatment of NASH. To the best of authors' knowledge this is the first study in the literature investigating the changes related to DPPIV in NASH patients. The liver, serum and urine samples have been investigated in order to elucidate the rational of treatment with DPPIV inhibitors in NASH.

## Methods

The study protocol has been approved by the institutional ethic committee of Hacettepe University. Information was given to all patients before the biopsy about the specimens' use in an experimental study and written consent was obtained. All procedures were in full compliance with the Helsinki Declaration of Human Rights. Thirty one patients with clinical diagnosis of NASH were recruited from the gastroenterology policlinic of Hacettepe University Hospital. After exclusion of viral, autoimmune, alcoholic, metabolic and toxic causes of liver disease, patients were given advice on diet and exercise. Those patients with malignancy, diabetes or using hepatotoxic drugs were not included in order to eliminate concomitant factors effecting liver enzyme or DPPIV levels. Anthropometric measurements (weight, height, waist and hip circumference) were done. All the patients underwent 75 mg Oral Glucose Tolerance Test (OGTT). Blood and urine samples were collected from 31 NASH patients and 17 healthy volunteers whose body mass index (BMI), serum transaminase levels and abdominal ultrasonography were normal. The fasting serum samples were used

for measurements of liver enzymes, glucose, insulin, C-peptide and lipids. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as  $\text{insulin (pmol/L)} \times \text{glucose } (\mu\text{mol/L})/22.5$ . HOMA-IR values equal to or greater than 3.0 were considered to be indicative of insulin resistance.

## Biochemical analyses

Serum measurements for liver enzymes, glucose, lipids, and urine measurements for creatinine were assayed immediately with Roche/Hitachi Modular Analytics (Tokyo, Japan). Reagents and calibrators from the same manufacturer (Roche Diagnostics, Mannheim, Germany) were used for each assay, and instrument operations and calibrations were performed as the instructions from manufacturer.

DPPIV measurements were performed on samples which were kept frozen at  $-70^{\circ}\text{C}$ . In order to minimize of the analytical variability, all serum and urine specimens were analyzed at the same day. Determination of DPPIV activities in serum and urine were assayed according to the colorimetric method of Nagatsu.<sup>17</sup> This method uses the chromogenic substrate glycyl-L-proline-*p*-nitroanilide. Briefly, 50  $\mu\text{L}$  of serum was incubated with 200  $\mu\text{L}$  of 71 mM glycine/NaOH buffer (pH 8.7) and 50  $\mu\text{L}$  of 3 mM glycylproline *p*-nitroanilide at  $37^{\circ}\text{C}$  for 30 min. The reaction was stopped by addition of the 2.7 mL acetate buffer (1 M, pH 4.5). The absorption at 385 nm was measured in order to detect *p*-nitroanilide. For calculations, *p*-nitroanilide was used as a standard and dipeptidyl peptidase from porcine kidney was used as a control. One unit of activity per liter was defined as the enzyme activity which produces 1 mol of *p*-nitroanilide in 1 min under the assay conditions. Urinary DPPIV activity was corrected with urinary creatinine. All chemicals were obtained from Sigma Chemical Co.

## Pathological and immunohistological evaluation

All patients with persistently more than two fold elevations of transaminases after 3 months of life style changes, underwent liver biopsy. The ultrasound guided liver biopsies were done by using 14 G, 15 cm true-cut needle. Conventional pathological examination was performed on 2 cm of liver specimens that were paraffin-embedded, cut and stained with haematoxylin & eosin. Histopathological classification for NASH was made according to the Brunt's criteria.<sup>18</sup> Approximately 0.5 cm biopsy material was used for CD26 immunostaining. Briefly whole specimen was immediately frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  until the immunolabeling procedure. Cryostat sections (6-8  $\mu\text{m}$  thick) were obtained on gelatin-coated slides. Indirect immunoperoxidase and indirect immunofluorescent (FITC) procedures used in this study were described in detail previously.<sup>19</sup> In brief, sections

