Abnormal serum alkaline and acid phosphatase isoenzymes in female breast cancer patients

E.O. Agbedana[1] and M.O. Ebesunun
[1]Department of Chemical Pathology, University of Ibadan, Nigeria and University College Hospital, Ibadan, Nigeria.

Summary
Serum total, different isoforms of both alkaline and acid phosphatases, liver function enzymes, calcium, inorganic phosphate, heamatocrit, white blood cells and platelet counts were determined in 50 female patients suffering from breast cancer. The serum total alkaline and total acid phosphatases within the breast cancer group were variable with significant elevation of both enzymes compared with the corresponding control values. The activities of alanine and aspartate transferases were higher than the control values, while the decreases in serum albumin and heamatocrit were statistically significant. In the breast cancer patients, the increases in the activities of both heat and urea labile alkaline phosphatases were significant. Similarly, in the patients, the tartrate-labile acid phosphatases activity was significantly elevated while the difference in tartrate resistant activity was not significant.

In 9 patients (18%), both total alkaline and acid phosphatases were excessively raised when compared with the control. The increased activities of urea-labile and heat-labile alkaline phosphatases as well as tartrate-resistant acid phosphatases are suggestive of increased activities of osteoclast and osteoblasts associated with bone metastasis. A possible diagnostic importance of this observation deserves further investigation, using monoclonal antibody techniques.

Keywords: *Serum Phosphatases, isoenzymes, breast cancer.*

Résumé
Le total du serum, les differents isofomes de la phosphatase alkaline et acide, la function des enzyme du foie, le calcium, le phosphate inorganique, l'heroatocrite, les compte des glubules blanc et des plate lettes ont été determineé chez 50 patietns femelles souffrant du cancer du sein. Le total du serum alkalin et de la phosphatase acide dans le groupe des femmes ayant le cancer du sein a été variable avec une elevation significative du deux enzymes par rapport a ceux des valeurs des controles. Les activités de l'alanine et de l'aspartate transferase ont été grande chez les controles alorsque une baisse significative du albumine serine, et l'hematocrite a été observé chez les patients souffrant du cancer du sein. Chez les meme patietns les activités du stress l'uréc labile et l'alkaline phosphatase ont été significanivement éléves, alaorsque la difference dans l'eactivité resistante au stress n'a pas été significative. Similairement, chez ces patients l'activité de la tartrate-labile acide phosphatase a été significativement élevés alorsque la difference dans la resistance de l'activitité de à la tartate a été statiquement significative.

Chez 9 (18%) des patients, les phosphatases alkalines et acides ont été excessivement élevé l'orsque comparé aux controles. L'augmentation des activitités de l'uree labile et de la phosphatase alkaline labile au stress de meme que cells de phosphatase acide resistante à la tartrate suggerent une augmentationd es activitités de l'osteocolastes associe aux metastates des OS. Ces observations represent une possibilite importante de diagnostique. Une investigation plus profonde en utilisant la technique des anticorps monclones est necessaire.

Correspondence: Prof. E.O. Agbedana, Department of Chemical Pathology, University of Ibadan, Nigeria.

Introduction

Cancer of the breast is a global problem and a large number of new cases are adequately diagnosed every year [1]. Bone metastasis is frequently observed in breast cancer patients and this is often associated with a higher mortality rate [2,3].

It has been suggested that in cancer patients with bone metastasis, increased activities of osteoclasts and osteoblasts can affect specific biochemical components of the bone [5,6]. This is supported by the work by Coleman and Reuben [7], who demonstrated increased bone isoenzymes of acid (ACP) and alkaline (AP) Phosphatases in breast cancer patients with bone metastasis. Also Wada *et al* [8] found increased activities of total acid and alkaline phosphatases as well as lactate dehydrogenase in breast cancer patients with bone metastasis, while other workers recorded altered serum calcium, inorganic phosphate, osteocalcin and hyroxyproline concentrations as well [5,6]. The stage at which these biochemical changes occur has not been well documented. Mamiya *et al* [9] found a significant elevation of alkaline phosphatases activity in 64.8% of their breast cancer patients in whom bone metastasis was not established. This leads one to speculate that changes in certain biochemical parameters may occur in breast cancer patients before the clinical manifestations of bone metastasis become obvious. In this study, we therefore evaluate the activities of serum AP and ACP isoforms using heating or specific inhibitors as well as other biomedical parameters in female breast cancer patients at different stages, but without any identifiable clinical manifestations of bone metastasis.

Materials and methods

Subjects

Fifty female patients, aged 20-62 years (mean ± SE = 45 ± 1.9) who were either attending the Surgical Outpatients' Clinic or were on admission at the University College Hospital, Ibadan and diagnosed as having cancer of the breast were studied. Their ages, body weights and duration of disease were recorded. The patients were clinically assessed and divided into the different stages of disease progression by the attending surgeon.

