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.

تأثير مدة مرض السكري النمط الثاني على الطرح الكلوي للكلوكوز لغاية الحد الاعلى لامتصاص الكلوكوز

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الملخّص
تتناول هذه الدراسة قياس كمية الكلوكوز المطروح في الادرار لمرضى السكري النمط الثاني مع الاخذ بالاعتبار مدة المرض. لغرض تقييم الاختلاف ، قمنا بدراسة العلاقة بين كمية الكلوكوز في الدم والإدرار لـ 302 مريض وقد تم تصنيف المرضى حسب مدة المرض . وقد تم قياس نسبة الكلوكوز في مصل الدم والإدرار ونسبة الكرياتنين في مصل الدم وكذلك تم حساب معدل الترشيح التنافذي وحمولة الكلوكوز وكمية الكلوكوز في الإدرار. بعدها قمنا بإجراء التحاليل الإحصائية للقيم ومقارنة النتائج للمجاميع حسب التقسيم أعلاه . وقد لوحظ أن القابلية القصوى لاسترجاع الكلوكوز في أنابيب الكلى وكمية الكلوكوز في الإدرار مختلفة بين مرضى السكري النمط الثاني ولغرض تقييم هذا الاختلاف قمنا بتحليل التباين للمجاميع كما قمنا بقياس ا لانحدار الخطي للقيم وقد وجد أن القابلية القصوى لاسترجاع الكلوكوز في أنابيب الكلى تزداد وان كمية الكلوكوز في الإدرار تقل بازدياد المدة 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 measurment

Reaction principle

Glucose + O2 + H2O $\xrightarrow{GOD}$ gluconic acid + H2O2

2H2O2 + 4-aminophenazone + phenol $\xrightarrow{POD}$ quinoneimine + 4H2O

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 (Butller, 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)

$$eCcr = \frac{(140 - age\ (year))X(mass\ (kg))X(0.85\ (if\ female))}{72\ X\ Serum\ creatinine\ mg/dl}$$

2- TGL mg/min. = $\frac{\text{GFR } (\frac{\text{ml}}{\text{min.}})\text{X Serum Glucose Concentration}(\frac{\text{mg}}{\text{dl}})}{100}$

3- UGE mg/min = $\frac{\text{Urine glucose concentration } (\frac{\text{mg}}{\text{dl}})\text{X Urine volume}(\frac{\text{ml}}{min.})}{100}$

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 TGL mean value from diabetic patients with 10 years and more duration of disease as shown in the table (4).

2- Urine Glucose Excretion (UGE)

a- Male with T2DM

It revealed that diabetic patients with (<2) years duration of disease had a 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) duration of disease and had a significant difference regarding UGE mean value from diabetic patients with 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).

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

** mean calculated value	Table (1)		
Parameters	(<2) years (n=46)	(2-<10) years n=101	10 years and more n=39
	Mean ± SD	Mean ± SD	Mean ± SD
RBS (mg/dl)	289.83±84.47	300.15±101.43	307.17±96. 59
Urine sugar (mg/dl)	578.59±464.54	397.93±454.90	241.14±280. 55
TGL (mg/min)**	312.31±83.46	303.29±101.83	276.51±100.97
UGE (mg/min)**	17.85±22.52	11.23±18.75	4.66±7.53
Serum Creatinin (mg/dl)	0.86±0.13	0.96±0.13	1.08±0.14
GFR (ml/min)**	109.24±14.02	101.98±12.72	89.02±11.39
Urine Volume (ml/min.)	2.32±1.21	1.89±1.06	1.68±0.68

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

Table (2)			
Parameters	(<2) years (n=25)	(2-<10) years n=50	10 years and more n=41
	Mean ± SD	Mean ± SD	Mean ± SD
RBS (mg/dl)	260.95±70.26	336.31±109.54	328.15±89.25
Urine sugar (mg/dl)	434.77±509.44	496.88±474.00	279.08±275.44
TGL (mg/min)**	280.20±93.31	320.00±114.02	290.84±103.43
UGE (mg/min)**	12.70±19.39	18.46±23.10	5.00±8.08
Serum Creatinin (mg/dl)	0.77±0.097	0.84±0.10	0.82±0.10
GFR (ml/min)**	106.36±14.66	95.09±11.42	86.91±12.35
Urine Volume (ml/min.)	2.20±1.01	2.52±1.47	1.4±0.48

Table (3):- Comparing UGE regarding the duration of illness (ANOVA test)

Table (3)			
Duration of illness	comparing	Sig. (male)	Sig. (female)
Few months up to two years (1)	1,2	0.006	0.425
Two years up to ten years (2)	2,3	0.228	0.135
Ten years and more (3)	1,3	0.000	0.016

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

Duration of illness	Interval code	Sig. (male)	Sig. (female)
Few months up to two years (4)	4,5	0.60	0.130
Two years up to ten years (5)	5,6	0.164	0.590
Ten years and more (6)	4,6	0.077	0.248
Table (4)			

