

Anti-Elastin Antibodies and Elastin Turnover in Normal Pregnancy and Recurrent Pregnancy Loss

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Keywords

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Problem

The aim of this study was to investigate elastin turnover and autoimmunity in patients with a history of recurrent pregnancy loss (RPL) and during normal pregnancy.

Method of study

Anti- α -elastin and anti-tropoelastin IgG and IgM antibodies were measured by a home-made ELISA in serum samples of 60 medically and obstetrically normal pregnant women, classified to three trimester groups, 18 female patients with RPL and 18 healthy non-pregnant women with a history of successful pregnancies. One way analyses of variance and Least Significant Difference method were used for a statistical analysis.

Results

Anti- α -elastin IgG autoantibodies were significantly decreased in the third trimester pregnant women. IgM anti- α -elastin autoantibodies were significantly decreased in all pregnancy groups compared with the controls. Synthesis/degradation ratio of elastin was significantly increased in the third trimester pregnancy group, suggesting decreased elastin degradation during this period of pregnancy. Comparing the RPL patients with the healthy non-pregnant controls showed a significantly increased anti- α -elastin IgG antibody and significantly decreased synthesis/degradation ratio in the patient's group, suggesting increased elastin degradation in RPL.

Conclusion

Elastin degradation is decreased during normal pregnancy. Increased anti-elastin IgG antibodies may contribute to the pathogenesis of pregnancy losses.

Introduction

Elasticity within distensible tissues such as arterial wall, skin and lung is associated with the presence

of elastic fibers in the extracellular matrix. Mature elastic fibers that impart elasticity consist of two morphologically distinct components, an amorphous material containing elastin and microfibrillar compo-

ment, which is present around the periphery of the amorphous material and interspersed within it.¹ Elastin is formed from the soluble precursor molecule tropoelastin (single linear polypeptide chain of 800 amino acid residue length), which becomes cross-linked by lysine-derived crosslinks, such as desmosine, isodesmosine, dehydrolysylnorleucine, or lysylnorleucine to form an elastic matrix that is capable of undergoing elastic recoil.² Elastin is one of the most hydrophobic polypeptides, the least soluble protein in the body and extremely durable, lasting the lifetime of the organism. The metabolic turnover of mature elastin fibers in adults is relatively slow. Degradation of elastic fibers is mediated by elastolytic enzymes, which are liberated from different cells including granulocytes, monocytes, lymphocytes, skin fibroblasts, cancer cells and others. These enzymes include: elastase from neutrophils and platelets, cathepsin G, metalloproteinases (MMPs) and the macrophage metalloelastase (MMP-12).³ Although only small amounts of elastin are degraded normally, increased degradation and fragmentation of elastic fibers may play a significant role in disease processes.

The uterus would appear to have specific features, which allow a degree of dramatic remodeling during pregnancy to accommodate the growing fetus. Qualitative observation of elastin distribution in the non-pregnant uterus revealed a decreasing gradient from the outer towards the inner myometrium, and the presence of elastin in the endometrial arterioles.⁴ The distribution of elastic components in the human uterus studied by immunocytochemical localization of elastin shows that within the endometrium elastin is present in the basal portions of the spiral arterioles and as traces in the basal lymphoid aggregates.⁴ According to the same study, within the myometrium, elastin was present in the perivascular tissue, particularly near the large vessels and within the smooth muscle of the outer myometrium. The markedly uneven distribution of elastin components in non-pregnant uterus and its decreasing gradient from outer to inner myometrium was confirmed by others.⁵

Guinja-Smith and Wossener reported that during pregnancy, the elastin content of the human uterus increases four- to fivefold and that in non-gravid uteri, the elastin content increases with parity.⁶ Uterine elastin contains 2.4 residues of desmosine plus isodesmosine in 1000 residues of amino acids. This value falls to 0.95 at term, indicating that synthesis of desmosines does not keep pace with the

synthesis of elastin and the cross-links remain constant during this change.⁷ Uterine artery also shows an adaptive media remodeling during pregnancy. The morphological changes in the arterial wall of the uterine artery in response to blood flow induced by pregnancy on a rabbit model showed significant media thickening and elastin laminae degradation.⁸ Uterine elastin metabolism appears to be unlike that of other elastic tissues, e.g. lung and large blood vessels – it is continuously expressed, and appears to be in continual cycle of degradation and replacement.⁹

