

Serum Relaxin and Renal Vascular Resistance in Diabetic and Non-Diabetic Patients with Different Grades of Chronic Kidney Disease

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Abstract Objectives: To evaluate serum relaxin and renal vascular resistance in diabetic and non-diabetic patients with different grades of chronic kidney disease and to correlate serum relaxin to various clinical and Doppler renal hemodynamic parameters. **Materials and methods:** Sixty patients with chronic kidney disease 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. Resistive index and pulsatility index were measured by Doppler ultrasonography of renal arteries. Serum relaxin was measured with ELISA method, and correlated with clinical and Doppler parameters. **Results:** Serum relaxin levels of groups 1 and 2 were significantly lower than group 3 and that of group 1 were significantly lower than group 2. Resistive index and pulsatility index of groups 1 and 2 were significantly higher than group 3, but there was no significant difference between group 1 and 2. There was an inverse correlation between serum relaxin levels, and serum creatinine in groups 1 and 2. In contrast, there was a positive correlation between serum relaxin and creatinine clearance in groups 1 and 2. There was an inverse correlation between serum relaxin levels, and both resistive index and pulsatility index, in group 2 but not in group 1. Finally, serum relaxin was significantly lower in diabetic compared to non-diabetic patients. **Conclusions:** We found that serum relaxin levels are significantly lower in patients with advanced stages of chronic kidney disease, as well as, in diabetic compared to non-diabetic patients. Also, serum relaxin is negatively correlated with patients' age, resistive index and pulsatility index in patients with chronic kidney disease.

Keywords: relaxin, diabetes mellitus, renal vascular resistance, diabetic nephropathy

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1. Introduction

Relaxin (RLX) is a naturally occurring hormone that inhibits organ fibrosis. Normally, it is associated with reproduction, and has been implicated in a number of pregnancy related functions. 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 [1,2]. Collagen accumulation is often a balance between synthesis and degradation by collagenases/gelatinases, RLX having a role in both processes. Secretion and activity of a variety of Matrix Metalloproteinases (MMPs), including MMP-1 and its rat

analog MMP-13, MMP-2, and MMP-9, are increased by RLX in several situations, whereas expression of their tissue inhibitors Tissue Inhibitor of Metalloproteinases (TIMPs) is decreased. In addition, RLX limits the *de novo* synthesis of collagen by inhibiting both fibroblast proliferation and expression of Smooth Muscle Actin (α -SMA) [3,4].

In the female, circulating RLX is produced by the ovarian luteal cells. In the male, RLX is produced by the prostate and is found in seminal fluid, but is not generally detected in the circulation. However, recent evidence suggests that relaxin may be produced locally to act in an autocrine or paracrine manner in some tissues, including kidneys [5,6].

Growing evidence suggests that the kidney is both a therapeutic target and potential source of relaxin.

Although the expression of renal relaxin is low, endogenous relaxin appears to play an important role in connective tissue homeostasis within the kidney, whereas exogenous relaxin has been shown to consistently and rapidly abrogate renal fibrosis at many levels, primarily through an ability to interfere with the actions of Transforming Growth Factor β -1 (TGF β -1). Furthermore, the vasodilatory and angiogenic properties of relaxin, in addition to its ability to improve renal function in humans, have contributed to its therapeutic significance in renal disease [7].

While the kidney and other vascular organs are known to dilate during pregnancy, the factors responsible for this dilation were poorly understood, until Conrad and co-workers elucidated that relaxin was the hormone involved in renal vasodilation and hyperfiltration. Relaxin was shown to increase effective renal plasma flow and Glomerular Filtration Rate (GFR), attenuate the renal circulatory response to angiotensin II and reduce plasma osmolality regardless of gender [8].

Doppler ultrasonography was used to assess intrarenal hemodynamics. Resistive Index (RI) and Pulsatility Index (PI), calculated from blood flow velocities in vessels, reflect renovascular resistance. These indices which are measured in renal arteries are reported as reliable measurements of down-stream renal resistance, and in patients with chronic kidney disease (CKD) [9,10].

