Differential Expression of Dipeptidyl Peptidase-IV (DPP-IV) in Indian Type-2 Diabetic Population

Allenki Venkatesham*, Martha Srinivas*, Devarakonda R Krishna*, Pantam Narayana**

Abstract
Objectives: Dipeptidyl peptidase IV (CD26; E.C. 3.4.14.5) is plasma membrane glycoprotein exopeptidase. There are no data on both phenotypic or genotypic expression and polymorphism of DPP-IV in Indian type-2 diabetic patients. Therefore we estimated the dipeptidyl peptidase IV (DPP-IV) levels in Indian type 2 diabetic population.

Methods: Selection of study group was as follows: 1. Twenty seven nondiabetic subjects (control group). 2. Twenty five newly detected diabetic patients (without treatment). 3. Fifty four diabetic patients on drug treatment (metformin and glibenclamide) for more than 3 yrs. Again this group was subdivided on the basis of HbA1c level (25 subjects only) into, 3a.moderately controlled (HbA1c 6-8%), 3b.uncontrolled (HbA1c >8%). In all the subjects both fasting and post-prandial blood sugar was estimated and ADA criteria was taken for the diagnosis. In the same subjects fasting and post-lunch dipeptidyl peptidase IV (DPP IV) enzyme levels were estimated. HbA1c levels moderately controlled group showed significant p value regarding fasting and postprandial DPP-IV levels.

Results: Fasting DPP-IV levels in different groups were statistically significant. Similarly post-prandial DPP-IV levels in different groups were statistically significant, except between the non diabetic Vs anti diabetic drug treated group. On comparison the ratio of fasting and post-prandial DPP IV levels is <1 in non-diabetic and newly detected diabetic groups and in anti-diabetic drug treated group (3a and b) the ratio is >1. Based on HbA1c levels moderately controlled group showed significant p value regarding fasting and postprandial DPP-IV levels.

Conclusions: Fasting DPP IV levels were raised in newly detected and drug-treated diabetic subjects in comparison to normal subjects and postprandial levels are markedly raised in newly detected diabetic group only. Fasting and post-prandial DPP IV levels varied significantly in moderately controlled diabetic group (HbA1c 6-8%) basing on p value fasting 0.001 and post-prandial 0.005. Fasting and post-prandial DPP IV ratio is >1 in antidiabetic drug treated group, whereas it is <1 in normal control group and newly detected diabetic group. This change may be attributed to chronicity of diabetes or uncontrolled diabetic status or due to effect of metformin on post-prandial DPP IV levels.

Introduction
Type 2 diabetes is characterized by peripheral insulin resistance and progressive failure of pancreatic β cell function that leads to inadequate insulin secretion.1 Glucagon-like peptide-1 (GLP-1) secretion and biosynthesis. 4 In addition, synthetic GLP-1 has potential in the treatment of diabetes mellitus.9 therefore GLP-1 they offer the rationale for evaluating the peptide’s therapeutic secretion. 5 It may also enhance glucose uptake in peripheral tissues,6 control of gastric emptying, antroduodenal motility 7 and satiety.8 these combined effects improve glucose tolerance.14 Dipeptidyl peptidase IV (CD26; E.C. 3.4.14.5) is plasma membrane glycoprotein exopeptidase that belongs to the prolyl oligopeptidase family.15 there are no data on both phenotypic or genotypic expression and polymorphism of DPP-IV in Indian type-2 diabetic patients. Therefore, we determined to study the DPP-IV levels in fasting / post-prandial states in different groups of subjects.

Materials and Methods
Dipeptidyl peptidases-IV (enzyme) and glycine-proline P-nitroanilide (substrate) were procured from Sigma, St. Louis, MO, USA. Tris HCL and phosphate buffers (pH7.6) were procured from E. Merck Ltd, Mumbai, India. Glucose kit was procured from Excel Diagnostics, Hyderabad, India. Metformin and glibenclamide combination tablets were procured from Medibast Pharma Ltd, Chennai, India. Metformin tablets were procured from Kare labs Pvt Ltd, Goa.