Autoantibodies to α -elastin (elastin breakdown product) and tropoelastin (elastin precursor) are found in the serum of the healthy human subjects^{10,11} and correlate with their respective serum peptide levels. These physiologic autoantibodies are assumed to be a part of a homeostatic mechanism, which clears altered elastin structures *via in situ* destruction or *via* opsonization of the products of degradation. Serum antibodies to tropoelastin (reflecting elastin synthesis) and α -elastin (reflecting elastin destruction) appear to correlate with the production and breakdown respectively of the elastic tissue.¹² Several studies established that abnormal variations in elastin metabolism may be detected by measuring ratios of α - and tropoelastin IgG antibodies as markers of elastin degradation and synthesis.^{12–14}

An increased elastin turnover can occur in several disorders, but its role is not well known. It seems that elastin degradation plays a role in the pathogenesis of a variety of autoimmune diseases where abnormal serum levels of anti-tropoelastin and anti- α -elastin are seen.¹⁴ A marked increase in pathologic anti- α -elastin autoantibodies was found in patients with systemic lupus erythematosus, scleroderma and polyarteritis nodosa and their role in the pathogenesis of autoimmune alterations has been suggested.^{14–16}

Still, elastin turnover and anti-elastin autoantibodies have not been an object of investigation in normal pregnancy trimesters and patients with recurrent pregnancy loss.

The aim of this study was to investigate the role of elastin autoimmunity for the occurrence of recurrent pregnancy loss (RPL) by determination of serum anti- α -elastin and anti-tropoelastin antibodies in patients with a history of RPL and the healthy non-pregnant controls. Additionally, we aimed to investigate elastin turnover during normal pregnancy by determination of the ratio of anti-tropoelastin to anti- α -elastin antibodies in the sera of first, second and third trimester pregnant women.

Materials and methods

Subjects

This is a prospective study carried out on three subjects groups. Ethical permission was obtained from the local ethical committee, and informed consent was obtained from all women prior to entry into the study. Venous blood samples were obtained from the following groups:

Group 1 comprised 60 pregnant women of a mean age of 24.3 (range 18–37) years attending prenatal care unit prior to termination of pregnancy. The inclusion criteria were: medically and obstetrically healthy pregnant women, first singleton pregnancy conceived normally, no history of miscarriage, or autoimmune disease. At the time of the blood sample obtaining, there were three subgroups of women, according to the gestational age: group 1a – first trimester pregnancy ($n = 20$), group 1b – second trimester pregnancy ($n = 20$) and group 1c – third trimester pregnancy ($n = 20$).

Group 2 comprised 18 non-pregnant women of a mean age of 34.6 (range 27–39) years with a previous history of recurrent pregnancy loss, defined as two or more consecutive pregnancy losses before 10 weeks of gestation. All women had gone through at least two unexplained previous miscarriages, with the group having had a mean of 2.07 (2–4) previous miscarriages and no live births. There were no karyotype abnormalities. At the time of sampling, it had been at least 1 month since any of the women had suffered a miscarriage.

Group 3 comprised 18 healthy non-pregnant women of a mean age of 29.4 (range 23–39) years with no history of miscarriage, or autoimmune disease. All of the women had already had at least one previous successful pregnancy.

At the time of the subject inclusion, 10 mL of blood sample was obtained by peripheral venipuncture, in a dry tube, for subsequent serum antibody assaying. Serum was stored at -70°C until analysed using home-made enzyme-linked immunosorbent assay (ELISA).¹⁵

ELISA for Determination of Anti- α -elastin and Anti-tropoelastin IgG and IgM Antibodies

The serum levels of anti- α -elastin (IgG and IgM) and anti-tropoelastin (IgG and IgM) antibodies were measured by a home-made ELISA.¹⁵ Microtiter 96-well

plates (MICROLON, U-bottom, high binding, Greiner Bio One, Frickenhausen Germany) were coated with α -elastin (for anti- α -elastin antibody determination) and tropoelastin (for anti-tropoelastin antibody determination) by adding 100 μL of a solution of human aortic α -elastin (kindly presented by Prof. Keith K. Colburn, JL Pettis Memorial Veterans Medical Center, Loma Linda Medical School, Loma Linda, CA, USA) or porcine aortic tropoelastin from a copper-deficient swine (kindly presented by Lawrence B Sandberg, JL Pettis Memorial Veterans Medical Center, Loma Linda Medical School, Loma Linda, CA, USA), (10 $\mu\text{g}/\text{mL}$ dissolved in 0.05 M carbonate buffer, pH 9.6) to each well and incubating for 2 hr at 37°C and overnight at 4°C . Wells were washed with a solution of PBS, containing 0.05% Tween 20 (PBS-Tween) and then blocked by incubation for 1 hr with 1% bovine serum albumin in PBS-Tween. After washing with PBS-Tween, 100 μL of patient sera, diluted 1:5 in PBS-Tween was added. The plates were incubated for 1 hr at 37°C . The wells were then washed with PBS-Tween, incubated with a peroxidase-linked anti-human IgG and IgM (Bul Bio, National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria) diluted 1:6 400 for IgG and 1:12 800 for IgM in 1% human serum albumin in PBS-Tween) and after the washings reacted with *o*-phenylenediamine plus 0.1% H_2O_2 as colorimetric substrate. The reaction was terminated by 50 μL 8 M H_2SO_4 and the absorbance was read at 492 nm on automatic micro-ELISA plate reader.