Diabetes Mellitus type 2 (DM2) is a chronic, debilitating, and costly disease caused by glucose-induced damage to cells, tissues, and eventually whole organ systems. One of the most frequent and serious complications of diabetes is the accumulation of excessive connective tissue (pathological fibrosis). This is a major feature in diabetic nephropathy [11].

However, despite the availability of modern therapies to control hyperglycemia and blood pressure, many patients with diabetes mellitus continue to show progressive organ damage. Thus, further examination of the mechanisms underlying the development of elevated blood glucose and more direct treatment strategies that target connective tissue (collagen) turnover are warranted [12].

Therefore, the aim of the current study was to evaluate serum relaxin in diabetic and non-diabetic patients with different grades of chronic kidney disease. Also, was to correlate serum RLX to various clinical and Doppler renal hemodynamic parameters.

2. Patients and Methods

This study included 60 patients with CKD, divided into two equal groups. Group 1 (30 patients with creatinine clearance less than 30 ml/min i.e. severe CKD), and group 2 (30 patients with creatinine clearance more than 30 ml/min and less than 90 ml/minute i.e. mild to moderate CKD) [13]. In addition, group 3 (included 20 controls, matched for age and gender). We exclude all patients with CKD who started dialysis, and those with known or suspected collagen disease. The cause of CKD was DM2 and/or hypertension.

All subjects were recruited from Internal Medicine Department, Faculty of Medicine, Menoufiya University, Egypt. The study was approved by our Ethical Committee

of Faculty of Medicine, and informed consents were taken from all subjects.

2.1. 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 Quantikine Human Relaxin kit can be used to generate relative mass values for naturally occurring human Relaxin. This assay employs the quantikine sandwich enzyme immunoassay technique. A monoclonal antibody specific for Relaxin-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Relaxin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody for Relaxin is added to the wells. Following a wash to remove any inbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportional to the amount of Relaxin bound in the initial step. The color development is stopped and the intensity of color is measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The concentration of in the samples is then determined by comparing the O.D. of the samples to the standard curve [14].

2.2. Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Relaxin-2 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

2.3. Measurement of Renal Vascular Resistance Using Renal Arterial Doppler Ultrasonography

No patient preparation was needed. Subjects were in supine, right & left decubitus positions. We used Hitachi Sequoia C512 with a 4C2 probe (Hitachi Medical Systems, Japan) with pulsed wave Doppler at 2 MHz, imaging at 2–4 MHz, and high pulse repetition frequency mode, using a flank approach. As per the renal protocol, a full renal grey scaled study of both kidneys to include the longest renal length, renal width, cortical thickness, echogenicity and

corticomedullary differentiation; were performed before spectral and color Doppler imaging was acquired.

The PI and the RI were computer estimated by the Hitachi software. The PI and the RI were calculated as: $PI = (PSV-MDV)/MV$ and $RI = (PSV-MDV)/PSV$ where PSV = peak systolic velocity, MDV = minimum diastolic velocity, and MV = mean velocity (time averaged velocity) [9,10].

2.4. Exam of Intra-Renal and Extra-Renal Vessels

Intra-renal Evaluation: Segmental artery spectral waveforms were recorded from 3 locations within the kidney, upper, mid and lower poles. Then appropriate sample volume size was selected. The angle was low as possible, preferably below 20 degrees. Then increase spectral waveforms without producing aliasing. Measurement of RI and PI, was done. Sweep speed was adjusted to increase the width of the waveform for acceleration times. Acceleration times and acceleration indices, were acquired. Changes in velocity, changes in time, and acceleration between end diastole and peak systole, were documented.

Extra-renal Evaluation: The entire external portion of the renal artery was assessed using color Doppler in long axis. Spectral Doppler measurements of blood flow velocity were sampled from the origin of the artery to the renal hilum. The angle should be as low as possible and not exceed 60 degrees. Then, maximal peak systolic velocity was recorded at the origin/proximal, mid, and distal renal artery, followed by acquirement of Doppler spectral recordings. A measurement of the peak systolic velocity in the aorta was acquired near the origin of the SMA (Used for the Renal-Aortic Ratio).