were fixed in cold acetone for 10 minutes and air-dried for at least 30 minutes. Then, they were incubated with monoclonal Ig G antibody against human CD26 (#MCA1317, clone number M-A261, mouse anti-human Ig G<sub>1</sub>, Serotec, UK) for 60 minutes at room temperature in humidity chambers. After washing with 0.01 M phosphate buffered saline at pH = 7.4, a group of sections were covered with FITC conjugated anti-mouse Ig G (1:20) (#F0261, Dako, USA) containing 0.2% bovine serum albumin and 1% normal human serum. Other groups of slides were covered with 3-3' diaminobenzidine tetrahydrochloride (DAB) conjugated anti-mouse screening kit (Serotec, UK) for peroxidase activity following the manufacturers' instructions. All antibodies were diluted in a background reducing buffer solution in 0.05 M TrisHCl containing 0.1% tween (#S3022, Dako, USA). A counterstaining with propidium iodide (Anchor, USA) was performed for FITC-labeled sections; and with Mayers' haematoxylin for DAB-labeled sections. Control staining was performed by omitting the initial primary antibody staining step and using a control mouse Ig G. Frozen thymus sections were used as positive control following manufacturers' instructions. Stained sections were examined in a random order and blinded fashion and scored by two investigators. For each observer all sections were evaluated in one sitting using the same microscope at the same magnification. The reported histological score is the average of these observations. Each section was graded for immune reaction on hepatocytes for each acinar zone (1, 2, and 3) on a scale of 0 to +++. 0 was given to no immune reactivity, + to weak but continuous reactivity, ++ for moderate but continuous reactivity, and +++ to intense but continuous immunostaining of hepatocytes for each zone. Data were documented with a Leica DMR microscope (Germany); images were captured via Leica DC500 digital camera (Germany).

### Statistical methods

Statistical analyses were done by using SPSS 10.0 program. The statistical significance was accepted as p values less than 0.05. Independent samples t test was used for parametric measurements, and  $\chi^2$  test, Mann-Whitney U test or Kruskal Wallis test were used for non-parametric variables. The related variable groups were compared by Friedman test. The associations of ordinal variable were analyzed by Kendall's tau-c test.

## Results

### 1. Characteristics of patients with NASH

Out of 31 patients with NASH, 14 (45%) were female and the mean age was  $46 \pm 11$  (Table I). Obesity, especially central obesity was present in most of the patients; 12 (38.9%) had BMI > 30 kg/m<sup>2</sup> and 28 (90.3%) had in-

creased waist to hip ratio. The mean fasting insulin level and HOMA-IR were  $84.8 \pm 40.8$  pmol/L and  $2.8 \pm 1.4$ , respectively. The evidence of insulin resistance was detected as impaired fasting glucose 5 (16.1%), impaired OGTT 6 (19.4%), increased fasting insulin level 10 (32.1%) or high HOMA-IR index 13 (41.9%).

There was no patients with simple hepatosteatosis and the class of NAFLD were of class 2, 3, 4 in 6.5%, 64.5% and 29.0% patients, respectively (Table II). The liver disease was graded as mild (45.2%), moderate (22.6%) or severe (32.3%) according to the intensity of portal inflammation, hepatosteatosis, ballooning degeneration and lobular inflammation. The majority of the patients had mild portal inflammation (80.6%), mild hepatosteatosis (54.8%), ballooning degeneration (93.5%) and lobular inflammation (96.8%). The stage of fibrosis was mild to moderate in all patients, except one with severe fibrosis.

### 2. Immunohistological evaluation of CD26

All of the NASH patients expressed CD26 of variable intensity on liver parenchymal cells (hepatocytes), stromal fibroblasts, and some capillary endot-

**Table I.** Characteristics of NASH Patients.

	NASH (n = 31)
Female: Male	45:55
Age (yr)*	$46 \pm 11$
Obesity	
Normal (BMI < 25.0 kg/m <sup>2</sup> )	16.1%
Overweight (BMI 25.0-29.9 kg/m <sup>2</sup> )	45.2%
Obese (BMI $\geq$ 30 kg/m <sup>2</sup> )	38.7%
Central obesity	
Waist circumference ( $\geq$ 102 cm for men or $\geq$ 88 cm for women) <sup>†</sup>	45.2%
Waist/hip ratio ( $\geq$ 0.9 cm for men or $\geq$ 0.8 cm for women) <sup>††</sup>	90.3%
Insulin resistance	
Impaired fasting glucose (110-125 mg/dL)	16.1%
Impaired OGTT (postload glucose 140-200 mg/dL)	19.4%
Increased fasting insulin (>100 pmol/L)	32.1%
HOMA-IR $\geq$ 3	41.9%
Hypertension ( $\geq$ 130/85 mmHg)	25.8%
Hypertriglyceridemia ( $\geq$ 150 mg/dL)	45.2%
Low HDL (< 40 mg/dL for men or < 50 mg/dL for women)	19.4%
LDL (mg/dL)*	$126.2 \pm 37.6$
AST (U/L)	$49.1 \pm 20.8$
ALT (U/L)*	$75.9 \pm 24.1$
GGT (U/L)*	$99.1 \pm 82.6$
CRP (mg/L)*	$0.6 \pm 0.5$

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, c-reactive protein; GGT, gamma-glutamyl transpeptidase; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein; OGTT, oral glucose tolerance test.

