

Serum Relaxin Levels and Importance in Systemic Sclerosis Patients

Abdel Megid MH¹, Saied EA¹, Abdel Atti E³, Azab N⁶, Hamad YH², Nkedy AM⁴, El Beily DA⁵, Abo Gabal AM⁴

¹Rheumatology, ²Physical Medicine Rheumatology and Rehabilitation, ⁴Radiology and ⁵Clinical Pathology Departments. Faculty of Medicine, Alexandria University. ³Internal Medicine and ⁶Chest Departments, Faculty of Medicine, Menofiya University. chab_abdelatty@hotmail.com

Abstract: Systemic Sclerosis (SSc) is a connective tissue disorder characterized by fibrosis of the skin and may involve the heart, lungs, kidneys, and gastrointestinal tract. Relaxin, a peptide hormone of the insulin superfamily, is involved in the promotion of extracellular matrix remodeling. **Aim of the work:** Measuring serum relaxin level, studying its antifibrotic, vasodilator, and proangiogenic effects and its association with disease activity in SSc patients. **Methods:** This study included 40 patients with SSc, who met ACR Criteria for the classification of SSc, and 40 age-matched healthy persons as a control group. All patients were subjected to: history taking with particular stress on the duration of the disease, presence of Raynaud's disease, finger tip ulcers, renal, heart, and lung affection, Modified Rodnan Skin Score (M.R.S.S.) using ultrasound, 2D-Echocardiography to measure systolic pulmonary arterial pressure (sPAP), computerized tomography (CT) of the chest, renal function tests, measurement of serum relaxin and serum vascular endothelial growth factor (sVEGF) using ELISA technique. **Results:** The level of sPAP, sVEGF, serum relaxin and urinary protein to creatinine ratio of the patients with SSc was significantly higher than controls ($P=0.0001$). There was no significant difference between patients with and without finger ulcers and interstitial pulmonary fibrosis (IPF) regarding sPAP, M.R.S.S and urinary protein to creatinine ratio. However, the level of sVEGF of the patients with finger ulcers and IPF was significantly higher and serum level of relaxin was significantly lower than patients without finger ulcers and/or IPF ($P=0.0001$). There was a strong positive correlation between M.R.S.S, and both serum relaxin and sVEGF, but not between serum relaxin and sVEGF. A non significant positive correlation between sVEGF and sPAP existed in patients with SSc ($r=+0.27$, $P=0.06$). **Conclusion:** Serum relaxin can be considered as a biomarker in SSc which correlates with disease activity and severity. Since it is a natural suppressor of age-related fibrosis in a number of tissues, including the skin, lung, kidney, and heart it can show efficacy in the prevention and treatment of fibrosis due to SSc. [Abdel Megid MH, Saied EA, Abdel Atti E, Azab N, Hamad YH, Nkedy AM, El Beily DA, Abo Gabal AM. **Serum Relaxin Levels and Importance in Systemic Sclerosis Patients.** *J Am Sci* 2012;8(9):253-260]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 37

Key words: systemic sclerosis, relaxin, VEGF.

1. Introduction

Scleroderma or systemic sclerosis is a complex, chronic connective tissue disease, which primarily causes skin thickening and hardening in addition to interstitial fibrosis of various internal organs (lung, heart, kidney, gastrointestinal tract, blood vessels, etc). The associated fibrosis is potentially driven by several independent factors involving immunological disorders, enhanced workload, hypertrophy, disturbed injury-repair mechanisms and/or metabolic defects, which eventually lead to the proliferation and differentiation of matrix-producing fibroblasts. These cells, when stimulated by a number of cytokines and growth factors, differentiate into myofibroblasts and synthesize large amounts of matrix proteins, mainly collagen, leading to tissue scarring and thickening. The symptoms of scleroderma vary between individuals, can develop in any age group and, if not properly treated, can lead to irreversible tissue damage⁽¹⁾.