Special Hints: Multiplicity of renal arteries to each kidney is common. Therefore, we firstly established the number of renal arteries, if possible. (This is also why we did accelerations from 3 areas: upper pole, inter-polar and lower pole-to determine if there was an evidence of different flow pattern to any particular area [15].

2.5. Statistical Analysis

Results are presented as mean \pm standard deviation (SD) unless otherwise stated. The ANOVA test with post hoc was used to compare among group 1, 2 and controls unless otherwise was stated. Fisher Exact analysis was also applied to compare proportions between groups. Pearson coefficient was used to study correlations between relaxin and different parameters. 5% was chosen as a level of significance for the used statistical tests. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) software version 10.

3. Results

The study included 60 patients with renal impairment. The patients are divided into two groups. Group 1 consists of 30 patients with creatinine clearance less than 30 ml/min. They were 20 male and 10 female and their age is ranged between 29 and 70 years. Group 2 consists of 30 patients with creatinine clearance ranged between 30 and 90 ml/min. They were 18 male and 12 female and their age is ranged between 32 and 65 years. Group 3 consists of 20 healthy individuals (control group). Twelve patients (40%) have DM2 in group 1 and 18 (60%) in group 2. Twenty six patients (87%) have hypertension in group 1 and 24 (80%) in group 2.

Table 1. Characteristics of the studied population (Mean \pm SD)

Parameter	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 20)	P-value
Gender (male %)	66.6% (n = 20)	60% (n = 18)	60% (n = 12)	> 0.05
Age (years)	43.4 \pm 17.4	50.4 \pm 10.5	47.6 \pm 9.1	0.25
SBP (mm Hg)	150 \pm 26.7	136 \pm 19.4	120.5 \pm 9	0.0001*
DBP (mm Hg)	90.7 \pm 14.6	85 \pm 10.4	74.5 \pm 7.9	0.0001*
Disease duration (year)	1.2 \pm 1.7	0.8 \pm 1.4		0.02*
Serum albumin (gm/dl)	3.1 \pm 0.4	3.2 \pm 0.3	4.3 \pm 0.4	0.0001*
Blood urea (mg/dl)	197 \pm 75.6	96.9 \pm 39.2	32.5 \pm 6.5	0.0001*
Serum creatinine (mg/dl)	12.6 \pm 5.2	4.5 \pm 1.3	0.8 \pm 0.1	0.0001*
RI	0.73 \pm 0.11	0.76 \pm 0.1	0.56 \pm 0.04	0.0001*
PI	1.49 \pm 0.49	1.62 \pm 0.28	0.91 \pm 0.08	0.0001*
Serum relaxin (pg/ml)	2.22 \pm 0.38	2.84 \pm 0.56	4.66 \pm 1.41	0.0001*

SBP = Systolic blood pressure, DBP = Diastolic blood pressure, RI = resistive index, PI = pulsatility index

* Statistically significant test

Table 2. Correlation between serum relaxin level and different parameters in group 1 and group 2

Parameter	Group 1 (n = 30)		Group 2 (n = 30)	
	r	P-value	r	P-value
Age (years)	-0.34	0.04*	-0.37	0.03*
SBP	0.23	0.22	0.22	0.25
DBP	0.28	0.12	0.11	0.57
Disease duration (year)	0.07	0.7	-0.36	0.04*
Serum albumin (gm/dl)	0.16	0.4	-0.27	0.15
Blood urea (mg/dl)	-0.6	0.0001*	-0.36	0.03*
Serum creatinine (mg/dl)	-0.8	0.0001*	-0.34	0.04*
Creatinine clearance (ml/min)	0.77	0.0001*	0.6	0.0001*
RI	0.03	0.86	-0.65	0.0001*
PI	-0.26	0.22	-0.59	0.01*

SBP = Systolic blood pressure, DBP = Diastolic blood pressure, RI = resistive index, PI = pulsatility index

