DETECTION OF SERUM COLLAGEN TYPE IV AND ELASTIN DERIVED PEPTIDES IN PATIENTS WITH BREAST CANCER

George Nicoloff¹, Tashko Deliyski², Asparuh Nikolov³

Key words: breast cancer, elastin-derived peptides, collagen type IV derived peptides, ELISA

SUMMARY

Breakdown of basement membrane is believed to be an essential step for tumor invasion and metastasis. The interaction between tumor cells and extracellular matrix can also result in the induction of basement membrane and elastin synthesis by tumor and stromal cells. The aim of our study was to detect serum concentrations of collagen type IV and elastin in patients with breast cancer, and to test the possible relationship of elastin-derived peptides (EDP) and collagen type IV derived peptides (CIVDP) with breast cancer. Serum levels of CIVDP and EDP were measured by ELISA in 39 breast cancer patients. These values were compared with serum levels in 25 age- and sex-matched controls. In patient group, EDP levels were independently associated with age (r=0.36; P=0.003) and tumor size (r=0.48; P=0.034), while CIVDP correlated with breast cancer stage (r=0.35; P=0.04). Our data suggested the possible correlation between changes in serum levels of EDP and CIVDP and breast cancer. Higher concentrations of EDP and CIVDP correlated with tumor pathology in breast cancer. Prospective and longer studies of larger populations are needed to identify the role of EDP and CIVDP as potential markers in breast cancer pathology.

INTRODUCTION

Breast cancer is an important disease because of its high frequency, which is continuously growing, its high death rate and the fact that this type of cancer more often affects women under 45. In 2003, the breast cancer morbidity and death rates in Bulgaria were 87.4 /100000 and 28.3 /100000, respectively (1). This morbidity rate is comparable with that of industrially developed countries. The higher mortality rate is a consequence of disease detection only in advanced stages.

Elastin is one of the major structural matrix proteins of the arterial wall (2-4). Mature elastin is composed of soluble elastin subunits, which are intermolecularly cross-linked (desmosine and isodesmosine formation) into a fibrous network that results in a highly polymerized insoluble protein. Elastin is composed largely of glycine, valine, proline and other hydrophobic residues that cluster in distinct domains that
contribute to protein elasticity. Multiple lysine residues in alanine-rich sequences are the source of the lysine-derived crosslinks such as desmosines that link the individual polypeptide chain into an elastic network (5).

During aging, the elasticity of connective tissue is reduced (6). Hornebeck et al. (7) studied the mechanism of the continual decrease of the elastin content of the vessel wall with age as compared to the elastin content of other organs and, especially, of breast cancers. Relatively large amounts of elastin were demonstrated in some human breast carcinomas (8).

Basement membrane acts as a barrier separating the epithelium from the surrounding stroma. Type IV collagen (CIV) constitutes the major component of basement membranes (9). The most widely expressed form is composed of two $\alpha_{1}(IV)$ chains and one $\alpha_{2}(IV)$ chain, and is found in the basement membrane of virtually all blood vessels. Degradation of CIV can occur under both physiological and pathological conditions (10). Type IV collagen degradation products also play an important role during angiogenesis (11), tissue remodeling and cancer progression (12,13). Verhoeven et al. (14) found in 34 patients with breast cancer that CIV accumulated beneath the basement membrane in some of the tumors. On the other hand, in mammary carcinomas, the first step of tumoral invasion is characterized by the loss of basement membrane components, particularly type IV collagen and laminin (15).

Measurement of serum antibodies to fragments of elastin (16-19) and CIV (20,21) is now possible and enables changes in degradation of the parent proteins to be detected. To monitor the levels of basement membrane components in biological fluids, several immunological methods have been developed (22). In view of the variation of the long-term prognosis in patients with breast cancer, early detection of patients at high risk of developing breast tumors would be of clinical significance.

Breakdown of basement membrane is believed to be an essential step for tumor invasion and metastasis. The presence of elastic fibers in human breast cancers was first observed by Letulle (23) in infiltrating epitheliomas of the mammary gland. Fibroblasts are able to synthesize elastin both in cell and tissue culture. This fibrous protein is not or hardly present in normal breast tissue but is present in some breast cancers. Its quantity varies between 0.6% and 9.2% of the tissue dry weight depending of the tumor sample.