Determination of Elastin Synthesis to Degradation Ratio

The elastin synthesis/degradation ratio for each subject was counted by dividing the mean value of anti-tropoelastin IgG antibody by the mean value of anti- α -elastin IgG antibody. The elastin synthesis/degradation ratio for each study group was determined by calculating the mean value and standard deviation of all ratios of the subjects in the group.

Statistical Analysis

Differences in anti- α -elastin and anti-tropoelastin IgG and IgM autoantibodies as well as in the elastin synthesis/degradation ratio between the groups were analysed for statistical significance ($P < 0.05$) with one-way analysis of variance (ANOVA) and multiple comparison test – Least Significant Difference (LSD)

method) using the statistical package SPSS, v. 13 (SPSS Inc., Chicago, IL, USA).

Results

Elastin Turnover in Normal Pregnancy

Results from determination of anti- α -elastin and anti-tropoelastin IgG and IgM autoantibodies in the three trimester pregnant women and the healthy non-pregnant controls are shown in Table I. Mean values of optical density and standard deviations for each study group are shown. The differences between pregnant women groups and the healthy non-pregnant controls were examined by ANOVA. *P* value versus controls is presented for each pregnancy group. Significant differences of anti- α -elastin IgG autoantibodies were established between third trimester pregnant women and controls – IgG was significantly decreased in relation to controls (*P* = 0.043). Anti- α -elastin IgM autoantibodies were significantly reduced in all pregnancy groups in relation to controls: *P* < 0.001 (1st trimester), *P* = 0.003 (2nd trimester) and *P* = 0.002 (3rd trimester). Comparison of anti-tropoelastin antibodies among the three trimesters and controls does not show significant differences neither for IgG nor for IgM isotypes. Synthesis/degradation ratio was significantly increased in third trimester pregnancy group compared with non-pregnant controls, suggesting decreased elastin degradation during this period of normal pregnancy.

The analysis of differences between the three trimester pregnancy groups are shown in Table II. Statistically significant difference was established only between first and third trimester anti- α -elastin IgG antibody – in third trimester group it was significantly decreased compared with the first trimester, suggesting reduced elastin degradation in the third trimester of pregnancy.

Elastin Turnover in RPL Patients

Comparison between RPL patients and the healthy non-pregnant controls showed significantly increased anti- α -elastin IgG antibody in the patient group (Sig. = 0.042). Analysis of anti- α -elastin IgM and anti-tropoelastin IgG/IgM did not show significant differences between patients and controls. Synthesis/degradation ratio in RPL patients was significantly decreased compared with the controls, suggesting an increased elastin degradation in RPL.

Discussion

Woessener and Brewer reported in 1963 that during pregnancy the human uterus increases at least 11-fold in wet weight, sevenfold in collagen and five- to sixfold in elastin content.¹⁷ After parturition, there is a rapid involution which is 75% complete by 8–11 days *post partum*. Collagen and elastin undergo rapid breakdown during this process. When involution is complete, the uterus is enlarged and contains

Table I Serum Levels (Average Optical Density Values) of Anti- α -Elastin and Anti-Tropoelastin Antibodies and Synthesis/Degradation Ratios in the Study Groups

