Abstract

Objective: To evaluate serum parathyroid hormone-related protein (PTHrP) as a marker of hypercalcemia in leukemic patients.

Design and Methods: We measured the serum levels of PTHrP, lactate dehydrogenase (LDH) and calcium in three patients with hypercalcemia due to leukemia.

Results: Serum levels of PTHrP, LDH and calcium were elevated at admission in all patients, and these levels were reduced to within the normal range after chemotherapy. However, normalization of serum PTHrP concentration occurred more rapidly than normalization of serum LDH levels after chemotherapy. The increase in serum PTHrP concentration accompanied leukemic cell proliferation and preceded the increases in serum LDH and calcium. Serum LDH concentration increased, but serum PTHrP concentration did not after administration of granulocyte colony-stimulating factor.

Conclusion: These findings suggest that serum PTHrP may be a more useful marker than serum LDH or calcium in assessing the status of leukemic patients with hypercalcemia.

Introduction

Parathyroid hormone-related protein (PTHrP) is the main cause of humoral hypercalcemia of malignancy (1, 2). PTHrP detection by RIA (3) or two-site IRMA kits (4) has been recently developed. It has been reported that serum PTHrP concentrations are increased in adult T-cell leukemia (ATL) patients with hypercalcemia (5). Imamura et al. (6) found that urinary excretion of PTHrP could be used as a predictor of hypercalcemia in ATL. However, the clinical utility of serum PTHrP measurements during the clinical course of leukemic patients with hypercalcemia is not known. Consequently, we measured serum PTHrP concentrations in leukemia patients with hypercalcemia during the course of their illness to investigate whether they could be useful in determining disease state.

Methods

Serum calcium levels were measured using the o-cresolphthalein complexing method. The normal range of serum calcium is 2.10–2.54 mmol/l. Serum calcium concentration was corrected as follows: 'corrected calcium (mg/dl) = measured calcium (mg/dl) + 0.8 (4.0–serum albumin (g/dl))'. The unit of mg/dl was changed to the molar unit of mmol/l. Serum lactate dehydrogenase (LDH) levels were measured using the Technicon auto analyzer method (Tarrytown, NY, USA) (normal range 50–107 mU/ml). Serum PTHrP levels were measured by using a PTHrP kit (Daich Radioisotope Institute, Tokyo, Japan) (3). This kit is composed of antibody raised against human PTHrP (109–141 amino acids), 125I-labeled [Tyr108]PTHRP (108–141 amino acids) and synthetic human PTHrP (109–141 amino acids) as a standard. The minimal level of detection is 10 pmol/l, and the measurable range is 10–1000 pmol/l. Serum with levels above 1000 pmol/l PTHrP was diluted with serum containing no measurable PTHrP. Serum PTHrP was stable under the conditions of sample handling. The normal value ranged from 10 to 60 pmol/l. Intra-assay and interassay variations were 1.9–5.9% and 2.4–3.9% respectively. The normal range of serum creatinine is 0.061–0.114 mmol/l.
Case 1
A 51-year-old woman was admitted to our hospital because of hypercalcemia (3.79 mmol/l). B-cell acute lymphocytic leukemia (peroxidase-negative, CD 19.20, SlgK, IgM-positive) was diagnosed by bone marrow aspiration. An anti-human T-cell leukemia/lymphoma virus type 1 (HTLV-1) antibody titer was negative. Physical examination was normal.

Case 2
A 60-year-old man was admitted because of hypercalcemia (4.32 mmol/l) and leukocytosis (17 000 cells/ml). He was found to have ATL by proliferation of CD4-positive lymphocytes, positive anti-HTLV-1 antibody titer and monoclonal integration of proviral DNA. His physical examination was normal.

Case 3
A 49-year-old woman was admitted because of leukocytosis. She was found to have ATL by proliferation of CD4-positive lymphocytes, positive anti-HTLV-1 antibody titer and monoclonal integration of proviral DNA. She went into remission with chemotherapy and was discharged. Three months later she complained of nausea and vomiting, and was readmitted for treatment of hypercalcemia (4.32 mmol/l).