(ANOVA test)

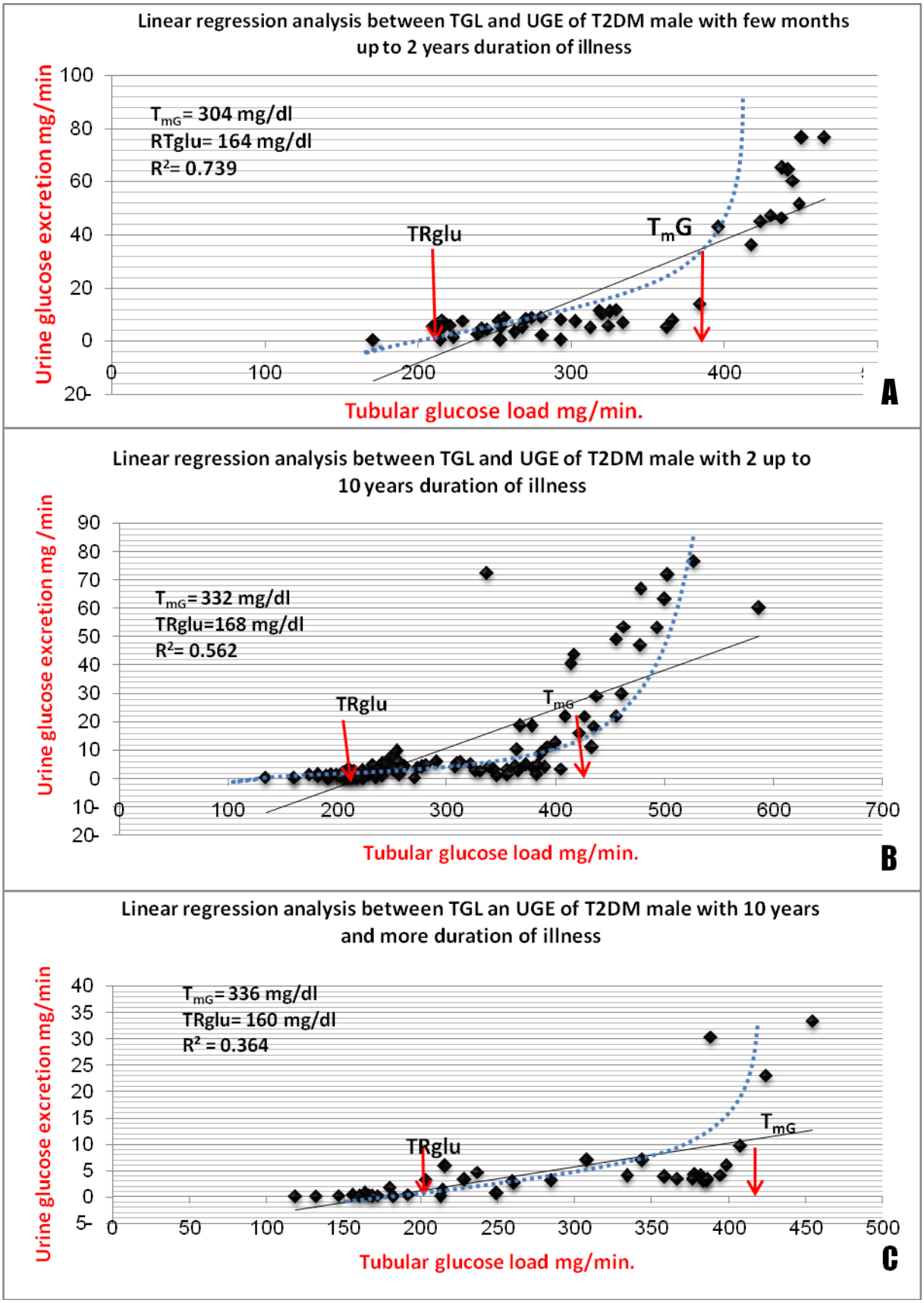
Linear regression analysis between TGL and UGE of T2DM male with few months up to 2 years duration of illness
Urine glucose excretion mg/min
T_{mG}= 304 mg/dl
RTglu= 164 mg/dl
R^2= 0.739
TRglu
T_mG
Tubular glucose load mg/min.
A
Linear regression analysis between TGL and UGE of T2DM male with 2 up to 10 years duration of illness
Urine glucose excretion mg /min
T_{mG}= 332 mg/dl
TRglu=168 mg/dl
R^2= 0.562
TRglu
T_{mG}
Tubular glucose load mg/min.
B
Linear regression analysis between TGL an UGE of T2DM male with 10 years and more duration of illness
Urine glucose excretion mg/min
T_{mG}= 336 mg/dl
TRglu= 160 mg/dl
R^2 = 0.364
TRglu
T_{mG}
Tubular glucose load mg/min.
C