The interaction between tumor cells and extracellular matrix can also result in induction of basement membrane and elastin synthesis by tumor and stromal cells. The aim of this study was (i) to detect serum concentrations of CIV and elastin in patients with breast cancer, and (ii) to test the possible relationship of elastin-derived peptides (EDP) and CIV-derived peptides (CIVDP) with breast cancer.

**MATERIAL AND METHODS**

**Subjects**

Study group consisted of 39 patients with breast cancer (mean age 61.7±9.5 years, age range 40-78 years) and control group of 25 age-matched healthy controls (56.9±7.9 years, age range 43-73 years) with no family history of diabetes, atherosclerosis or emphysema. All subjects were non-smokers.

The diagnosis was based on clinical palpation, bilateral mammography and ultrasound. Pathologic diagnosis with core needle or fine needle biopsy was obtained prior to any surgical procedure. Final pathological diagnosis was made according to the WHO classification (TNM), analyzing all tissue removed. Based on H&E staining, standardized grading, description of histologic type, extent of vessel invasion and of resection margins was reported.

Immunohistochemical (IHC) staining for estrogen receptor (ER), progesterone receptor (PR) and HER-2 was done by using the following antibodies from DAKO (DakoCytomation, Glostrup, Denmark): ER (ER, m7047, 1:50 dilution), PR (PR, m3529, 1:10 dilution) and HER-2 (A0485, 1:50 dilution). Nuclear staining for hormone receptors and membranous staining for HER-2 were evaluated.
For hormone receptors, a semiquantitative method for evaluation of immunoreactivity was utilized. This method took two factors into account: fraction of positively stained cells in the sample by high-power microscopy (no staining = 0, <1% = 1, 1-10% = 2, 11-33% = 3, 34%-66% = 4 and 67%-100% = 5) and intensity of staining measured on a 0-3+ scale. On this scale, 0 implies no staining, 1+ weak, 2+ moderate and 3+ strong staining. Using this system, the maximum score is 8. Score >3 is considered positive.

Scoring of HER-2 results was also done with a semiquantitative method using the following categories: 0-negative results or membrane staining <10% tumor cells, 1+ weak and incomplete membrane staining in >10% tumor cells, 2+ weak or moderate complete membrane staining in >10% tumor cells, and 3+ strong complete membrane staining in >10% tumor cells. Scores 2+ and 3+ are considered positive.

Routine staging examinations included physical examination, whole blood counts, and routine chemistry including liver enzymes, alkaline phosphatase, and calcium; assessment of menopausal status was also used. Chest x-ray, abdominal ultrasound and isotopic bone scan were included.

Ethical approval was obtained from the institutional research ethics committee and all patients and controls gave their written informed consent prior to enrolment in the study.

Antigen and preparation of immune sera

Soluble α-elastin was prepared from human cadaver aortas of young, healthy trauma victims (24). Elastin purity was confirmed by amino acid analysis by Prof. Robert Mecham (Washington University, St. Louis, USA).

Young male sheep and male rabbits were immunized subcutaneously with human aortic α-elastin (5 mg in 0.5 mL saline, emulsified in an equal volume of Freund’s complete adjuvant, for each animal). The antigen was administered at several sites on the back of the animals. There were 5 immunizations (with one week interval between applications). One week after the last inoculation, the titer of the anti-elastin antibodies was determined and the animals were bled. The immunoglobulins were obtained by salt precipitation (NH₄)₂SO₄ at 35% saturation and, after 48 h dialysis against saline, applied to a column of CL-Sepharose 6B (Pharmacia, Uppsala, Sweden) in a gel bed of 2.6×80 cm. Elution was carried out with saline (0.15 M NaCl) at a flow rate of 30 mL/h and 5-mL fractions were collected. The IgG fraction was identified as the third peak. The fractions from this peak were pooled and concentrated up to 10 mg protein per mL. Protein concentrations were determined according to Lowry et al. (25).

Detection of elastin-derived peptides (26)

Elastin-derived peptides were detected by an enzyme-linked immunosorbent assay (ELISA).

