Effect of T2DM progressing on renal glucose excretion till the $T_{mg}$

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Abstract
In this study, an attempt has been made to measure the amount of glucose excreted in urine regarding the duration of T2DM. In order to achieve that 302 T2DM patients have been included and then classified into 3 groups according to duration of illness. T2DM patients have been tested for random blood glucose, urine glucose concentration and serum creatinine while glomerular filtration rate (GFR), tubular glucose load (TGL) and urine glucose excretion (UGE) have been calculated. Then an analytical tests were applied to analysis these parameters and comparing the results of different groups according to classification of this study. The maximum transport of glucose ($T_{mg}$), and UGE till the $T_{mg}$ (splay region) are varied between T2DM patients. In order to evaluate the validity, ANOVA test for groups variance, and linear regression were applied. According to this study the $T_{mg}$ increased and the UGE till the $T_{mg}$ decreased as the duration of illness increased.

تأثٍر مذة مرض السكري النمط الثانً على الطرح الكلوي للكلوكوز لغاٌة الحد

الأعلى لامتصاص الكلوكوز

لؤي احمذ محمذ الجلبً        شاكر محمود سلٍمان

الملحِص

تتناول هذه الدراسة قياس كمية الكلوكوز المطرح في الإدراز لمرضى السكري النمط الثاني مع الاخذ بالاعتبار مدة المرض. لغرض تقييم الاختلاف ، فما لدراسة العلاقة بين كمية الكلوكوز في الدم والإدراز لـ 302 مرضي و قد تم تصنيف المرضى حسب مدة الإدراز . وقد تم قياس نسبة الكلوكوز في مصل الدم والإدراز ونسبة الكرياتين في مصل الدم وكذلك تم حساب معدل الترشيح التنافتي وحولية الكلوكوز وكمية الكلوكوز في الإدراز. بعدها فما بإجراء التحاليل الإحصائية لقيم ومقارنة النتائج للمجموعات حسب التقسيم أعلاه . وقد لوحظ أن الفاًية القصوى لاسترجاع الكلوكوز في أدابب الكلى وكمية الكلوكوز في الإدراز مختلفة بين مرضى السكري النمط الثاني ولغرض تقييم هذا الاختلاف فما بتحليل التباين للمجموعات كما فما بقياس الاندماج الحسي للقيم وقد وجد أن الفاًية القصوى لاسترجاع الكلوكوز في أدابب الكلى تزداد وان كمية الكلوكوز في الإدراز تقل بازياد مدة الزمنية للمرض.
Introduction
Glucose is freely filtered at the glomerulus and completely reabsorbed at the level of the proximal convoluted tubule (Rave et al., 2006). When the blood glucose rising the reabsorption of filtered glucose in the proximal convoluted tubule increases until a maximum value is reached (Marsenic et al., 2009). Any further increase in blood glucose results in the excretion of glucose in urine. The appearance of glucose in urine before this point is reflected in the concept of a renal threshold for glucose excretion (Rave et al., 2006). According to this concept, no glucose should be detectable in urine at sub-threshold blood glucose levels.

Reabsorption of glucose from glomerular filtrate occurs by means of sodium–glucose co-transporters (SGLTs) in the proximal convoluted tubulae. There are six members of this family. Approximately 90% of glucose is reabsorbed by SGLT2 (Kanai et al., 1994; Write, 2001), a high-capacity low-affinity glucose transporter. SGLT2 is thought to be located exclusively on the luminal surface of the epithelial cells lining the S1 and S2 segments of the proximal tubule. Transport of sodium and glucose by SGLT2 occurs in a 1:1 ratio (Kleta et al., 2004; Kim et al., 2010; Fujimori et al., 2009). The remaining 10% of glucose reabsorption is mediated by SGLT1, a high-affinity, low-capacity glucose/galactose transporter sodium/glucose coupling ratio = 2:1, located on the luminal surface of epithelial cells lining the S3 segment of the proximal tubule (Pajor et al., 2008; Del Valle et al., 2002). Glucose reabsorbed from the proximal tubules by SGLTs is then released into the circulation through the action of facilitative glucose transporters (GLUTs) at the basolateral membrane of the epithelial cells lining the proximal tubules (GLUT2 in the S1/2 segments and GLUT1 in the S3 segment). In contrast, GLUTs facilitate passive transport (equilibration) of glucose across membranes and do not require an energy source (Uldery and Thorens, 2004).