\* Data are presented as mean  $\pm$  SD.

<sup>†</sup> female vs. male p=0.052.

<sup>††</sup> female vs. male p = 0.297.

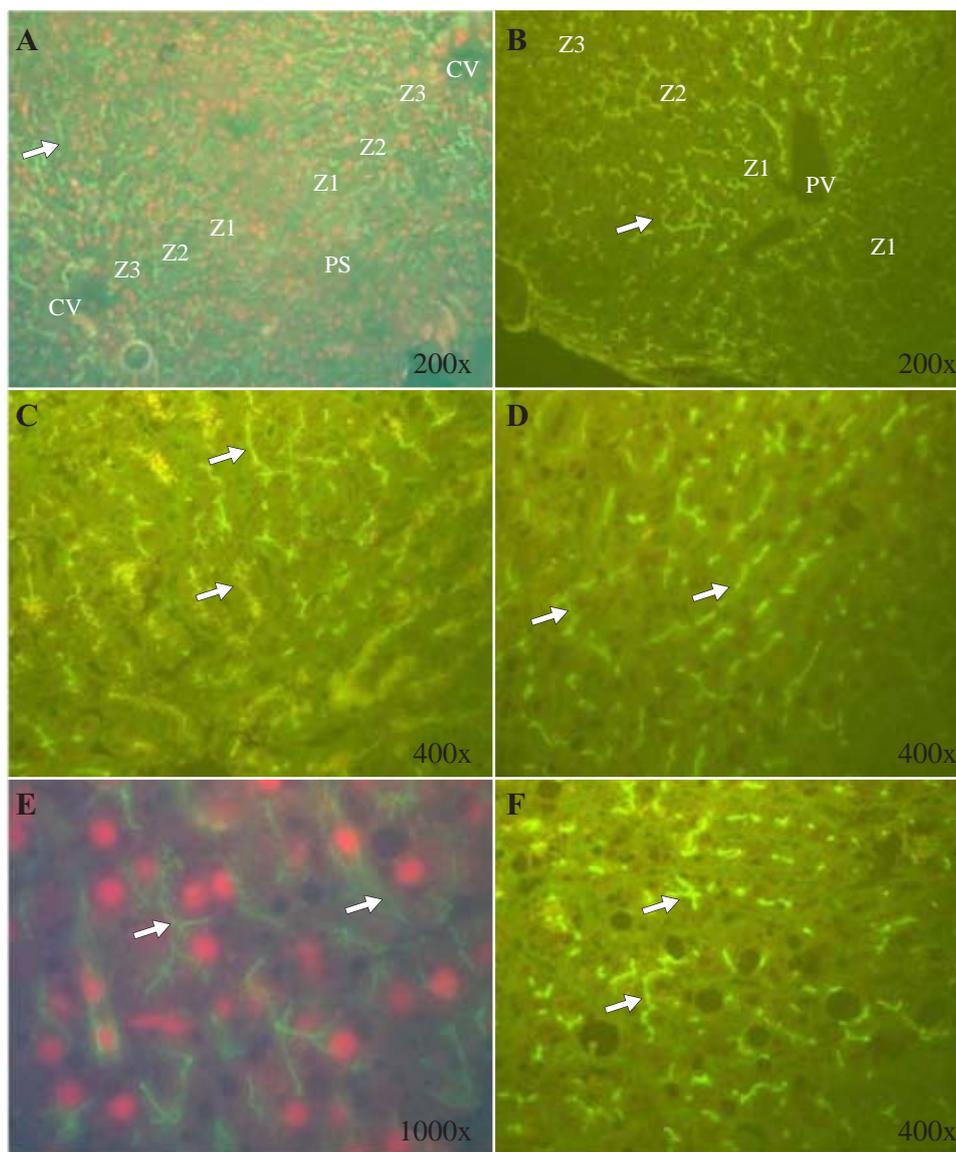
**Table II.** Histopathological features of patients.