* Statistically significant correlation

Comparison among group 1, 2 and 3 (Table 1) showed that there was no significant difference with regard to

gender and age (P > 0.05). There was a significant difference among group 1, 2 and 3 with regard SBP, DBP,

serum albumin, blood urea, serum creatinine, creatinine clearance, RI, PI and serum relaxin ($P < 0.05$). SBP and DBP of group 1 were significantly higher than group 3 ($P = 0.0001$ for both). SBP and DBP of group 2 were significantly higher than group 3 ($P = 0.004$ and 0.0001 respectively). SBP of group 1 was significantly higher than group 2 ($P = 0.04$) but there was no significant difference with regard DBP ($P = 0.2$). Serum albumin level of group 1 and 2 was significantly lower than group 3 ($P = 0.0001$ for both) but there was no significant difference between group 1 and 2 ($P = 0.6$). Blood urea and serum creatinine of group 1 and 2 were significantly higher than group 3 ($P = 0.0001$ for all). Blood urea and serum creatinine of group 1 were significantly higher than group 2 ($P = 0.0001$ for both). RI and PI of group 1 and 2 were significantly higher than group 3 ($P = 0.0001$ for all) but there was no significant difference between group 1 and 2 ($P > 0.05$). Serum relaxin level of group 1 and 2 was significantly lower than group 3 ($P = 0.0001$ for both). Serum relaxin of group 1 was significantly lower than group 2 ($P = 0.0001$). Disease duration was significantly longer in group 1 than in group 2 ($P = 0.02$).

Table 2 shows correlation between serum relaxin level and different parameters in group 1 and group 2. There was no correlation between serum relaxin level and different parameters in group 1 and 2 with regard, SBP, DBP, serum albumin. There was an inverse significant correlation between serum relaxin level and disease duration in group 2 ($P = 0.04$) but not in group 1 ($P = 0.7$). There was an inverse correlation between serum relaxin level and age of the patients in group 1 ($P = 0.04$) and group 2 ($P = 0.03$). There was an inverse correlation between serum relaxin level and blood urea in group 1 ($P = 0.0001$) and group 2 ($P = 0.03$). There was an inverse correlation between serum relaxin level and serum creatinine in group 1 ($P = 0.0001$) and group 2 ($P = 0.04$). There was a positive correlation between serum relaxin level and creatinine clearance in group 1 ($P = 0.0001$) and

group 2 ($P = 0.0001$). There was an inverse correlation between serum relaxin level and both RI and PI in group 2 ($P = 0.0001$ and 0.01 respectively) but not in group 1. (Figure 1-Figure 2).

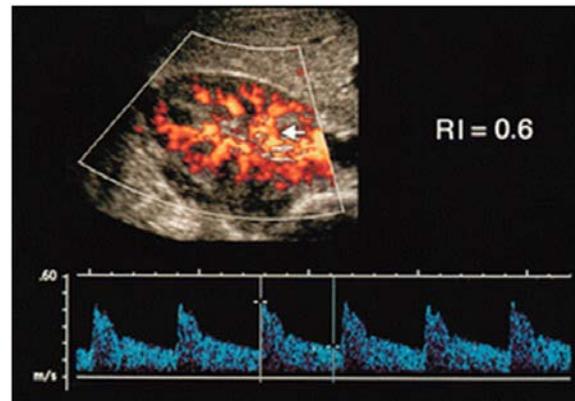


Figure 1. Normal resistive index in a 25-year-old healthy woman. Color Doppler sonogram is used to identify inter-lobar artery (arrow); waveform is maximized using lowest pulse repetition frequency possible

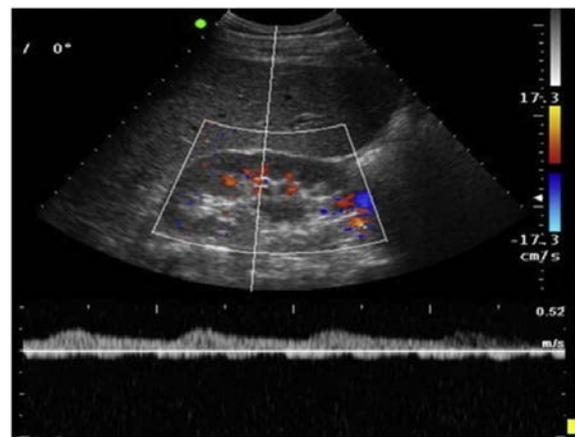


Figure 2. Tardus–parvus waveform in a patient with nephropathy and renal artery stenosis. Note the delayed and dampened upstroke yielding a rounded appearance to the waveform. RI = 0.9