Relaxin, a naturally occurring protein, is structurally related to the insulin family of peptides

⁽²⁾. In females, it is produced by the ovarian luteal cells and/or placenta during pregnancy, while in males, relaxin is produced by the prostate, is found in seminal fluid, but is not generally detected in the circulation. Recent evidences suggest that relaxin may be produced locally to act in an autocrine or paracrine manner in some tissues⁽³⁾.

Fibrosis is a condition characterized by excessive accumulation of extracellular matrix components, particularly the fibrillar collagens (such as types I and III). This accumulation is primarily due to activation of fibroblasts to a myofibroblastic phenotype characterized by accelerated fibrillar collagen production⁽⁴⁾. Furthermore, there is a decrease in the clearance of extracellular matrix due to decreased secretion of the matrix metalloproteinases (MMPs) that degrade collagen, and an increase in their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs)⁽⁴⁾.

Relaxin has antifibrotic properties; it downregulates collagen production and increases collagen degradation⁽⁵⁾. Relaxin acts directly on

transforming growth factor -1-stimulated fibroblasts to decrease myofibroblast differentiation and collagen secretion while increasing expression of the metalloproteinases enzymes that are responsible in part for collagen degradation^(6, 7). Relaxin acts in synergy with interferon to reduce collagen overexpression by fibroblasts isolated from patients with scleroderma⁽⁷⁾. In addition, recombinant human relaxin prevents the development of bleomycin-induced pulmonary fibrosis in rodents⁽⁸⁾.

Relaxin treatment of normal dermal fibroblasts or scleroderma fibroblasts resulted in a marked decrease in collagen secretion and increase in its degradation^(9, 10).

Unfortunately, treatment of scleroderma in humans has produced mixed results. Early studies in the late 1950s and early 1960s generally demonstrated a benefit of partially purified porcine relaxin for many symptoms of scleroderma, but further studies were delayed for many years due to concerns about use of impure relaxin preparations in humans^(11, 12).

Relaxin treatment did not reverse advanced stages of fibrosis⁽¹³⁾ due to either, downregulation of relaxin signaling at the doses used, or the development of relaxin autoantibodies, which were detected at both low and high relaxin doses⁽¹⁴⁾. Therefore, the evidence that far casts doubt on the utility of relaxin in the treatment of advanced scleroderma but further studies on the use of relaxin in the treatment of less severe scleroderma may be warranted.

The lung has emerged as a relaxin target organ. Relaxin treatment of human lung fibroblasts resulted in a reduction in the expression of collagen types I and III and fibronectin in response to transforming growth factor beta (TGF β), a potent fibrogenic agent, and furthermore promoted extracellular matrix degradation by increasing the levels of MMPs⁽¹⁵⁾. Relaxin treatment dramatically decreased bleomycin-induced collagen content in the lung, alveolar thickening, and improved the overall fibrosis score⁽¹⁵⁾.

Relaxin has antifibrotic effects on renal tissues. In cultured renal fibroblasts, cortical-epithelial cells and mesangial cells, relaxin decreased TGF- β -induced fibronectin levels, and promoted fibronectin degradation⁽¹⁶⁾. Using renal fibroblast cell lines and primary cortical fibroblasts, relaxin inhibited TGF- β -induced fibroblast-myofibroblast transition, contractility, collagen I and fibronectin secretion, and increased MMP secretion^(17, 18). In addition, relaxin decreased the phosphorylation and nuclear localization of Smad2 and association of Smad2 with Smad3⁽¹⁷⁾. Because Smad2 nuclear translocation and association with Smad3 are critical to many of the

profibrotic effects of TGF- β , they provide a possible mechanism for relaxin's effects on fibrotic pathways triggered by TGF- β . These findings were very recently confirmed, and in addition relaxin was shown to act through activation of the nitric oxide/cGMP pathway⁽¹⁹⁾.

Relaxin treatment of primary atrial and ventricular fibroblasts caused alterations in fibrotic markers including decreased collagens types I and III, fibroblast to myofibroblast transition and cell proliferation, and increased MMP secretion⁽²⁰⁾. Male, but not female, relaxin developed increased left ventricular collagen content and collagen type I expression with aging, which was reversed with exogenous relaxin treatment^(20, 21). Nevertheless, substantial evidence exists suggesting the possible use of relaxin in the treatment of cardiac fibrosis.

