CROATICA CHEMICA ACTA
CCACAA **78** (3) 427–432 (2005)

ISSN-0011-1643
CCA-3031
Original Scientific Paper

Clinical Relevance of the Serum Dipeptidyl Peptidase IV (DPP IV/CD26) Activity in Adult Patients with Crohn's Disease and Ulcerative Colitis*

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RECEIVED FEBRUARY 21, 2005; ACCEPTED MAY 10, 2005

Dipeptidyl peptidase IV (DPP IV/CD26), a serine-type protease, is a membrane-anchored enzyme widely expressed in many cell types. DPP IV is also present in a soluble form in the serum. The aim of this study was to evaluate the clinical relevance of the changes in serum DPP IV activity in adult patients with inflammatory bowel diseases – IBD (Crohn's disease – CD and Ulcerative colitis – UC). Both serum DPP IV activities in patients with CD (36.8±1.5 μmol min^{-1} dm^{-3}) and UC (33.9±2.1 μmol min^{-1} dm^{-3}) were statistically significantly decreased compared to healthy controls (48.4±1.1 μmol min^{-1} dm^{-3}), (P<0.001). The values correlated inversely with the disease severity for both diseases. No statistically significant difference in the serum DPP IV activity in patients with CD and UC was found and therefore it cannot be a good marker for a differential diagnosis. However, it appears to be useful as an available non-invasive marker in the diagnosis of disease activity.

Keywords
• dipeptidyl peptidase IV (DPP IV/CD26)
• inflammatory bowel disease
• Crohn's disease
• ulcerative colitis

INTRODUCTION

Inflammatory bowel diseases (IBDs) are characterized by chronic, relapsing intestinal inflammation of obscure origin. Two distinct disorders, Crohn's disease (CD) and ulcerative colitis (UC), have been identified, each of which may be a heterogeneous group of diseases.[1] The etiology and pathogenesis of IBD is not well understood, but each disorder appears to have its own constellation of genetic factors, immunological characteristics, and pathological findings.[2,3]

Dipeptidyl peptidase IV (DPP IV/CD26; EC 3.4.14.5) is an abundant, widely distributed serine protease able to cleave the N-terminal dipeptides from polypeptides. Cleavage by DPP IV can result in activation, inactivation or even significant change in the activity of physiological peptides.[4,5] This membrane-bound glycoprotein is a unique multifunctional protein, acting as a receptor, binding and proteolytic molecule, also expressed on the surface of various cell types, including epithelial and endothelial cells and lymphocytes. Proteolytic cleavage of the membrane bound DPP IV results in a soluble form that mi-

* Dedicated to Professor Željko Kućan on the occasion of his 70th birthday.
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grates as a homodimer with a molecular weight range of 210–290 kDa.[6] It has previously been shown that the DPP IV plays a significant role in the pathophysiology of IBD.[7]

The aim of this study was to evaluate the clinical relevance of changes in serum DPP IV activity in adult patients with inflammatory bowel diseases (CD and UC) compared to the levels in healthy controls. We hypothesized that changes in DPP IV serum activity could relate to the disease activity together with other inflammatory parameters. In clinical practice, the differential diagnosis of CD and UC is often difficult. Different biochemical, clinical, endoscopic, pathological and histological features should be combined in order to allocate the appropriate diagnosis. However, a precise diagnosis is not possible in about 10 to 20 % of patients with chronic colitis, which results in the designation »indeterminate colitis«.[8]

The immunopathological bases of CD and UC pathogenesis are different. The Th1-inflammatory response induces a transmural CD-like inflammatory reaction while a Th2-cytokine profile induces a UC-like inflammation.[9] The CD26/DPP IV signal transduction function is directly related to its expression level on human Th1 and Th2, enhancing Th1 cytokine response.[4,10] Based on these findings, we furthermore hypothesized that the serum DPP IV activity could be a potential distinguishing marker between Crohn's disease and ulcerative colitis.