The patients were classified into different stages, using Manchester classification, as follows:

Stage 1: The tumour is confined to the breast and is attached to the underlying muscle with no axillary node involvement. Skin attachment or ulceration when present must be in continuity with the tumour and extended beyond it.

Stage 2: As in Stage 1 but with mobile axillary lymph node involvement.

Stage 3: i. Skin movement is beyond periphery of the tumour, or
ii The tumour is attached to the underlying muscle, or
iii. Axillary lymph nodes are mobile or fixed but not distant metastatses.

Stage 4: a. Lymphatic spread is beyond the ipsilateral axilla or
b. Distant blood borne metastases are present.

None of the patients had clinical manifestations of bone disease and all patients clinically assessed to suffer from any liver disease, or bone disease or leukaemia were not included in the study. All investigations were performed before surgical operation and/or commencement of treatment with either chemotherapy or radiotherapy. Twenty-five apparently healthy women within the same age range served as the control group.

Blood sampling

Blood samples were collected by venepuncture from all subjects into plain bottles. Serum was isolated by centrifugation at 3,000rpm at ambient temperature. Samples were stored at –40°C until the different biochemical parameters were analysed.

Analytical procedure

Heat and urea enzyme inhibition

Total and heat stable serum AP activities, using the modified method of Kind and King [10] were measured before and after incubation of serum for 15 minutes at 56°C in a well-regulated temperature-controlled water bath. The heat-labile AP activity was calculated as the difference between total and heat stable activities. Total and urea-stable [12] serum AP activities were measured [10] after incubation of serum for 18 minutes at 37°C in the presence or absence of 2mol/L urea solution (1:10 v/v) [12]. The urea-labile activity was calculated as the difference between total and urea-stable activities.

Tartrate enzyme inhibition

Total and tartrate stable serum ACP activities were measured using the method of King and Jegatheesan [13] after incubation of serum for 60 minutes at 37°C in the presence or absence of 1 m/L L+tartrate solution. The difference between total and tartrate stable activities was regarded as tartrate labile ACP.

Serum Transferases

Aspartate (AST) and alanine tranferases (ALT) were assayed at 37°C using the modified method [14] of Reitaan and Franke [15].

Serum proteins

Serum total protein was assayed using the Wilt and Trendlenberg method [16] while the Bromocresol dye-binding technique was used for the quantitation of albumin [17].

Other biochemical determinations

Serum calcium and inorganic phosphate concentrations were carried out using automation technique (Hitachi 740, Tokyo, Japan).

Statistical analysis

Statisitcal analyses were performed using Chi-square test, ANOVA, Pearson correlation and Students t-test. $P < 0.05$ was regarded as significant.

Results

Table 1 shows the mean ± SE values for the biochemical and haematological parameters in all subjects. Total protein, inorganic phosphate, calcium, platelet and white blood cell counts were not significantly altered in the breast cancer patients when compared with the corresponding control values. On the other hand, the mean serum albumin and haematocrit levels were significantly ($P < 0.05$) reduced, while the activities of ALT and AST were increased ($P < 0.01$) in breast cancer patients when compared with the corresponding control values.

Table 1: Biochemical and haematological parameters (Means ± S.E) in all subjects

Parameters	Patients N = 50 Mean ± S.E	Controls N = 25 Mean ± S.E.	P-value
PCV %	33.92 ± 0.06	37.88 ± 0.24	0.05
WBC (cm^3)	4.606 ± 106.7	3.934 ± 89.74	ns
Platelet (cm^3)	210.160 ± 9825.45	100.800 ± 890.25	ns
ALT (i.u)	18.20 ± 2.00	13.72 ± 0.66	0.04
AST (i u)	44.86 ± 4.84	25.52 ± 2.7	0.002
Total protein (g/L)	71.06 ± 2.01	72.44 ± 1.09	ns
Albumin (g/L)	32.75 ± 1.68	39.68 ± 0.66	0.005
Calcium (mg/100ml)	8.33 ± 0.24	9.12 ± 0.17	ns
Inorganic phosphate (mg/100 ml)	2.99 ± 0.12	3.34 ± 0.9	ns

N = number of subjects
S.E = Standard error of mean
PCV = Packed cell volume
WBC = White blood cell count
ALT = Alanine transferase
AST = Asparate transferase

Table 2 shows the mean activities of the different isoforms of both AP and ACP in all the subjects. In the breast cancer patients, the respective activities for both serum total AP and ACP ranged from normal to high levels with mean values significantly elevated when compared with the corresponding control levels ($P < 0.02$; $P < 0.001$), respectively. Also, the activities of both heat-and urea-labile AP were increased ($P < 0.02$), while the change in the heat resistant AP was not significant when compared with the corresponding control values. However, the percentage contribution of the heat-resistant AP to the total activity was significantly reduced in the breast cancer patients (20.4 vs

44.1%) ($P < 0.001$). As for ACP, the mean value for the tartrate-labile fraction was significantly elevated in the breast cancer patients ($P < 0.002$).

