Measurement of GLP-1 in Impaired Glucose Tolerance Subjects in Comparison to Type 2 Diabetes Patients and Healthy Subjects

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Abstract

Background: Incretin therapy of type 2 diabetes patients is based on the fact that Incretin Effect is diminished in those patients. The objective is to measure glucagon-like peptide-1 (GLP-1) levels in impaired oral glucose tolerance (IGT) subjects and compare them to those of type 2 diabetes patients and healthy subjects. If the incretin hormone (GLP-1) is established to be diminished in IGT subjects, future study may assess effectiveness of incretin therapy to prevent or delay diabetes in IGT subjects.

Patients and methods: GLP-1 was measured by ELISA test at 0, 30 and 120 minutes in accordance with OGTT in three groups: type 2 diabetes groups including 24 patient, impaired glucose tolerance group including 24 subject and healthy control group including 24 subject as control. Patients were classified according to the WHO criteria for diabetes diagnosis.

Results: Fasting GLP-1 levels were none significantly different between the studied groups. One the other hand, GLP-1 response at 30' was significantly diminished in diabetics when compared with IGT and controls. GLP-1 levels at 120' were significantly reduced in type 2 diabetes patients when compared with IGT and controls and significantly diminished in IGT when compared with controls.

Conclusions: The study indicates that the GLP-1 levels are diminished in impaired glucose tolerance subjects when it’s compared to normal subjects.

Keywords: impaired glucose tolerance, diabetes, GLP-1

1. Introduction

1.1 Type 2 Diabetes Disease Burden

It was expected that the number of People with diabetes would increase from 171 million in 200 to reach 366 million in 2030 but the prevalence globally already reached 371 by 2012 as indicated by the International Diabetes Federation (IDF), and the calculated projections globally indicate that the prevalence of diabetes will reach 530 million people in 2030 (IDF, 2012). In 2012, Egypt came in the top ten countries in the prevalence of diabetes occupying the seventh place with 16.9 % of population between 20-79 (48,305,000) which is as many as 7,548,750 type 2 diabetes patient, and an estimated undiagnosed patients reaching 4,207,295, where between 6.8% and 7.5% are impaired glucose tolerance subjects accounting for about 3,304,696 this contributed to as many as 65003 cases with diabetes related deaths. Surprisingly the diabetes related expenditure per person with diabetes were estimated only 136 USD per year (IDF, 2012).

Many treatment regimens were applied for treatment and only the newly introduced incretin therapy can claim the ability to change the nature of diabetes form a progressive disease to static disease through their assumed ability to preserve b-cell function (Farilla et al., 2003). Incretins are gastrointestinal hormones secreted in response to nutrient ingestion. They work on the beta cells in the pancreas stimulating glucose-dependent insulin secretion. The incretin hormones responsible predominantly for this enteral-insulin relationship are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino tropic peptide (GIP). Also GLP-1 secretion is regulated by neural and endocrine factors (Drucker & Nauck, 2006).
In addition to their ability to stimulate glucose secretion in a glucose dependent manner, GLP-1 is able to exert other metabolic properties that control glucose homeostasis, that includes suppression of glucagon from the alpha cells in pancreas, improvement of glucose utilization and disposal and slowing of gastric emptying (D’Alessio & Vahl, 2004; Zander, Madsbad, Madsen, & Holst, 2002). By slowing the gastric absorption of nutrients and promoting a feeling of satiety GLP-1 can lead to weight loss in overweight individuals (Gautier, Fetita, Sobngwi, & Saliou-Martin, 2005). Studies in both animals and humans revealed that GLP-1 may also have protective effects on the cardiovascular system (Drucker & Nauck, 2006). Furthermore, in animal studies, both GLP-1 and GIP were able to increase beta-cell mass, by promoting islet cell proliferation and inhibition of apoptosis (Brubaker & Drucker, 2004). Based on the previous data, enhancing of incretin action has emerged as a potential therapeutic goal in the development of new therapies for the management of type 2 diabetes.

