Original Research Article

Significance of Serum Relaxin in Diabetic and Non-Diabetic Patients with Chronic Kidney Disease

Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both. The aim of the work is to evaluate significance of serum relaxin in diabetic and non-diabetic patients with chronic kidney disease. 60 subjects aged from (18years to 60 years) in internal medicine department at Tanta University hospital (outpatient – inward). This study was carried out from between March 2020 to March 2021. There was insignificant difference between groups according to age There was an inverse significant correlation between serum relaxin level and HBA1C in group 2 .but not in group 3 . There was an inverse significant correlation between serum relaxin level and creatinine in group 2 .and in group 3. the best cut of level of relaxin hormone in discriminating normal individuals fron CKD patients was 150 ng/dl with an area under the curve(AUC) of 0,988 yeilding sensitivity of 93%, specifity of 95% , positive predictive value (PPV) 97%, negative predictive value(NPV) 86% and accuracy of 93%. Serum RLX levels are significantly lower in patients with CKD than healthy subjects . Also, they are significantly lower in diabetic patients compared to non-diabetic patients.. This study highlights that RLX may be a valuable therapeutic strategy for limiting the progression of established fibrosis in diabetic nephropathy. Being a naturally occurring physiological hormone, RLX has an excellent safety profile with potentially fewer side effects than conventional treatments. However, this needs further work.

Keywords: Serum Relaxin ,Diabetic , Chronic Kidney Disease

Introduction:

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both (1).

Chronic hyperglycemia in synergy with the other metabolic aberrations in diabetic patients can cause damage to various organ systems, leading to the development of disabling and life-threatening health complications, most prominent of which are micro vascular (retinopathy, nephropathy, and neuropathy) and macro vascular complications leading to a 2-fold to a 4-fold increased risk of cardiovascular diseases. In 2015, the International Diabetic Federation estimated that the prevalence of diabetes was 8.8% from ages 20 to 79 years affecting a population of approximately 440 million people (2).

One of the most important clinical features of diabetes is its association with chronic tissue complications. A short-term increase in hyperglycemia does not result in serious clinical complications. The duration and severity of hyperglycemia is the major causative factor in initiating organ damage. Early morphological signs of renal damage include nephromegaly and a modified Doppler, but the degree of damage is best ascertained from proteinuria and Glomerular filtration rate (GFR) (2-3).

The average incidence of diabetic nephropathy is high (3% per year) during the first 10 to 20 years after diabetes onset. Typically, it takes 15 years for small blood vessels in organs like kidney, eyes and nerves to get affected. It

is estimated that more than 20 and up to 40% of diabetic patients will develop chronic kidney disease (CKD) depending upon the population, with a significant number that develop end stage renal disease (ESRD) requiring renal replacement therapies such as kidney transplantation. Incidentally, diabetes with no clinical sign of kidney damage during the initial 20 to 25 years is significantly less likely (1% a year) to cause major renal complication later in life (4-6).

Relaxin (RLX) is mainly known as a reproductive hormone which is produced by the corpus luteum and/or placenta in many species. The relaxin peptide family in humans consists of seven members, relaxin-1, -2 and -3 and insulin-like (INSL) peptides 3, 4, 5 and 6. (7-8).

Relaxin regulates cardiovascular (CV) functions modulating blood pressure (BP), inflammation, cell injury/death, fibrosis, and angiogenesis. It also induces vasodilation ameliorating endothelial dysfunction in hypertension (HTN) (9).

The aim of the work is to evaluate significance of serum relaxin in diabetic and non-diabetic patients with chronic kidney disease.

Patients and Methods:

The study was carried out on: 60 subjects aged from (18years to 60 years) in internal medicine department at Tanta University hospital (outpatient –

inward). This study was carried out from between March 2020 to March 2021. Study design: It is cross-sectional study. Study Approval:

(A) Ethics:

Permission obtained from Research Ethics Committee as a part of Quality Assurance Unit in Faculty of Medicine at Tanta University to conduct this study and to use the facilities in the hospital.

Any unexpected risks appeared during the course will be cleared to participants and the ethical committee on time.

(B) Consent:

Informed written consent was obtained from all patients after full explanation of benefits and risks of the study. Privacy of all patients' data is granted by a special code number for every patient file that includes all investigations.

Inclusion criteria:

1. Patients with chronic kidney disease with diabetes.
2. Patients with chronic kidney disease without diabetes.
3. Age from 18-60 years.