Fig. 1

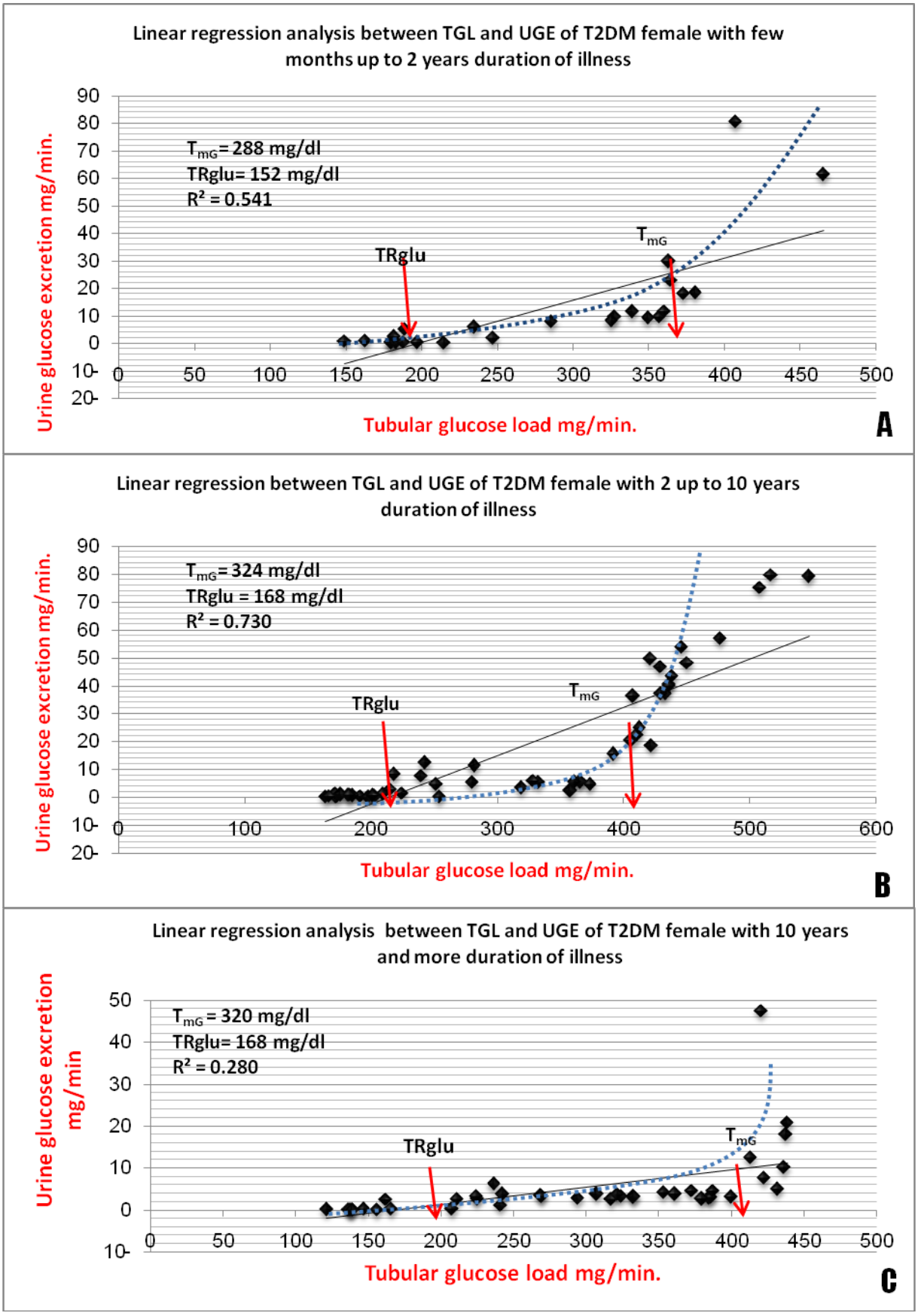
Linear regression analysis between TGL and UGE of T2DM female with few months up to 2 years duration of illness
T_{mG}= 288 mg/dl
TRglu= 152 mg/dl
R^2 = 0.541
TRglu
T_{mG}
Urine glucose excretion mg/min.
Tubular glucose load mg/min.
A
Linear regression between TGL and UGE of T2DM female with 2 up to 10 years duration of illness
T_{mG}= 324 mg/dl
TRglu = 168 mg/dl
R^2 = 0.730
TRglu
T_{mG}
Urine glucose excretion mg/min.
Tubular glucose load mg/min.
B
Linear regression analysis between TGL and UGE of T2DM female with 10 years and more duration of illness
T_{mG} = 320 mg/dl
TRglu= 168 mg/dl
R^2 = 0.280
TRglu
T_{mG}
Urine glucose excretion mg/min
Tubular glucose load mg/min.
C

Fig.2

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) inversively.
2- Duration of T2DM of both male and female can affect T_{mG} ejectively.

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