

## INCREASED SERUM LEVELS OF ANTI-ELASTIN ANTIBODIES IN PATIENTS WITH PEYRONIE'S DISEASE

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### ABSTRACT

The cause of Peyronie's disease is unknown. Immunological mechanisms in the pathogenesis have been previously suggested. Antibodies to elastin are present in all individuals. However, abnormal serum levels of anti-tropoelastin (reflecting elastin synthesis) and anti- $\alpha$ -elastin (reflecting elastin destruction) are seen in a variety of autoimmune diseases. We show that patients with Peyronie's disease have higher levels of antibodies to tropoelastin ( $p < 0.047$ ) and  $\alpha$ -elastin ( $p < 0.012$ ) than age-matched controls, suggesting an increase in elastin synthesis and breakdown, respectively. These findings suggest the presence of autoimmune mechanisms in the pathogenesis of Peyronie's disease, which may have future diagnostic and therapeutic implications.

KEY WORDS: penile induration, antibodies, elastin, tropoelastin, autoimmunity

Peyronie's disease has no known cause and is characterized by palpable plaques within the penis, penile angulation with erection and painful penile erections. Reported associated factors include trauma to the corporeal bodies and histocompatibility antigens of the B7 cross-reacting group.<sup>1</sup> However, the HLA-B7 relationship was not confirmed in another study.<sup>2</sup> There is a higher incidence of Dupuytren's contractures in men with Peyronie's disease<sup>3</sup> and occasional spontaneous regression or disappearance of penile plaques has been reported.<sup>4</sup> A local manifestation of a generalized etiology or response is suggested.

Elastin is an extracellular matrix protein that provides compliance to organs, such as skin, blood vessels, lungs and other connective tissues.<sup>5-7</sup> The synthesis and breakdown of elastin occur throughout a lifetime.<sup>8,9</sup> Products of the biosynthesis and degradation of elastin are detected in the serum by measuring antibodies to elastin peptides of tropoelastin and  $\alpha$ -elastin, respectively.<sup>10,11</sup> We show that there is a marked increase of serum antibodies to tropoelastin and  $\alpha$ -elastin in patients with Peyronie's disease compared with healthy individuals, suggesting an increased production and degradation of elastin in this disease.

### MATERIALS AND METHODS

**Subjects.** Sera were obtained from consecutive patients with Peyronie's disease from the urology clinics at Loma Linda University and Loma Linda Veterans Administration Hospital. Sera from 8 patients with Peyronie's disease were compared with 8 age-matched, otherwise healthy patients with degenerative arthritis. Degenerative arthritis does not alter normal semen levels of anti-elastin antibodies (unpublished observation). Sera were stored at  $-70^{\circ}\text{C}$  until tested.

**Measurement of anti-elastin antibodies.**  $\alpha$ -Elastin, soluble peptides of insoluble elastin, was prepared from human cadaver aorta according to the method described by Mecham and Lange.<sup>12</sup> Porcine aortic tropoelastin was prepared and purified from copper deficient swine by a modified method described by Sandberg et al.<sup>13</sup> Tropoelastin and  $\alpha$ -elastin purity was greater than 95% as verified by amino acid analyses.<sup>14</sup> An enzyme-linked immunosorbent assay modified from that of Giro et al.<sup>15</sup> for detection of anti-tropoelastin and anti- $\alpha$ -elastin antibodies in plasma samples was performed. Microtiter 96 well plates were coated with 200  $\mu\text{l}$ . antigen (tropoelastin or  $\alpha$ -elastin) dissolved in carbonate buffer (pH 9.6) at a concentration of 15 mg./ml., with 200 ml. of 0.1% bovine serum albumin in phos-

phate buffered saline (pH 7.2) serving as a negative control for wells containing tropoelastin or  $\alpha$ -elastin. The plates were sealed and incubated overnight at  $4^{\circ}\text{C}$ . The plates were then washed 3 times with phosphate buffered saline-Tween (0.05%) before adding 100 ml. of patient serum diluted at 1:100 with phosphate buffered saline containing 10% heat inactivated fetal calf serum. Phosphate buffered saline buffer (100 ml.) with 10% fetal calf serum was added to each control well. Plates were sealed and incubated overnight at  $4^{\circ}\text{C}$ . The wells were washed 3 times with phosphate buffered saline-Tween. Then, 100 ml. goat anti-human IgG coupled to phosphatase diluted with phosphate buffered saline-Tween at 1:1,000 was added to each well and incubated at  $37^{\circ}\text{C}$  for 1 hour. The wells were again washed 3 times with phosphate buffered saline-Tween and then 200 ml. para-nitrophenyl phosphate, made up in diethanolamine buffer (pH 9.6) at a concentration of 1 mg./ml., were added to each well and incubated for 15 minutes to 2 hours. The results were recorded as absorbance  $A_{405\text{ nm}}$  for the conversion of p-nitrophenyl phosphate to p-nitrophenol, expressed as the difference between the test sample and the serum response to bovine serum albumin alone. All sera were assayed simultaneously on a Titertek Multiskan MC enzyme-linked immunosorbent assay reader.

