Serum protein changes in breast cancer: a prospective study

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SUMMARY Sixty women admitted to the King's College Hospital group for biopsy of a lump in the breast have been followed sequentially for one year. Thirty women had early operable breast cancer and 30 had benign breast disease. Each patient had 10 serum proteins measured preoperatively and postoperatively at three months and at one year. The patients with breast cancer had significantly higher levels of β_2 glycoprotein preoperatively and caeruloplasmin at one year postoperatively than those with benign breast disease. There were a number of significant correlations between serum protein levels and the progression of breast cancer as measured by the clinical score. There were significant correlations with caeruloplasmin preoperatively and at three months postoperatively. Prealbumin and haemopexin showed correlations preoperatively; α_1 antitrypsin and β_2 glycoprotein only correlated at three months postoperatively. A longer follow-up will be required to establish the value of serum protein changes which could predict the development of metastases in patients with breast cancer.

Changes in the various fractions of serum proteins, particularly alpha glycoproteins, have been reported many times in cancer patients (Winzler and Smyth, 1948; Bacchus *et al.*, 1967; Snyder and Ashwell, 1971), and although similar changes are seen in many non-malignant conditions, eg, inflammation, the measurement of specific glycoproteins has indicated that specific protein profiles may exist for malignant and non-malignant disease, and even for different types of malignancy (Snyder and Ashwell, 1971; McPhedran *et al.*, 1972; Douma and Van Dalen, 1974).

Unfortunately, in spite of a considerable number of serum protein surveys performed in cancer patients, many of the reports are not comparable or conflict with each other. Many studies have not been confined to a single type of cancer nor taken account of the extent of the disease; the nature, age, and state of health of the control subjects has usually not been considered, and no study has reported serum protein changes followed serially in the same subjects over an extended period of time.

This study was undertaken to determine (a) whether a diagnostic profile of plasma protein changes occurs in early breast cancer compared to non-malignant breast disease; and (b) whether plasma

protein changes in breast cancer patients followed prospectively could be correlated with the spread of the tumour. The results of the first year are reported here.

Patients and methods

Sixty women admitted to King's College Hospital for biopsy of a lump in the breast have been studied. Thirty of these women were found to have breast cancer and 30 to have non-malignant breast disease. All the women were aged less than 70 years and had breast lumps less than 5 cm in diameter, with or without palpable ipsilateral axillary glands. Those with breast cancer therefore had tumours which fell into clinical stage I or II of the Manchester classification (Wise *et al.*, 1971) and had no evidence of clinically occult metastases as shown by routine chest x-ray, full blood count, and serum chemistry (serum bilirubin, alkaline phosphatase, aspartate transaminase and hydroxybutyrate dehydrogenase, calcium, phosphate, uric acid, sodium, potassium and urea) or bone scanning.

The two groups of women had venous blood sampled the day before operation and at three months and one year postoperatively.

A careful clinical record has been kept of each cancer patient and of the patients with benign

breast disease. A system of quantifying the approximate tumour mass in each cancer patient was devised to produce the clinical score, so that correlations between the protein changes and extent of the cancer could be performed. This scoring system was based upon the diameter of the breast tumour measured postoperatively, and additional points were given for evidence of histological spread of the tumour to involve skin, pectoral muscles or lymph nodes. Postoperatively the score was increased for local recurrence of the tumour in the scar or lymph nodes and for the development of each metastatic lesion confirmed by biopsy. Equal weighting was given to proven metastases irrespective of site, and the score was modified if a lesion increased or decreased in size. A reduced weighting was given to symptoms or investigations which suggested metastatic spread before a lesion was confirmed, eg, persistent backache¹.

Serum was separated from the blood samples, divided into aliquots, coded, and stored at -20°C . Samples were randomised and analysed in batches of 50.

Estimations of 10 serum proteins were made using single radial immunodiffusion on commercial Tripartigen plates (Hoechst Ltd). These were albumin, pre-albumin, α_1 acid glycoprotein, α_1 antitrypsin, α_2 HS glycoprotein, β_2 glycoprotein, caeruloplasmin, haptoglobin, haemopexin, and transferrin.

STATISTICAL METHODS

The serum protein levels for each patient were coded onto punched cards and analysed by computer. The distributions of most of the serum proteins were found to be log-normal, and statistical comparisons with such proteins were made on logarithmic data.

Results

COMPARISON BETWEEN THE TWO DIAGNOSTIC POPULATIONS

Preoperatively β_2 glycoprotein was significantly higher in the cancer patients ($P < 0.015$) but none of the other protein levels was significantly different in the two diagnostic groups (Table 1).

At three-month follow-up there were no significant differences between the mean levels of any of the proteins in the two diagnostic groups (Table 1).

At one year follow-up the mean level of caeruloplasmin was significantly higher in the cancer patients than in the benign group ($P < 0.031$) (Table 1).

¹Further details of the scoring system may be obtained from the authors.