 Exclusion criteria:

1. Auto-immune diseases (SLE, thyroiditis)
2. Liver diseases.
3. Pregnancy.
4. Type 1 diabetes mellitus.

5. Patients with eGFR<15 ml/min/1.73 m2 or on dialysis.

- Subjects will be divided into three groups:
- Group (1): 20 healthy individuals (control group).
- Group (2): 20 chronic kidney disease patients with diabetes.
- Group (3): 20 chronic kidney disease patients without diabetes.
- All included cases will be subjected to the following:

1. Complete history taking.
2. Complete clinical examination.
3. Laboratory investigation:

- Blood glucose level (fasting – 2 hours postprandial).
- HbA1c.
- Serum creatinine.
- eGFR (will be calculated using MDRD equation).
- GFR (mL/min/1.73m2) =
- Serum relaxin level.

- Methodology:
- Principle of the assay:

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for RLN2 has been pre-coated onto a micro plate. Standards and samples are pipetted into the wells with a Horseradish

Peroxidase (HRP) conjugated antibody specific for RLN2. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of RLN2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

- Sample collection and storage:

Serum Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g, 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

- Measurement of Serum Relaxin Using ELISA Technique:

The Quantikine® ELISA Human Relaxin–2 Immunoassay (R & D Systems; USA & Canada R & D Systems, Inc.) in a 4.5 hour solid phase ELISA designed to measure human Relaxin in cell culture supernates, serum, and plasma. It contains E. coil–expressed recombinant human Relaxin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Relaxin showed linear curves that were parallel to the

standard curves obtained using the Quantikine standards. These results indicated that the Quantakine Human Relaxin kit can be used to germinate relative mass values for naturally occurring human Relaxin.

- Regent preparation:

Kindly use graduated containers to prepare the reagent. Bring all reagents to room temperature (18-25°C) before use for 30min. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. Wash Buffer(1x):

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 300 ml of Wash Buffer (1 x).

2. Standard

Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute each lyophilized Standard with 0.5 ml of ddH2O. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to use.

- Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the Ziploc, store unused wells at 4°C.

3. Add 50µl of Standard or Sample per well. Standard need test in duplicate.

4. Add 50µl of HRP-conjugate to each well. Mix well and then incubate for 1 hour at 37°C.

5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 50µl of Substrate A and 50µl of Substrate B to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

7. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing. 8. Determine the optical density of each well within 10 minutes, using a micro plate reader set to 450 nm..

Statistical analysis:

The sample size was calculated using Epi-Info software statistical package created by World Health organization and center for Disease Control and Prevention, Atlanta, Georgia, USA version 2002. The criteria used for sample size calculation (n>33) were 95% confidence limit, 80% power of the study, expected outcome in in treatment group 90% compared to 60% for control groups.

Analysis of data were performed by SPSS v25 (SPSS Inc., Chicago, IL, USA). Quantitative parametric variables (e.g. age) were presented as mean and standard deviation (SD). They were compared between the two groups by unpaired student's t- test and within the same group by paired T test. Quantitative non-parametric variables (e.g. VAS) were presented as median and range and compared between the two groups by Mann Whitney (U) test and within the same group by Wilcoxon test. P value < 0.05 was considered significant.

Results:

This study was conducted in Tanta University hospital on 60 subjects.

Subjects divided to 3 groups.

- **Group 1** consists of 20 healthy subjects (control group) age ranged from 29-60 years old with a mean value of 50.90±8.58.
- **Group 2** consists of 20 chronic kidney disease (CKD) diabetic patient age ranged from 36-60 years old with amean value of 51.25±7.28.
- **Group 3** consists of 20 chronic kidney disease (CKD) non-diabetic patients age ranged from 33-60 years old with amean value of 52.20±7.34.

There was insignificant difference between groups according to age (p=0.861).Table (1)

Table (1): Age distribution in studied groups

Groups	Age (years)		ANOVA	
	Range	SD ± Mean	F	P-value
Group 1	**29-60**	**50.90±8.58**	**0.150**	**0.861**
Group 2	**36-60**	**51.25±7.28**		
Group 3	**33-60**	**52.20±7.34**		

* Statistically significant at $p \leq 0.05$

F (ANOVA)

Gender distribution in studied groups

- **Group 1:** (healthy) included 8 females (40 %) and 12 males (60 %).
- **Group 2:** (CKD Diabetic patients) included 6 females (30%) and 14 males (70%).
- **Group 3**: (CKD non-Diabetic patients) included 8 females (40 %) and 12 males (60 %).