Aim of the work

This work aimed at measuring serum relaxin, studying its antifibrotic, vasodilator, and proangiogenic effects and its association with disease activity in SSc patients.

2. Subjects and Methods

Forty patients with SSc who met ACR criteria for the classification of SSc and 40 healthy persons with matched age as controls were included in the study.

After an informed consent has been taken, all patients were subjected to the following:

- 1- Full medical history with particular stress on the duration of the disease, presence of Raynaud's disease, finger tip ulcers, renal, heart, and lung affection.
- 2-Modified Rodnan Skin Score (M.R.S.S.) measurement of skin thickness using (U/S)⁽²²⁾.
- 3-2D-Echocardiography to measure systolic pulmonary arterial pressure in patients and controls.
- 4- CT chest.
- 5-Renal function tests: Urea, Creatinine, and Protein/Creatinine ratio in 24 hours urine collection (P/C) Ratio.

Laboratory Workup:

I- Measurement of Serum Relaxin -2 using ELISA technique⁽²³⁾.

The Quantikine Human Relaxin -2 Immunoassay in a 4.5 hour solid phase ELISA designed to measure human Relaxin -2 in cell culture supernates, serum, and plasma. It contains *E.coli* - expressed recombinant human Relaxin -2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Relaxin -2 showed linear curves that were parallel to the standard curves obtained using the Quantikine standards. These results indicated that the Quantikine

Human Relaxin -2 kit can be used to germinate relative mass values for naturally occurring human Relaxin -2⁽²³⁾.

II- Measurement of sVEGF in SSc pts and controls using ELISA technique⁽²⁴⁾.

Blood samples were collected from both groups, patients and controls, left to clot, serum separated, kept under -20°C until the date of assay to measure serum Relaxin and sVEGF (...24...).

Statistical analysis:

Data were processed and analyzed using a computer based program (SPSS software version 10). The results were expressed as mean and standard deviation. For comparison of two means, the paired Student t-test was used. Pearson coefficient was used to study correlations between different parameters. Fisher Exact analysis was also used to compare proportions between groups. A *p*-value <0.05 was considered significant.

3. Results

The study was performed on 40 patients with SSc (mean age ± standard deviation, SD: 39.3 ± 6.7 years). All patients [34 women and 6 men] were enrolled among those followed in Rheumatology Department in the last 5 years. Mean duration of the disease was 2.7 ± 0.4 years.

Patients were compared with 40 age and sex matched controls [36 women and 4 men, mean age 36.1 ± 7.9 years) from the medical and nursing staff of our hospital. Patients and controls were informed about the study plan and gave their written consent.

Table I describes the characteristics of the studied population (patients and controls). There was no significant difference between patients and controls regarding age and gender (*p*>0.05). Levels of sPAP, sVEGF, serum relaxin and protein to creatinine ratio of the patients with SSc were significantly higher than controls (*p*=0.0001 for all).

Table II shows a comparison between female patients with SSc and female controls. There was no significant difference between female patients and controls regarding age (*p*=0.1). Levels of sPAP, sVEGF, serum relaxin and protein to creatinine ratio of female patients with SSc were significantly higher than female controls (*p*=0.0001 for all).

Table III shows a comparison between male patients with SSc and male controls. There was no significant difference between male patients and controls regarding age (*p* =0.5). Levels of sPAP, sVEGF, serum relaxin and protein to creatinine ratio of male patients with SSc were significantly higher than male controls (*p*=0.01 for all).

Table IV shows a comparison between female and male patients with SSc. There was no significant difference between female and male patients with SSc regarding age, sPAP, serum relaxin, disease

duration, skin thickness score, protein to creatinine ratio and presence of finger ulcers and presence of interstitial pulmonary fibrosis (IPF) (*p* > 0.05). Level of sVEGF of male patients with SSc was significantly higher than female patients with SSc (*p*=0.002) (Figure 2).