Table 2: Acid and alkaline phosphatase activities in all subjects

Parameters	Patients N = 50 Mean ± S.E	Controls N = 25 Mean ± S.E	P-value
Total AP (i.u)	97.95 ± 19.78	38.16 ± 1.58	0.002
Heat resistant AP (i.u)	17.96 ± 2.54	38.16 ± 1.58	ns
Heat labile AP (i.u)	79.99 ± 17.24	23.14 ± 0.79	0.002
% Heat resistant AP	20.35 ± 1.44	44.11 ± 0.69	0.001
Urea labile AP (i.u)	73.44 ± 16.0	24.76 ± 2.60	0.002
Total ACP (i.u)	43.34 ± 3.73	24.76 ± 0.69	0.01
Tartrate resistant ACP (i.u)	26.64 ± 3.26	14.28 ± 1.04	0.002
Tartrate labile	17.70 ± 0.16	10.48 ± 0.7	0.002
% Tartrate resistant ACP	6.15 ± 4.4	58.33 ± 2.9	0.001

ACP = Acid phosphatase
AP = Alkaline phosphatase.

As shown in Table 3, nine patients (18%) had elevated serum total AP activity (> 108 i.u) with a mean level of 274.5 ± 90.0 i.u., while the remaining 41 patients (82%) had normal total AP activities with a mean value of 58.9 ± 3.4 i.u. The inorganic phosphate concentration, the activities of the liver enzymes and the different isoforms of AP were significantly elevated in the 'high' total AP, while the corresponding value in the 'normal' total AP group was about 41%. The mean calcium concentrations were not significantly different in both groups.

Table 3: Biochemical parameters in 'high' and 'normal' total alkaline phosphatase groups

Parameters	High AP > 108 i.u N = 9 mean ± S.E	Normal AP < 108 i.u N = 41 mean ± S.D	P-value
Total AP (i.u)	274.52 ± 90.50	58.87 ± 3.41	0.001
Heat resistant AP (i.u)	22.88 ± 0.25	7.36 ± 0.53	0.001
Urea resistant AP (i.u)	69.66 ± 19.66	20.00 ± 1.24	0.001
Total ACP (i.u)	32.25 ± 6.31	42.12 ± 4.52	0.001
Tartrate resistant ACP (i.u)	31.66 ± 5.27	24.92 ± 3.80	0.001
Calcium (mg/100ml)	8.6 ± 0.30	8.49 ± 0.22	ns
Inorganic phosphate (mg/100ml)	4.17 ± 0.6	2.60 ± 0.13	0.001
ALT (i.u)	20.66 ± 7.42	15.09 ± 1.09	0.01
AST (i.u)	50.66 ± 12.57	44.02 ± 06	0.01
Urea labile AP (i.u)	216.74 ± 70.32	38.87 ± 1.91	0.02
Heat labile AP (i.u)	251.32 ± 80.20	51.51 ± 1.91	0.02

Similarly, when the patients were classified as having normal or high levels of total AP (Table 4), 32 patients (65%) had high total AP (> 28 i.u) with a mean value of 55.9 ± 4.2 i.u, while 18 patients (36%) had normal values (< 28 i.u) with a mean level of 18.5 ± 0.6 i.u. The activities of ALT and the isoenzymes of both AP and ACP were significantly elevated in the 'high' total ACP group when compared with the corresponding values of in the 'normal' total ACP group ($P < 0.01$; $P < 0.001$; $P < 0.002$). The AST activity, calcium and inorganic phosphate concentrations were not significantly different in both groups.

Table 4: Biochemical parameters in 'high' and 'normal' total acid phosphatase groups

Parameters	High AP > 28 i.u N = 32 mean ± S.E	Normal AP < 28 i.u mean ± S.D	P-value
Total AP (i.u)	55.90 ± 4.15	18.46 ± 0.57	0.001
Tartrate resistant ACP (i.u)	32.21 ± 3.91	9.23 ± 1.28	0.001
Total AP (i.u)	113.27 ± 25.82	40.07 ± 4.83	0.001
Heat labile AP (i.u)	89.20 ± 20.80	34.07 ± 3.01	0.02
Urea labile AP (i.u)	75.74 ± 9.20	23.31 ± 2.24	0.001
Heat resistant AP (i.u)	24.07 ± 2.27	6.0 ± 0.61	0.001
Urea resistant AP (i.u)	37.53 ± 0.19	2.72 ± 0.22	0.001
Calcium (mg/100ml)	8.79 ± 0.19	7.86 ± 0.42	ns
Inorganic phosphate (mg/100ml)	3.09 ± 0.20	2.72 ± 0.22	ns
ALT (i.u)	18.79 ± 2.63	14.38 ± 0.97	0.00
AST (i.u)	40.05 ± 4.89	40.38 ± 7.87	ns

The analysis of variance showed that the concentration of total ACP, total AP, calcium and inorganic phosphate were independent of the stage of progression of the breast cancer (Table 5).

