Measurement of Serum N-Acetyl β Glucosaminidase Activity in Patients with Breast Cancer

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N-acetyl-β-glucosaminidase (NAG) activity measured in sera from 129 breast cancer patients was elevated (mean 18.2 units/l) compared with that in sera from 28 healthy women (11.6 units/l) ($p = 0.001$). There was a weak correlation between NAG activity and carcinoembryonic antigen (CEA) and CA-153, but no relationship to age, menopausal status, node status, stage, histology of tumour or to steroid receptors. NAG, CEA and CA-153 were measured in periodic follow-up samples taken after surgery (up to 26 months) from 17 patients. NAG activity fluctuated within a narrow range, unlike CEA and CA-153. In 70% of cases the pattern was similar to at least one of the other markers, and was generally maintained at a higher level in patients who suffered relapse compared with those who remained disease-free up to the last follow-up, but was not significantly altered before relapse. The measurement of NAG activity is unlikely to be of value in predicting time or occurrence of relapse or of clinical utility in post-surgical therapy. Increased appearance in serum may aid metastasis by degrading the extracellular matrix and it may be better investigated as a predictor of progression from in situ to invasive and metastatic cancer.

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Treatment decisions in the management of, in particular, post-surgical cancer patients are dependent on a variety of clinical factors including the measurement of proteins that are over-expressed and released from tumour cells into the vascular system. These so-called tumour markers (in the case of breast malignancies, carcinoembryonic antigen (CEA) and (CA153) are general routinely evaluated during patient follow-up. The effectiveness of chemotherapy in suppressing metastatic growth may be reflected in the fluctuating levels of these markers. They are considered as useful indicators of the reappearance of cancer cells prior to detection by clinical tests such as bone scan.

Tumour metastasis, which involves the dissemination of malignant cells by penetration through the basement membrane into the vascular system, contributes to the circulating pool of these and other markers. In this context considerable interest has been focused on enzymes involved in degradation of the extracellular matrix and particularly in the catabolism of glycosaminoglycans to the lower viscosity metabolites which effectively reduce mechanical barriers normally impeding cellular invasion. Lysosomal enzymes such as N-acetyl β glucosaminidase (NAG:EC 3.2.1.30) are widely distributed in human cells and have been implicated in a variety of diseases including microangiopathy and diabetes.

In their recent studies Severini and colleagues (1, 2) have suggested that there is an elevation in the activity of NAG in the sera of cancer patients, particularly those with malignancies of the stomach, liver and pancreas. They also reported significantly high levels of activity in the majority of the breast cancer patients enrolled into their study.

In the study described here we have evaluated NAG activity in the sera of 129 breast cancer patients in Kuwait and compared it with measurements of CEA and CA-153 as well as examining its relationship to other clinico pathological features of breast cancer patients.

MATERIAL AND METHODS

Patients

Blood samples were taken from 129 consecutive patients (over a period of 18 months) prior to breast surgery, and also from 17 patients during the course of their subsequent treatment up to about 26 months. The clinical details of the patients in the study are recorded in Table 1. For the

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control group, blood was taken from 28 healthy female volunteers, ranging in age from 24–57 years; 23 were below 50 years of age, 20 were premenopausal and 8 were postmenopausal. Sera were separated from clotted blood by centrifugation at 1000 g for 20 min and either analysed immediately or within a month of storage at −20°C.

NAG enzyme assay

NAG activity was determined in 0.05 ml of serum in a reaction volume of 1 ml in citrate buffer containing 3-cresolsulphonphthaleinyl-N-acetyl-β-D-glucosaminide as substrate, according to the procedure described by the manufacturer, Boehringer (Mannheim, Germany). The increase in absorbance of the hydrolysis product, 3-cresolsulphonphtalein, was measured at 580 nm following incubation at 37°C for 15 min. A reagent blank was also included in each run. The reaction was stopped by adding 2 ml sodium carbonate buffer. Thus the volume activity was calculated (given that the extinction coefficient of 3-cresolsulphonphthalein is 40.67 (1 $mmol^{-1} \times cm^{-1}$) as;

$$\frac{1000 \times 3.05}{40.67 \times 1 \times 0.05 \times 15} \times \text{O.D.}_{\text{sample}} = 100 \times \text{O.D.}_{\text{sample}} \text{ Units/l}$$

Samples were assayed in duplicate and sometimes repeated in different runs. Interassay variation was found to be less than 5%. To determine whether storage of samples affected NAG activity, samples of normal serum were divided into a number of aliquots and frozen, and then measured for periods of up to 1 month. Loss of activity was not significant. For purposes of analyses, the median value was used to divide patients into high or low-expressing groups.