Table 3. Comparison between patients with and without DM2 in group 1

Parameter	Diabetic Patients (n = 12)	Non-diabetic Patients (n = 18)	P-value
Age (years)	60.5 ± 4.7	40.7 ± 17.2	0.003*
SBP (mm Hg)	150 ± 10.4	150 ± 228.4	0.86
DBP (mm Hg)	85.1 ± 5.2	91.5 ± 15.4	0.19
Disease duration (year)	1.2 ± 0.8	1.2 ± 1.8	0.19
Serum albumin (gm/dl)	3.1 ± 0.1	3.1 ± 0.4	0.7
Blood urea (mg/dl)	213.5 ± 32.9	194.5 ± 80.1	0.13
Serum creatinine (mg/dl)	15.3 ± 5.5	15.6 ± 9.7	0.46
RI	0.69 ± 0.07	0.73 ± 0.11	0.06
PI	1.07 ± 0.12	1.49 ± 0.49	0.02*
Serum relaxin (pg/ml)	2.1 ± 0.42	2.3 ± 0.37	0.04*

DM2 = Diabetes mellitus type 2, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, RI = resistive index, PI = pulsatility index
* Statistically significant test

Table 4. Comparison between patients with and without DM2 in group 2

Parameter	Diabetic Patients (n = 18)	Non-diabetic Patients (n = 12)	P-value
Age (years)	52.4 ± 8.7	47.3 ± 12.4	0.33
SBP (mm Hg)	143.3 ± 18.5	125 ± 15.7	0.009*
DBP (mm Hg)	88.3 ± 9.2	80 ± 10.4	0.06
Disease duration (year)	0.4 ± 0.2	1.5 ± 2.1	0.4
Serum albumin (gm/dl)	3.1 ± 0.2	3.5 ± 0.3	0.0001*
Blood urea (mg/dl)	98.5 ± 48.8	94.5 ± 18.8	0.13
Serum creatinine (mg/dl)	4.2 ± 1.5	4.9 ± 0.9	0.3
RI	0.75 ± 0.04	0.76 ± 0.16	0.3
PI	1.5 ± 0.33	1.7 ± 0.23	0.12
Serum relaxin (pg/ml)	2.81 ± 0.54	2.86 ± 0.61	0.018*

DM2 = Diabetes mellitus type 2, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, RI = resistive index, PI = pulsatility index
* Statistically significant test

Table 3 and Table 4 show a comparison between diabetic and non-diabetic patients in group 1 and 2. There was no significant difference between diabetic and non-diabetic patients in both groups as regards DBP, disease duration, blood urea, serum creatinine, and RI ($P > 0.05$).

Diabetic patients were significantly older than patients without DM2 in group 1 ($P = 0.003$). PI of diabetic patients was significantly lower than patients without DM2 in group 1 ($P = 0.02$). Serum relaxin was significantly lower in diabetic patients compared to non-diabetic patients ($P = 0.04$).

There was no significant difference between patients with and without DM2 in group 2 with regard age, RI and PI ($P > 0.05$). Patients with DM2 have significantly higher SBP than patients without DM2 in group 2 ($P = 0.009$). Serum albumin level of patients with DM2 was significantly lower than patients without DM2 in group 2 ($P = 0.0001$). Serum relaxin was significantly lower in diabetic patients compared to non-diabetic patients ($P = 0.018$).