EXPERIMENTAL

Patients

The study was performed on 62 patients, 38 with CD (mean age ± SD: 42.7 ± 14.4; 19 males, 19 females), and 24 with UC (mean age ± SD: 45.6 ± 17.6; 13 males, 11 females). All patients were admitted to the Department of Gastroenterology, Clinical Hospital Centre Rijeka. Diagnoses of CD or UC were established on the basis of clinical history, laboratory, endoscopic and histological data. The control group included 65 healthy donors (mean age ± SD: 41.6 ± 12.1; 32 males, 33 females). The CD activity was evaluated using the Crohn's Disease Activity Index (CDAI),[11] while the UC activity was evaluated according to the Truelove and Witts' (TW) classification.[12] Localization of the disease was determined according to the Wienna classification[13] for CD while UC was divided into proctosigmoiditis, left-side colitis and pancolitis. Blood samples were obtained after all patients and controls signed informed consents under the protocols approved by the Ethics Committee.

Determination of Serum DPP IV/CD26 Activity

Blood samples for the analysis of DPP IV activity were collected between 8:00 and 10:00 a.m. Sera were separated from fasting blood samples and stored at –80 °C until thawed for enzyme activities. Serum DPP IV activity was measured by detection of free 4-nitroaniline in an assay mixture containing 0.1 M Tris-HCl, (pH = 8.0), 2×10^{-3} M Gly-Pro *p*-nitroanilide as the substrate (Sigma Chemical, Germany), and enzyme in a total volume of 0.20 ml. After 30 min of incubation at 37 °C, the reaction was stopped by addition of 800 ml of sodium acetate buffer (1 M, pH = 4.5). All measures were performed in duplicate at 405 nm using a Varian Cary UV/Vis Spectrophotometer according to the protocol of Kreisel *et al.*[14] Enzyme activities are expressed as international units per liter of serum, which corresponds to the hydrolysis of 1 μmol min^{-1} dm^{-3}.

Statistical Analyses

Data are expressed as mean ± SE. Statistical analyses were performed as a cross-sectional analysis of all data obtained from the blood samples collected from patients and controls. STATISTICA version 6.1 (StatSoft Inc.) was used for all calculations. The following tests were applied: descriptive statistics, one-way ANOVA with *post hoc* comparison (Scheffe), linear correlation (Pearson r and Spearman R) and Student's t test. The level of $P<0.05$ was defined as statistically significant.

RESULTS AND DISCUSSION

By dividing patients with CD on the basis of the Crohn's Disease Activity Index (CDAI <150; 150–250 and >250) and patients with UC using the Truelove and Witts' classification (TW-mild and TW-severe), three and two groups of patients were formed, respectively. Both disease severity indices were applied as the most commonly used and recommended ones, even if no clinical index is superior.[15]

Data on routine laboratory parameters for specific groups (ESR – erythrocytes sedimentation rate, CRP – C-reactive protein, erythrocytes, leukocytes, lymphocytes, PLT – platelets, immunoglobulin fractions, Fe, TIBC – total iron binding capacity, total proteins) are given in Tables I and Table II.

Statistically significant differences were found for the following parameters among patients with CD: CRP ($P = 0.015$), ESR ($P = 0.003$), erythrocytes ($P = 0.019$), TIBC ($P = 0.007$), PLT ($P = 0.006$) (Table I). In patients with UC, statistically significant differences were found in the following parameters: CRP ($P < 0.001$), ESR ($P < 0.001$), Fe ($P = 0.025$), TIBC ($P < 0.008$), PLT ($P < 0.001$) (Table II). The inflammatory parameters correlated with the severity of the disease, as it is well known and expected. Consequently, it is not possible to distinguish between CD and UC on the basis of routine laboratory parameters and sometimes neither after sophisticated endoscopic and histological analyses, which is a frequently encountered problem in clinical practice. Signs of general inflammation, such as ESR or CRP, are less frequently found in UC when compared to CD, but the differences are not sufficient for a differential diagnosis. Folic acid deficiency, albumin deficiency, hypomagne-