Results

Case 1 (Fig. 1A)
On admission, the concentrations of serum PTHrP, LDH and calcium were elevated to 1080 pmol/l, 1 050 mU/ml and 3.79 mmol/l respectively, but normalized after chemotherapy. However, normalization of serum PTHrP occurred more rapidly than that of serum LDH. At 8 days after admission, the serum PTHrP concentration (25 pmol/l) decreased to within the normal range. However, serum LDH activity at this time remained high (430 mU/ml) (Fig. 1B). At 10 days after admission, bone marrow aspiration revealed hypocellular bone marrow. At 18 days after admission, leukemic cells in the bone marrow began to proliferate, and serum PTHrP concentration increased before the increase in serum LDH. That is, at 20 days after admission, serum PTHrP level was elevated above the normal range (92 pmol/ml), but serum LDH level (103 mU/ml) remained within the normal range (Fig. 1C). Serum creatinine concentration did not increase above 0.114 mmol/l at all during the hospitalization. The serum 1,25-dihydroxyvitamin D3 and interleukin-1β and interleukin-6 were 22 pg/ml (normal range 15–70 pg/ml), 14.6 pg/ml (normal range 0–15.6 pg/ml) and 35.5 pg/ml (normal range 0–54.9 pg/ml) respectively.

Case 2 (Fig. 2A)
On admission, levels of serum PTHrP (1020 pmol/l), LDH (393 mU/ml) and calcium (4.32 mmol/l) were increased. At 10 days after admission, serum PTHrP (48 pmol/l) and calcium levels had normalized, but serum LDH remained elevated (132 mU/ml) (Fig. 2B). Serum PTHrP concentration rapidly decreased after chemotherapy as compared with serum LDH. After administration of granulocyte colony-stimulating factor (G-CSF), serum LDH increased, but PTHrP levels remained normal (Fig. 2C). At this time there was no evidence of proliferation of leukemic cells. The serum creatinine concentration did not increase above 0.116 mmol/l at all during the hospitalization. Serum 1α,25-dihydroxyvitamin D3 concentration was 18 pg/ml.

Case 3 (Fig. 3A)
Increased levels of serum PTHrP (308 pmol/l), LDH (209 mU/ml) and calcium (4.32 mmol/l) were present on admission. After chemotherapy, these concentrations decreased to within the normal range. However, normalization of serum PTHrP concentration preceded that of serum LDH activity (Fig. 3B). At 20 days after admission, leukemic cells in the peripheral blood reached their nadir. When the leukemic cells in the peripheral blood began to proliferate, the serum PTHrP concentration increased to 70 pmol/l. At that time, however, serum LDH and calcium levels remained within the normal range (Fig. 3C). Serum creatinine levels remained less than 0.114 mmol/l except for the period from day 48 to 50 (maximum creatinine level was 0.168 mmol/l). Serum 1α,25-dihydroxyvitamin D3 concentration was 22 pg/ml. To provide a comparison with the leukemic patients, we also evaluated a patient with multiple myeloma with bone metastasis (case 4) and a breast cancer patient with liver metastasis (case 5). Although radiology and chemotherapy (except for steroids and calcitonin) were not used in the latter two cases during the period of investigation, an increase in serum PTHrP concentration still preceded increases in serum calcium and LDH levels (Fig. 4A). However, this differed somewhat from the leukemic patients in that the decrease in serum PTHrP concentration was more gradual than the decrease in calcium and LDH in case 5 (Fig. 4B).

Discussion
PTHrP is the cause of humoral hypercalcemia of malignancy (1, 2). Two groups have recently developed an RIA and two-site IRMA for the detection of circulating PTHrP (3, 4). It has been reported that approximately 32% of patients with ATL develop hypercalcemia (7). Imamura et al. (6) found that the urinary excretion of PTHrP in patients with ATL
correlated with serum LDH levels. In this study we measured PTHrP in the serum of three patients with leukemia by RIA and found remarkable changes in PTHrP levels during the acute phase. It is well known that hypercalcemia occurs in ATL (7) but rarely occurs in acute leukemia (8). One of our patients (case 1) had acute B-cell lymphocytic leukemia. B-cell lymphocytic leukemia with hypercalcemia due to PTHrP is rare (9). Although we did not confirm the expression of PTHrP mRNA in the leukemic cell, serum concentrations of 1α,25-dihydroxyvitamin D3 and interleukin-1β and interleukin-6 were within the normal range. Moreover, PTHrP levels paralleled the number of leukemic cells, suggesting that PTHrP was produced by the leukemic cell itself.