CORRELATIONS OF SERUM PROTEIN LEVELS WITH PATIENT'S AGE

Although the mean ages of the two diagnostic groups differed significantly (benign 44 years; cancer 52 years), correlation analysis between serum protein levels and the ages of the patients in both groups failed to reveal any statistically significant relationship at any time.

CORRELATION OF INDIVIDUAL SERUM PROTEINS WITH TIME

Correlation analysis between the serum protein levels at the different time points revealed generally good correlations between levels of single proteins at the different times, particularly among the benign group (Table 2). Haptoglobin, β_2 glycoprotein, and caeruloplasmin levels showed the most internal consistency in both groups of patients.

α_1 Antitrypsin and transferrin levels were marginally more consistent in the cancer group than in the benign, but prealbumin, α_1 acid glycoprotein, α_2 HS glycoprotein, and haemopexin levels were less internally consistent in the cancer group.

CORRELATION BETWEEN DIFFERENT SERUM PROTEINS AT DIFFERENT TIMES

There were many correlations between different proteins at the various times but few were consistent with time or seen in both groups. The detailed correlation matrices are too extensive for inclusion in this paper.

The proteins that differed when the two populations were compared, ie, β_2 glycoprotein and caeruloplasmin, did not show any interrelationship.

CORRELATION OF SERUM PROTEIN LEVELS WITH CLINICAL SCORE

Correlations between the various serum proteins and the clinical scores of the cancer patients at the same time points are shown in Table 3. There were significant correlations with caeruloplasmin preoperatively and at three months postoperatively. Pre-albumin and haemopexin showed correlations only preoperatively, and α_1 antitrypsin and β_2 glycoprotein correlated only at three months postoperatively.

The distribution of clinical scores of the cancer patients at the three-month and one-year postoperative follow-up times is narrow and skewed as few patients have developed high scores by one year (Figure). This distribution probably accounts for the alteration in the direction of some of the correlations which is seen at different times.

Table 1 Comparison of serum protein levels between patients with breast cancer and those with benign breast tumours

Proteins	Cancer		Benign tumour		P
	Mean	SD	Mean	SD	
Serum albumin*					
Preop.	3302.50	1407.23	3615.00	1419.39	NS
3 mth	3359.64	1000.05	3406.88	825.60	NS
1 yr	4981.43	1661.28	5133.91	1501.21	NS
Pre-albumin†					
Preop.	1.45 (28.50)	0.21	1.45 (28.18)	0.17	NS
3 mth	1.44 (27.38)	0.16	1.47 (29.51)	0.13	NS
1 yr	1.39 (24.78)	0.14	1.38 (23.99)	0.15	NS
α_1 Acid glycoprotein†					
Preop.	1.78 (60.3)	0.15	1.79 (61.7)	0.21	NS
3 mth	1.77 (58.9)	0.18	1.72 (52.5)	0.26	NS
1 yr	1.91 (81.3)	0.20	1.81 (64.6)	0.19	NS
α_1 Antitrypsin†					
Preop.	2.17 (147.9)	0.25	2.28 (190.6)	0.19	NS
3 mth	2.18 (151.4)	0.23	2.19 (154.9)	0.14	NS
1 yr	2.28 (190.6)	0.17	2.22 (166.0)	0.18	NS
α_2 HS Glycoprotein*					
Preop.	68.54	21.75	72.06	19.17	NS
3 mth	66.18	21.10	70.25	19.42	NS
1 yr	67.18	20.92	65.38	25.41	NS
β_2 Glycoprotein†					
Preop.	1.30 (19.95)	0.09	1.23 (16.98)	0.11	< 0.015
3 mth	1.36 (22.91)	0.14	1.36 (22.91)	0.16	NS
1 yr	1.20 (15.85)	0.17	1.14 (13.80)	0.14	NS
Caeruloplasmin†					
Preop.	1.45 (28.18)	0.20	1.38 (23.99)	0.20	NS
3 mth	1.55 (35.48)	0.11	1.54 (34.67)	0.10	NS
1 yr	1.52 (33.11)	0.15	1.42 (26.30)	0.19	< 0.031
Haemopexin†					
Preop.	1.78 (60.26)	0.13	1.75 (56.24)	0.11	NS
3 mth	1.85 (70.79)	0.10	1.86 (72.44)	0.07	NS
1 yr	1.87 (74.14)	0.11	1.83 (67.61)	0.15	NS
Haptoglobin†					
Preop.	2.34 (218.77)	0.24	2.29 (194.98)	0.26	NS
3 mth	2.27 (186.20)	0.20	2.18 (151.36)	0.21	NS
1 yr	2.38 (239.86)	0.20	2.32 (208.92)	0.21	NS
Transferrin†					
Preop.	2.36 (229.08)	0.16	2.33 (213.78)	0.18	NS
3 mth	2.53 (338.82)	0.17	2.47 (295.12)	0.18	NS
1 yr	2.55 (354.80)	0.08	2.54 (346.73)	0.07	NS

*Proteins with normal distributions have their mean values expressed as mg/100ml.