For our purposes, the modified version of Baydanoff et al. (24) was used with the following reagents: rabbit immune serum towards human aortic α-elastin (IgG fraction), human aortic α-elastin as reference antigen, sheep IgG towards human aortic α-elastin, and anti-sheep IgG peroxidase immunoconjugate (Sigma, USA).

- The immunoconjugate was diluted 1:1000 in diluting buffer (phosphate-buffered saline, containing 1% bovine serum albumin and 0.05% Tween 20).
- As a substrate solution, we used o-phenylenediamine (0.4 mg/mL in 0.05 M citrate buffer, pH 5.0) and 0.01% H₂O₂.
- The polystyrene plates were coated with 100 µL of rabbit anti-elastin IgG (10 µg/mL in 0.05 M carbonate buffer, pH 9.6) by incubation for 3 h at 37 °C and overnight at 4 °C.
- Before use, the plates were washed 3 times with diluting buffer to remove unbound protein.
- Blockade of the remaining “active” centers of the polystyrene wells was done by polystyrene plate incubation for 24 h with 1% solution of bovine serum albumin (BSA) (Sigma, USA) in phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 20.
The wells were then filled with 100 µL of human sera (diluted 1:5) to be tested and incubated for 1 h at 37°C.

After washing, the plates were incubated with sheep anti-elastin IgG (10 µg/mL) for 1 h at 37°C.

After washing with the diluting buffer, the plates were incubated 3 times for 1 h each with immunoconjugate in diluting buffer. Incubation with the substrate solution was carried out in the dark for 30 min at room temperature (20°C).

The reaction was then stopped by adding 50 µL of 4 M H₂SO₄. Absorbance readings were made using a Microelisa Reader 210 (Organon Teknika, Belgium) at a wavelength of 492 nm.

The following controls for the reaction were employed:

(i) substrate control: only substrate solution was added to the polystyrene wells, coated with rabbit anti-elastin IgG;

(ii) immunoconjugate control: the immunoconjugate was added directly to the wells coated with rabbit anti-elastin IgG, and the wells were then incubated with substrate solution (this control served as a reference for the measurement of the extinction values of the tested samples);

(iii) control of the rabbit immune serum: rabbit anti-elastin IgG was replaced with normal rabbit IgG;

(iv) negative controls of the specificity of the reaction: the assay was carried out according to the usual protocol, but the tested samples were replaced with standard human albumin solution (Difco Lab; USA), standard collagen type IV from human placenta (type VI, SIGMA., USA), human fetus (20 weeks of gestation), and saline extracts from multiple organs of a 30-year-old healthy subject (killed in an accident); aorta, heart, lung, brain, liver, spleen, kidney, testicle, thymus, skin and cross-striated muscles (all with concentrations 10 µg/mL); (v) positive control: the tested sample was replaced with human aortic α-elastin with concentration 10 µg/mL.

Detection of collagen type IV derived peptides

Serum concentrations of CIVDP were measured by a sandwich enzyme-linked immunosorbent assay (ELISA).

- Each well of the microtiter plate was sensitized with 100 µL of 10 µg/mL of anti-human CIV monoclonal antibody (C1926, produced in mouse, Immunogen: human collagen type IV. The antibody recognizes an epitope located on the α1 and/or α2 chains of human collagen type IV; Sigma, USA) at room temperature for 3 h; followed by overnight incubation at 4°C.

- Blockade of the remaining ‘active’ centers of the polystyrene wells was done by polystyrene plate incubation for 24 h with 1% solution of bovine serum albumin (BSA) (Sigma, USA) in phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 20.

- Then 100 µL of serum sample (diluted 1:5), or of purified human pepsin digested CIV (0-600 ng/mL) (Sigma, USA) was placed in each well of a microtiter plate and incubated for 1 h at 37°C.

- 100 µL of the second antibody (mouse anti-human CIV monoclonal antibody) conjugated with horseradish peroxidase (HRPO) according to Wilson and Nakane (28) (diluted 1:2 000) (Sigma, USA) was allowed to react in each well at 37 °C for 1 h. o-Phenylenediamine (0.4 mg/mL) was added to citrate buffer, and 100 µL of this solution was added to each well and allowed to react for 30 min.