There was insignificant difference according to gender (P = 0.750).Table (2)

Table (2): Gender distribution in studied groups

	Sex	Group 1	Group 2	Group 3	Total
Male	**N**	12	14	12	38
	%	60.0%	70.0%	60.0%	63.3%
Female	**N**	8	6	8	22
	%	40.0%	30.0%	40.0%	36.7%
Total	**N**	20	20	20	60
	%	100.0%	100.0%	100.0%	100.0%
Chi-square	**x^2****				**0.574**
	P-value				**0.750**

* Statistically significant at p ≤ 0.05

x^{2**}(Chi square test)

Table (3): Clinical Data of the studied groups**.**

		Range			Mean	±	S. D	F. test	p. value		
BMI (kg/ m²)	**Group 1**	23	–	38	29.92	±	4.48	1.014	0.369	P1	0.271
	Group 2	25	–	40	31.53	±	4.76			P2	0.190
	Group 3	24.8	–	40	31.84	±	4.45			P3	0.831
Systolic BP (mmHg)	**Group 1**	90	–	140	111.75	±	14.07	5.136	0.009*	P1	0.017*
	Group 2	90	–	220	135.00	±	35.02			P2	0.004*
	Group 3	90	–	220	141.00	±	37.42			P3	0.573
Diastolic	**Group 1**	60	–	90	77.75	±	9.93	2.995	0.048*	P1	0.026*

BP (mmHg)	**Group 2**	60	–	180	90.25	±	24.84			P2	0.061
	Group 3	60	–	110	88.25	±	13.70			P3	0.717
Disease duration (yr.)	**Group 1**							T: 1.381	0.175		
	Group 2	1	–	10	5.0	±	2.35				
	Group 3	1.5	–	8	4.10	±	1.72				

BMI=body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure

P1 comparison between group 1&2

P2 comparison between groyp 1&3

P3 comparison between group 2&3

* Statistically significant at $p \leq 0.05$

F (ANOVA)

Table (3) showed the clinical data of all studied subjects, there was no significant difference according to BMI and disease duration (P > 0.05).

There was a significant difference among group 1, 2 and 3 according to SBP and DBP.

SBP is significally higher in group 2 & 3, there was significant differance between group 1(control group) and group 2 (CKD diabetic patient) (P =0.017) and between group 1(control group) and group 3(CKD non diabetic patients)(P =0.004) but there was no significant difference between group 2(CKD diabetic patient) & 3(CKD non diabetic patient) (P =.573).

DBP is significally higher in group 2 & 3, there was significant difference between group 1 (control) and group 2 (CKD diabetic patient) (P=.026) but there was no significance difference between group 1(control) and group 3(CKD non diabetic patients) (P =.061)and also no significant difference between group 2 (CKD diabetic patients)&group 3(CKD non diabetic patients) (P= .717)

Table (4): Laboratory investigations of the studied groups.

				Range	Mean	±	S. D	F. test	p. value		
Hb (g/dl)	**Group 1**	10	–	14.1	11.83	±	1.26	1.006	0.372	P1	0.161
	Group 2	9	–	13	11.22	±	1.38			P2	0.474
	Group 3	8	–	15	11.52	±	1.43			P3	0.488
Fasting Blood Glucose (mg/dl)	**Group 1**	75	–	98	85.55	±	6.54	297.50	0.001*	P1	0.001*
	Group 2	140	–	225	184.00	±	20.69			P2	0.328
	Group 3	70	–	120	90.05	±	12.35			P3	0.001*
Post Prandial blood glucose (mg/dl)	**Group 1**	78	–	128	99.45	±	13.92	161.23	0.001*	P1	0.001*
	Group 2	200	–	370	270.40	±	50.36			P2	0.032*
	Group 3	90	–	175	122.15	±	21.85			P3	0.001*
HbA1C (%)	**Group 1**	4	–	6	5.05	±	0.68	43.006	0.001*	P1	0.001*
	Group 2	4.6	–	9.6	8.03	±	1.17			P2	0.001*
	Group 3	5.3	–	8.7	6.74	±	1.13			P3	0.001*
Serum albumin (gm/dl)	**Group 1**	3.8	–	5.2	4.40	±	0.36	1.570	0.217	P1	0.452
	Group 2	3.5	–	5.2	4.28	±	0.51			P2	0.083
	Group 3	3.2	–	5.3	4.12	±	0.61			P3	0.317

Hb=Hemoglobin, HbA1C= Hemoglobin A1C

P1 comparison between group 1&2

P2 comparison between groyp 1&3

P3 comparison between group 2&3

* Statistically significant at $p \leq 0.05$

F (ANOVA)

Table (6): showed Laboratory investigations of the studied groups.

There was no significant difference according to HB and serum albumin level ($P > 0.05$).