Study design
The study included as follows:
Table 1: Clinical characteristics of subjects participating in the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-Diabetic</th>
<th>Newly Detected</th>
<th>Oral Anti-Diabetic (Combination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.4 ± 4.46</td>
<td>48.2 ± 6.40</td>
<td>54 ± 8.16</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>29(17M/12F)</td>
<td>25(13M/12F)</td>
<td>54(25M/29F)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 ± 2.78</td>
<td>23.32 ± 4.57</td>
<td>23.49 ± 3.32</td>
</tr>
<tr>
<td>Fasting glucose (mg%)</td>
<td>80.3 ± 8.43</td>
<td>146.4 ± 30.91</td>
<td>150.72 ± 86.41</td>
</tr>
<tr>
<td>Postprandial glucose (mg%)</td>
<td>110 ± 18.25</td>
<td>232.6 ± 58.44</td>
<td>245.6 ± 8.96</td>
</tr>
<tr>
<td>Fasting DPP-IV (U/l)</td>
<td>22.89 ± 3.75</td>
<td>45.78 ± 8.8</td>
<td>35.71 ± 6.13</td>
</tr>
<tr>
<td>Postprandial DPP-IV (U/l)</td>
<td>26.54 ± 5.13</td>
<td>60.87 ± 13.11</td>
<td>30.94 ± 4.94</td>
</tr>
<tr>
<td>DPP-IV (U/l)</td>
<td>0.86</td>
<td>0.75</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Data values were expressed as mean ± SD.

Table 2: Statistical significance levels of DPP-IV in different groups

<table>
<thead>
<tr>
<th>DPP-IV (Dipeptidyl Peptidase-IV)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Non-diabetic Vs Newly detected</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Non-diabetic Vs anti-diabetic (combination) treated</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-diabetic (combination) treated Vs Newly detected</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Data values were expressed as mean ± SD. P- value less than 0.05 are considered as statistically significant. (NS – Non significant)

Group 1. Twenty seven non-diabetic subjects (control).

Group 2. Twenty five newly diagnosed type 2 diabetes without treatment.

Group 3. Fifty four diabetic patients on oral antidiabetic drug treatment (metformin and glibenclamide combination) for more than 3 yrs.

3a. Moderately controlled group (HbA1c 6-8%) - 10 patients.

3b. Uncontrolled diabetes (HbA1c >8%) – 15 patients.

All patients were recruited at the Department of General Medicine, Mahatma Gandhi Memorial Hospital, Warangal, Andhra Pradesh, India from March 2005 to April 2006. All subjects were attending general health check up at our outpatient department (Thursday-Diabetic Care Programme) in MGM Hospital. Subjects were excluded if they had chronic gastrointestinal diseases associated with chronic pancreatitis, history of any malignant disease, history of alcohol abuse, kidney or liver failure and other diseases affecting carbohydrate metabolism. Fasting as well as post-prandial blood samples were collected from all subjects and fasting and post-prandial serum glucose and DPP-IV levels were estimated. Serum glucose levels were estimated by glucose oxidase/ peroxidase (GOD/POD) method. Different concentrations of DPP-IV (10, 20, 30, 40, 50, 60, 70 and 80 U/l) in serum were prepared for calibration curve. DPP-IV activity was measured by a colorimetric assay. Gly-Pro-4 p-nitroanilide, a chromogenic substrate of DPP-IV, is hydrolysed into dipeptide Gly-Pro and product 4-nitroaniline, the rate of appearance of which could be measured spectrophotometrically.

The study was approved by institutional ethics committee (Kakatiya Medical College, Warangal) and informed consent was obtained from each subject according to the principles of the declaration of Helsinki.

Statistical analysis

All variables are expressed as mean ± SD. Group differences of continuous variables were compared using ANOVA followed by Student - Newman Keuls post hoc test. For all analyses, a P value < 0.05 was considered to be statistically significant. All analyses were performed using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA). As HbA1C levels were estimated in small group (25 subjects), for statistical significance Pearson correlation co-efficient was used.