Table V shows a comparison between patients with and without finger ulcers and IPF. There was no significant difference between patients with and without finger ulcers and IPF regarding sPAP, M.R.S.S and protein to creatinine ratio (*p*>0.5). Level of sVEGF in patients with finger ulcers and IPF was significantly higher than patients without finger ulcers and IPF (*p*=0.0001). Patients with finger ulcers and IPF had significantly lower serum level of relaxin than patients without finger ulcers and IPF (*p*=0.0001). Patients with finger ulcers and IP are significantly older than patients without finger ulcers and IPF (*p*=0.03). Disease duration of the patients with finger ulcers and IPF was higher than patients without finger ulcers and IPF but this did not reach a significant value (*p*=0.06).

Table VI shows a correlation between serum relaxin, sVEGF and different parameters in patients with SSc. There was no correlation between serum relaxin and age (*r*=+0.2, *p*=0.3), sPAP (*r*=-0.13, *p*=0.7), disease duration (*r*=+0.09, *p*=0.6) and protein to creatinine ratio (*r*= -0.05, *p*=0.7). Also There was no correlation between serum relaxin and sVEGF (*r*=-0.02, *p*=0.3). There was a strong positive correlation between M.R.S.S and both serum relaxin and sVEGF (*r*=+0.7, *p*=0.0001 for both). There was a positive correlation between sVEGF and both age (*r*=+0.3, *p*=0.03) (Figure 5) and disease duration (*r*=+0.4, *p*=0.01) (figure 6) in patients with SSc.

There was a positive correlation between sVEGF and sPAP in patients with SSc but it did not reach a significant value(*r*=+0.27, *p*=0.06).

Table I. Characteristics of the studied population (patients with SSc and controls) (data expressed as mean±SD):

	Patients with SSc (n=40)	Controls (n=40)	<i>p</i> -value
Gender (female %)	85% (n=34)	90% (n=36)	0.7
Age (years)	39.3±6.7	36.1±7.9	0.06
sPAP (mmHg)	52.6±12.1	23.5±5.5	0.0001*
sVEGF (pg/ml)	1241.7±562	36.2±4.1	0.0001*
Serum relaxin (pg/ml)	150.5±39.4	4.7±1.4	0.0001*
Protein to creatinine ratio (gm/24 hours)	0.6±0.4	0.1±0.03	0.0001*

*=Significant n=number

sPAP=pulmonary systolic pressures VEGF=vascular endothelial growth factor

SSc=systemic sclerosis

Table II. Comparison between female patients with SSc and female controls (data expressed as mean±SD):

	Female patients (n=34)	Female controls (n=36)	p-value
Age (years)	38.8±6.5	35.9±8.3	0.11
sPAP (mmHg)	53±11.8	23.4±5.8	0.0001*
sVEGF (pg/ml)	1126±492.6	36.6±4	0.0001*
Serum relaxin (pg/ml)	144.6±40	4.7±1.3	0.0001*
Protein to creatinine ratio (gm/24 hours)	0.6±0.4	0.1±0.04	0.0001*

*=Significant n=number
 sPAP=pulmonary systolic pressure
 sVEGF=vascular endothelial growth factor
 SSc=systemic sclerosis

Table III. Comparison between male patients with SSc and male controls (data expressed as mean±SD):

	Male patients (n=6)	Male controls (n=4)	P-value
Age (years)	42±7.3	37.5±2.9	0.5
sPAP (mmHg)	50±14.7	24±1.2	0.01*
sVEGF (pg/ml)	1897.4±505	32.1±2.4	0.01*
Serum relaxin (pg/ml)	183.8±2.6	4.5±2.3	0.01*
Protein to creatinine ratio (gm/24 hours)	0.4±0.05	0.1±0.01	0.01*

*=Significant n=number
 sPAP=pulmonary systolic pressure
 sVEGF=vascular endothelial growth factor
 SSc=systemic sclerosis

Table IV. Comparison between female and male patients with SSc (data expressed as mean±SD):