Table (5): Kidney function tests of the studied groups

		Range			Mean	±	S. D	F. test	p. value		
Creatinine(mg/dl)	**Group 1**	0.5	–	1.4	0.90	±	0.27	74.307	0.001*	P1	0.001*
	Group 2	2.5	–	6.2	4.14	±	1.07			P2	0.001*
	Group 3	1.9	–	6	3,71	±	1.13			P3	0.036*
Urea(mg/dl)	**Group 1**	18	–	42	28.90	±	7.30	142.748	0.001*	P1	0.001*
	Group 2	57	–	88	70.10	±	9.87			P2	0.001*
	Group 3	49	–	76	62.94	±	7.28			P3	0.008*
GFR (mL/min/1.73m^2)	**Group 1**	92	–	115	101.76	±	6.99	39.951	0.001*	P1	0.001*
	Group 2	18	–	82	48.70	±	25.78			P2	0.001*
	Group 3	21	–	80	56.60	±	22.74			P3	0.222

GFR=glomerular filteration rate

P1 comparison between group 1&2

P2 comparison between group 1&3

P3 comparison between group 2&3

* Statistically significant at $p \leq 0.05$

F (ANOVA)

Table (5) showed kidney function tests of the studied groups, There was a significant difference between the studied group according to blood urea and serum creatinine and GFR. The highest value of urea and creatinine were in group 2 and the lowest values were in group 1.But according to GFR the highest value in group 1 and lowest value in group 2.

There was a significant increase in Serum Creatinine in group 2 (CKD diabetic patient) compared to group 1(control)(P=.001) and a significant increase in group 3(CKD non diabetic patient) compared to group 1(control)(P=.001) and there was also significant difference between group 2(CKD diabetic patient) and group 3(CKD non diabetic patient) (P=.0.036*).

There was a significant increase in serum urea in group 2 (CKD diabetic patient) compared to group 1(control)(P=.001) and a significant increase in group 3(CKD non diabetic patient) compared to group 1(control)(P=.001) and between group 2(CKD diabetic patient) and group 3(CKD non diabetic patient) (P=.008).

There was a significant decrease in GFR in group 2 (CKD diabetic patient) compared to group 1(control)(P=.001) and significant decrease in group 3(CKD non diabetic patient) compared to group 1(control)(P=.001) but there was no significance between group 2(CKD diabetic patient) and group 3(CKD non diabetic patient) (P=.222).

Table (6): Serum Relaxin level within the studied groups.

		Range			Mean	±	S. D	F. test	p. value		
S. Relaxin	**Group 1**	144	–	318	252.6	±	55.59	126.486	0.001*	P1	0.001*
	Group 2	40	–	115	74.00	±	20.93			P2	0.001*
	Group 3	88	–	170	130.85	±	20.45			P3	0.001*

P1 comparison between group 1&2

P2 comparison between groyp 1&3

P3 comparison between group 2&3

* Statistically significant at $p \leq 0.05$

F (ANOVA)

Table (6) showed Serum relaxin level within the studied groups, there was a significant difference between the studied group according to serum relaxin.

The highest value was in group 1 and lowest value was in group 2. There was a significant decrease in Serum Relaxin in group 2 (CKD diabetic patient) compared to group 1(control)(P=.001) and also significant decrease in group 3(CKD non diabetic patient) compared to group 1(control)(P=.001) and also in group 2(CKD diabetic patient) combared to group 3(CKD non diabetic patient) (P=.001).

Table (7): Relation between serum Relaxin and other parameters within the studied groups.

	S. Relaxin			
With	**Group 2**		**Group 3**	
	r **	P	r **	p
Age (yr.)	-0.446	0 .049*	-0.085	0.722
Hb (g/dl)	0.250	0.288	0.361	0.118
BMI (kg/ m²)	0.074	0.756	-0.009	0.971
SBP(mmHg)	-0.643	0.002*	-0.656	0.002*
DBP(mmHg)	-0.338	0.145	-0.586	0.007*
Fasting blood glucose (mg/dl)	0.061	0.797	0.425	0.062
Post prandial blood glucose (mg/dl)	-0.142	0.551	0.275	0.241
HbA1c (%)	-0.493	0.027*	-0.212	0.368
.Serum albumin (g/dl)	-0.170		-0.171	

		0.473		0.470
Creatinine (mg/dl)	-0.437	0.042*	-0.705	0.001*
Urea (mg/dl)	-0.527	0.017*	-0.532	0.016*
Disease duration (yr.)	-0.635	0.003*	-0.493	0.027*
GFR (mL/min/1.73m^2)	0.791	0.001*	0.719	0.001*