Hormone receptor assays

Frozen tissue was pulverized into fine powder and resuspended, by homogenization, in ice-cold buffer containing 10 mM Tris HCl pH 7.4, 1 mM EDTA, 0.5 mM dithiothreitol and 10% glycerol. Following a 30 min incubation at 20°C, the suspension was centrifuged at 600 g for 10 min. The supernatant fraction was re-centrifuged at 105000 g for 60 min and the resulting 'cytosol' supernatant was used for the receptor assays. Aliquots were adjusted to a protein concentration of 1–2 mg/ml just prior to assay. Both oestrogen receptor (ER) and progesterone receptor (PR) values were determined by an enzyme immunoassay (EIA) technique using the kits from Abbott Laboratories (Chicago, IL, USA) as described previously (3). The remaining cytosol was stored at −80°C.

Determination of CEA and CA-153

Both of these proteins were determined in serum samples using the automated Microparticle Enzyme Immunoassay

Table 1

Relationship between NAG activity and clinical/pathological parameters

Parameter	Total no. patients	Low NAG < 17.4 units/l	High NAG > 17.4 units/l	p-value
Age				
< 50 years	81	41	40	NS
> 50 years	22	9	13	
Unknown	3	3		
Menopausal status				
Pre	68	36	32	NS
Post	32	14	18	
Unknown	6	3	3	
Nodal status				
−ve	37	21	16	NS
+ve	65	28	37	
Unknown	4	4		
Stage				
T_1	12	7	5	NS
T_2	46	23	23	
T_3	11	6	5	
T_4	29	11	18	
Unknown	8	6	2	
Histology				
Infiltrating ductal	75	35	40	NS
Infiltrating lobular	6	4	2	
Medullary	4	1	3	
Other	21	13	8	

NS: not statistically significant.

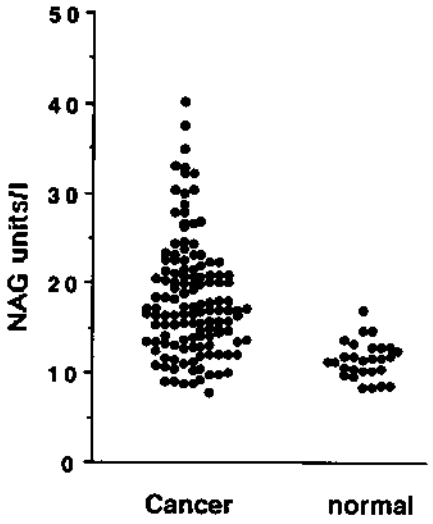

Fig. 1. NAG activity, measured as described in Methods, in sera from 129 pre-surgical breast cancer patients and 28 apparently healthy women. Median value in cancers (17.4 units/l) was significantly higher than that in normals (11.5/l); $p = 0.001$.

from Abbott Laboratories (Chicago, IL, USA). For purposes of analysis the following cut-off points (recommended by Abbott, and in line with generally used values) were used to indicate positivity in a sample: 3.2 ng/ml for CEA and 30 Units/ml for CA-153.

Statistical analyses

Data were analysed using the SPSS statistical computer software package. A non-parametric test for sample groups with unequal variance and non-normal distribution was used to compare means and median values of different groups. The χ^2 test was used to compare percentages.

RESULTS

NAG activity was measured in the sera taken from 129 patients who subsequently underwent surgery for breast cancer. The mean total activity was 18.2 Units/l with SD of 6.38 and SEM of ± 0.56. The median value was 17.4 Units/l while the 25th, 75th and 90th percentile levels were 13.6, 20.8 and 24.6, respectively. This was significantly greater than the mean and median values of 11.6 and 11.5 Units/l (SD $\pm$ 2.1 and SEM $\pm$ 0.39) that were observed for the 28 normal individuals ($p < 0.001$); the 25th, 75th and 90th percentile levers were 10.3, 12.9 and 13.7, respectively. The range of values obtained is illustrated in Fig. 1; 8–40 Units for the cancers and 8–17 Units for the normal group. Taking the upper limit of the normal range as 15.8 Units/l (mean $\pm$ 2 SD), 76 patients would be considered to have pathological NAG activity.

In the 106 patients for whom information was available, we found no correlation between NAG activity and age, menopausal status, nodal status, stage or tumour histology, using the median to segregate samples into high- and low-activity groups (Table 1). NAG activity was also unrelated to either oestrogen or progesterone receptor status of the tumour. We did find a significant relationship with the serum levels of CA-153 ($p = 0.008$) but not of CEA, using the consensus cut-off points for these markers (Table 2). CEA and CA-153 were significantly related to each other ($p = 0.001$). When considered as continuous variables, there was a significant correlation between NAG and both CEA and CA-153 ($p = 0.013$ and $p = 0.025$, respectively) and also between CEA and CA-153 ($p = 0.01$) (Fig. 2).

