CASE REPORTS

A variant alkaline phosphatase found in a case of gastric carcinoma with super bone scan

F Kobayashi, T Ikeda, S Tozuka, O Noguchi, T Fukuma, S Sakamoto, F Marumo, T Komoda, Y Sakagishi, C Sato

Abstract

A rare case of gastric carcinoma associated with increased serum variant alkaline phosphatase activities is presented. A 54 year old man had extremely high serum alkaline phosphatase activity (18 607 U/l) with normal calcium and phosphate concentrations. His bone scintigram showed abnormal findings, 'super bone scan'. He was diagnosed as having Borrmann type 4 gastric carcinoma with diffuse bone metastases by examinations of the upper gastrointestinal tract and iliac bone biopsy. The alkaline phosphatase isozyme of this patient was of the bone type as measured by cellulose acetate membrane electrophoresis and the placenta/bone type by agarose gel electrophoresis, respectively. Immunoelectrophoresis and the immunoprecipitation method using monoclonal antibodies against various alkaline phosphatase isozymes, however, showed that his serum alkaline phosphatase had the liver type antigenicity. Furthermore, it had a larger molecular size and different sugar chains compared with the common liver type alkaline phosphatase. These findings suggest that a unique variant alkaline phosphatase was produced by gastric cancer cells, which is possibly an explanation for the high serum alkaline phosphatase activities in this patient.

(Gut 1995; 36: 299–302)

Keywords: alkaline phosphatase, gastric carcinoma, super bone scan.

Localised bone metastasis is occasionally seen in patients with gastric carcinoma.^{1,2} Diffuse metastases to the entire skeletal system, however, in which bone scintigraphy shows abnormal findings, for example super bone scan, is uncommon.^{3,4} In gastric carcinoma with super bone scan, several clinical features including high serum alkaline phosphatase (ALP) activities have been recognised.⁵ High serum ALP activities in those patients are thought to arise from the bone, although ALP producing gastric carcinoma has also been reported.⁶ We describe a patient with gastric carcinoma with super bone scan, in whom serum ALP was recognised as the bone type by

conventional electrophoresis, but after further investigation, was identified to be the liver type like ALP with a larger molecular size and different sugar chain structures compared with the common liver type ALP. It is suggested that high serum ALP activities in this patient were derived from the liver type like ALP in gastric carcinoma.

Case report

On 22 May 1991, a 54 year old man presented to our hospital because of increasing lumbago and back pain since late April. He had also lost 8 kg in weight a month. Physical examination showed significant anaemia, gingival bleeding, systolic heart murmur, and hepatomegaly, and he was admitted for further examinations.

Laboratory examinations showed: anaemia (haemoglobin 4.46 mmol/l), thrombocytopenia (42 000/ μ l), the appearance of immature granulocytes and erythroblasts in the peripheral blood, increased serum activities of lactate dehydrogenase (823 U/l), γ glutamyltransferase (348 U/l), aspartate aminotransferase (90 U/l), and alanine aminotransferase (50 U/l). The serum ALP value was extremely high (18 607 U/l, reference range: 140–248 U/l). Renal function and serum electrolytes including calcium and phosphate were normal. Prothrombin time was normal, but partial prothrombin time was slightly prolonged and the fibrin degenerative product was increased (11.5 μ g/dl). Serum carcinoembryonic antigen and ferritin concentrations were within normal ranges. Aspiration of bone marrow of the sternum showed a dry tap.

A barium meal study and gastrofibrescopy showed Borrmann type 4 gastric carcinoma. Apart from a compression fracture of the 8th thoracic vertebra, no other abnormalities such as lytic or blastic bone lesions were detected by magnetic resonance images and bone tomography.

The whole body scan was performed three hours after intravenous administration of 0.4 mCi/kg technetium-99m (^{99m}Tc)-methylene diphosphate, using a Hitachi Gamma View-F RC-1C-1635LF camera equipped with a low energy and high resolution collimator. The window centre setting and the window width were adjusted to 141 KeV and 20%, respectively, and the scan was performed from

Department of Internal Medicine, Yokosuka Kyosai Hospital, Kanagawa, Japan
F Kobayashi
T Ikeda
S Tozuka
O Noguchi
T Fukuma
S Sakamoto

Second Department of Internal Medicine and Division of Health Science, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan
F Marumo
C Sato

First Department of Biochemistry, Saitama Medical School, Saitama, Japan
T Komoda
Y Sakagishi

Correspondence to: Dr C Sato, Second Department of Internal Medicine, Faculty of Medicine, Tokyo Medical and Dental University, 5-45, Yushima 1-chome, Bunkyo-ku Tokyo 113, Japan.