TABLE I. Laboratory and immune parameters (mean ± SE) for different groups of patients with Crohn's disease

Laboratory and immune parameters	CDAI[a]			
	< 150	150–250	> 250	P[b]
ESR[c]/(mm/h)	16.6 ± 3.8	32.2 ± 4.8	56.5 ± 11.5	0.003*
CRP/(mg/dm^3)	1.9 ± 0.9	12.8 ± 2.8	27.4 ± 11.7	0.015*
Erythrocytes/ (10^{12}/dm^3)	4.9 ± 0.3	4.7 ± 0.1	3.9 ± 0.2	0.019*
Leukocytes/(10^9/dm^3)	7.2 ± 0.9	9.6 ± 1.3	6.1 ± 1.4	0.163
Lymphocytes/(10^9/dm^3)	10.8 ± 3.3	8.5 ± 2.3	14.5 ± 6.7	0.521
PLT[c]/(10^9/dm^3)	268.4 ± 9.9	324.5 ± 24.4	460.3 ± 71.2	0.006*
IgG/(g/dm^3)	11.9 ± 1.4	11.8 ± 1.2	12.5 ± 1.8	0.946
IgA/(g/dm^3)	3.3 ± 0.5	2.9 ± 0.4	4.0 ± 0.7	0.297
IgM/(g/dm^3)	1.1 ± 0.2	1.4 ± 0.3	1.0 ± 0.1	0.577
Fe/(μmol/dm^3)	10.8 ± 1.7	8.7 ± 1.3	6.6 ± 1.4	0.249
TIBC[c]/(μmol/dm^3)	54.1 ± 3.4	46.1 ± 2.2	37.4 ± 3.7	0.007*
Total proteins/(g/dm^3)	77.3 ± 2.8	70.4 ± 3.7	69.8 ± 3.0	0.287

[a] CDAI, Crohn's Disease Activity Index,[11] CDAI ≤ 150 – remission, CDAI >150 – active disease.
[b] Statistically significant difference, *P<0.05.
[c] ESR, erytrocytes sedimentation rate; CRP, C-reactive protein; PLT, plateletes; TIBC, total iron binding capacity.

TABLE II. Laboratory and immune parameters (mean ± SE) for different groups of patients with ulcerative colitis

Laboratory and immune parameters	TW-mild[a]	TW-severe[a]	P[b]
ESR[c]/(mm/h)	15.9 ± 2.5	42.1 ± 7.8	< 0.001**
CRP[c]/(mg/dm^3)	1.9 ± 0.7	105.3 ± 41.8	< 0.001**
Erythrocytes/(10^{12}/dm^3)	4.3 ± 0.1	4.1 ± 0.2	0.513
Leukocytes/(10^9/dm^3)	7.4 ± 0.7	9.5 ± 0.9	0.083
Lymphocytes/(10^9/dm^3)	10.8 ± 2.9	10.2 ± 2.5	0.896
PLT[c]/(10^9/dm^3)	240.6 ± 18.6	398.0 ± 28.6	< 0.001**
IgG/(g/dm^3)	12.3 ± 0.9	11.5 ± 0.7	0.571
IgA/(g/dm^3)	2.5 ± 0.4	3.6 ± 0.7	0.133
IgM/(g/dm^3)	1.1 ± 0.1	0.8 ± 0.2	0.074
Fe/(μmol/dm^3)	10.7 ± 1.9	3.8 ± 1.3	0.025*
TIBC[c]/(μmol/dm^3)	50.4 ± 2.9	35.1 ± 4.6	0.008*
Total proteins/(g/dm^3)	72.4 ± 6.4	66.8 ± 18.3	0.307