	Female patients (n=34)	Male patients (n=6)	P-value
Age (years)	38.8±6.5	42±7.3	0.3
sPAP (mmHg)	53±11.8	50±14.7	0.9
sVEGF (pg/ml)	1126±492.6	1897.4±505	0.002*
Serum relaxin (pg/ml)	144.6±40	183.8±2.6	0.2
Disease duration (years)	2.7±0.4	2.8±0.3	0.5
Finger ulcer (present)	82%(n=28)	100%(n=6)	0.6
Presence of IPF	82%(n=28)	100%(n=6)	0.6
M.R.S.S	2.4±0.5	3±0	0.2
Protein to creatinine ratio (gm/24 hours)	0.6±0.4	0.4±0.5	0.9

*=Significant n=number
 sPAP=pulmonary systolic pressure
 sVEGF=vascular endothelial growth factor
 IPF=interstitial pulmonary fibrosis
 M.R.S.S = Modified Rodnan skin Score
 SSc=systemic sclerosis

Table V. Comparison between SSc patients with and without finger ulcers and IPF (data expressed as mean±SD):

	Patients with finger ulcers and IPF (n=34)	Patients without finger ulcers and IPF (n=6)	P-value
Age (years)	40.4±6.3	33.3±5.2	0.03*
sPAP (mmHg)	51.7±12.7	57.3±6.3	0.5
sVEGF (pg/ml)	1386.2±479.2	422.5±25.7	0.0001*
Serum relaxin (pg/ml)	143.2±38.3	192±2.9	0.0001*
Disease duration (years)	2.8±0.3	2.3±0.5	0.06
M.R.S.S	2.6±0.5	3±0	0.2
Protein to creatinine ratio (gm/24 hour)	0.6±0.4	0.4±0.09	0.3

*=Significant n=number
 sPAP=pulmonary systolic pressure
 sVEGF=vascular endothelial growth factor
 M.R.S.S = Modified Rodnan skin Score
 IPF=interstitial pulmonary fibrosis
 SSc=systemic sclerosis

Table VI. Correlation between serum relaxin, sVEGF and other parameters in patients with SSc

	Serum relaxin		sVEGF	
	r	P-value	r	P-value
Age (years)	+0.2	0.3	+0.3	0.03*
sPAP (mmHg)	-0.13	0.7	+0.27	0.06
Disease duration (years)	+0.09	0.6	+0.4	0.01*
M.R.S.S	+0.7	0.0001*	+0.7	0.0001*
Protein to creatinine ratio (gm/24 hours)	-0.05	0.7	+0.2	0.3

*=Significant n=number
 sPAP=pulmonary systolic pressure
 sVEGF=vascular endothelial growth factor
 M.R.S.S = Modified Rodnan skin Score
 SSc=systemic sclerosis

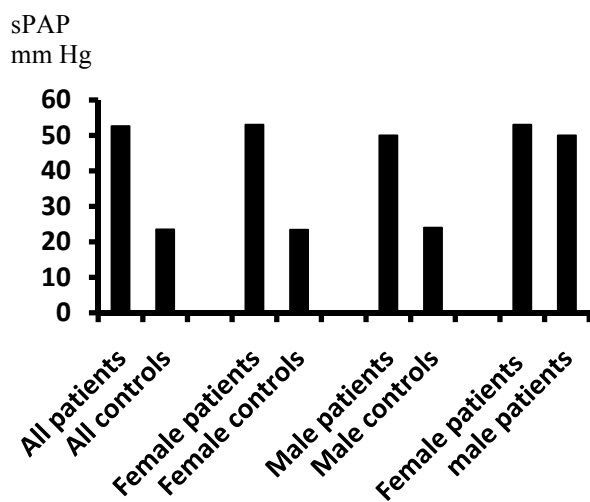


Figure 1. sPAP in all patients with SSc and controls (males and females)