BMI=body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, Hb=Hemoglobin, HbA1C= Hemoglobin A1C

* Statistically significant at $p \leq 0.05$

**r: Pearson correlation

Table (7) showed relation between serum relaxin and other parameters within group 2(CKD diabetic patient) and group 3(CKD non diabetic patient). There was no correlation between serum relaxin level and different parameters in group 2 and 3 with regard, HB, BMI, serum albumin, fasting blood glucose and post prandial blood glucose. There was an inverse significant correlation between serum relaxin level and age in group 2 (P = 0.049) but not in group 3 (P = 0.722). There was an inverse significant correlation between serum relaxin level and SBP in group 2 (P = 0.002) and in group 3 (P = 0.002). There was an inverse significant correlation between serum relaxin level and DBP in group 3 (P = 0.007) but not in group 2 (P = 0.145). There was an inverse significant correlation between serum relaxin level and HBA1C in group 2 (P = 0.027) but not in group 3 (P = 0.368). There was an inverse significant correlation between serum relaxin level and creatinine in group 2 (P = 0.042) and in group 3 (P = 0.001). There was an inverse significant correlation between serum relaxin level and urea in group 2 (P = 0.017) and in group 3 (P = 0.016). There was an inverse significant correlation between serum relaxin level and disease duration in group 2 (P = 0.003) and in group 3 (P = 0.027). There was a positive correlation between serum relaxin level and GFR in group 2 (P = 0.001)and in group 3(P = 0.001).

Table (8): Sensitivity, specificity for serum relaxin in healthy control subjects & CKD cases.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
S. Relaxin	150	0.988	93	95	97	86	93

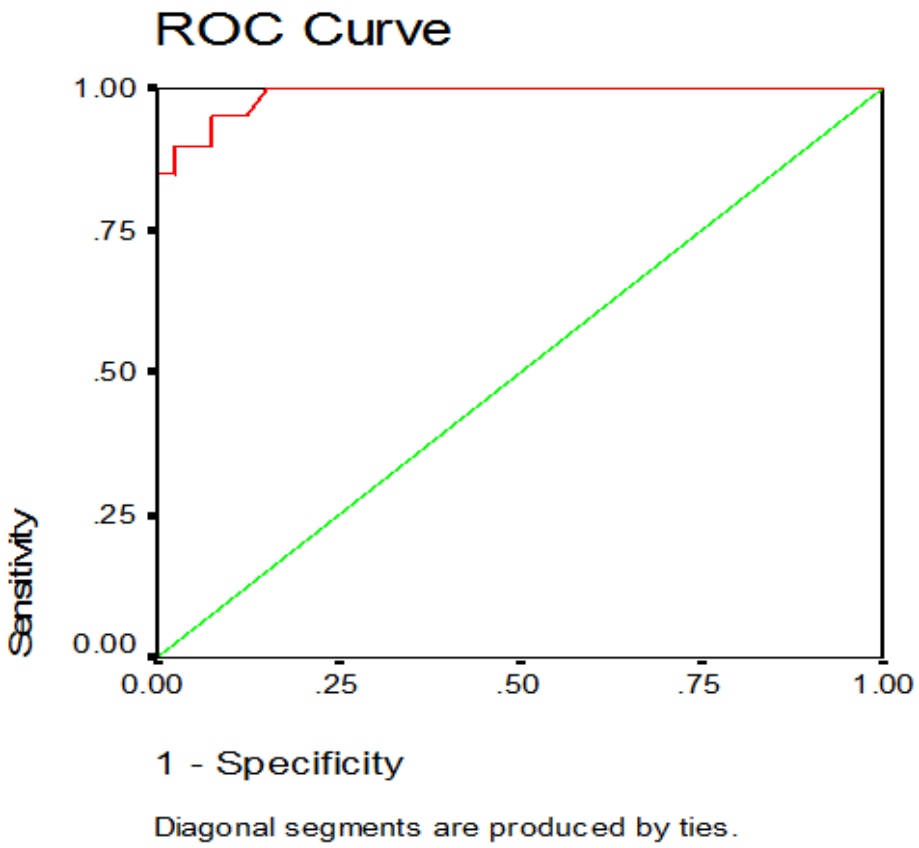

Figure 1): ROC curve between healthy control group & both (diseased) groups (CKD patients).

AUC: Area under the curve.

PPV; Postive predictive value.

NPV; Negative predictive value.