Study group (N)	Anti- α -elastin antibody		Anti-tropoelastin antibody		Synthesis/degradation ratio (<i>P</i> versus controls)
	IgG (O.D) (<i>P</i> versus controls)	IgM (O.D) (<i>P</i> versus controls)	IgG (O.D) (<i>P</i> versus controls)	IgM (O.D) (<i>P</i> versus controls)	
Pregnant 1st trimester (<i>n</i> = 20)	0.397 ± 0.148 (<i>P</i> = 0.974)	0.243 ± 0.047 (<i>P</i> * = 0.000)	0.599 ± 0.204 (<i>P</i> = 0.318)	0.200 ± 0.050 (<i>P</i> = 0.512)	1.55 ± 0.405 (<i>P</i> = 0.429)
Pregnant 2nd trimester (<i>n</i> = 20)	0.365 ± 0.108 (<i>P</i> = 0.427)	0.254 ± 0.052 (<i>P</i> * = 0.003)	0.593 ± 0.155 (<i>P</i> = 0.379)	0.211 ± 0.056 (<i>P</i> = 0.956)	1.62 ± 0.384 (<i>P</i> = 0.062)
Pregnant 3rd trimester (<i>n</i> = 20)	0.318 ± 0.092 (<i>P</i> * = 0.043)	0.252 ± 0.029 (<i>P</i> * = 0.002)	0.525 ± 0.161 (<i>P</i> = 0.691)	0.195 ± 0.028 (<i>P</i> = 0.353)	1.72 ± 0.456 (<i>P</i> * = 0.049)
RPL patients (<i>n</i> = 18)	0.477 ± 0.137 (<i>P</i> * = 0.042)	0.279 ± 0.045 (<i>P</i> = 0.198)	0.546 ± 0.143 (<i>P</i> = 0.999)	0.190 ± 0.052 (<i>P</i> = 0.241)	1.16 ± 0.210 (<i>P</i> * = 0.047)
Controls (<i>n</i> = 18)	0.396 ± 0.094	0.299 ± 0.047	0.546 ± 0.134	0.212 ± 0.081	1.40 ± 0.45

P value versus non-pregnant controls is shown for each group.

**P* < 0.05 was considered significant.

Table II One Way Analysis of Variance of the Differences of Anti- α -Elastin and Anti-Tropoelastin Antibodies and Synthesis/Degradation Ratios Between the Study Pregnancy Groups

Study group	Sig. (<i>P</i> value) versus 3rd trimester				
	Anti- α -elastin		Anti-tropoelastin		Synthesis/ degradation ratio
	IgG	IgM	IgG	IgM	
Pregnant 1st trimester (<i>P</i> value)	0.035*	0.515	0.153	0.778	0.230
Pregnant 2nd trimester (<i>P</i> value)	0.211	0.885	0.193	0.376	0.992
Pregnant 1st trimester versus 2nd trimester (<i>P</i> value)	0.397	0.432	0.912	0.543	0.277

P value versus each trimester is shown.

**P* < 0.05 was considered significant.

more elastin and collagen. In non-gravid uteri that have undergone six or more pregnancies, elastin is five times the values found for nulliparous uteri.¹⁷

It could be suggested that the homeostasis of elastin synthesis/degradation ratio according to the gestation is strongly required for the uterine remodeling during pregnancy to accommodate the growing fetus. Growth and remodeling of elastin must involve highly coordinated interactions between cells, cytokines, proteinases, proteinase activators and inhibitors as well as the extracellular matrix itself. Alterations of elastin metabolism during pregnancy could induce increased production of either anti-elastin and/or anti-tropoelastin autoantibodies. Once increased, these autoantibodies could enhance the metabolism alteration or cause autoimmune reactions, which are potentially harmful for the pregnancy.

Anti-elastin antibodies together with anti-tropoelastin antibodies and elastin-derived peptides, established in sera of healthy subjects,^{10,11} are markers of elastin degradation and synthesis. Anti-elastin antibodies may play a physiologic role in the remodeling of the injured or senescing elastic structures by binding and clearance of elastin degradation products. Wei et al. established that monoclonal and polyclonal anti-human aorta elastin antibodies stained elastic fibers on tissue sections, suggesting that the epitopes recognized are available on the native fibers for reactions with the antibodies.¹⁸ Abnormal variations in elastin metabolism were established in a variety of autoimmune disorders by measuring ratios of anti- α - and anti-tropoelastin IgG antibody.^{14–16}

To our knowledge, this is the first study on elastin turnover during the three trimesters of normal pregnancy. We used the serum levels of anti-tropoelastin

and anti- α -elastin antibodies and their ratio as markers of elastin synthesis and degradation. In all trimester groups, we established significantly decreased levels of anti- α -elastin IgM antibodies compared with the controls and significantly reduced anti- α -elastin IgG during the third trimester. Synthesis/degradation ratio was significantly increased during the last trimester. These findings all together suggest decreased elastin degradation during pregnancy in terms of constant synthesis as the levels of anti-tropoelastin antibodies did not show significant differences in relation to non-pregnant controls. We could speculate that decreased elastin degradation has a protective role in pregnancy. This hypothesis is in accordance with the following findings: (1) Products of elastin fibers degradation – serum elastin-derived peptides (EDPs) have distinct cellular effects as they interact with cell membrane receptor complex including a 67-kDa elastin-binding protein (EBP) identified as an enzymatically inactive spliced variant of human β -galactosidase.^{19,20} These interactions activate intracellular signaling pathways that lead to diverse cellular events²¹ such as increased elastase production, free radical release, cell proliferation, and chemotactic activity for monocytes, fibroblasts, and tumor cells.²² Thus, the disruption of elastin is not just an end product of elastin turnover but it may be an important contributor to the pathogenesis of tissue injury. (2) It was established that elevated levels of EDPs contribute to a Th1 phenotype polarization of human peripheral blood lymphocytes (PBLs) by interaction and activation of the S-gal-elastin receptor associated with functional differentiation or shift towards Th-1 phenotype and cytokine production.²³ According to the debated Wegmann's hypothesis, 'pregnancy is a