In past studies, (Toft-Nielsen et al., 2001) and (Vilsboll, Krarup, Deacon, Madsbad, & Holst, 2001), Toft-Nielsen et al. (2001) reported ~20 and ~30% lower postprandial GLP-1 levels in IGT subjects and patients with type 2 diabetes compared with normal oral glucose–tolerant subjects, respectively. In line with these data, Vilsboll et al. (2001) found not only total but also intact GLP-1 levels to be reduced in patients with type 2 diabetes. However, in subsequent studies, other investigators failed to detect such differences in GLP-1 levels in another group of patients with type 2 diabetes (Ryskjaer et al., 2006).

1.2 Aim of Work

The aim is to compare the GLP-1 levels between IGT subjects, type 2 diabetes patients and healthy subjects. If the GLP-1 levels were found to be diminished in IGT subjects that will help us consider incretin based therapy effectiveness in preventing or delaying diabetes.

2. Patients and Methods

This study was conducted on 72 adults, during the period from June 2012 till January 2013, who were classified into three groups based on their performance in a standard Oral Glucose Tolerance Test (OGTT) (WHO, 2006). GLP-1 was measured by ELISA at 0, 30 and 120 Minutes in accordance with the OGTT in the tree groups. Patients were recruited in diabetes outpatient clinic at Mansoura University, and laboratory analysis was performed in biochemistry department in Al Azhar College of Pharmacy. The study population was selected from type 2 diabetes patients’ families and friends who usually escort them to the hospital. In the hospital, those relatives and friend were offered to participate in the study.

Patient demographic for each study member were recorded including age (in years) and Body Mass Index (BMI) as (kg/m²).

1. Type 2 Diabetes Group

This group includes 24 patients. A screening process was performed to select group subjects. Diabetes group were chosen to be newly diagnosed and drug naïve neither taking oral therapy nor insulin, to limit the effect of type 2 diabetes disease length on the hormone level if any, bearing in mind that a patient with 10 to 15 years of type 2 diabetes may have different hormone profile than a patient with shorter duration of disease. Patient were included in this group if fasting plasma glucose \(\geq 7.0 \text{ mmol/l} (126 \text{ mg/dl})\) and \(2\text{-h plasma glucose} \geq 11.1 \text{ mmol/l} (200 \text{ mg/dl})\).

2. Impaired Glucose Tolerance Group

This group included 24 patients. While the impaired glucose tolerance group was expected to take long time to complete the patient recruitment but it did not, taking only 10 days, giving an idea about how much prevalent is the disease in Egypt. Patients were included in this group if their fasting plasma glucose level \(\geq 6.1 \text{ mmol/l} (110 \text{ mg/dl})\) and \(< 6.9 \text{ mmol/l} (125 \text{ mg/dl})\) and \(2\text{-h plasma glucose} \geq 7.8 \text{ and < 11.1 mmol/l} (140 \text{ mg/dl and 200 mg/dl})\).

3. Healthy Control Group

This group included 24 subjects with normal fasting plasma glucose as \(< 5.6 \text{ mmol/l},\) and a normal \(2\text{-h plasma glucose as < 7.8 mmol/l} (140 \text{ mg/dl})\). An indicator of the stealthy nature of the disease, another aspect was noticed while recruiting patients. In the diabetes group of the 24 subject, 4 patients were diagnosed with diabetes who were otherwise completely healthy and self-reported no complain of type 2 diabetes symptoms, and were primarily expected to be in the control group, while of 24 subjects in the impaired glucose tolerance (prediabetes) group, 14 subjects self-reported no complain of any type 2 diabetes symptoms and completely healthy and also were expected to be in the control group.
2.4 Blood Samples Withdrawal
We collected the blood samples into centrifuge-safe lavender vacutainer tubes, which contain EDTA for blood anticoagulation and Aprotinin (0.6 TIU/ml of blood) to inhibit the activity of proteinases and can collect 7 ml blood/tube. Then we gently rock the centrifuge-safe lavender vacutainer tubes several times immediately after collection. After that we centrifuge the blood at 1,600-x g for 15 minutes at 4 °C after that we collected the plasma. Plasma was then kept at -70 °C.