Accepted for publication 29 April 1994

Figure 1: ^{99m}Tc methylene diphosphate bone scintigram showing so called super bone scan. Note the considerably increased uptake in axial skeletons and the absence of renal images.

anterior and posterior projections. The image showed a homogeneously and symmetrically increased uptake of the bone tracer without renal images, that is to say, super bone scan (Fig 1). The biopsy specimen from the iliac bone showed the infiltration of poorly differentiated adenocarcinoma cells, which were similar to those seen in biopsy gastric specimens.

Abdominal ultrasonography and computed tomography showed hepatosplenomegaly, but space occupying lesions or other abnormalities were not detected.

The diagnosis of advanced gastric carcinoma with diffuse bone metastases was made and chemotherapy with cisplatin and UFT (Taiho, Tokyo, Japan: an oral drug containing tegafur and uracil) and radiation therapy for the thoracic and lumbar vertebrae were started. As the serum ALP activity decreased to 6100 U/l and the pain was well controlled by morphine, he was discharged from the hospital on 17 August.

Chemotherapy with mitomycin C and UFT was continued but serum ALP and fibrin degenerative product values gradually increased again, bleeding tendency appeared, and he was readmitted on 11 October. Although he was treated with cisplatin and UFT again and treatment for disseminated intravascular coagulation were performed, his status deteriorated, and he died on 6 December.

Methods

The characteristics of the patient's serum ALP were investigated biochemically and immunologically.

For the agarose gel electrophoresis, Gel 8 for ALP isozyme and a high resolution ALP staining kit were obtained from Ciba Corning Diagnostics Co (Palo Alto, CA, USA). Electrophoresis and the staining of the gel plate were performed according to the manufacturer's instructions. To remove the glycanphosphatidylinositol-anchor moiety or sialic acid in the ALP molecule, the ALP sample was treated at room temperature for three hours

with 0.1 U/ml phosphatidylinositol specific phospholipase C (PIPLC, EC 3.1.4.10, Funakoshi Pharmaceutical Co, Tokyo, Japan) and 0.1 U/ml sialidase (*Vibrio cholerae*, Koch-Light Ltd, UK), respectively, as reported earlier.^{7,8} The resulting aliquot was used for the electrophoresis. The liver, bone, placental, and intestinal ALP controls were prepared as described previously.⁹

To determine the ALP antigenicity, the ALP sample (9 μl) was preincubated for one hour at room temperature with 1 μl of monoclonal antibodies against human liver (LAP 1/9 clone), bone (BAP 1/9 clone), term placental (H7 clone), placental like (F11-clone), and intestinal (D 98 clone) ALPs.^{7,8} The monoclonal antibodies of BAP and LAP¹⁰ were gifts from Drs E M Bailyes and J P Luzio, University of Cambridge, UK, those of H7 and F11^{11,12} were from Dr J L Millán, La Jolla Cancer Research Foundation, CA, USA, and the monoclonal antibody against intestinal ALP¹³ was from Dr H Harris, University of Pennsylvania, PA, USA. In our experience, each monoclonal antibody had no significant cross reactions with other types of ALP except for antibone ALP, which had a little but negligible interaction with liver ALP. Subsequently, the resulting ALP isozyme anti-ALP antibody complex in the incubation mixture was electrophoretically isolated with a polyacrylamide disc gel (Alpkhor, Johkoh Co, Tokyo).

Twenty μl of the ALP sample was incubated overnight at 4°C with 2 μl of monoclonal antibodies against human liver, bone, term placental, and adult intestinal ALPs. The mixture was then incubated with protein A (BDH Chemicals Ltd, Dorset, UK) and left to stand for one hour at room temperature, and centrifuged at 2000 $\times g$ for five minutes. The supernatant was assayed for ALP activity.^{7,8} Control assay was carried out with saline in place of the monoclonal antibodies.