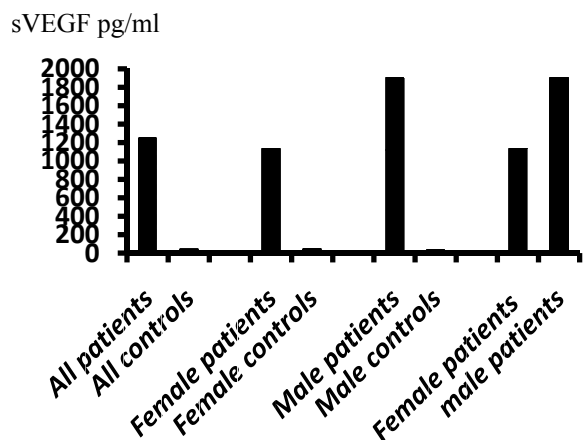


Figure 2. Level of sVEGF in all patients with SSc and controls (males and females)

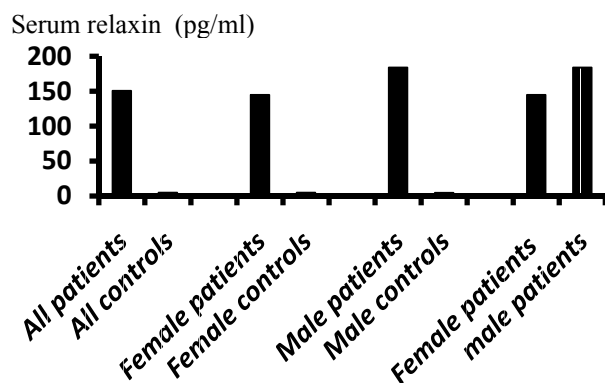


Figure 3. Level of serum relaxin in all patients with SSc and controls (males and females)

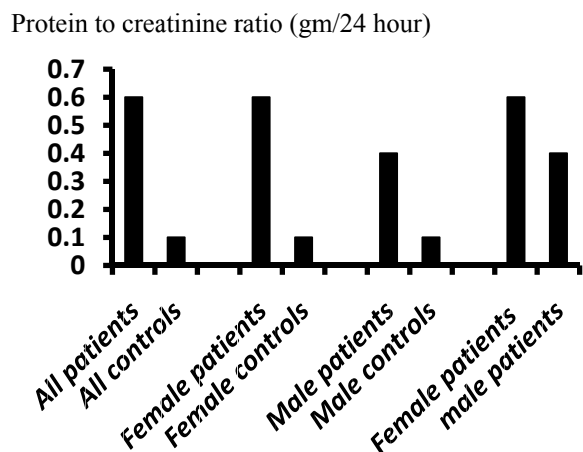


Figure 4. Level of protein to creatinine ratio in all patients with SSc and controls (males and females)

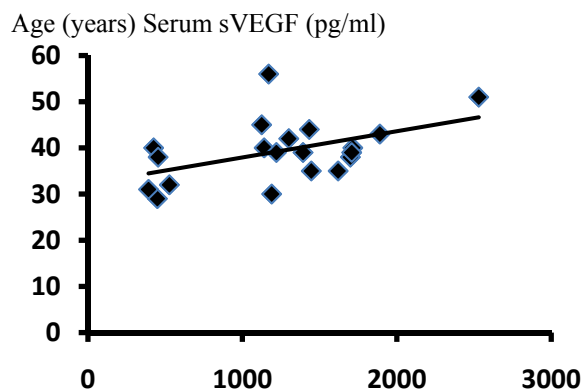


Figure 5. Correlation between level of serum sVEGF and age in patients with SSc ($r=+0.3$, $p=0.03$)

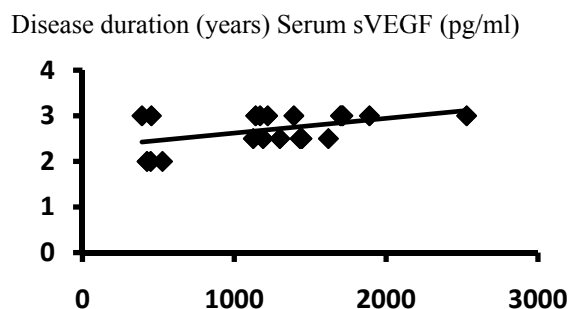


Figure 6. Correlation between level of serum sVEGF and disease duration in patients with SSc ($r=+0.4$, $p=0.01$)

4. Discussion

Systemic sclerosis is an autoimmune connective tissue disorder of unknown cause characterized by humoral and cellular immune dysregulation, prominent alterations in the microvasculature and most characteristically by excessive deposition of collagen and other extracellular macromolecules in the skin and visceral organs⁽²⁵⁾.