	Number	Percentage
Class of NAFLD		
1/2/3/4	0/2/20/9	0.0/6.5/64.5/29.0
Grade of NASH		
Mild/moderate/severe	14/7/10	45.2/22.6/32.3
i. Portal inflammation		
Absent/mild/moderate/sever	5/25/1/0	16.1/80.6/3.2/0.0
ii. Hepatosteatosi		
Mild/moderate/sever	17/4/10	54.8/12.9/32.3
iii. Ballooning degeneration		
Absent/persent	2/29	6.5/93.5
iv. Lobular inflammation		
Absent/Present	1/30	3.2/96.8
Stage of NASH		
1/2/3	22/8/1	71.0/25.8/3.2

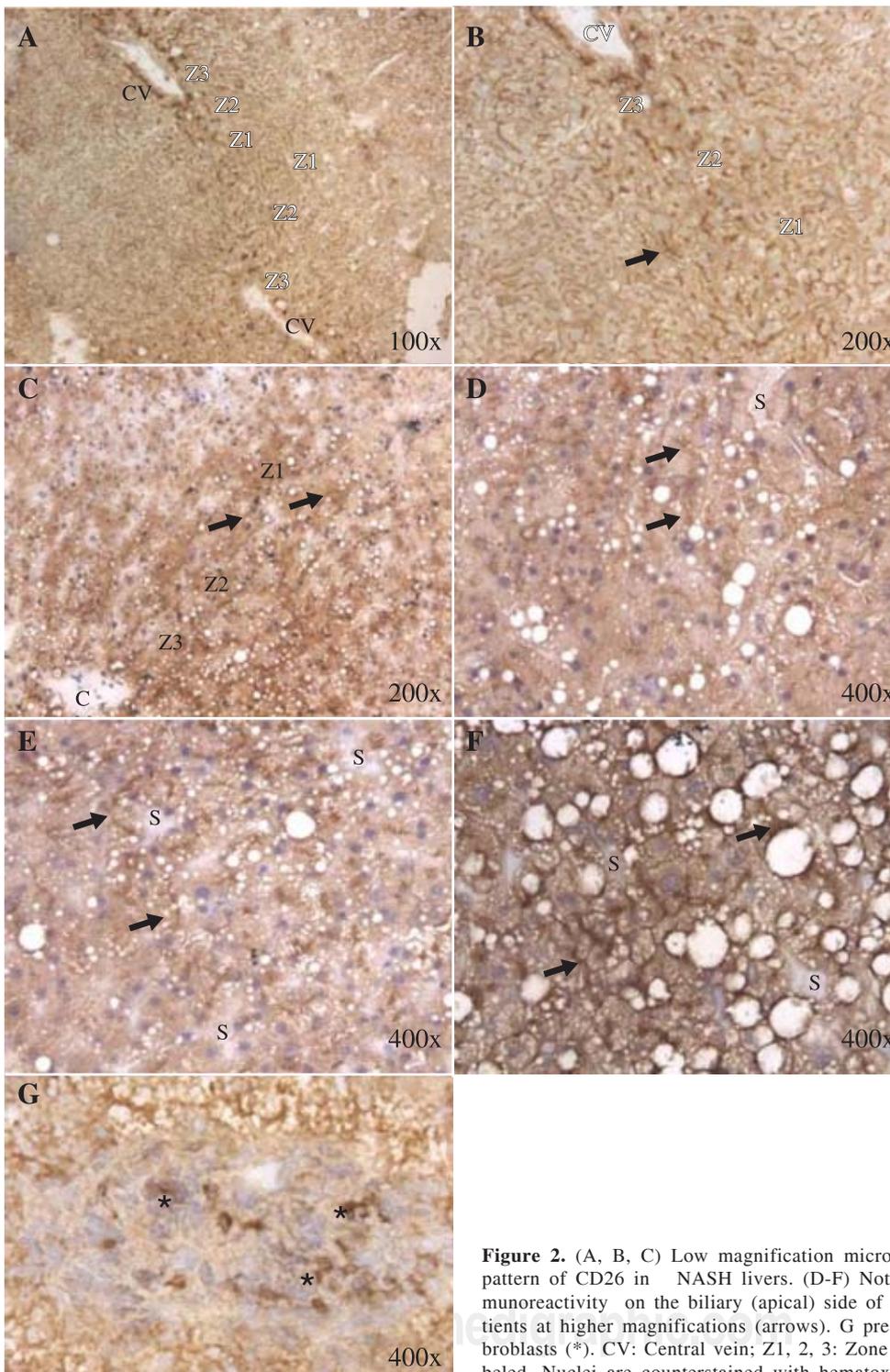
NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

helia. A more diffuse and strong immunolabeling was observed on hepatocytes (*Figure 1*). CD26 immunoreactivity was strictly located on the apical (biliary) side of hepatocyte in all NASH specimens (*Figure 2*). Immunoreaction was not present at the basolateral (sinusoidal) compartment in any of the cases. Hepatocytes expressed CD26 in all acinar zones. Though the intensity of CD26 immunostaining was higher in zone 3 when compared to that of the zone 1 in several subjects, the difference was not statistically significant regarding the zonal distribution pattern ( $p = 0.076$ , *Figure 3*). This may probably relate to the limited number of patients.