Table 5: Serum calcium levels and disease state in breast cancer patients

Source of variation	Sums of square	Df	Mean square	Fobs	P
Column: Disease Stages III - IV	0.07	1	0.07	0.046	ns
Row: Age of patients	0.06	1	0.06	0.40	ns
CXR	1.04	1	1.04	0.689	ns
Within	49.73	33	1.5		
Total	4990	36			

ns = not significant.

Similarly, the duration of disease and the presence of advanced stages of breast cancer were independent of each other (Table 6).

Table 6: Disease stage and duration of disease in breast cancer patients

Duration	Disease III	Stages IV	Total
Before 6 months	N = 11 (8.84) 28.94%	N = 3 (5.15) 7.89%	N = 14 36.83%
After 6 months	N = 13 (15.15) 34.21%	N = 11 (8.84) 28.94%	N = 24 63.15%
Total	N = 24 63.15%	N = 14 36.83%	N = 38 100%

The number in parentheses are the expected cell frequencies.
($X^2 = 2.30$; df = 1; $P < 0.05$)

There were no significant correlations between total AP, ACP and calcium or phosphate except for that between total ACP and calcium concentration in the cancer patients ($P < 0.05$). The urea and heat-labile AP activities were significantly correlated with the activities of tartrate-resistant ACP only in the cancer patients.

Discussion

In this study, we measured some biochemical parameters in breast cancer patients who had no recognisable clinical manifestation of bone metastasis. We have demonstrated in the patients that both total AP and ACP showed large variations with mean activities significantly higher than those of the corresponding control levels.

Elevation of serum ACP and AP is closely associated with the presence of bone metastasis in cancer patients [7,8]. Although accuracy of prediction of the presence of bone metastasis using biochemical parameters has not been well defined. Several studies [8,18] demonstrate a high heat-labile AP activity in breast cancer patients in whom bone metastasis has been radiologically established. Similar to these earlier studies, we found that both heat-and urea-labile AP as well as tartrate-resistant ACP were elevated in our patients. This may reflect increased activities of osteoclasts and osteoblasts associated with bone metastasis. In fact, the study of frequency et al [19] strongly suggested that bone related isoforms of alkaline phosphatase can be used in the early detection and follow-up of bone metastasis in breast cancer patients. Therefore, it is reasonable to assume that similar changes in ACP and AP can occur in patients with sub-clinical or early stage bone metastasis. Using the highest enzyme activity in the control group as the cut-off point, 9 patients (18%) had a high AP (> 108 i.u), while the remaining 41 patients (82%) belonged to the normal AP group. In the 'high' AP group, the major increase (5-fold) was due to an increase in the bone-related isoform. It was also significant that the tartrate-resistant ACP in the 'high' AP group was high while the corresponding value was actually reduced in the 'normal' AP group. In both groups, ALT and AST were within the reference ranges for this community, but the inorganic phosphate concentration was significantly increased in the 'high' AP group.

When the patients were classified as having high or normal total ACP using the highest activity in the control group as cut-off point, 32 patients (64%) had high, while 18 patients (36%) had normal total ACP levels. Both tartrate-labile and resistant ACP, total, heat-labile and heat-resistant AP were significantly elevated in the high ACP group when compared with normal ACP group. It was also significant that all patients who had high AP belonged to the high ACP group. The changes in the different biochemical parameters were independent of age, duration as well as the progression of the disease. The study by Desoize et al [20,21] suggests that increased tartrate-resistant ACP, heat-and urea-labile AP activities are markers of the activities of osteoclasts and osteoblasts respectively. Therefore, one possibility which needs further evaluation is that the biochemical changes observed in the high AP group are early signs of sub-clinical/ early stage bone or liver metastasis. However, the changes in serum liver enzymes were within the normal range of values for normal individuals in this community and therefore suggest minimal liver damage, if any at all, in these patients. We can therefore exclude the presence of liver metastasis. Further studies using monoclonal antibodies prepared against specific isoenzymes of AP and ACP should be carried out in breast cancer patients to assess the diagnostic value of these enzymes in the early detection of sub clinical/early stage bone metastasis in such patients.

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