Th2 phenomenon' as in normal pregnancy, there is a shift in the cytokine pattern from Th1 towards Th2.²⁴ (3) An increased elastin degradation may play a role in the pathogenesis of a variety of autoimmune diseases,^{14–16} where a marked increase in pathologic anti- α -elastin autoantibodies was found.

To evaluate the protective role of the established elastin turnover changes during normal pregnancy, we aimed to investigate elastin turnover in RPL patients. Our previous study showed increased anti- α -elastin IgG autoantibodies in a smaller group ($n = 9$) of RPL patients.²⁵ This study confirmed our previous results. Anti- α -elastin IgG autoantibodies in RPL patients were significantly increased compared with the healthy non-pregnant controls, anti-tropo-elastin IgG antibodies did not differ and synthesis/degradation ratio in RPL patients was significantly decreased. These results are evidence of increased elastin degradation in RPL patients, which is in accordance with similar results established in some autoimmune disorders.^{12–16}

The question is – if the elevated pathogenic anti- α -elastin IgG antibodies in RPL are result of the increased elastin degradation in RPL, what is the trigger mechanism for the degradation? Elastin is intertwined with other matrix components therefore its degradation involves cooperation of multiple proteinases to uncover macromolecules that mask the elastic fibers.²⁶ Degradation of elastin may be localized to the pericellular sites, where proteinases are protected from inhibitors and where potentially surface-bound enzymes may be concentrated. Elastin degradation mediated by macrophages and trophoblasts is confined to the immediate pericellular environment. Destruction of mature elastin by elastases is probably the result of an imbalance in the normal inhibitor-proteinase ratio.²⁷ The major plasma inhibitors contributing to the regulatory balance are alpha 1-proteinase inhibitor (alpha 1-PI) and alpha 2-macroglobulin. It has been established that in certain pathologic circumstances, an imbalance between the neutrophil serine proteinase elastase and its major plasmatic inhibitor alpha 1-PI leads to abnormal tissue destruction and disease development (rheumatoid arthritis, glomerulonephritis, adult respiratory distress symptom, psoriasis, cancer).²⁸ During pregnancy, elevated maternal plasma levels of elastase were established in both early and late onset forms of pre-eclampsia, and circulatory neutrophils have been reported to be activated – more acute in the former than in the latter.²⁹

Whatever the elastin degradation triggering mechanism is, once elevated, serum anti- α -elastin IgG autoantibodies could contribute to autoimmune reactions causing disturbances of the delicate immunoregulation of pregnancy. One possible autoantibody induced mechanism of pregnancy loss is inappropriate complement activation and complement-mediated immune attack at the feto-maternal interface. Though activated complement components are present in normal placentas^{30,31} in successful pregnancy it seems that uncontrolled complement activation is prevented by regulatory proteins present on the trophoblast membrane.^{32,33} Experiments performed in a mouse model of APS showed that activation of the complement cascade is required to induce pregnancy loss and thrombosis.³⁴ Activation of the complement pathway amplifies autoantibody effects by the generation of potent mediators of effector cell activation, including C3a, C5a, and C5–9 MAC, causing thrombosis, tissue hypoxia, and inflammation within the placenta, and ultimately leading to fetal injury.

In conclusion, our study refers to two assumptions: first that elastin degradation is protectively decreased during normal pregnancy and second: that increased anti-elastin IgG antibodies could contribute to the pathogenesis of pregnancy losses. These findings need further confirmation by other methods (i.e. measurement of serum EDP, anti-fibrin-1 antibody, etc). Understanding of elastin turnover and autoimmunity during normal pregnancy could be important for the therapeutic approaches in RPL. Our recent study established that physiologic anti-elastin and anti-anti-elastin autoantibodies were identified in different IVIg lots.²⁵ The presence in IVIg of anti-idiotypes against anti-elastin autoantibodies from patient's sera may provide an additional mechanism for the beneficial effect of IVIg in RPL and supports the concept of a functional idiotypic network regulating autoimmune responses in humans.

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