[a] Truelove and Witts' classification.[12]
[b] Statistically significant difference; *P<0.05, **P<0.001.
[c] ESR, erytrocytes sedimentation rate; CRP, C-reactive protein; PLT, plateletes; TIBC, total iron binding capacity.

siemia and hypocalcaemia can be found in both conditions. Elevated liver enzymes occur with both diseases. They may be due to associated reactive hepatitis, pericholangitis or primary sclerosing cholangitis. Some new serological markers, such as perinuclear antineutrophile cytoplasmic antibodies (pANCA) and anti-*Saccharomyces cerevisiae* antibody (ASCA) were given in order to help differentiate UC from CD. Combination of both tests has insufficient sensitivity and specificity for differential diagnosis, but adds pieces of the mosaic together with other features.[16]

As it was previously shown that DPP IV plays a significant role in the pathophysiology of IBD,[7] we investigated the clinical relevance of serum DPP IV activity in IBD as a possible distinguishing marker between CD and UC. Serum DPP IV activities in patients with CD and UC and controls are presented on Figure 1. Both serum DPP IV activities in patients with CD (36.8±1.5 μmol min^{-1} dm^{-3}) and UC (33.9±2.1 μmol min^{-1} dm^{-3}) were statistically significantly decreased compared to healthy controls (48.4±1.1 μmol min^{-1} dm^{-3}), (P<0.001). When comparing serum DPP IV activities in patients with CD and patients with UC using the Scheffe *post hoc* test, no statistically significant differences between the two groups of patients with IBD was found.

The DPP IV activity correlates inversely with the CDAI score (r = –0.2863) in patients with CD, as shown in Figure 2. By evaluating the interdependence of DPP

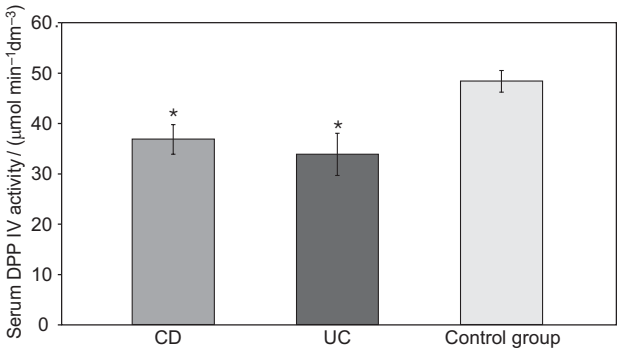

Figure 1. Serum DPP IV activity in patients with ulcerative colitis (UC) and Crohn's disease (CD) compared to healthy donors (control group). Box whisker plot represents mean ± 95 % confidence interval (95 % CI) *Statistically significantly different compared to control group (P<0.001)

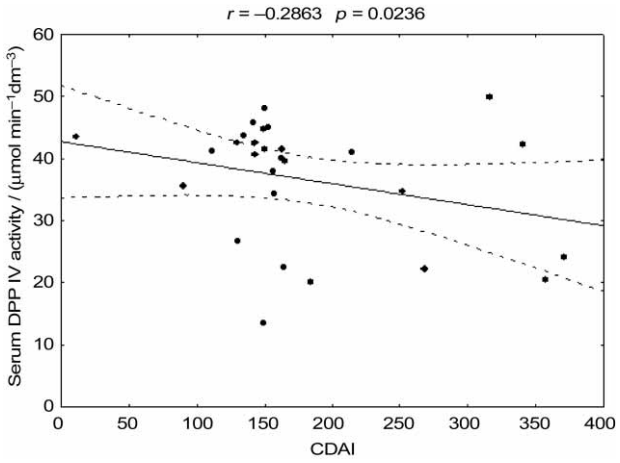

Figure 2. Correlation between the serum DPP IV activity and Crohn's Disease Activity Index (CDAI).