There was correlation between CD26 immunoreactivity with histopathological grade of NASH ( $p = 0.001$ ) and hepatosteatosi ( $p = 0.003$ ) by kendal's tau test. Patients with higher grade or more sever ste-



**Figure 1.** (A, B) Low magnification micrographs showing the zonal expression pattern of CD26 in NASH livers. (C-F) CD26 immunoreactivity is obviously observed on the biliary (apical) side of the hepatocytes at higher magnifications (arrows). PS: Portal space; CV: Central vein; PV: Portal vein branch; Z1, 2, 3: Zone 1, 2, 3. All micrographs are FITC labeled, Nuclei are counterstained with propidium iodide in A and E.



**Figure 2.** (A, B, C) Low magnification micrographs showing the zonal expression pattern of CD26 in NASH livers. (D-F) Note the sinusoids (S) and the CD26 immunoreactivity on the biliary (apical) side of the hepatocytes of several NASH patients at higher magnifications (arrows). G presents CD26 expression on stromal fibroblasts (\*). CV: Central vein; Z1, 2, 3: Zone 1, 2, 3. All micrographs are DAB labeled. Nuclei are counterstained with hematoxylin.

atosis had poorer CD26 immunoreactivity (*Figure 4*). Statistically significant correlation was not detected between CD26 immune reaction or portal inflammation ( $p = 0.713$ ), ballooning degeneration ( $p = 0.158$ ), lobular inflammation ( $p = 0.318$ ), stage ( $p = 0.610$ ) and class ( $p = 0.956$ ) parameters of NASH (*Table III*).

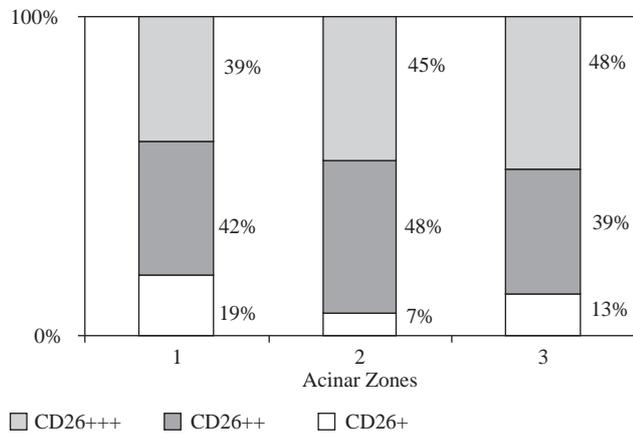
The correlation between zonal CD26 staining and clinical (sex, age, anthropometric measurements) or laboratory (liver enzymes, lipid levels, fasting glucose, OGTT, HOMA, CRP) parameters were searched with Pearson correlation. There were inverse correlation with body weight ( $p = 0.008$ ). Higher GGT levels were associated with +2 or +3 immunoreactivity of CD26 at all zones ( $p = 0.046$ ).

### 3. Serum DPPIV enzyme activity

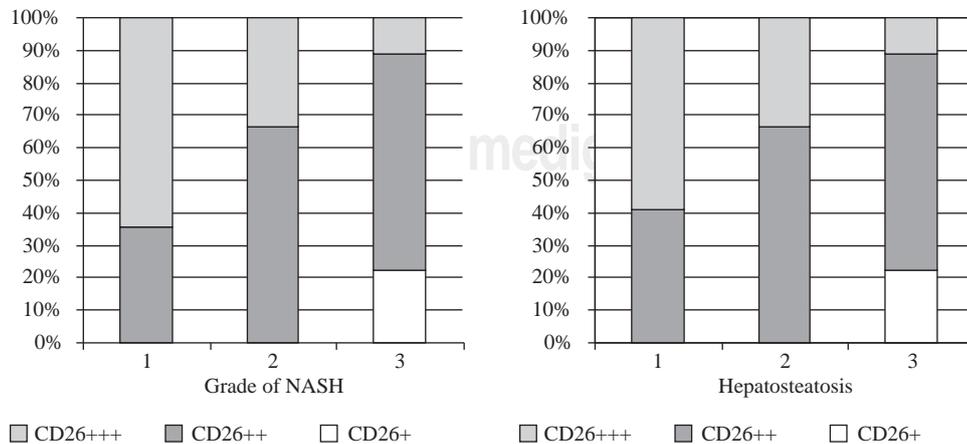
Serum DPPIV activity was significantly higher in patients with NASH ( $57.3 \pm 7.8$  U/L) than in controls ( $43.6 \pm 10.6$  U/L) ( $p < 0.000$ , *Figure 5*). Serum DPPIV activity correlated with grade ( $p = 0.038$ ,  $r = 0.373$ ) and steatosis ( $p = 0.018$ ,  $r = 0.423$ ). But there was no association with zonal DPPIV staining ( $p = 0.743$ ), stage ( $p = 0.286$ ) or class ( $p = 0.286$ ). 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 ( $p = 0.045$ ,  $r = 0.363$ ).