We examined the relationship between serum PTHrP, LDH and calcium levels in three leukemic patients without renal failure, and found that normalization of

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Figure 1** (A) Clinical course of case 1. Serum levels of PTHrP (●), LDH (○) and calcium (□) are shown. During the clinical course, creatinine concentration was less than 0.114 mmol/l. (B) After chemotherapy, serum PTHrP levels normalized more rapidly than serum LDH levels. (C) When leukemic cells in the bone marrow began to proliferate, serum PTHrP levels increased before serum LDH levels. The shaded areas represent the normal range of serum PTHrP and LDH. (B) and (C) are enlarged from (A).
serum PTHrP concentration preceded that of serum LDH levels after chemotherapy (cases 1, 2 and 3). We also found that elevation of the serum PTHrP level preceded elevation of the serum LDH level (cases 1 and 3) with leukemic cell proliferation. Serum LDH activity is commonly used as an indicator of tumor burden in leukemic patients. These findings suggest that serum PTHrP levels may be more useful than serum LDH levels for confirming remission or for predicting relapse in leukemic patients with hypercalcemia. It has been reported that serum LDH activity increases after G-CSF therapy in some patients (10, 11). In patient 2 serum LDH concentration increased after the administration of G-CSF, but serum PTHrP concentration did not, and leukemic cells were not detectable in the peripheral blood at that time. These findings suggest that the normal leukocyte response to G-CSF affects serum LDH levels, while the serum PTHrP level may

Figure 2 (A) Clinical course of case 2. Serum levels of PTHrP (●), LDH (□) and calcium (■) are shown. During the clinical course, creatinine concentration was less than 0.116 mmol/l. (B) After chemotherapy, serum PTHrP levels normalized more rapidly than serum LDH levels. (C) After administration of G-CSF, serum LDH activity was elevated but serum PTHrP concentrations remained normal. The shaded areas represent the normal range of serum PTHrP and LDH levels. (B) and (C) are enlarged from (A).
better reflect the status of the leukemia. Nakamura et al. (12) have reported that the serum PTHrP concentration started to increase in parallel with tumor enlargement and before an increase in serum calcium. Our finding that elevation of the serum PTHrP concentration correlates better with leukemic cell proliferation than with serum calcium concentration indicates that serum PTHrP concentration may be useful in assessing disease status in leukemic patients with hypercalcemia. Acute severe hypercalcemia in these patients is an important factor in their survival, and if therapy for hypercalcemia can be started earlier, it may result in improved survival.

Analyses of patients with renal failure using RIA have demonstrated elevated serum C-terminal PTHrP levels,

Figure 3 (A) Clinical course of case 3. Serum levels of PTHrP (●), LDH (○) and calcium (□) are shown. During the clinical course, creatinine concentration was less than 0.114 mmol/L except for the period from 48 to 50 days. (B) After chemotherapy, serum PTHrP levels normalized more rapidly than serum LDH levels. (C) When leukemic cells in the peripheral blood began to proliferate, serum PTHrP levels increased before serum LDH and calcium levels. The shaded areas represent the normal range of serum PTHrP and LDH levels. (B) and (C) are enlarged from (A).
while serum C-terminal PTHrP levels were not affected in patients with a creatinine clearance greater than 20 ml/min (13) or a serum creatinine concentration of less than 0.168–0.175 mmol/l (14). In our study, serum creatinine levels were never above 0.168 mmol/l. Therefore it is unlikely that the serum C-terminal PTHrP levels measured in this study were affected by renal function. Indeed, two ATL patients with hypercalcemia and renal failure were excluded from the study.

On the basis of our results, we can speculate that PTHrP is produced in leukemic cells, while serum LDH elevation is the result of destruction of leukemic cells. Further investigation is required to delineate these distinct mechanisms.

**Figure 4** Clinical courses of cases 4 and 5. Serum levels of PTHrP, LDH and calcium are shown. During these clinical courses, creatinine concentration was less than 0.114 mmol/l. (A) Patient with multiple myeloma with bone metastasis (case 4). Serum PTHrP and LDH levels changed in parallel, but the increase in serum calcium was slightly delayed. (B) Patient with breast cancer with liver metastasis but without bone metastasis (case 5). This case was different. When serum LDH was normalized, serum PTHrP concentration was still elevated (Aug. 29).
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References


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