Blood was also collected for Blood Glucose (BG), insulin and HbA1c measurement. BG measured at 0, 30 and 120 minutes in accordance with the OGTT in the tree groups. Insulin was measured at 0 and 120 minutes and HbA1c at 0 minute.

2.5 Extraction of GLP-1 Peptide From Plasma
We first acidified the plasma with an equivalent amount of buffer A. Then we mixed and centrifuged it at 6000 to 17000-x g for 20 minutes at 4 °C. After that we equilibrated a SEP-COLUMN that contains 200 mg of C18 via washing with buffer B (1 ml once) then by buffer A (3ml, 3 times).

After this step no pressure was applied to the column. Then we loaded the acidified plasma solution onto the pretreated C18 SEP-COLUMN and slowly washed the column with buffer A (3 ml twice) and we discarded the wash. Then we eluted the peptide slowly using buffer B (3 ml once) and collected the eluant in a polystyrene tube then we evaporated the eluant to dryness in a centrifugal concentrator.

The dried extract was then kept at -20 °C and we performed the assay instantly where the assay buffer was used to reconstitute the dried extract.

The absorbance was read in each well at 450 nm. And GLP-1 was measured as ng/ml.

The GLP-1 measuring kit is provided by DRG International Inc., USA. Fax: (908) 233-0758, e-mail: corp@drg-international.com, website: www.drg-international.com and its catalogue details under the name DRG® Glucagon-Like Peptide-1 (Human, Rat, Mouse) ELISA (EIA-4141).

2.6 Statistical Methods
Data management and analysis were performed using Data Analysis and Statistical Software for Professionals (STATA) vs. 12. Numerical data were summarized using means and standard deviations or median & ranges. Comparisons between the 3 groups were performed using one way ANOVA for continuous data and Chi-Square for nominal data. All p-values are two-sided. P-values < 0.05 were considered significant.

2.7 Ethical Considerations
This study was conducted according to Helsinki’s declaration and the guidelines for Good Clinical Practice. The local ethics committees approved the protocol, and informed consent was obtained from all patients before study entry.

3. Results
3.1 Patients’ Characteristics

<table>
<thead>
<tr>
<th></th>
<th>People with Diabetes (n=24)</th>
<th>IGT (n=24)</th>
<th>People without Diabetes (n=24)</th>
<th>One Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.3 ± 11.9</td>
<td>46.9 ± 14.8</td>
<td>39.2 ± 13.5</td>
<td>3.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 3.4</td>
<td>25.9 ± 2.6</td>
<td>26.1 ± 3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 18 (75.0 %)</td>
<td>16 (66.7 %)</td>
<td>17 (70.8 %)</td>
<td>X²</td>
</tr>
<tr>
<td></td>
<td>Female 6 (25.0 %)</td>
<td>8 (33.3 %)</td>
<td>7 (29.2 %)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Chi-square test

As shown in Table 1, no statistically significant differences were found among the studied groups regarding age, BMI and sex distribution.
3.2 Clinical and Pathological Characteristics

Table 2. Comparison between BG levels in the studied groups, measured as (mg/dl) at 0 (Fasting BG), 30 and 120 minutes

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Fasting BG</td>
<td>210.0 ± 53.3</td>
<td>81.0 ± 2.7</td>
<td>77.0 ± 2.7</td>
<td>143.9</td>
</tr>
<tr>
<td>BG at 30'</td>
<td>261.2 ± 38.4</td>
<td>142.6 ± 6.0</td>
<td>120.7 ± 7.6</td>
<td>260.9</td>
</tr>
<tr>
<td>BG at 120'</td>
<td>328.0 ± 16.9</td>
<td>183.4 ± 10.9</td>
<td>98.0 ± 7.9</td>
<td>2071.1</td>
</tr>
</tbody>
</table>

Post-hoc analysis

- GI Vs GII P
- Fasting BG
- People with Diabetes Vs IGT 0.0001*
- People with Diabetes Vs People without Diabetes 0.0001*
- IGT Vs People without Diabetes 0.0001*
- BG at 30'
- People with Diabetes Vs IGT 0.0001*
- People with Diabetes Vs People without Diabetes 0.0001*
- IGT Vs People without Diabetes 0.0001*
- BG at 120'
- People with Diabetes Vs IGT 0.0001*
- People with Diabetes Vs People without Diabetes 0.0001*
- IGT Vs People without Diabetes 0.0001*