4. Discussion

Fibrosis is increasingly recognized as a ubiquitous cause of organ dysfunction and failure in a diverse range of circumstances [16]. Therefore, the study of diabetic fibrosis is relevant to our understanding of both diabetic complications and progressive organ failure in general. However, despite the significance of progressive fibrosis, therapeutic strategies for its treatment remain elusive. Although renin-angiotensin system blockers are now viewed as first-line treatment for diabetic nephropathy and cardiomyopathy [17], and have clearly been shown to confer organ protection, these treatments fail to halt the progression of diabetes-induced fibrosis in most patients. Even more elusive has been the search for therapies that may modify or reduce existing scarring, the final outcome in many diabetic complications. The clinical reality is that most patients present with established pathology. Currently, there are no treatments that consistently and effectively reverse established collagen deposition. Therefore, it is imperative that novel interventions that can halt the progression of diabetic nephropathy are identified [18].

In the current study, serum RLX levels were significantly lower in group 1 (advanced CKD) compared to group 2 (mild renal impairment).

Fibrosis is a condition marked by excessive accumulation of extracellular matrix components. This accumulation is primarily due to activation of fibroblasts to a myofibroblastic phenotype characterized by accelerated fibrillar collagen production. Furthermore, there is a decrease in the clearance of extracellular matrix due to decreased secretion of the MMPs that degrade collagen, and an increase in their endogenous inhibitors, the TIMPs. In many types of fibrosis, there are few options for treatment. Therefore, agents that reduce collagen production and increase its clearance are in demand to treat fibrotic diseases. RLX is one of these candidate agents [19].

RLX has antifibrotic effects in experimental models of renal fibrosis. In cultured renal fibroblasts, cortical-

epithelial cells and mesangial cells, relaxin decreased TGF- β -induced fibronectin levels, and promoted fibronectin degradation [20]. In other studies using renal fibroblast cell lines and primary cortical fibroblasts, RLX inhibited TGF- β -induced fibroblast-myofibroblast transition, contractility, collagen I and fibronectin secretion, and increased MMP secretion [21,22]. In addition, RLX decreased the phosphorylation and nuclear localization of S mad 2, and association of S mad 2 with S mad 3. Because S mad 2 nuclear translocation and association with S mad 3 are critical to many of the profibrotic effects of TGF- β , these studies provide a possible mechanism for relaxin-related effects on fibrotic pathways triggered by TGF- β . These results were very recently confirmed, and in addition relaxin was shown to act through activation of the nitric oxide/cGMP pathway [23].

In the current study, RLX was significantly lower in diabetic patients compared to non-diabetics in both groups. In line with our work, Szepletowska et al [24] 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.

Decrease of RLX level in diabetic patients may play a role of renal fibrosis and renal failure. Several studies have also shown that RLX inhibits TGF- β or angiotensin II-induced myofibroblast differentiation, which may be the case for the actions of RLX in the mRen-2 rat model because both these profibrotic factors are up-regulated during the pathogenesis of diabetes [22,25].

Transforming growth factor- β (TGF- β) promotes renal cell hypertrophy and stimulates extracellular matrix accumulation, the 2 hallmarks of diabetic renal disease. In tissue culture studies, cellular hypertrophy and matrix production are stimulated by high glucose concentrations in the culture media. High glucose, in turn, appears to act through the TGF- β system because high glucose increases TGF- β expression, and the hypertrophic and matrix-stimulatory effects of high glucose are prevented by anti-TGF- β therapy. In experimental diabetes mellitus, several reports describe overexpression of TGF- β or TGF-beta type II receptor in the glomerular and tubulointerstitial compartments. As might be expected, the intrarenal TGF- β system is triggered, evidenced by activity of the downstream S mad signaling pathway. Treatment of diabetic animals with a neutralizing anti-TGF- β eta antibody prevents the development of mesangial matrix expansion and the progressive decline in renal function. This antibody therapy also reverses the established lesions of diabetic glomerulopathy. Finally, the renal TGF- β system is significantly up-regulated in human diabetic nephropathy. Although the kidney of a nondiabetic subject extracts TGF- β from the blood, the kidney of a diabetic patient actually elaborates TGF-beta1 protein into the circulation. The data presented here strongly support the consensus that the TGF- β system mediates the renal hypertrophy, glomerulosclerosis, and tubulointerstitial fibrosis of diabetic kidney disease [26].

In addition to its antifibrotic effects, RLX also possesses potent vasodilatory properties that have been demonstrated in humans [27].