Patients, Materials and Methods
Subjects involve in the current study are composed of 302 diabetes mellitus type 2 patients 40 years and above 186 male and 116 female attending to alwaffa diabetic center and Ibn sena teaching hospital. The patient selected are varied from newly to previously diagnosed as T2DM, with negative UTI test (Mavrakanas et al., 2009), normal blood pressure, not take aminoglycoside antibiotics at least two weeks before participate in this study (Takamoto et al., 2003), not on insulin treatment (Chin et al., 1997) and if female she must be not pregnant (Alto, 2005). Blood samples were obtained from all diabetic type 2 patient by antecubital venepuncture between 9-10 a.m. after taking breakfast at 7 o’clock. Urine samples were obtained from all patients by asking them to void and empty their bladders completely immediately after blood samples were taken. By this method we obtained the first sample which is discarded, the urine volume of the second sample which is obtained by voiding after an hour of time was measured in a graduated cylinder to calculate urine excretion per minute for each patient, this sample is used to measure urine glucose concentration. (Note: GUE was done first to exclude sample with UTI).

Methods
We can illustrate the methodology in this study as follows:
1- Serum Glucose and Urine glucose measurement

Reaction principle

\[ \text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{GOD} \text{gluconic acid} + \text{H}_2\text{O}_2 \]
\[ 2\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + \text{phenol} \xrightarrow{POD} \text{quinoneimine} + 4\text{H}_2\text{O} \]

Colored result was measured at 500 nm by using APEL spectrophotometer (Jian et al., 2011; Ahmed et al., 2010; Iqbal, Kalsoom, and Jafri, 2011). RANDOX kit made in united kingdom was used.

2- Serum Creatinine

Creatinine in alkaline picrate solution forms a color complex (Butler, 1975) detected at 520 nm by using APEL spectrophotometer. SYRBIO kit made in france was used.

Calculated parameters

1- Glomerular Filtration Rate (GFR) ml/min.

A commonly used surrogate marker for estimate of creatinine clearance is the Cockcroft-Gault formula (Poggio et al., 2005), which in turn estimates GFR (Melloni et al., 2008)

\[ e\text{Cr} = \frac{(140 - \text{age (year)})\times \text{mass (kg)}\times 0.85 \text{ (if female) \times } 72 \times \text{Serum creatinine mg/dl}}{100} \]

2- TGL mg/min.

\[ \frac{\text{GFR (mL/min)} \times \text{Serum Glucose Concentration (mg/dL)}}{100} \]

3- UGE mg/min

\[ \frac{\text{Urine glucose concentration (mg/dL)} \times \text{Urine volume (ml/min)}}{100} \]

Results

1- Tubular Glucose Load (TGL)

a. Male with T2DM

It revealed that diabetic patients with (<2) years duration of disease had a non-significant difference regarding TGL mean value from diabetic patients with (2-<10) and 10 years and more duration of the disease. And diabetic patients with (2-<10) years duration of disease had a non-significant difference regarding TGL mean value from diabetic patients with 10 years and more duration of disease as shown in the table (4).

b. Female with T2DM

It revealed that diabetic patients with (<2) years duration of disease had a non-significant difference regarding TGL mean value from diabetic patients with (2-<10) and 10 years and more duration of disease. and diabetic patients with (2-<10) years duration of disease had a non-significant difference regarding UGE mean value from diabetic patients with (2-<10) and 10 years and more duration of disease. and diabetic patients with (2-<10) years duration of disease had a non-significant
difference regarding UGE mean value from diabetic patients with 10 years and more duration of disease as shown in the table (3).

b- Female with T2DM

It revealed that diabetic patients with (<2) years duration of T2DM had a non-significant difference regarding UGE mean value from diabetic patients with (2-<10) years duration of disease and had a significant difference regarding UGE mean value from diabetic patients with (2-<10) years duration of disease. and diabetic patients with (2-<10) years duration of disease had a non significant difference regarding UGE mean value from diabetic patients with 10 years and more duration of disease as shown in the table (3).