- The reaction was stopped by adding 50 µL 4 M H₂SO₄ to each well and optical density was measured with a Microelisa Reader 210 (Organon Teknika, Belgium) at a wavelength of 492 nm.
Statistical analysis

Statistical modules in Excel (Microsoft Corporation, Redmond, WA, USA) and Statgraphics Plus (Manugistics, Rockville, MD, USA) for Windows were used to calculate statistics on all data. Values are expressed as mean (x) ± standard deviation (SD). Student’s T-test and F ratio, in one-way analysis of variance (ANOVA), were used to assess differences between study groups. Least significant difference, Tukey honest significant difference, Scheffe, Bonferroni, Newman-Keuls, and Duncan – normal distribution, and Kruskal-Wallis (K-W) Test – non-normal distribution. For selected data sets, correlation analysis was performed and data were considered significant at P value of less than 0.05.

RESULTS

In healthy control subjects, the mean concentration of EDP was 51.2±21.7 ng/mL. Significantly higher levels of EDP were observed in patients in comparison with controls (97.7±38.9 ng/mL vs. 51.2±21.7; P=0.001), range 35-200 ng/mL in patients and 10-94 ng/mL in controls. A similar tendency was found in CIVDP concentrations, which were significantly higher in patients as compared with controls (106.9±25.6 ng/mL vs. 74.6±15.8; P=0.001), range 61-170 ng/mL in patients and 44-101 ng/mL in controls.

In patient group, EDP levels correlated with age (r=0.36; P=0.003) and tumor size (r=0.48; P=0.034), while CIVDP correlated with the stage of breast cancer (r=0.35; P=0.04). There was strong correlation between EDP and CIVDP (r=0.387; P=0.001).

Negative correlation was detected between the presence of estrogen receptor and patient age (r=0.45; \(P=0.0047\)). In the present study, there was no correlation between hormone receptors (ER, PR and HER2) and changes in the levels of CIVDP and EDP in patients with breast cancer. Twenty-four patients were positive for ER, 23 for PR and 19 for HER2.

There was a correlation between lymphatic status (N) and tumor size (r=0.56; P=0.0004).

Significant changes were found in serum EDP concentration: significantly lower in T1 than in T3 and significantly higher than in controls; significantly lower in T2 than in T3 and significantly higher than in controls; and significantly higher in T3 than in all other groups (Table 1, Fig. 1). Significant changes

Table 1. Circulating elastin-derived peptides (EDP) obtained by ‘sandwich’ version of ELISA

<table>
<thead>
<tr>
<th>Study group</th>
<th>EDP (ng/mL)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (n=16)</td>
<td>89±33</td>
<td>-</td>
<td>NS</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T2 (n=19)</td>
<td>102±31</td>
<td>NS</td>
<td>-</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T3 (n=4)</td>
<td>145±48</td>
<td>P&lt;0.05</td>
<td>-</td>
<td>P&lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>Control (n=25)</td>
<td>51±22</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are mean±SD; T1 = tumor size ≤2 cm; T2 = tumor size >2 cm ≤5 cm; T3 = tumor size >5 cm; NS = non-significant; LSD test was used for significant between-group differences.

Figure 1. Mean serum elastin-derived peptide (EDP) levels in 39 study patients with breast cancer (divided according to tumor size) and 25 healthy controls. Serum EDP was significantly lower in T1 than in T3 and significantly higher than in controls; significantly lower in T2 than in T3 and significantly higher than in controls; and significantly higher in T3 than in all other groups.
were also observed in CIVDP concentrations: significantly lower in stage 1 than in stage 3 and significantly higher than in controls; significantly higher in T2 than in controls; and significantly higher in stage 3 than in stage 1 and controls (Table 2, Fig. 2).

### DISCUSSION

It is well known that elastic fibers are an integral component of the blood vessel wall (29). Elastin is a fibrous protein constituent of the extracellular matrix, degradation of which may be detected by the presence of serum EDP in the circulation. Presently, two major antigenic classes are recognized on the elastin molecule, one species specific and the other with broad species cross-reactivity (30). The levels of circulating soluble elastin peptides in healthy subjects has been suggested as an indicator of aging (24,31). EDP levels were also significantly elevated in patients with acute aortic dissection (32). Thus, soluble elastin levels in circulating blood may be informative as to pathological conditions involving connective tissues (31).