The sugar chain heterogeneity of ALP isozymes^{14,15} was determined by lectin affinity chromatographies according to the methods described previously.⁸ The following lectins were purchased from E Y Lab (San Mateo, CA, USA): concanavalin A, phytohaemagglutinin E, pea lectin, and wheat germ agglutinin. Briefly, the ALP preparation with a specific activity of 1 $\mu\text{mol}/\text{min}$ was applied on a concanavalin A column, and allowed to stand at room temperature for three hours. The three fractions were obtained using two different concentrations (0.01 and 0.5 mol/l) of a-methylmannoside: an unbound fraction (fraction I), a weakly bound fraction (fraction II), and a strongly bound fraction (fraction III). These fractions were further applied on the phytohaemagglutinin E, pea lectin, and wheat germ agglutinin columns. The unbound and bound fractions were separated on the respective columns using *N*-acetyl-D-glucosamine at 0.5 mol/l, a-methylmannoside at 0.5 mol/l as the elution buffer. Each value shown represents the enzyme activity of one of the following fractions as a percentage of the total activity: Ia, the fraction passing through both the concanavalin A and phytohaemagglutinin E column; Ib, the

Figure 2: ALP isozyme patterns with agarose gel electrophoresis.

1-3; patient's ALP (1, untreated; 2, sialidase treated; 3, phosphatidylinositol specific phospholipase C treated); 4, liver ALP; 5, bone ALP; 6, placental ALP; 7, intestinal ALP.

Figure 4: Molecular sizes of various alkaline phosphatase isoenzymes. 1, bone ALP; 2, liver ALP; 3, patient's ALP; 4, intestinal ALP. All the ALPs were pretreated with sialidase. The comparative molecular size of ALP isozymes was determined by 5.0% polyacrylamide slab gel electrophoresis with 0.1% sodium dodecylsulphate under non-reducing conditions.

fraction passing through the concanavalin A and binding to the phytohaemagglutinin E column; IIa, the fraction binding weakly to concanavalin A column and passing through the pea lectin column; IIb, the fraction binding weakly to concanavalin A and binding to the pea lectin column; IIIa, the fraction binding strongly to concanavalin A and passing through the wheat germ agglutinin column; IIIb, the fraction binding strongly to both the concanavalin A and wheat germ agglutinin column. The yield of the enzyme activity from respective lectin columns was more than 90%.

The enzyme sample was treated for three hours with 0.1 U/ml PIPLC and 0.1 U/ml sialidase for apparent molecular size determinations. The resulting aliquot was run on 5% polyacrylamide slab gel with 0.1% sodium dodecylsulphate under the non-reducing condition.^{8,16} The enzyme active band was detected by 5-bromo-3-indolylphosphate p-toluidine salt containing 10 μ mol/l of $\text{Zn}(\text{CH}_3\text{COO})_2$ and 1 mmol/l MgCl_2 . The comparative molecular size was estimated from the mobility using standard molecular size markers (Pharmacia, Uppsala, Sweden).

Figure 3: ALP isozyme patterns of the patient's serum incubated with or without the monoclonal antibodies against various ALP isozymes. The supporting medium used was 7.7% polyacrylamide disc gel.

1, untreated; 2, with antihuman liver ALP (LAP 1/9 clone); 3, antihuman bone ALP (BAP 1/9 clone); 4, antihuman placental ALP (H7 clone); 5, antihuman placental like ALP (F 11 clone); 6, antihuman intestinal ALP (D98 clone).

Results

With conventional electrophoresis on a cellulose acetate membrane, the patient's ALP isozyme was shown to be the bone type. By electrophoresis on an agarose gel, his ALP was found at the placenta/bone ALP region (Fig 2). In addition, the molecule of the patient's ALP had sialic acid, but no glycan-phosphatidylinositol-anchor moiety (Fig 2). Using polyacrylamide disc gel electrophoresis, his ALP was found at the intestinal ALP region (data not shown).

As Fig 3 shows, the ALP reacted mainly with the monoclonal antibody against human liver ALP isozyme (LAP 1/9 clone). By an immunoprecipitation method, the amount of the patient's serum ALP isozyme was estimated as follows: the liver type 92%, the bone type 6%, the intestinal type 2%. The apparent molecular size of his ALP (168 kDa) determined by sodium dodecylsulphate-polyacrylamide gel electrophoresis was larger than that of common liver type (154 kDa) or the bone type (165 kDa) (Fig 4).

As the Table shows, the sugar chain moiety of the patient's ALP had more fractions Ia (multiantennary complex type sugar chain) and Ib (complex sugar chain bearing bisecting GlcNAc) than those of healthy adult serum, human bone, and human liver ALPs.