IV and CDAI using Student's t test, this correlation was found to be statistically significant (P = 0.024). Furthermore, by using the one-way ANOVA test, a significant difference was found in the serum DPP IV activity between the groups of patients having the CDAI score less than 150 and more than 250 (P = 0.023). The serum DPP IV activities in different groups of CD patients are shown in Figure 3. The CDAI is routinely used for the determination of the Crohn's disease activity, but its limitation is the individual subjective experience of each patient and also the nature of the disease itself.[15] Therefore, in clinical practice it is possible to encounter cases with relatively low CDAI values, but with chronic active disease. Some of younger patients with chronic active disease and with the presence of perianal complications showed very low DPP IV activity in serum; they even had lower CDAI indexes. This indicates that the DPP IV activity in serum could be an additional marker that confirms the inflammatory process in the bowel.

No correlation was found between the serum DPP IV activity and routine laboratory parameters in either disease. In addition, no statistically significant differences were found in the serum DPP IV activity relating to the location and extension of the pathological lesions either in patients with CD or UC. In patients with CD, no statistically significant differences were found regarding the age of the patients or the period of illness.

In patients with UC, a statistically significant difference was found between the two groups (TW-mild and TW-severe, P = 0.035). Likewise in CD, the serum DPP IV activity correlated inversely with the disease activity, showing lower serum DPP IV activity values in patients with TW-severe, as shown in Figure 4. There is a statistically significant difference in the serum DPP IV activity between male and female patients (P = 0.006).

The role and origin of soluble DPP IV is not completely understood, but it is clear that the enzymatic activity or at least the catalytic domain of DPP IV is indeed involved in the immune regulation by cleaving cytokines and influencing T-cell activation and costimulation.[17,18]

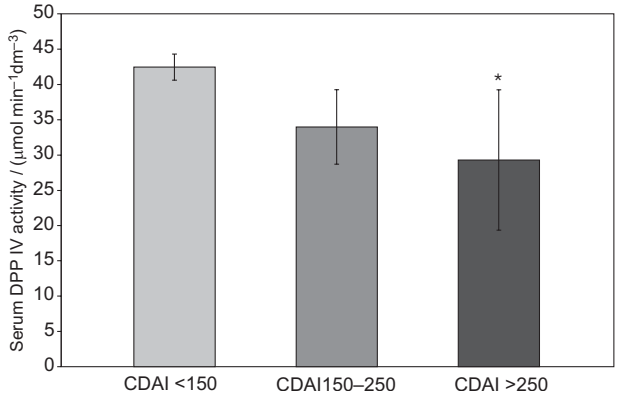

Figure 3. Serum DPP IV activity in three groups of patients with Crohn's disease. Box whisker plot represents mean ± 95 % confidence interval (95 % CI) *statistically significantly different compared to CDAI<150 (P = 0.023). CDAI, Crohn's Disease Activity Index:[11] CDAI ≤ 150 – remission, CDAI >150 – active disease.

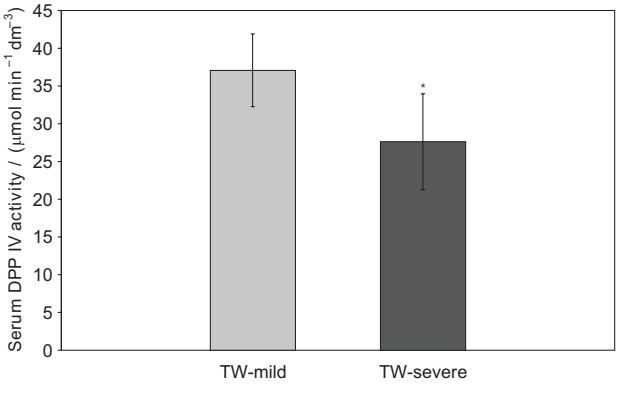

Figure 4. Serum DPP IV activity in two groups of patients with ulcerative colitis. TW-mild and TW-severe, Truelove and Witts' classification;[12] box whisker plot represents mean ± 95 % confidence interval (95 % CI); *statistically significant compared to TW-mild (P = 0.035)