### 4. Urinary DPPIV activity

Urinary DPPIV activity was similar in patients with NASH ( $1.52 \pm 0.94$  U/mmol creatinine) and controls ( $1.37 \pm 0.68$  U/mmol creatinine) ( $p < 0.63$ , *Figure 6*). There was no association with zonal CD26 staining ( $p = 0.861$ ), grade ( $p = 0.311$ ), stage ( $p = 0.541$ ) or class ( $p = 0.541$ ).



**Figure 3.** The distribution of CD26 immunostaining intensity in acinar zones.



**A**

**B**

**Figure 4.** The intensity of CD26 immunostaining according grade of NASH (A) and hepatosteatosis (B).

### Discussion

The presented data suggest that the high serum DPPIV activity is an indicator of NASH. The serum activity and staining intensity of DPPIV correlate with hepatosteatosis and grade indicating the necro-inflammatory activity in the liver.

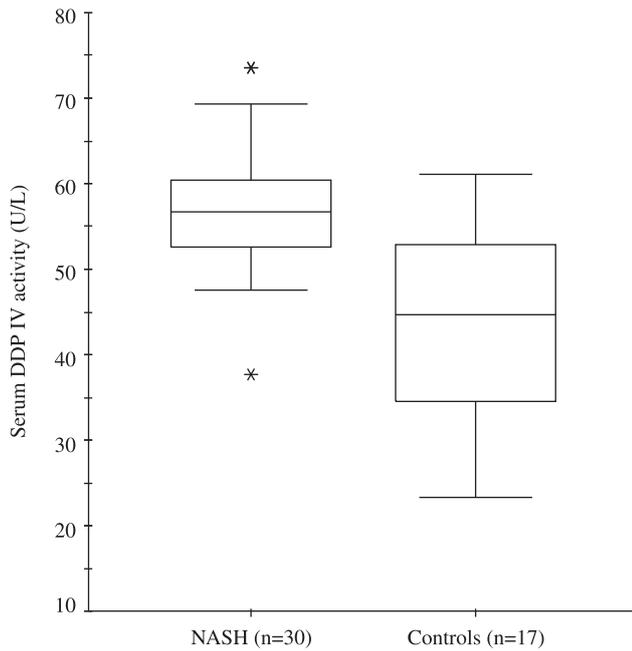
DPPIV may act by several possible mechanisms in NASH pathogenesis: First, it might be regulating the insulin resistance of liver which determines the steatosis in liver. Second, DPPIV might direct the immune response towards proinflammatory Th1 type rather than anti-inflammatory Th2 type which subsequently 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. These possible mechanisms will be discussed in details.

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 life style modification or drugs.<sup>20</sup> Incretins; namely glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are hormones stimulating glucose-dependent insulin secretion. Incretins improve  $\beta$  cell function and survival in an insulin dependent man-

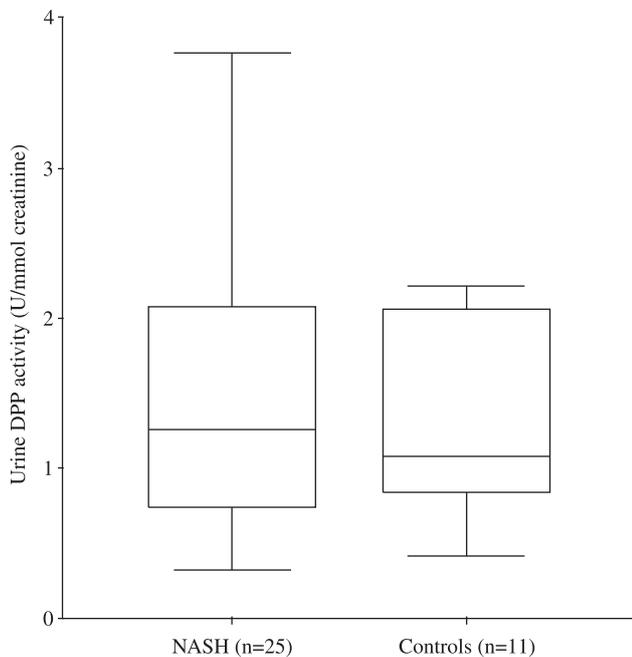
**Table III.** Association of CD26 immunostaining with histopathological parameters.