Table (1): Biochemical measurement regarding Duration of illness of male T2DM

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(&lt;2) years Mean ± SD</th>
<th>(2-&lt;10) years Mean ± SD</th>
<th>10 years and more Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBS (mg/dl)</td>
<td>289.83±84.47</td>
<td>300.15±101.43</td>
<td>307.17±96.59</td>
</tr>
<tr>
<td>Urine sugar (mg/dl)</td>
<td>578.59±464.54</td>
<td>397.93±454.90</td>
<td>241.14±280.55</td>
</tr>
<tr>
<td>TGL (mg/min)**</td>
<td>312.31±83.46</td>
<td>303.29±101.83</td>
<td>276.51±100.97</td>
</tr>
<tr>
<td>UGE (mg/min)**</td>
<td>17.85±22.52</td>
<td>11.23±18.75</td>
<td>4.66±7.53</td>
</tr>
<tr>
<td>Serum Creatinin (mg/dl)</td>
<td>0.86±0.13</td>
<td>0.96±0.13</td>
<td>1.08±0.14</td>
</tr>
<tr>
<td>GFR (ml/min)**</td>
<td>109.24±14.02</td>
<td>101.98±12.72</td>
<td>89.02±11.39</td>
</tr>
<tr>
<td>Urine Volume (ml/min.)</td>
<td>2.32±1.21</td>
<td>1.89±1.06</td>
<td>1.68±0.68</td>
</tr>
</tbody>
</table>

Table (2): Biochemical measurement regarding Duration of illness of female T2DM

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(&lt;2) years Mean ± SD</th>
<th>(2-&lt;10) years Mean ± SD</th>
<th>10 years and more Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBS (mg/dl)</td>
<td>260.95±70.26</td>
<td>336.31±109.54</td>
<td>328.15±89.25</td>
</tr>
<tr>
<td>Urine sugar (mg/dl)</td>
<td>434.77±509.44</td>
<td>496.88±474.00</td>
<td>279.08±275.44</td>
</tr>
<tr>
<td>TGL (mg/min)**</td>
<td>280.20±93.31</td>
<td>320.00±114.02</td>
<td>290.84±103.43</td>
</tr>
<tr>
<td>UGE (mg/min)**</td>
<td>12.70±19.39</td>
<td>18.46±23.10</td>
<td>5.00±8.08</td>
</tr>
<tr>
<td>Serum Creatinin (mg/dl)</td>
<td>0.77±0.097</td>
<td>0.84±0.10</td>
<td>0.82±0.10</td>
</tr>
<tr>
<td>GFR (ml/min)**</td>
<td>106.36±14.66</td>
<td>95.09±11.42</td>
<td>86.91±12.35</td>
</tr>
<tr>
<td>Urine Volume (ml/min.)</td>
<td>2.20±1.01</td>
<td>2.52±1.47</td>
<td>1.4±0.48</td>
</tr>
</tbody>
</table>
Table (3):- Comparing UGE regarding the duration of illness (ANOVA test)

<table>
<thead>
<tr>
<th>Duration of illness</th>
<th>comparing</th>
<th>Sig. (male)</th>
<th>Sig. (female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Few months up to two years</td>
<td>1,2</td>
<td>0.006</td>
<td>0.425</td>
</tr>
<tr>
<td>Two years up to ten years</td>
<td>2,3</td>
<td>0.228</td>
<td>0.135</td>
</tr>
<tr>
<td>Ten years and more</td>
<td>1,3</td>
<td>0.000</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Table (4):- Comparing TGL regarding the duration of illness