Table (9): Sensitivity, specificity for serum relaxin in healthy control subjects & CKD non diabetic patients.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
S. Relaxin	160	0.976	95	90	90	95	93

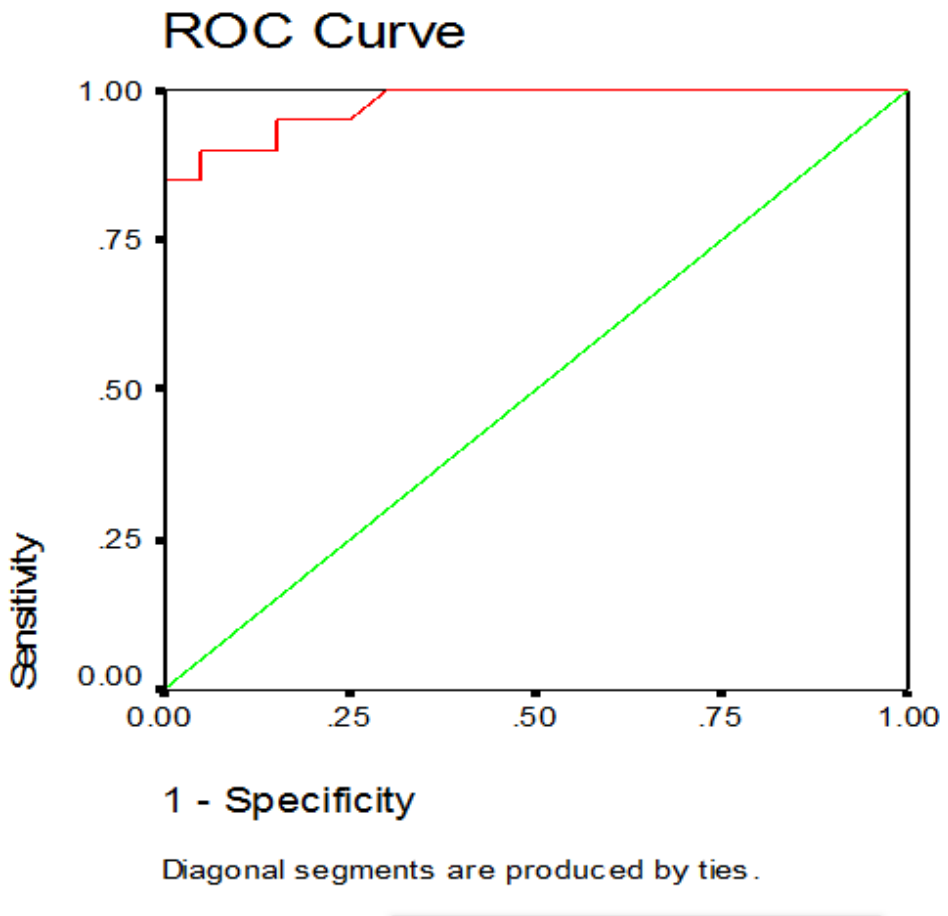

Figure (2): ROC curve between healthy control group & CKD non diabetic patient.

AUC: Area under the curve.

PPV; Postive predictive value.

NPV; Negative predictive value.

Table (10): Sensitivity, specificity for serum relaxin in CKD diabetic patients & CKD non diabetic patients.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
S. Relaxin	110	0.963	90	85	86	89	88