**Measurement of antibodies to collagen types 1 and 3.** Collagen type 1 and type 3 were solubilized in carbonate buffer at 15  $\mu\text{g.}/\text{ml}$ . at pH 9.6 and used to coat 96 well microtiter plates. An enzyme-linked immunosorbent assay measuring antibodies to collagen type 1 and type 3 was done otherwise as described previously for elastin.

**Statistics.** The patient groups were compared to the control group nonparametric statistics using the Wilcoxon matched pairs test.

### RESULTS

Table 1 shows that serum antibodies to tropoelastin are much higher in patients with Peyronie's disease than in age-matched controls ( $1.053 \pm 0.142$  versus  $0.811 \pm 0.144$ ,  $p < 0.047$ ). Antibodies to  $\alpha$ -elastin were higher in serum from Peyronie's disease

TABLE 1. Serum anti-tropoelastin and anti- $\alpha$ -elastin antibodies in 8 patients with Peyronie's disease compared with 8 age-matched controls

Antibodies	Optical Density $\pm$ SEM*		p Value
	Healthy Controls	Peyronie's Disease	
Anti-tropoelastin	$0.811 \pm 0.144$	$1.053 \pm 0.142$	$<0.047$
Anti- $\alpha$ -elastin	$0.979 \pm 0.051$	$1.377 \pm 0.078$	$<0.012$

\* Measured by enzyme-linked immunosorbent assay.

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patients than from age-matched controls ( $1.377 \pm 0.078$  versus  $0.979 \pm 0.051$ ,  $p < 0.012$ ). Antibodies to collagen type 1 and type 3 in serum from patients with Peyronie's disease (table 2) were not statistically different than those from the control population ( $0.638 \pm 0.066$  versus  $0.537 \pm 0.055$ ,  $p = 0.13$  and  $1.166 \pm 0.099$  versus  $1.049 \pm 0.099$ ,  $p = 0.2$ , respectively).

#### DISCUSSION

The pathogenesis of Peyronie's disease is not well understood. The association with Dupuytren's contractures and cross-reacting B7 antigens suggests that a metabolic or autoimmune derangement may be involved in the disease process. We decided to study autoimmune abnormalities in patients with Peyronie's disease, since the major discernible defect appears to be dense collagenous penile plaques or strands of dense fibrous tissue within the tunica albuginea,<sup>4</sup> not unlike destructive changes in connective tissue seen in collagen-vascular diseases. The most predominant forms of collagen in soft tissues are types 1 and 3. Our study shows that antibodies to collagen types 1 and 3 are present in patients with Peyronie's disease but at no different amounts than in healthy age-matched controls (table 2). It appears that collagen antibodies may not have a major role in the pathogenesis of Peyronie's disease.

Serum antibodies to elastin are found in all individuals but their function is not clear. Some investigators hypothesize that anti-elastin antibodies may participate in some forms of atherosclerosis, since immune complexes containing anti-elastin antibodies are present in the atherosclerotic plaques.<sup>16-18</sup> Recent studies demonstrated that antibodies to tropoelastin peptides (the precursor to elastin) and  $\alpha$ -elastin peptides (a breakdown product of elastin) correlate closely with the production and destruction of elastin in the body.<sup>11</sup> Our study shows that patients with Peyronie's disease appear to have a marked increase in elastin production as demonstrated by high levels of serum anti-tropoelastin antibodies and an increased level of antibodies to  $\alpha$ -elastin, indicative of elastin destruction. Both findings suggest a high turnover rate of elastin in Peyronie's disease. Microscopic examination of plaques in Peyronie's disease shows dense packing of collagen consistent with the displacement of elastin and other structures similar to that seen in the skin of scleroderma patients. The antibody response to elastin might possibly participate in the pathogenesis of Peyronie's disease or it may be merely a marker of elastin production and degradation as a result of a different disease mechanism. Autoimmune mechanisms shown in our study to be present in Peyronie's disease may or may not be the predominant feature in the pathogenesis of this condition but they may have therapeutic implications. As demonstrated by the large number of therapeutic modalities attempted in Peyronie's disease, the effectiveness of treatment is at best limited.

The destruction of elastin and the increase in collagen packing in penile plaques appear similar to mechanisms seen in scleroderma and other connective tissue disease. Although advances in treatment in connective tissue diseases have been

slow, progress is being made. Our study suggests that there may be a rationale to consider therapy focused on the immune system in patients with more severe involvement with Peyronie's disease using immunosuppressive agents, such as cytotoxic drugs. Hopefully, specific immunotherapy with agents such as monoclonal antibodies aimed at a specific, as yet undiscovered, antigen or cell surface marker responsible for Peyronie's disease will be possible in the future. Anti-elastin antibodies or elastin derived peptides may be involved in the pathogenesis of Peyronie's disease as represented by changes reported in our study. However, much work must be done to establish a connection between elastin and Peyronie's disease.

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TABLE 2. Serum antibodies to types 1 and 3 collagen in 8 patients with Peyronie's disease compared with 8 age-matched controls

Anti-Collagen Antibodies	Optical Density $\pm$ SEM*		p Value
	Healthy Controls	Peyronie's Disease	
Type 1	$0.537 \pm 0.055$	$0.638 \pm 0.066$	= 0.13
Type 2	$1.049 \pm 0.099$	$1.166 \pm 0.099$	= 0.2

\* Measured by enzyme-linked immunosorbent assay.