<table>
<thead>
<tr>
<th>Duration of illness</th>
<th>Interval code</th>
<th>Sig. (male)</th>
<th>Sig. (female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Few months up to two years</td>
<td>4,5</td>
<td>0.60</td>
<td>0.130</td>
</tr>
<tr>
<td>Two years up to ten years</td>
<td>5,6</td>
<td>0.164</td>
<td>0.590</td>
</tr>
<tr>
<td>Ten years and more</td>
<td>4,6</td>
<td>0.077</td>
<td>0.248</td>
</tr>
</tbody>
</table>
Fig. 1

**A**
Linear regression analysis between TGL and UGE of T2DM male with few months up to 2 years duration of illness

- $T_{mg} = 304 \text{ mg/dl}$
- $RTglu = 164 \text{ mg/dl}$
- $R^2 = 0.739$

**B**
Linear regression analysis between TGL and UGE of T2DM male with 2 up to 10 years duration of illness

- $T_{mg} = 332 \text{ mg/dl}$
- $TRglu = 168 \text{ mg/dl}$
- $R^2 = 0.562$

**C**
Linear regression analysis between TGL an UGE of T2DM male with 10 years and more duration of illness

- $T_{mg} = 336 \text{ mg/dl}$
- $TRglu = 160 \text{ mg/dl}$
- $R^2 = 0.364$
Fig. 2

Linear regression analysis between TGL and UGE of T2DM female with few months up to 2 years duration of illness

- $T_{mg} = 288\, \text{mg/dl}$
- $T_{Rglu} = 152\, \text{mg/dl}$
- $R^2 = 0.541$

Linear regression between TGL and UGE of T2DM female with 2 up to 10 years duration of illness

- $T_{mg} = 324\, \text{mg/dl}$
- $T_{Rglu} = 162\, \text{mg/dl}$
- $R^2 = 0.730$

Linear regression analysis between TGL and UGE of T2DM female with 10 years and more duration of illness

- $T_{mg} = 320\, \text{mg/dl}$
- $T_{Rglu} = 168\, \text{mg/dl}$
- $R^2 = 0.280$
**Discussion**
At very high filtered glucose loads, the rate of glucose reabsorption reaches a constant maximal value, called the tubular transport maximum (Tm) for glucose. This value increased as the disease progressing, that can be seen in the figures (1,2) this finding is supported by Arakawa et al., 2001; who suggesting that enhanced glucose reabsorption in diabetic mice represents a mechanism that defeats blood glucose homeostasis in the diseased state. Likewise Kamran et al., 1997; who claimed Tm of glucose is greater in animal models with type 1 and type 2 diabetes. While Rahmoune et al., 2005; who demonstrated there are significantly elevated SGLT2 levels and glucose uptake are seen in human exfoliated proximal tubular epithelial cells isolated from the urine of type 2 diabetic individuals with disease progressing. this finding is countered by Bank and Aynedjian, 1990; whom demonstrated that high glucose in the proximal tubule directly stimulates sodium reabsorption via sodium-glucose cotransport and indirectly via increased expression of the sodium-glucose cotransporters, SGLT1 and SGLT2. In this study it was observed the urine glucose excretion till the transport maximum (splay region) for male and female are decreased as the duration of illness increase as shown in the tables (3, 4) that mean the splay value for newly diagnosed Type 2 diabetic patient is more than that for patient with chronic illness this finding can supported by Freitas et al., 2008; who claimed that the Renal SGLT2 mRNA expression has also been reported to be elevated in chronic diabetic rats. Likewise Rahmoune et al., 2005; who demonstrated there are significantly elevated SGLT2 levels and glucose uptake are seen in human exfoliated proximal tubular epithelial cells isolated from the urine of type 2 diabetic individuals with disease progressing.

**Conclusions**

1- Duration of T2DM of both male and female can significantly affect UGE till the $T_{mg}$ ( Splay region ) inversely.

2- Duration of T2DM of both male and female can affect $T_{mg}$ ejectively.

**References**


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