3.3 Insulin and HbA1c Levels

Table 3. Comparison between other laboratory findings in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>People with Diabetes (n=24)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>7.03 ± 1.97</td>
<td>7.02 ± 1.38</td>
<td>7.05 ± 1.64</td>
<td>0.002</td>
</tr>
<tr>
<td>Insulin at 120'</td>
<td>61.5 ± 15.3</td>
<td>56.6 ± 14.2</td>
<td>49.3 ± 15.5</td>
<td>3.95</td>
</tr>
<tr>
<td>HbA1C</td>
<td>7.75 ± 0.76</td>
<td>6.12 ± 0.19</td>
<td>5.27 ± 0.23</td>
<td>167.6</td>
</tr>
</tbody>
</table>

Post-hoc analysis

- GI Vs GII P
- HbA1C
- People with Diabetes Vs IGT 0.0001*
- People with Diabetes Vs People without Diabetes 0.0001*
- IGT Vs People without Diabetes 0.0001*

Table 2 shows that type 2 diabetes patients had significantly higher fasting BG, BG at 30' and BG at 120' than IGT subjects and controls. Also IGT subjects had significantly higher fasting BG, BG at 30' and BG at 120' than controls. While table 3 shows that type 2 diabetes patients had significantly higher HBA1C than IGT subjects and controls. Also, IGT subjects had significantly higher HBA1C than controls. In addition, it has been shown that diabetics had significantly higher insulin levels than controls at 120'. In spite of the fact that diabetics had higher insulin levels than IGT subjects and IGT subjects had significantly higher insulin levels than controls at 120', the difference is statistically short of significance.
3.4 GLP-1 Level

Table 4. Comparison between GLP-1 levels between the studied groups

<table>
<thead>
<tr>
<th></th>
<th>People with Diabetes (n=24)</th>
<th>IGT (n=24)</th>
<th>People without Diabetes</th>
<th>One Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting GLP-1</td>
<td>0.58 ± 0.16</td>
<td>0.66 ± 0.24</td>
<td>0.58 ± 0.19</td>
<td>F= 1.5</td>
</tr>
<tr>
<td>GLP-1 at 30'</td>
<td>0.79 ± 0.08</td>
<td>0.96 ± 0.28</td>
<td>1.02 ± 0.15</td>
<td>F= 9.4</td>
</tr>
<tr>
<td>GLP-1 at 120'</td>
<td>0.64 ± 0.07</td>
<td>1.06 ± 0.2</td>
<td>1.2 ± 0.18</td>
<td>F= 9.4</td>
</tr>
<tr>
<td>Repeated measures ANOVA</td>
<td>F= 17.0</td>
<td>F= 19.0</td>
<td>F= 107.0</td>
<td>P= 0.0001*</td>
</tr>
<tr>
<td>Post-hoc analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI Vs GII</td>
<td>People with Diabetes Vs IGT</td>
<td></td>
<td></td>
<td>0.019*</td>
</tr>
<tr>
<td>GLP-1 at 30'</td>
<td>People with Diabetes Vs IGT</td>
<td></td>
<td></td>
<td>0.0001*</td>
</tr>
<tr>
<td>GLP-1 at 120'</td>
<td>People with Diabetes Vs IGT</td>
<td></td>
<td></td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

The analysis as in Table 4 showed no significant differences between the studied groups regarding fasting GLP-1 levels. On the other hand GLP-1 response at 30' was significantly diminished in type 2 diabetes when compared with IGT and controls. GLP-1 at 120' levels were significantly reduced in type 2 diabetes patients when compared with IGT and controls and in IGT when compared with controls. In the same time Repeated measures analysis showed significant differences of GLP-1 levels in all groups throughout the study intervals.