Results

Upon statistical analysis (ANOVA), DPP-IV levels in fasting/ postprandial states in different groups were compared (non-diabetic, newly detected and anti diabetic drug treated for >3yrs). In anti-diabetic drug treated group 25 subjects were selected for HbA1C estimation. Ten subjects are moderately controlled (HbA1c 6-8%) and remaining 15 subjects were grouped as uncontrolled (HbA1c > 8%). Table 1 represents the clinical characteristics of all group subjects DPP-IV levels in antidiabetic (combination) drug treated group showed significantly higher (35.71 ± 6.13 and 34.2 ± 8.6 U/l) than in non-diabetic subjects (25.89 ± 3.75 U/l) and lower than newly diagnosed type-2 diabetic patients (45.78 ± 8.8 U/l) in fasting stage.

Statistical significant levels of DPP-IV in different groups presented in Table 2. The results of present study indicate that fasting DPP-IV levels in different groups were statistically significant (P<0.05). Similarly post-prandial DPP-IV levels in different groups were statistically significant (P<0.05), except between the non-diabetic Vs anti-diabetic drug treated group.

Discussion

In comparison to non-diabetic group (22.89 ± 3.75) fasting DPP IV levels in newly detected diabetics (45.78 ± 8.8) are significantly increased and moderately raised in treated group (35.71 ± 6.13). Regarding post-prandial DPP IV, significantly increased in newly detected group (60.87 ± 13.11) but not much elevated in treated group (30.94 ± 4.94). Table 2 shows fasting DPP IV levels were statistically increased in all the groups. Post-prandial DPP IV levels were also significantly increased in all the groups except non-diabetic vs treated group. Table 3 shows comparison of HbA1c levels with fasting and post-prandial DPP IV levels.

The results were elaborated on Pearson correlation co-efficient and the p value was calculated.

In comparison to non-diabetic patients both fasting and post-prandial DPP IV levels increased in moderately controlled and uncontrolled group. But when compared to normal and treated uncontrolled group, significant p values were observed in treated moderately controlled group. One interesting observation is that, post-prandial DPP IV levels were increased in comparison to fasting DPP IV levels, in normal subjects and exaggerated in newly detected group but not increased, on the other hand decreased than fasting DPP IV in chronic diabetics with treated...
group (as shown in Fig. 1). Ratio of fasting/post-prandial DPP IV is <1 in normal controls and newly detected diabetics without treatment, but the same ratio is noted >1 in chronic drug-treated group. This observation may be due to chronicity of diabetes mellitus or with metformin treatment or uncontrolled metabolic status in spite of DPP IV being low. High levels of DPP IV (fasting and post-prandial) in early stage of DM (newly detected) show a reactive phase in the evolution of diabetes mellitus. This reactive phase is whether due to increased incretin response of the gut due to food ingestion or due to inflammatory response of the gut in the early onset of diabetes is unknown.

**Conclusions**

Indian ethnic groups show increased post-prandial response of DPP-IV in normals and still exaggerated response in newly detected diabetic group without treatment. Fasting DPP IV levels show positive correlation with duration of diabetes. But post-prandial DPP IV levels are significantly higher in newly detected group when compared to normal controls. This is a contradictory finding to the conclusion of Jacob et al. where they showed plasma DPP IV activity in patients with type-2 diabetes mellitus correlates positively with HbA1c levels, but is not acutely affected by food intake. This special reactive phase is seen in Indian ethnic groups. Further studies are required in this line.

Reversal of fasting/post-prandial DPP IV ratio in the course of diabetes mellitus can be taken as a parameter for therapeutic intervention either by multiple drugs or insulin. This reversal of ratio might also be revealing failure of gut incretin response.

**Acknowledgement**

The first author is very thankful to Dr. C. Raghu Ram, Superintendent, Mahatma Gandhi Memorial Hospital, Warangal – 506 002, Andhra Pradesh, India, for providing necessary facilities to carry out this study effectively. The authors are very grateful to the diabetic patients who attended Diabetic O.P. for giving constant cooperation in smooth conduct of this study.

**References**