Human breast tumors are often associated with a fibrotic reaction termed desmoplasia. Tumor cells may indirectly modulate the composition of the extracellular matrix by influencing fibroblast properties. There has been considerable interest in determining the possible significance of elastosis in breast cancer outcome since Shivas and Douglas (33) reported a high correlation between focal elastosis and prognosis in breast cancer patients. This conclusion received support from several other studies, all of which measured focal periductal elastosis, which appeared to correlate with estrogen receptor positive status (34,35). We did not find a correlation in our study between the presence of ER and the soluble fragments of elastin and CIV.

Indirect support for the epithelial production of elastin in breast cancer has come from in vitro experiments with breast cancer cell lines (36). There is evidence that elastin synthesis by fibroblasts is stimulated by contact with components of the extracellular matrix of breast cancers and a start has been made to examine the interaction of breast cancer cell lines with elements of normal and cancerous matrices. These investigators showed that fibroblasts increased elastin synthesis when grown on a matrix preformed by breast cancer cells, but there was no increase in the small amounts of elastin synthesized by breast cancer epithelial cell lines grown in this system. Using ELISA, we also found an increased production of elastin in cells from patients with breast cancer.

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**Table 2. Circulating collagen type IV derived peptides (CIVDP) obtained by 'sandwich' version of ELISA**

<table>
<thead>
<tr>
<th>Study group</th>
<th>CIVDP (ng/mL)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I (n=11)</td>
<td>94±19</td>
<td>-</td>
<td>NS</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Stage II (n=19)</td>
<td>107±25</td>
<td>NS</td>
<td>-</td>
<td>NS</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Stage III (n=9)</td>
<td>120±31</td>
<td>P&lt;0.05</td>
<td>NS</td>
<td>-</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Control (n=25)</td>
<td>74±16</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are mean±SD; NS = non-significant; LSD test was used for significant between-group differences.*

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**Figure 2. Serum collagen type IV derived peptide (CIVDP) levels in 39 study patients with breast cancer (divided according to stage of the disease) and 25 healthy controls. Serum CIVDP was significantly lower in stage 1 than in stage 3 and significantly higher than in controls; significantly higher in stage 2 than in controls; and significantly higher in stage 3 than in stage 1 and controls.**
The reason for increased EDP levels in patients with breast cancer is either increased elastin synthesis or degradation. The production of elastic fibers provokes stromal fibroblasts within the adjacent breast cells to produce elastic fibers (37-39).

Polymeric insoluble elastin was isolated and characterized from 34 human breast cancers (40). Its amino acid composition showed great similarity with human aorta or ligamentum nuchae elastins. Meanwhile, it differs from these mature elastins by its degree of crosslinking; the amount of Desmosine + Isodesmosine expressed as Des + IDes/ Lys was about 4 times lower than in polymeric aorta elastin. These results suggested that breast cancer elastin is closely related to newly synthesized aorta elastin. It appears therefore that the neosynthesis of this matrix component is accelerated with age. It is difficult to discuss our data and the results of Hornebeck et al. (40) because the age of the study subjects, the methods and antigens were all different. However, we found that EDP of our patients correlated with tumor size and patient age.

An elastinolytic activity (elastase) was found in human breast cancer extracts. This activity increased with the elastin content of the tumors.

Increased EDP levels in breast cancer patients may be the result of increased elastinolytic activity in tumor cells. Probably, this is the result of increased elastase activity. Our hypothesis is supported by the data reported by Hornebeck et al. (7,40).

Kao and Stern (41) studied elastases in human breast carcinoma cell lines. They found that tumor cells had 10- to 30-fold elastase activity present in fibroblasts. This result confirms again our speculation on increased elastolytic activity. Kao et al. (42) found elastin degradation by proteases from cultured human breast cancer. The desmoplastic reaction to human breast cancer contains both collagen and elastin. We agree with the speculation of the authors that breast tumor cells themselves may modulate the turnover of these stromal proteins.