	Zone 1*	Zone 2*	Zone 3*	Overall*
Grade	0.002	0.000	0.004	0.001
Hepatosteatosis	0.035	0.001	0.000	0.003
Portal inflammation	0.400	0.740	0.600	0.713
Ballooning degeneration	0.327	0.791	0.174	0.158
Lobular inflammation	0.421	0.314	0.327	0.318
Stage	0.726	0.476	0.750	0.610
Class	0.933	0.481	0.917	0.956

\* p values.



**Figure 5.** Serum DPP IV activity in NASH patients and controls.



**Figure 6.** Urinary DPP IV activity in NASH patients and controls.

ner; while as an insulin independent function they cause hepatic and peripheral energy disposal which in turn increase insulin sensitivity. DPP IV degrades incretins and so regulates endocrine arm of entero-insulin axis which relays presence of nutrients in intestine to endocrine pancreas. Experiments on DPP IV mutated animal models have been demonstrated that DPP IV mutation is compatible with life.<sup>15</sup> Inhibition of DPP IV stimulates insulin se-

cretion, inhibits glucagon release, slows gastric emptying, promotes satiety and reduces body weight resulting in improved glucose tolerance.<sup>12-14</sup> In human studies, oral DPP IV inhibitors have been shown to prevent the transition from prediabetic to diabetic state as well as shown to be effective in treatment of diabetes without causing hypoglycemia or another serious side effect including hepatotoxicity.<sup>9-11,15</sup> In this study, serum DPP IV activity of NASH patients were increased without any correlation with parameters of insulin resistance. Therefore, other mechanisms might cause the beneficial effects of DPP IV inhibition in NASH patients.

CD26, which is identical to DPP IV, is considered as a general marker for cellular activation in the immune system. Antigenic stimulations induce CD26 expression on T cells, B cells, NK cells as well as on a subpopulation of dendritic cells.<sup>6-7,21</sup> Since antigen presentation without costimulatory signal induces anergy or tolerance in T cells, co-stimulatory signals are essential for T cell activation. The association of ADA expressing dendritic cells and CD26 expressing T cell functions as a costimulatory signal during engagement between T cell antigen receptor and CD3 complex.<sup>22</sup> The costimulatory potential of T cells is directly related to the amount of CD26 on its surface. CD26 is three to six folds higher on Th1 cells than Th2 cells.<sup>23</sup> Thus CD26 promotes an augmented T cell activation for a Th1 type and pro-inflammatory cytokine production, such as IFN- $\gamma$ , IL-2, IL-6, IL-8, and TNF- $\alpha$  resulting in enhanced immune response. The percentage of CD26 positive peripheral lymphocytes has been found to be lower in primary biliary cirrhosis patients comparing to healthy controls, and CD26 positive lymphocyte number has been normalized after immune modulation with ursodeoxycholic acid treatment.<sup>24</sup> Inflammation and fibrosis are the basic pathologies differentiating simple steatosis and NASH. TNF- $\alpha$  is a well known pro-inflammatory mediator and it has been suggested to be most critical cytokine inducing liver injury in NASH.<sup>25</sup> DPP IV has a potential effect on inflammation pathway by mediating TNF- $\alpha$  synthesis in NASH patients. The CD26 profile of lymphocytes in NASH should be investigated for determining the immunological role of CD26.