The RI and PI have been used as pulsed-wave Doppler measurements of renal vascular resistance in CKD

patients. In our patients with CKD, serum RLX was found to be negatively correlated with patients' age, RI, and PI.

Aging involves normal endothelial function but blunts the physiological endothelium-dependent and -independent vasodilator response to RLX [28]. Compatible with our results, Petersen et al (1995) and Petersen et al (1997) found that RI and PI were associated with the severity of renal disease [10,29].

RLX may be equally efficient for relaxing efferent arteriolar tone in rats and humans because the hormone is a functional angiotensin II antagonist [30]. Actually, vascular gelatinase activity plays is known to play a pivotal role in the remodeling of the extracellular matrix and plays a pivotal role in the renal vasodilatory response to RLX [31]. In fact, RLX-induced collagenase activation could convert big endothelin ET 1 into bioactive ET 1-32, which in turn binds and activates ETB receptors, thereby inducing nitric oxide activation and release [32].

5. Conclusion

serum RLX levels are significantly lower in patients with advanced stages than those with early stages of CKD. Also, they are significantly lower in diabetic patients compared to non-diabetic patients. Moreover, serum RLX is negatively correlated with patients' age, renal vascular resistance (RI & PI) in patients with CKD. 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.

References

- [1] Sherwood OD. Relaxin's physiological roles and other diverse actions. *Endocr Rev* 2004; 25: 205-234.
- [2] Bathgate RAD, Hsueh AJ, Sherwood OD. Physiology and molecular biology of the relaxin peptide family. In: Knobil E, Neill JD, eds. *Physiology of reproduction*, 3rd ed. San Diego: Elsevier 2006; 679-770.
- [3] Samuel CS, Hewitson TD. Relaxin in cardiovascular and renal disease. *Kidney Int* 2006; 69: 1498-1502.
- [4] Nistri S, Bigazzi M, Bani D. Relaxin as a cardiovascular hormone: physiology, pathophysiology and therapeutic promises. *Cardiovasc Hematol Agents Med Chem* 2007; 5: 101-108.
- [5] Garber SL, Mirochnik Y, Brecklin CS, et al. Relaxin decreases renal interstitial fibrosis and slows progression of renal disease. *Kidney Int*. 2001; 59: 876-882.
- [6] Robert G. Bennett. Relaxin and its role in the development and treatment of fibrosis. *Transl Res*. 2009; 154 (1): 1-6.
- [7] Samuel CS, Hewitson TD. Relaxin and the progression of kidney disease. *Curr Opin Nephrol Hypertens*. 2009 Jan; 18 (1): 9-14.
- [8] Conrad KP. Mechanisms of renal vasodilation and hyperfiltration during pregnancy. *J Soc Gynecol Invest* 2004; 11: 438-448.
- [9] Platt JF, Ellis JH, Rubin JM, et al. Intrarenal arterial Doppler sonography in the detection of renal vein thrombosis of the native kidney. *Am J Roengenol* 1994; 162: 1367-1370.
- [10] Petersen LJ, Petersen JR, Ladefoged SD, et al. The pulsatility index and the resistive index in renal arteries in patients with hypertension and chronic kidney disease. *Nephrol Dial Transplant* 1995; 10: 2060-2064.
- [11] Asbun J, Villarreal FJ. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *J Am Coll Cardiol* 2006; 47: 693-700.
- [12] Svensson M, Sundkvist G, Arnqvist HJ, Björk E, Blohmé G, Bolinder J, Henricsson M, Nyström L, Torffvit O, Waernbaum I, Ostman J, Eriksson JW. Signs of nephropathy may occur early in young adults with diabetes despite modern diabetes management: results from the nationwide population-based Diabetes Incidence Study in Sweden (DISS). *Diabetes Care* 2003; 26: 2903-2909.