Table 2

Correlation between NAG activity and steroid receptor status and CEA and CA-153 levels

Parameter	Total no. patients	Low NAG < 17.4 units/l	High NAG ≥ 17.4 units/l	p-value
Oestrogen receptor*				
−ve	35	17	18	NS
+ve	35	15	20	
Unknown	36	21	15	
Progesterone receptor*				
−ve	32	14	18	NS
+ve	39	19	20	
Unknown	35	20	15	
CEA				
<3.2 ng/ml	69	36	33	NS
>3.2 ng/ml	32	12	20	
Unknown	5	5		
CA153				
<30 units/ml	69	39	30	0.008
>30 units/ml	32	9	23	
Unknown	5	5		

NS: statistically not significant. * +ve is ≥15 fmol/mg protein.

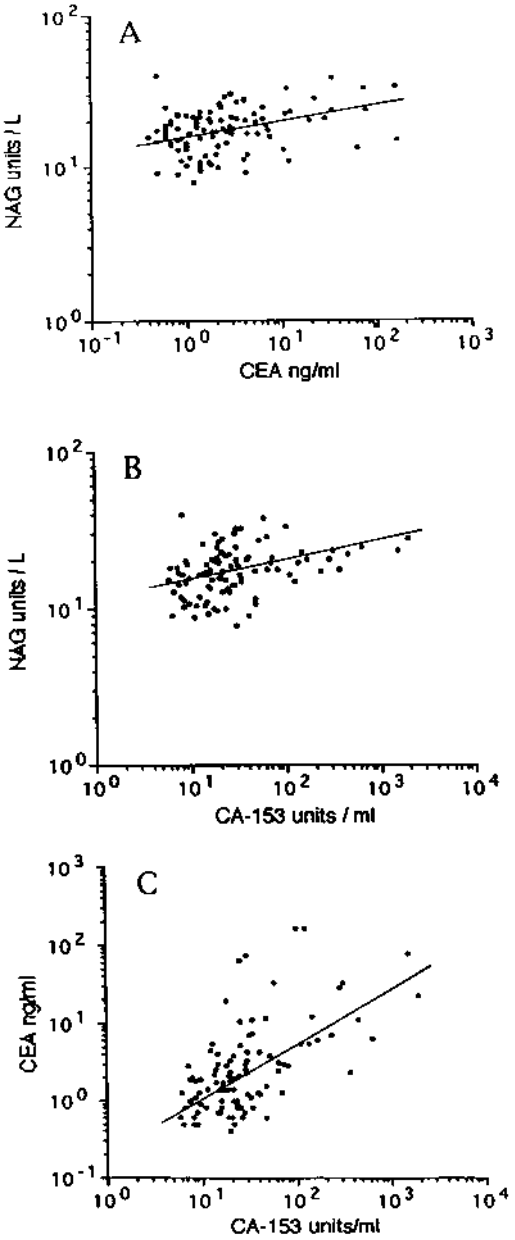

Fig. 2. Relationship between NAG activity and the levels of CEA ($p = 0.013$, $r = 0.245$) and CA-153 ($p = 0.025$, $r = 0.222$) and between CEA and CA-153 ($p = 0.01$, $r = 0.254$) in pre-surgical sera form breast cancer patients.

For 17 randomly selected patients we also measured NAG in periodic serum collections up to about 26 months post-surgery. These women were followed-up at regular intervals, undergoing routine isotope, x-ray and haematological tests. All received a combination of radiotherapy and chemotherapy and all but one patient were also given hormone therapy. In 7 cases the NAG activity followed the general pattern shown by CEA and CA-153 and in another 5 cases it was similar to at least one of these markers. In the remaining 5 cases we observed a similar fluctuation pattern in the levels of CEA and CA-153 but quite different from values of NAG activity (examples are charted in Fig. 3). Unlike the other two markers, NAG activity remained within a very narrow range in individual patients. Three of the four patients who were still disease-free at the time of the last follow-up had fairly constant NAG values at the upper end of the normal range. CEA levels in these patients were also normal over a long preceding period but were very high in case 14. CA-153 was greatly elevated in 3 of these cases. In 4 cases where the patients had relapsed but were still alive, NAG values tended to drift downwards but remained in the pathological range. Where death was recorded, both CEA and CA-153 levels were seen to rise in most cases, whereas NAG activity remained fairly stable throughout but in the pathological range.