Discussion

Super bone scan is defined as a homogeneously and symmetrically increased uptake of bone tracers in the skeleton compared with soft tissues. It may be misinterpreted as normal, and it is important for differential diagnosis that there is no visualisation of renal images despite normal renal functions.³ Although these scans are most commonly seen in patients with diffuse metastases of prostatic cancer or breast cancer,^{17,18} some cases of gastric carcinoma have also been reported.²⁻⁵

Comparative amounts of the six fractions of ALP isozymes by serial lectin affinity chromatographies

	% Of respective fractions of ALP activity					
	Ia	Ib	IIa	IIb	IIIa	IIIb
Patient's serum	20	11	28	12	1	28
Healthy adult serum	0	1	35	60	2	2
Human liver	1	0	31	35	1	32
Human bone	0	1	4	18	19	58

Percentages of the fraction of ALP activity obtained by serial lectin chromatography. See Methods section.

Therefore, gastric carcinoma should also be considered when super bone scan is seen.

In diffuse bone metastases of gastric carcinoma, specific clinical features are recognised.⁵ Common symptoms are anaemia, lumbago and back pain, and a bleeding tendency, and common laboratory findings are leukoblastic anaemia, data suggesting disseminated intravascular coagulation, high serum lactate dehydrogenase activities, and extremely high serum ALP values.

The origin of the ALP in diffuse bone metastases of gastric carcinoma has not been clarified. Uchida *et al*⁶ reported a necropsy case of occult gastric carcinoma with diffuse bone metastases, and extremely high serum ALP activities. They stained carcinoma cells immunohistochemically and assumed that some of the serum ALP might have originated from the carcinoma cells, although it might have been partially derived from the bone. They did not analyse serum ALP isozymes or examined the bone by scintigraphy.

ALP and its isozymes have been investigated in gastrointestinal malignancies as tumour markers.¹⁹⁻²³ It has been reported that the ALP produced by gastric carcinoma is the placental type, the placenta like type, or a hybrid form consisting of subunits of the intestinal type and the placental type.²¹ We characterised ALP isozymes in the patient biochemically and immunologically. The conventional electrophoresis on a cellulose acetate membrane showed that the patient's ALP was the bone type and the result of agarose gel electrophoresis was also the placenta/bone type. The result of polyacrylamide disc gel was, however, different, and the patient's ALP seemed to be the intestinal type. Immunoelectrophoresis and immunoprecipitation with monoclonal antibodies against various ALP isozymes showed that the patient's serum ALP had an antigenicity of the liver type ALP. Moreover, it is suggested that this antigenetically liver type ALP is a unique variant ALP because of its larger molecular size and the different sugar chains. The liver type or liver type like ALP producing gastric carcinoma has not been reported before. High serum ALP activities may result from the tumour production in our patient. Studies of the enzyme in the patient tissues would have been very helpful. The mechanism of the production of the variant liver ALP by cancerous cells remains to be investigated.

The prognosis of gastric carcinoma with super bone scan is usually poor. The mean

survival time varies from about one month to about five months.⁵ In this patient, treatments with cisplatin and UFT before overt disseminated intravascular coagulation were effective, and the patient survived more than six months despite the advanced carcinoma. Of particular importance is the correlation between the serum activity of ALP and the efficacy of the chemotherapy.