DPP IV also functions as a receptor providing the interaction between activated T cells, hepatocytes, hepatic satellite cells (HSC) and extracellular matrix proteins such as collagen and fibronectin.<sup>6,26</sup> Additionally, fibroblast activation protein (FAP) has been revealed to be exhibited DPP IV activity and its expression is limited to activated fibroblasts and HSC at sites of tissue injury and remodeling.<sup>7</sup> The role of DPP IV on necroinflammatory signal for the fibrosis in liver has been supported by the studies with thiazolidinediones (TZD) which are orally available, reversible DPP IV inhibitors. TZD retards the fibrosis in the liver by restoring PPAR $\gamma$  activity on HSC. During liver fibrosis reduced expression of PPAR $\gamma$  caus-

es activation of HSC which leads to transforming growth factor (TGF)  $\beta$ 1 induced collagen synthesis. Besides decreasing the insulin resistance, TZD have been shown to inhibit collagen and fibronectin synthesis in toxic and cholestatic models of liver fibrosis in rats.<sup>27</sup> TZD can normalize the liver enzymes levels; improve metabolic parameters and liver histopathology in NASH patients.<sup>20,28</sup> The potential of DDPIV inhibitors to improve liver fibrosis without causing hepatotoxicity makes them better candidate than TZD for the treatment of NASH.

CD26 expression patterns differ in rat and human liver tissue. Biliary side of hepatocytes and brush border of cholangiocytes are equally stained by OX-61 in tree acinar zones of adult rat liver.<sup>8,29</sup> However, CD26 expression with TaI, 1F7 and TS145 is restricted to acinar zones 2 and 3 in normal human liver.<sup>30</sup> Several theories have been proposed for zonal distribution of CD26 in human, such as different metabolic demand and “microenvironment heterogeneity”; hepatocyte differentiation and “the steaming liver”; and preferential zonal excretion of an unknown substrate together with DPPIV into the bile.<sup>30</sup> CD26 expression is distorted in cirrhosis so that zonal expression is lost and all hepatocytes become CD26 positive. The changing pattern of DPPIV expression has been proposed to be important for altered extracellular matrix (ECM) - parenchymal cell interaction that leads to loss of tissue architecture in development of cirrhosis. Additionally, CD26 expression alters from biliary side to basolateral side of hepatocytes during severe liver injury or allograft rejection.<sup>7,30</sup> M-A261 clone was used for the evaluation of CD26 distribution in the present study. In view of the fact that CD26 immunoreactivity was present in all zones at only biliary side of hepatocytes, we thought that metabolic changes in NASH or necro-inflammation initiated fibrogenesis may be altering the DPPIV/CD26 expression pattern in liver. This is supported by the finding that the intensity of staining was correlated with hepatosteatosis and the grade but not with stage. The absence of correlation between CD26 immunostaining and stage of NASH might be due to statistical reasons since, the liver fibrosis was mild in majority of patients. Alternatively, though the intensity of CD26 immunostaining was higher in zone 3 than zone 1 in several patients, the difference was not statistically significant by using non-parametric tests regarding the zonal distribution pattern. This may most likely relate to the limited number of patients. Ideally study for CD26 expression pattern should include sufficient number of liver biopsies with “normal” histology, simple hepatosteatosis and steatohepatitis.

The serum DPPIV activity invariably increases in cirrhosis and several liver diseases such as intra and extra hepatic cholestasis, primary biliary cirrhosis, toxic or alcoholic hepatitis and chronic hepatitis C. This increase is correlated with the prognosis of liver diseases.<sup>7,31-33</sup> Additionally, the serum DDPIV activity is lower in women,

elderly, obese people and diabetics, all of which are associated with increased insulin resistance. The reduced serum DDPIV activity in these conditions has been thought as an adaptive response for impaired insulin response of body. The lowered DDPIV activity reduces the degradation of incretins, thus incretin-mediated glucose-dependent insulin secretion is enhanced.<sup>34-35</sup> In this study, 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. Similar to the intensity of CD26 staining in liver, serum DPPIV activity was correlated with hepatosteatosis and grade but not with stage. These changes of DDPIV in NASH might result either primarily from underlying liver disease or secondary to insulin resistance. The origin of soluble DDPIV in the serum is not understood but the possible sources are endothelial, epithelial or T cells. The uncertainty about the source of increased serum DPPIV activity makes a weak point in this study. The cellular origin of the correlation between tissue and serum DPPIV activity remains to be determined with future studies.

Serum DPPIV level is strongly correlated with serum direct bilirubin level and so it is a valuable marker of cholestasis both in adults and children. Nevertheless, urinary DPPIV excretion is increased only in pediatric liver disease.<sup>31</sup> As a possible explanation for this discrepancy between adults and children, the restricted biliary excretion of DPPIV in adults has been proposed. This study also supported the biliary excretion of DDP IV, since urinary DPPIV activity in NASH patients were similar to controls in spite of increased serum activity.

In conclusion, 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. The current studies on NASH have been aimed to develop markers detecting patients with high risk of progressive fibrosis and to develop effective treatments. If the future studies confirm that the alterations related to DPPIV is 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.

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