Changes of DPP IV expression and serum activity appear to occur in several clinical and experimental situations of altered immune function. Current data suggest that the persisting immune dysbalance could have a significant impact on the pathogenesis of IBD.[19,20] Both diseases seem to result from the unrestrained activation of a non-specific inflammatory response in the intestine. Whether a Th1 or Th2 response is responsible for mucosal inflammation of the gastrointestinal tract has considerable impact on the nature of inflammation because Th1 responses are marked by transmural cellular infiltration (similar histopathologic picture is obtained in Crohn's disease) and Th2-mediated inflammations are, on the contrary, marked by more superficial cellular infiltrates (this situation is more akin to ulcerative colitis).[21] Based on the different pathogenesis of IBD, our hypothesis was that the serum DPP IV activity could be a possible different-diagnostic marker between CD and UC, reflecting the different T helper cytokine pattern. Meanwhile, the obtained results do not corroborate the hypothesis that the serum DPP IV enzymatic activity differs between patients with CD and patients with UC, thus reflecting the concept of different cytokine patterns in one or the other subtype of IBD. Consequently, it seems that the serum DPP IV activity could not be used as a specific differential diagnostic marker between CD and UC, but further investigations are necessary for differentiation of CD from UC. However, it is important to know that no measurements of immune parameters in the peripheral blood can reflect the immune activation in the mucosa. Furthermore, future studies of the DPP IV activity in samples obtained by biopsy of the intestine should be performed, in order to determine the significance of DPP IV at the mucosal level.

CONCLUSIONS

In our study we have confirmed the previously published results that the serum DPP IV activity is decreased in IBD patients.[7,20] The obtained results can suggest a functional compartmentalization of DPP IV, which can be interpreted as the adaptive systemic immune response to a local inflammatory reaction. The serum DPP IV activity in IBD patients correlated inversely with the grade of disease. Soluble DPP IV in serum seems to be involved in the pathophysiology of IBD and appears to be useful as an available non-invasive marker in the diagnosis of disease activity.

Acknowledgments. – This work was supported by grant No. 0062045 from the Ministry of Science and Technology of the Republic of Croatia. The authors thank Mrs. Dolores Kovačić of the Department of Chemistry and Biochemistry, School of Medicine, University of Rijeka, Croatia, for excellent technical assistance. Many thanks go to Mrs. Tatjana Pribanić Hero for collecting the clinical history data.

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SAŽETAK

Klinički značaj serumske aktivnosti dipeptidil peptidaze IV (DPP IV/CD26) u odraslih bolesnika s Crohnovom bolešću i ulceroznim kolitisom

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Serinska proteaza dipeptidil peptidaza IV (DPP IV/CD26) membranski je vezan enzim, izražen u brojnim vrstama stanica. Također je prisutna u topivome obliku u serumu. Cilj ovoga istraživanja bio je ispitati klinički značaj promjena serumske aktivnosti DPP IV u odraslih pacijenata s upalnim bolestima crijeva – IBD (Chronova bolest – CD i ulcerozni kolitis – UC). Obje vrijednosti serumske DPP IV aktivnosti u pacijenata s CD (36.8±1.5 μmol min^{-1} dm^{-3}) i UC (33.9±2.1 μmol min^{-1} dm^{-3}) bile su statistički značajno snižene u usporedbi s kontrolnom skupinom (48.4±1.1 μmol min^{-1} dm^{-3}), (P<0.001). Vrijednosti su bile obrnuto proporcionalne s aktivnošću obiju bolesti. Nije utvrđena statistički značajna razlika u serumskoj DPP IV aktivnosti između bolesnika s CD i UC, što upućuje na zaključak da DPP IV nije dobar marker za dijagnostičko razlikovanje tih dviju bolesti. Međutim, mogao bi poslužiti kao neinvazivan marker u dijagnostici aktivnosti bolesti.