Clinical hallmark of the disease is the name-giving skin thickening (scleroderma), which is usually quantified according to the (MRSS)⁽²⁶⁾.

Since its discovery in 1962, relaxin (RLX) has long been regarded as a hormone of pregnancy on the basis of its several actions that help to facilitate gestation, parturition and lactation⁽²⁷⁾. These actions were assumed to be achieved through extracellular matrix (ECM) turnover and collagen degradation. It is now becoming evident that relaxin's ability to reduce matrix synthesis and increase ECM degradation has important implications in several non reproductive organs, including the heart, lung, kidney, liver and skin⁽²⁸⁾. The hormone is present in both sexes and this emphasizes the pleiotrophic role of the hormone. This was recently approved by

Agoulnik (2010), who confirmed the importance of RLX in the development and anatomical position of the testis, the maintenance of spermatogenesis, as well as sperm functions⁽²⁹⁾.

The increased level of RLX in our SSc patients agrees with **Giordano et al.**, (2005)⁽³⁰⁾ who found that serum RLX was significantly increased in SSc patients than in controls. This literature was the only published research measuring serum RLX in SSc patients, but RLX has been evaluated in other pathological conditions such as breast cancer and chronic heart failure and was reported to be increased^(31, 32).

The importance of studying relaxin in SSc emerges from the fact that SSc is a connective tissue disease in which tissue fibrosis is the predominant clinical feature that affects skin and internal organs including the lungs, kidneys and heart⁽²⁷⁾. Relaxin is a peptide hormone that exhibits anti-fibrotic and anti-inflammatory actions, while promoting vasodilatation, wound healing, and angiogenesis⁽²⁸⁾.

Giordano et al. (2005) suggested that RLX overproduction and secretion in SSc might represent a defensive response against the fibrotic process due to the well known anti fibrotic activities of the hormone⁽³⁰⁾.

Recombinant H2 relaxin treatment with an early onset of dermal fibrosis resulted in the significant reduction and normalization of dermal fibroblast function and collagen concentration. In contrast, H2 relaxin had no significant effects on established dermal fibrosis and its ability to inhibit established pulmonary, renal and cardiac fibrosis ranging from 40% to 70% at this time point^(33, 34). Viscosity of the dermis, compared to the other organs and / or permanent damage to the internal structure of the skin, most likely explains why relaxin may not be responsive at this time point. These findings demonstrate that there is a narrow window within which relaxin can successfully be used as an anti-fibrotic therapy for dermal scarring.

It was recently demonstrated that relaxin was biologically active and well tolerated in humans⁽³⁵⁾. Although relaxin treatment was beneficial in some individuals only, the primary efficacy of relaxin as an antifibrotic agent was not met due to the addition of several patients with end-stage scleroderma that appeared to be untreatable. Potency and efficacy of relaxin as an antifibrotic therapy is diminished, when applied to more severe stages of dermal fibrosis⁽³⁵⁾.

Relaxin has been shown to act at multiple levels to decrease collagen accumulation in several organs, including the skin^(6, 8). Although limited studies have demonstrated that relaxin is able to bind to endothelial cells⁽³⁶⁾ and adipocytes⁽³⁷⁾, certain studies demonstrate that relaxin acts directly on

dermal fibroblasts to inhibit their differentiation, which would otherwise lead to the over-production of collagen (and other matrix proteins) in the skin⁽³⁵⁾.

Czirja 'K. (2008) stated that MRSS is considered to be the 'gold standard' for measuring the dermal skin thickness in SSc⁽³⁸⁾. The MRSS has been approved to be one parameter of the activity⁽³⁹⁾ and severity scales⁽⁴⁰⁾.