- [13] Uhlig K, Macleod A, Craig J, et al. Grading evidence and recommendations for clinical practice guidelines in nephrology. A position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006; 70: 2058-2065.
- [14] Hall ML. Serum Relaxin Concentrations in Systemic Sclerosis. *Br J Pharmacol*, 2007; 150: 677.
- [15] Ikee R, Kobayashi S, Hemmi N, et al. Correlation between the resistive index by Doppler ultrasound and kidney function and histology. *Am J Kidney Dis* 2005; 46: 603-609.
- [16] Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007; 117: 524-529.
- [17] Bell DS. Treatment of heart failure in patients with diabetes: clinical update. *Ethn Dis* 2002; 12 (suppl 1): S12-S18.
- [18] Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993; 329: 1456-1462.
- [19] Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat M-L, Gabbiani G. The Myofibroblast: One Function, Multiple Origins. *Am J Pathol*. 2007; 170: 1807-16.
- [20] McDonald GA, Sarkar P, Rennke H, Unemori E, Kalluri R, Sukhatme VP. Relaxin increases ubiquitin-dependent degradation of fibronectin in vitro and ameliorates renal fibrosis in vivo. *Am J Physiol Renal Physiol*. 2003; 285: F 59-67.
- [21] Heeg MH, Koziolok MJ, Vasko R, Schaefer L, Sharma K, Muller GA, et al. The antifibrotic effects of relaxin in human renal fibroblasts are mediated in part by inhibition of the S mad 2 pathway. *Kidney Int*. 2005; 68: 96-109.
- [22] Masterson R, Hewitson TD, Kelynack K, Martic M, Parry L, Bathgate R, et al. Relaxin down-regulates renal fibroblast function and promotes matrix remodelling in vitro. *Nephrol Dial Transplant*. 2004; 19: 544-52.
- [23] Mookerjee I, Hewitson TD, Halls ML, Summers RJ, Mathai ML, Bathgate RAD, et al. Relaxin inhibits renal myofibroblast differentiation via RXFP1, the nitric oxide pathway, and S mad 2. *FASEB J*. 2008: 120857.
- [24] Szepletowska B, Gorska M, Szlachowska M. Plasma relaxin concentration is related to beta-cell function and insulin sensitivity in women with type 2 diabetes mellitus. *Diabetes research and clinical practice* 2008; 79: e1-e3.
- [25] Samuel CS, Unemori EN, Mookerjee I, Bathgate RA, Layfield SL, Mak J, Tregear GW, Du XJ. Relaxin modulates cardiac fibroblast proliferation, differentiation and collagen production and reverses cardiac fibrosis in vivo. *Endocrinology* 2004; 145: 4125-4133.
- [26] Chen S, Jim B, Ziyadeh FNDiabetic nephropathy and transforming growth factor-beta: transforming our view of glomerulosclerosis and fibrosis build-up. *Semin Nephrol*. 2003 Nov; 23 (6): 532-43.
- [27] Erikson MS, Unemori EN 2001 Relaxin clinical trials in systemic sclerosis. In: Tregear GW, Ivell R, Bathgate RA, Wade JD, eds. *Relaxin 2000: Proceedings of the Third International Conference on Relaxin and Related Peptides*. Amsterdam: Kluwer; 373-381.
- [28] Van Drongelen J, Ploemen IH, Pertjjs J, et al. Aging attenuates the vasodilator response to relaxin. *Am J Physiol Heart Circ Physiol*. 2011; 300 (5): H1609-H1615.
- [29] Petersen LJ, Petersen JR, Talleruphuus U, et al. The pulsatility index and the resistive index in renal arteries. Associations with long-term progression in chronic kidney disease. *Nephrol Dial Transplant* 1997; 12: 1376-1380.
- [30] Debrah DO, Conrad KP, Jeyabalan A, et al. Relaxin increases cardiac output and reduces systemic arterial load in hypertensive rats. *Hypertension* 2005; 46: 745-750.
- [31] Jeyabalan A, Novak J, Danielson L, et al. Essential role for vascular gelatinase in relaxin-induced renal vasodilation, hyperfiltration and reduced myogenic reactivity of small arteries. *Circ Res* 2003; 93: 1249-1257.
- [32] Conrad KP, Novak J. Emerging role of relaxin in renal and cardiovascular function. *Am J Physiol Regul Integr Comp Physiol* 2004; 287: 250-261.