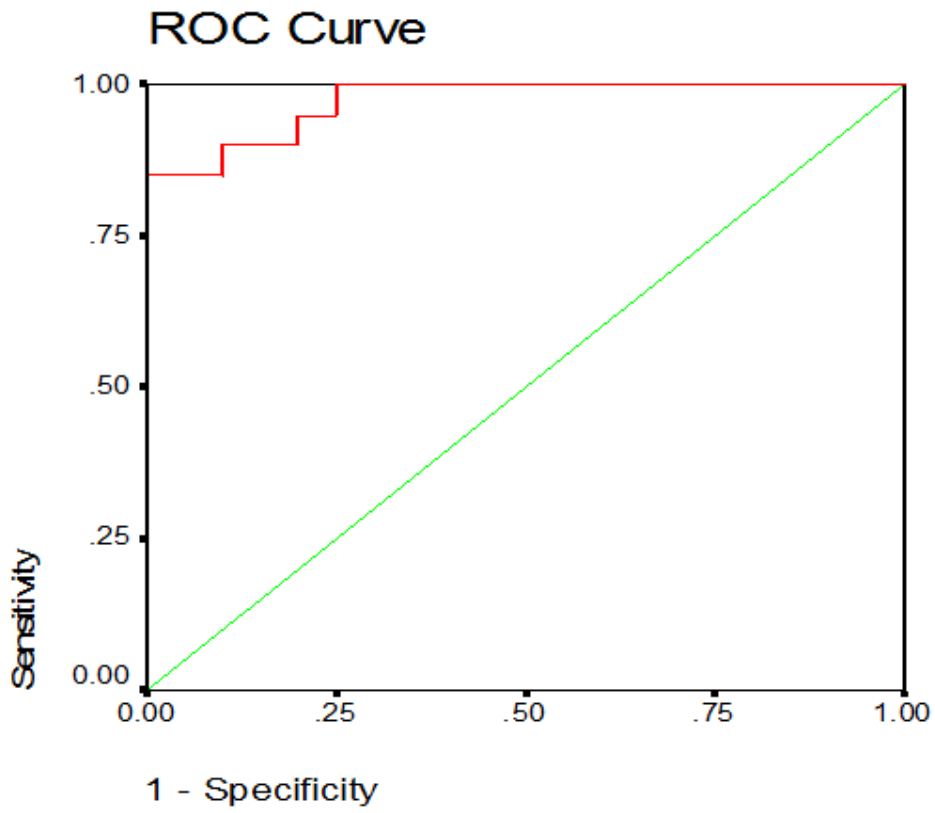

Figure (3): ROC curve between CKD diabetic patients & CKD non diabetic patient.

AUC: Area under the curve.

PPV; Postive predictive value.

NPV; Negative predictive value.

Table (8-10) showed Sensitivity, Specificity for serum relaxin in the 3 groups.

Interestingly, ROC curve (figure 2) illustrated the sensitivity and specificity of serum relaxin in healty control subjects and all CKD cases (group 2 and 3), the best cut of level of relaxin hormone in discriminating normal individuals fron CKD patients was 150 ng/dl with an area under the curve(AUC) of 0,988 yeilding sensitivity of 93%, specifity of 95% , positive predictive value (PPV) 97%, negative predictive value(NPV) 86% and accuracy of 93%.

Furthermore by ROC analysis of relaxin hormone in healthy control subjects and non diabetic CKD patients, the best cut off level of relaxin in discriminating normal individuals from non diabetic CKD patients was 160 ng/dl with an area under the curve (AUC) 0,976 yeilding sensitivity 95% ,specifity 90%, positive predictive value (PPV) 90% , negative predictive value 95 % and accuracy 93% (figure2).

Finally by ROC analysis of relaxin hormone in diabetic CKD patients and non-diabetic CKD patients the best cut off level of relaxin in discriminating non diabetic CKD patients from diabetic CKD patients was 110 ng/dl with an area under the curve (AUC) 0,963 yeilding sensitivity 90% ,specifity 85% ,positive predictive value (PPV) 86% , negative predictive value 89% and accuracy 88% (figure29).

DISCUSSION

Diabetes mellitus is a major health problem and a chronic metabolic disease. Diabetes mellitus (DM) is characterized by hyperglycemia due to a total or relative lack of insulin secretion and insulin resistance or both. The metabolic abnormalities involve carbohydrate, protein, and fat metabolism. DM affects all age groups but is more common in adults [10].

Renal damage is a major microvascular diabetic complication that could lead to death of diabetic patients. Hyperglycemia induces chronic kidney disease and renal damage by activation of protein kinase C, increased production of advanced glycosylation end products (AGEs), and diacylglycerol synthesis [11].

Glomerular filtration rate (GFR) is an ideal marker of kidney function. But its measuring is time consuming, and it is usually estimated from equations that depend on serum creatinine and cystatin C [12].

Microalbuminuria is a noninvasive biomarker available for the diagnosis of diabetic nephropathy. However, some patients with microalbuminuria have advanced renal pathological changes for which therapy is less effective than earlier stages of the disease. Therefore, novel biomarkers for earlier diagnosis of diabetic nephropathy are crucially required13

Relaxin (RLX) is a naturally occurring hormone that inhibits organ fibrosis. Normally associated with reproduction, RLX has been implicated in a number of pregnancy related functions, including softening the cervix and vagina at delivery, inhibiting cell apoptosis, and decreasing the total peripheral resistance through vasodilation [14].

The physiological actions of RLX may also have important implications elsewhere, having been shown repeatedly to inhibit excessive collagen accumulation in various cell culture and animal models of fibrosis.[15]

It was recognized that relaxin also plays a role in the cardiovascular system. Patients with chronic heart failure have increased myocardial relaxin gene expression and elevated plasma relaxin concentrations [16].

Relaxin stimulates cardiac ANP secretion and increases coronary blood flow through a nitric oxide-mediated mechanism [17]. It was furthermore shown that relaxin is a vasodilator of small systemic resistance arteries [18]. Relaxin is also involved in the regulation of cardiac[19] and renal collagen synthesis [20].