Relaxin level was found to be higher in patients having high MRSS. Moreover, serum RLX correlated positively and significantly to disease MRSS. It is logic to find high RLX level with increased MRSS which mean more skin fibrosis which can trigger more RLX production. Meanwhile, skin thickening is a relatively a direct measure of the underlying process of fibrosis⁽¹⁵⁾.

Hanitsch et al. (2009) stated that improvement of skin sclerosis, spontaneously or as a result of treatment, does not necessarily reflect improvement of organ fibrosis⁽²⁶⁾.

The primary receptor for relaxin, LGR7 has been identified in several tissues and is likely a key regulator of collagen turnover in the heart, kidney, and lung⁽⁴¹⁾. Also, plasma relaxin levels were found to correlate with Forced Vital Capacity Percent of predicted (FVC% predicted). This may explain the higher levels of relaxin in patients without IPF in this study. Since naturally occurring higher relaxin production in women may protect against fibrosis under pathological conditions suggesting it may be responsible for differences in IPF gender phenotypes it was expected to find less incidence of IPF in the female group of patients but this did not reach significance in the present work. Again, females had lower levels of relaxin which could result from consumption or may be due to over production in males with enhanced fibrosis to overcome the pathological cascade⁽⁴¹⁾.

Renal complications are common in SSc although they are not always clinically significant. There are some recognized abnormalities in SSc renal physiology that may have predisposed the SSc patients to such renal events. Many patients with SSc have reduced renal blood flow and higher plasma renin levels, either reclining or sitting at rest, after exposure to cold or with sodium depletion. This suggests that the endovascular systems of patients with SSc are sensitive to changes in blood flow and other stimuli and may have contributed to the new-onset hypertension and in some cases full-blown renal crisis⁽⁴²⁾.

Relaxin causes renal vasodilatation and hyperfiltration by increasing nitric oxide (NO) production via stimulation of type 2 NO synthase^(5, 43). In addition, relaxin acts on endothelin by binding to the endothelin B receptor, which is involved in

renal vasodilatation, hyperfiltration, and reduced myogenic reactivity of small renal arteries. Therefore, it was not unexpected that the effects of renal vasodilatation and hyperfiltration disappeared when relaxin was withdrawn.

Until more experience in healthy persons or in patients with circulatory or renal abnormalities will be available ⁽²⁸⁾, we recommend that the use of relaxin must be with caution. We suggest that patients who receive relaxin therapy should have their BP monitored daily during relaxin treatment and for several weeks following withdrawal of relaxin, as a means of monitoring for new-onset hypertension or acute renal impairment. When hypertension appears, serial measurement of serum creatinine and control of BP are mandatory. Relaxin induces the expression of an angiogenic agent, vascular endothelial growth factor (VEGF) ^(44,45).

Older age group in our research had high incidence of digital ulcers, IPF, PH, increased skin thickness, high level of VEGF and low level of RLX. This is due to the long duration of the disease which leads to more complications as fibrosis, vasculitic changes, and angiogenetic effect. This agrees with Bennett ⁽³⁾.

In our study there was a positive correlation between MRSS, RLX and VEGF and this agrees with **Palejwala et al.**, 2002 ⁽⁴⁵⁾.

Also in this study, there was no significant difference in PH, RFT, and skin thickness between our two groups of patients (group with digital ulcers and IPF and the group without). This means that both groups are under influence of the effect of RLX which monitors the process of fibrosis and angiogenesis independent from other factors mediated through the disease (SSc) itself.

Although relaxin treatment was suggested for pulmonary fibrosis treatment ⁽⁴⁶⁾, Recombinant Human Relaxin failed Phase III clinical trials and will not be pursued any further as a treatment for scleroderma ⁽⁴⁷⁾.

Conclusion

Relaxin can be considered as a biomarker or serological marker in SSC which correlates with disease activity and severity, and helps to predict disease outcome. It can be used to evaluate disease progression and /or patient's response to treatment.

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7/22/2012