DISCUSSION

NAG activity was elevated in the serum samples from women with breast cancer compared with those from an apparently healthy group. This finding is similar to that of

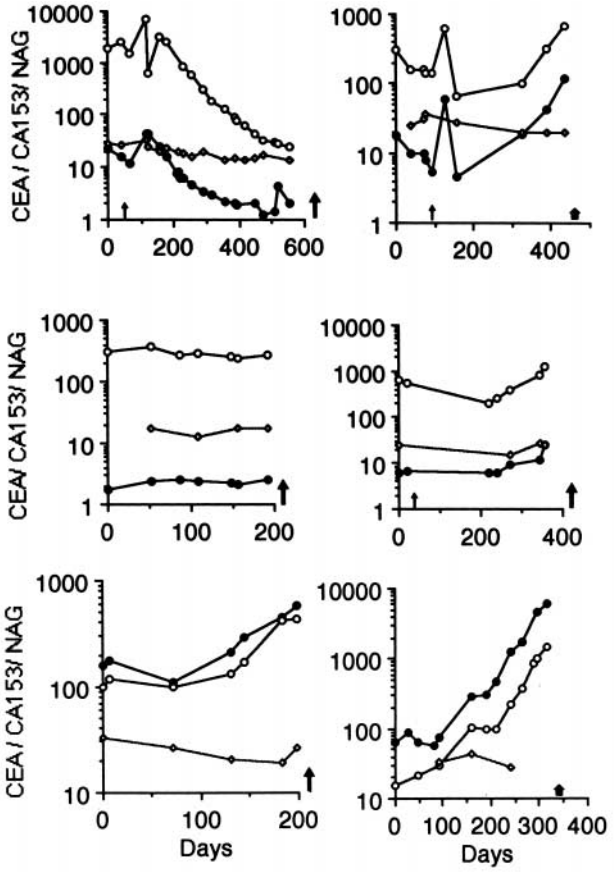

Fig. 3. Measurement of NAG activity (◇, units/l), CEA(●, ng/ml) and CA-153 (○, units/ml) in several randomly selected patients performed on serum samples taken at various times after primary breast surgery (day 0). The arrows on each graph indicate time of relapse (↑) or death (⬟) or whether the patient was still alive after the end of the study period (⬆).

Severini and colleagues (1, 2) who reported an elevation in NAG activity in 82–86% of patients with poorly or moderately differentiated tumours but in only 37% of those with well-differentiated tumours. We found pathological activity in 59% of our cases. Severini et al. also assayed three isoenzyme forms and found that in cancer patients the contribution of the B form had increased in contrast to the predominant A form. Their actual values for NAG activity (1) were much greater than ours, presumably because they used a different substrate in the assay. Gressner & Roebruck (4) reported a normal mean of 21 using a substrate similar to ours but with a ρ-nitrophenyl group, and Hashimoto and colleagues (5) a mean of 11.8 (no experimental details provided). Severini and colleagues (2) reported 14/18 sera samples from breast cancer patients to be 'positive' for NAG (presumably above the normal range). They also examined sera from patients with liver, pancreatic, gastric and colorectal cancers and found elevated NAG in 100% of the samples from the first 3 groups and 60–87% from the colorectal group. Increased activity was also found in 24% of patients with (unspecified) benign disorders but was not commented upon. This included 1/10 cases of benign breast disease.

We found no relationship between NAG activity and pathological features of the tumour. There was a weak correlation between NAG activity and the levels of CEA and CA-153, which are commonly used as tumour markers to monitor patients during post-surgical therapy. We followed all three proteins in 17 randomly selected patients. In about 70% of these the NAG activity roughly coincided with the levels of one or both of these markers during the period followed. It was generally maintained at a lower level in those patients who remained disease-free after primary surgery, but was consistently higher in those who subsequently relapsed and/or died. Taken alone however, NAG activity in the patient charts shown in Fig. 3 cannot be regarded as predictive of the timing of relapse. Follow-up on all the patients in this study will be needed in order to assess impact on survival.

The appearance of increased NAG activity may, however, have other biological implications. It is uncertain whether this is due to increased exocytosis resulting from an acceleration in cellular metabolism in cancer cells, or whether it is a consequence of decreased removal of NAG from the circulation by Kupfer cell endocytosis. It is not inconceivable that metastatic cells may be more active in secreting enzymes capable of degrading the surrounding extracellular matrix as a necessary pre-requisite to entering the circulation. It is interesting that the extracellular appearance of NAG is paralleled by simultaneous rise in β-glucoronidase, (another lysosomal enzyme) in cancers of the pancreas and liver and to a lesser extent of the colon and stomach, but much less so of the breast (2). This perhaps suggests that there may be different groups of enzymes responsible for aiding metastasis in different cancers. The fact that Severini and his colleagues (2) reported that 14/18 cases had elevated NAG but only 5/18 showed increased β-glucoronidase activity in their breast cancer cases also tends to argue in favour of a specific method of release rather than a result of cellular damage.

In conclusion, NAG activity was elevated in breast cancers and was related to the levels of CEA and CA-153 but not to any of the major clinico-pathological parameters. It may be useful to determine NAG activity in patients with in situ carcinoma; predictive factors for progression to the invasive, and eventually metastatic state are becoming increasingly necessary in such cases.

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