For this reason, our study was conducted to evaluate significance of serum relaxin in diabetic and non-diabetic patients with chronic kidney disease.

To the best of our knowledge, there is lack of human studies that evaluated the level of relaxin in both diabetic and non-diabetic CKD patients and one previous study has assessed the role of relaxin in diabetic and non-diabetic CKD patients as compared with the healthy controls.

This study was conducted in Tanta University hospital on 60 subjects. Subjects divided to 3 groups; group 1 that included 20 healthy subjects, group 2 that included 20 chronic kidney disease (CKD) diabetic patient and group 3 that included 20 chronic kidney disease (CKD) non diabetic patients.

In our study, there were no significant differences between all studied groups regarding demographic data (Age, sex and BMI), which ensures the comparability of the groups for further comparisons.

In our study, there is no statistically significant difference in the hemoglobin level and serum albumin level between the cases in the three studied groups.

This was in accordance with **Lee et al. (2020)** who showed that there was no statistically significant difference in the mean hemoglobin level, incidence of anemia and albumin level between the diabetic and non-diabetic CKD patients included in their study [21].

This disagreed with **Zhang et al. (2017)** who found that the incidence of anemia was higher in CKD patients with DM than in patients with CKD alone [22.

The difference could be explained due to small sample size among the cases included in our study.

In our study, both SBP and DBP were statistically significantly higher in the diabetic and non-diabetic CKD groups as compared to the control group. However, there was no

statistically significant difference in the mean SBP and DBP between the diabetic and non-diabetic CKD groups.

This agreed with **Abdelatti et al. (2014)** who included Sixty patients with chronic kidney disease and were divided into two groups: group 1 (30 patients with creatinine clearance of less than 30 ml/minute), and group 2 (30 patients with creatinine clearance of more than 30 ml/minute). Twelve patients (40%) had diabetes mellitus type 2 in group 1 and 18 (60%) in group 2. In addition, group 3 (20 healthy controls), compatible for age and gender, was included. They showed that there is no statistically significant difference in both SBP and DBP between diabetic and non-diabetic severe CKD patients and both groups had statistically significantly higher values as compared with the healthy control group [(23)].

This disagreed with **Zhang et al. (2017)** and **Ito et al. (2012)** who showed that both systolic blood pressure and the percentage of patients with hypertension-related complications were higher in CKD patients with DM than in those without DM [(141)]. Increased blood volume and vascular resistance resulting from insulin resistance in DM might contribute to the development of hypertension [(24)]. In patients with both DM and CKD, additional factors, such as sympathetic stimulation, renin–angiotensin–aldosterone system activation, water–sodium retention, and reductions in levels of vasoactive substances, may contribute to elevated blood pressure[(146)].

In our study, the mean serum creatinine and serum urea was significantly higher in the diabetic CKD patients compared to the other groups . However GFR was statistically significantly lower in the diabetic and non-diabetic CKD groups as compared to the control group without no statistically significant difference between the two groups.

This agreed with **Zhang et al. (2017)** who found that 24-h urinary protein and serum creatinine were higher in CKD patients with DM than in those without DM ($P < 0.001$), that indicating more severe kidney affection in the diabetic group [(25)].

In our study, was a significant decrease in serum relaxin in group 2 (CKD diabetic patient) compared to group 1(control)(P=.001) and between group 3(CKD non diabetic patient) compared to group 1(control)(P=.001) and between group 2(CKD diabetic patient) and group 3(CKD non diabetic patient) (P=.001)

This agreed with **Abdelatti et al. (2014)** who showed that RLX was significantly lower in diabetic patients compared to non-diabetics in both groups [(26)].

In line with our work, **Szepietowska et al.** proved that there was a positive correlation between RLX concentration and insulin sensitivity, meaning that in diabetic patients with high insulin resistance and low insulin sensitivity, RLX hormone level decreased [(27)].

Despite this, the study has some limitations as the small sample size included in each group and being a single center study, which decreases the power of the obtained results. Also, the study lacks the presence of a diabetic only group (Without CKD) that could present an additional diagnostic value in the patients with diabetes by early identification of their kidney affection.

Conclusions:

Serum RLX levels are significantly lower in patients with CKD than healthy subjects . Also, they are significantly lower in diabetic patients compared to non-diabetic patients.. This study highlights that RLX may be a valuable therapeutic strategy for limiting the progression of established fibrosis in diabetic nephropathy. Being a naturally occurring physiological hormone, RLX has an excellent safety profile with potentially fewer side effects than conventional treatments. However, this needs further work.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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