- Pagani JJ, Libshitz HI. Imaging bone metastases. *Radiol Clin North Am* 1982; 20: 545-60.
- Abe H, Nakamura M, Sakamoto K. The bone metastasis of gastric cancer-evaluated by bone scintigraphy. *J Med Imag* 1990; 10: 992-7 (in Japanese with an abstract in English).
- Fukuda T, Inoue Y, Ochi H, Nakazima H, Sawa H, Onoyama Y. Abnormally high diffuse activity on bone scintigram. The importance of exposure time for its recognition. *Eur J Nucl Med* 1982; 7: 275-7.
- Saphner T, Love RR, Perlman S. Super bone scan in metastatic stomach cancer. *Wis Med J* 1990; 89: 161-3.
- Yasutake K, Matsushita K, Imamura Y, Oya M, Hozumi T, Kato J, *et al*. A case of early gastric carcinoma with 'disseminated carcinomatosis of bone marrow'. *Jpn J Gastroenterol* 1989; 86: 1304-9 (in Japanese with an abstract in English).
- Uchida T, Shikata T, Shimizu S, Takimoto Y, Iino S, Suzuki H. Gonadotropin and alkaline phosphatase producing occult gastric carcinoma with widespread metastasis of generalized bone. *Cancer* 1989; 48: 140-50.
- Miura M, Matsuzaki H, Bailyes EM, Koyama I, Sakagishi Y, Sekine T, *et al*. Difference between human liver- and bone-type alkaline phosphatases. *Clin Chim Acta* 1989; 168: 177-88.
- Komoda T, Sato M, Furiya K, Sakagishi Y, Sekine T. Allelic and ectopic polymorphisms in human placental alkaline phosphatases: sugar chain heterogeneities. *Clin Chim Acta* 1989; 186: 203-10.
- Koyama I, Miura M, Matsuzaki H, Sakagishi Y, Komoda T. Sugar-chain heterogeneity of human alkaline phosphatases: differences between normal and tumor-associated isozymes. *J Chromatogr* 1987; 413: 65-78.
- Bailyes EM, Seabrook RN, Calvin J, Maguire GA, Price CP, Siddle K, *et al*. The preparation of monoclonal antibodies to human bone and liver alkaline phosphatase and their use in immunoaffinity purification and in studying these enzymes when present in serum. *Biochem J* 1987; 244: 725-33.
- Jemmerson R, Shah N, Fishman WH. Evidence for homology of normal and neoplastic human placental alkaline phosphatases as determined by monoclonal antibodies to the cancer-associated enzyme. *Cancer Res* 1985; 45: 3268-73.
- Jemmerson R, Fishman WH. Convenient selection of monoclonal antibodies to isoenzymes of placental alkaline phosphatase using the catalytic activity of the antigen in enzyme-antigen immunoassay. *Anal Biochem* 1982; 124: 286-92.
- Wray LK, Harris H. Demonstration using monoclonal antibodies of inter-locus heteromeric isozymes of human alkaline phosphatase. *J Immunol Methods* 1982; 55: 13-8.
- Cummings RD, Kornfeld S. Fractionation of asparagine linked oligosaccharides by serial lectin agarose affinity chromatography. *J Biol Chem* 1982; 257: 11235-40.
- Osawa T, Tsuji T. Fractionation and structural assessment of oligosaccharides and glycopeptides by use of immobilized lectins. *Ann Rev Biochem* 1987; 56: 21-42.
- Komoda T, Koyama I, Fujimori-Arai Y, Yamagishi F, Sakagishi Y, Hirota N. Lectin affinity chromatography as a useful tool for determining the heterogeneity of alkaline phosphatases - intestinal-like enzymes. In: Miyai K, Kanno T, Ishikawa, eds. *Progress in clinical biochemistry*. Shannon, Ireland: Elsevier, 1992: 345-9.
- Witherspoon LR, Blonde L, Shuler SE. Bone scan patterns of patients with diffuse metastatic carcinoma of the axial skeleton. *J Nucl Med* 1975; 17: 253-7.
- Thrupkaew AK, Henkin RE, Quinn JL. False negative bone scans in disseminated metastatic disease. *Radiology* 1974; 113: 383-6.
- Skinner JM, Whitehead R. Carcinoplacental alkaline phosphatase in malignant and premalignant conditions of the human digestive tract. *Virchows Arch (Pathol Anat)* 1981; 394: 109-18.
- Imanishi H, Hada T, Muratani K, Hirano K, Higashino K. An alkaline phosphatase reacting with monoclonal antibodies to intestinal and placental isoenzymes. *Clin Chim Acta* 1989; 186: 309-14.
- Imanishi H, Hada T, Muratani K, Hirano K, Higashino K. Expression of a hybrid form of alkaline phosphatase isoenzyme in a newly established cell line (HuG-1) from a gastric cancer patient. *Cancer Res* 1990; 50: 3408-12.
- Aizawa K, Motoyama T, Watanabe H. Placental alkaline phosphatase-like isoenzymes produced by human gastric cancer cells. *Acta Pathol Jpn* 1989; 39: 630-7.
- Watanabe H, Tokuyama H, Ohta H, Sotomura Y, Okai T, Ooi A, *et al*. Expression of placental alkaline phosphatase in gastric cancers. An immunohistochemical study using the prepared monoclonal antibody. *Cancer* 1990; 66: 2575-82.