4. Discussion

The observation that oral glucose triggers a higher insulin level than parenteral glucose at the same plasma glucose (PG) levels is called the incretin action. The incretin action is attributed to the two main incretin hormones known as glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (Holst & Gromada, 2004).

They are both secreted from the proximal part of the small intestine as response to food ingestion. They cause high insulin secretion a very glucose-dependent manner (Dube & Brubaker, 2004). The incretin action was proven to be markedly reduced in type 2 diabetes patients. This diminished incretin action come along with a reduced GLP-1 response to a meal with mixed content (Vilsboll et al., 2001), a decreased insulin secreting potency of GLP-1 (Kjems, Holst, Volund, & Madsbad, 2003) and nearly complete loss of insulin secretion in the late-phase in response to GIP (Vilsboll, Krarup, Madsbad, & Holst, 2002).
In addition, it had been proven that the inhibition of glucagon secretion is impaired through oral glucose tolerance tests (OGTTs) in contrast with isoglycemic parenteral infusion of glucose in patients with type 2 diabetes (Knop, Vilsboll, Madsbad, Holst, & Krarup, 2007). The key observation that the GLP-1 response is blunted and that the β-cell response to GIP is grossly impaired in diabetes has led to the notion that an impaired incretin effect contributes to the β-cell incompetence of diabetes (Vilsboll & Holst, 2004). Clinical data showing that GLP-1 analogs can normalize glycemic state by stimulating insulin secretion in type 2 diabetes patients has strengthened the incretin theory (Zander et al., 2002).

While the aim of the present study is to evaluate levels of GLP-1 in type 2 diabetes patients, IGT patients and non-diabetic healthy subjects, as expected from the study design, type 2 diabetes patients had significantly higher fasting BG, BG at 30’ and BG at 120’ than IGT and controls. Also IGT had significantly higher fasting BG, BG at 30’ and BG at 120’ than controls.

Regarding GLP-1 levels, it had shown no significant differences between the studied groups regarding fasting GLP-1 levels. Meanwhile GLP-1 response at 30’ was significantly diminished in type 2 diabetes when compared with IGT and controls. GLP-1 at 30’ levels were significantly reduced in type 2 diabetes when it’s compared with IGT and controls in IGT when compared with controls. Repeated measures analysis had shown significant differences of GLP-1 levels in all groups throughout the study intervals.

The present results are in agreement with Toft-Nielsen et al. (2001) and (Vilsbøll et al., 2001). Toft-Nielsen et al. (2001) reported ~20 and ~30% lower postprandial GLP-1 levels in IGT subjects and patients with type 2 diabetes compared with normal oral glucose–tolerant subjects, respectively. In line with these data, Vilsboll et al. (2001) found not only total but also intact GLP-1 levels to be reduced in patients with type 2 diabetes. However, in subsequent studies, other investigators failed to detect such differences in GLP-1 levels in another group of patients with type 2 diabetes (Ryskjaer et al., 2006). The reasons for the dissimilar results in different cohorts of patients are difficult to explain, but it is important to compare the subject characteristics in more detail including diabetes duration and glycemic control. Patients studied by Vilsboll et al. (2001) and Toft-Nielsen et al. (2001) had a longer diabetes duration and exhibited higher A1C levels. It is therefore possible that more defects in GLP-1 secretion develop later during the pathogenesis of type 2 diabetes. In this context, another factor with a potential impact on postprandial GLP-1 levels is the velocity of gastric emptying (Vozzo et al., 2002). Thus, any deceleration of gastric emptying might blunt the subsequent incretin responses (Gentilcore et al., 2006). Another possible factor that might impact on GLP-1 secretion in type 2 diabetes patients is the presence of hyperglucagonemia. In fact, that also appeared in previous studies where high glucagon levels were found to be associated with lower GLP-1 concentrations (Meier, Holst, Schmidt, & Nauck, 2007).

4. Conclusion

The study showed that GLP-1 levels in IGT subjects are diminished at certain points in their response curve especially at 120 min. Such a fact may suggest a future protocol to evaluate the effect of incretin therapy on the progression time to frank diabetes in IGT subjects, giving a new hope against that disease.

References


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