In our investigation, EDP showed a correlation with tumor size. One possible explanation is that elastin turnover is not as active during the early stages of breast cancer as that of collagen. This speculation is supported by a reduced elastin/collagen ratio (43).

Collagen IV is a major component of the extracellular matrix and vessel basement membranes. It has been demonstrated that a variety of diseases cause alterations in CIV synthesis, degradation, and release at the tissue level. Collagen IV serum levels are altered in many diseases, including liver fibrosis (44), autoimmune diseases like scleroderma (45), RA (46), SLE (47), etc.

Verhoeven et al. (14) studied the presence of basement membrane material in the areas of elastosis in 34 breast cancers. In some tumors, CIV accumulated beneath the basement membrane. Periductal elastosis in the areas of extensive fibrosis showed focal CIV immunoreactivity, indicating remnants of ducts. Each tumor showed CIV immunostaining of the elastotic areas, with various degrees of intensity. Excessive production of basement membrane material by the epithelial cells of the ducts leads to the formation of a CIV skeleton. This skeleton can act as a matrix for secondary deposition of elastic material. We also found a high production of CIV in our study patients. Moreover, CIVDP correlated with the stage of breast cancer.

Collagen IV has also been implicated in metastatic cancer. Research has shown that an alteration in CIV binding affects the metastasis of cancer cells (48).

Clavel et al. (15) underline the interactions between tumoral cells and the extracellular matrix in breast cancers with possible implications for diagnosis and prognosis. In mammary carcinomas, the first step of tumoral invasion is characterized by the loss of basement membrane components, particularly CIV and laminin.

It has been proposed that proteases secreted by cancer cells facilitate tumor invasion and metastasis by degrading the components of extracellular membranes. The lysosomal cysteine protease cathepsin L is synthesized in large amounts and secreted by many malignantly transformed cells in culture. The
secreted protease is potent in degrading collagen, laminin, elastin, and other structural proteins of basement membranes. Most breast cancers expressed elevated levels of cathepsin L. According to these authors, this enzyme may prove useful as a diagnostic or prognostic marker of human malignancy (49). Of course, proteolysis of collagen, and perhaps other extracellular matrix proteins, is of critical importance.

In our study, we used a pepsinized CIV extracted from human placenta. We did not use 7S antigen because its serum concentration is so low that the magnitude of change would be difficult to measure. This would not allow for making conclusions on a chronic pathogenic process in individual patients during a longitudinal study. Our data on healthy adults are similar to the results of Tamaro et al. (44). They used pepsinized CIV and ELISA like ours. They found that the mean serum level of CIV was higher than in our subjects (104±11.2 ng/mL vs. 74.6±15.8 ng/mL). According to these authors, CIV seems to be a useful marker for normal and pathological turnover, but patient age has to be considered for correct interpretation of the results.

In order to investigate the role of EDP and CIV in breast cancer, we studied 39 patients by ELISA. Our aim was to study the possible relationship of elastin and CIV (specific biological markers of extracellular matrix) with breast cancer. An important factor in the development of connective tissue alterations is abnormal degradation or synthesis of both proteins. As a result, collagen and elastin derived peptides are present in the circulation. We present the results of the determination of EDP and CIVDP in patients with breast cancer.

Increased synthesis or degradation could cause elevated serum levels of CIV found in individuals with disease. We tested whether serum levels of EDP and CIV are related to cancer; this study established normal serum levels of EDP and CIV in healthy controls and compared them to those in cancer patients. The patients had significant changes in the levels of EDP and CIV. The significant elevation of serum EDP and CIV levels found in this study could be a reliable marker of early stages of breast cancer. On the other hand, the level of CIV correlated with the stage of breast cancer. In breast cancer, it is probable that circulating CIV molecules may either be released from tissue during the synthesis, provided that the vessel wall allows for such leakage, be products of degradation of pre-existing basement membranes, or newly synthesized molecules (50). The published data describe an increased production of CIV and protease inhibitors, but a decreased collagenolytic activity, which suggests that the first possibility may be valid. That is why the synthesis of CIV is elevated. Serum CIV may be indicative of CIV degradation in the tissue and may correlate with metastasis. The quantitative measurement of CIV may reflect the disease severity and assist in the monitoring of disease progression and treatment effect.
REFERENCES