†Proteins with log-normal distributions have their mean values shown as logarithms to base 10 with the antilog of the mean expressed in mg/100 ml in parentheses.

NS = not significant.

Discussion

There appear to be differences in the serum protein levels between women with breast cancer and those with benign breast disease. These differences are seen preoperatively at the earliest time of clinical detection of a breast tumour and do not appear to be related to the age difference between the groups.

Raised serum caeruloplasmin has been reported previously in patients with both cancer and chronic inflammatory diseases (Sternlieb and Scheinberg, 1961; Snyder and Ashwell, 1971), but an increase in β_2 glycoprotein levels does not appear to have been reported before. In previous studies the levels have been either unchanged (Cleve, 1968) or reduced (Snyder and Ashwell, 1971). Our study, however, is confined to one type of cancer at an early stage and is not strictly comparable with the previous studies quoted.

Although comparison of the two groups of patients has revealed only differences in caeruloplasmin and β_2 glycoprotein levels, the correlation analyses suggest that the metabolism of serum glycoproteins may be altered more fundamentally.

Although caeruloplasmin and β_2 glycoprotein levels correlated with the clinical score at certain times, a number of other glycoproteins are also correlated with the spread of breast cancer. It would therefore be unwise to restrict further surveys in cancer patients only to those serum proteins which are different from those of patients with benign disease.

The clinical scoring system does attempt to quantify the tumour mass and thus enable correlation analysis to be performed, but it is impossible to detect subclinical spread of breast cancer, and the scoring system must clearly underestimate the tumour mass of many patients. At present few of

Table 2 Correlations between individual protein levels at different times

Group	Protein	Preoperative with 3 months postoperative	Preoperative with 12 months postoperative	3 months postoperative with 12 months postoperative
Benign	Albumin	0.04	0.10	0.06
	Prealbumin	0.29	0.49	0.42
	α_1 Acid glycoprotein	0.34	0.39	0.36
	α_1 Antitrypsin	0.34	0.31	0.12
	α_2 HS Glycoprotein	0.04	0.17	0.44
	β_2 Glycoprotein	0.64	0.51	0.27
	Caeruloplasmin	0.56	0.62	0.21
	Haemopexin	0.47	0.43	0.10
	Haptoglobin	0.74	0.50	0.50
	Transferrin	0.23	0.10	0.04
Cancer	Albumin	0.20	0.22	0.13
	Prealbumin	0.10	0.11	0.32
	α_1 Acid glycoprotein	0.12	0.01	0.58
	α_1 Antitrypsin	0.47	0.44	0.30
	α_2 HS Glycoprotein	0.03	0.23	0.04
	β_2 Glycoprotein	0.43	0.53	0.34
	Caeruloplasmin	0.40	0.62	0.14
	Haemopexin	0.02	0.09	0.18
	Haptoglobin	0.56	0.35	0.35
	Transferrin	0.30	0.16	0.29

Values shown are Pearson correlation coefficient *r*.

Table 3 Correlation between serum proteins and clinical scores in patients with breast cancer

Protein	Preoperative correlation	Postoperative —3 month correlation	Postoperative —1 year correlation
Albumin	0.15	—0.13	0.21
Prealbumin	—0.36*	—0.05	—0.16
α_1 Acid glycoprotein	—0.04	—0.32	0.12
α_1 Antitrypsin	0.16	0.41*	0.22
α_2 HS Glycoprotein	—0.13	0.28	—0.08
β_2 Glycoprotein	—0.23	0.36*	—0.16
Caeruloplasmin	—0.36*	0.36*	0.14
Haemopexin	—0.36*	0.05	0.17
Haptoglobin	—0.02	0.01	—0.29
Transferrin	—0.04	—0.03	—0.02

*Statistically significant

P < 0.05

Values shown are Pearson correlation coefficient *r*.

our patients have developed metastatic disease and hence high scores and it is too early to detect any clear or simple pattern of glycoprotein changes emerging which correlates with progression of breast cancer.

The biological functions of many of these glycoproteins are numerous and not all are known, neither is the mechanism by which changes are induced by cancer. It is possible that glycoproteins are synthesised directly by the tumour, but in view of the diversity of protein changes, it seems more likely that the malignant process is indirectly affecting the metabolism of these proteins. Their synthesis in the liver is known to be influenced by many hormones (Hoch-Ligeti and Irvine, 1954; Good *et al.*, 1971) and we are currently investigating

Figure Skewed distribution of clinical scores at different times, from a larger series of breast cancer patients, including those patients currently reported.

the relationship of hormone and protein changes in our cancer patients.

In view of the comparatively long natural history of breast cancer, the prospective follow-up of cancer patients needs to be several years longer to establish the value of any prognostic profile of serum protein changes. We are therefore continuing this comprehensive survey in order to investigate the relationship of serum protein changes to the pathogenesis of breast cancer.

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