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Evaluation of serum concentration of parathyroid hormone-related protein and its implication in hypercalcemia in squamous cell carcinoma of the head and neck

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Abstract. Hypercalcemia is a common and serious complication associated with squamous cell carcinoma (SCC) and is considered to be caused by a tumor-derived factor, parathyroid hormone-related protein (PTHrP). However, the correlation between serum levels of calcium and PTHrP and the kinetics of PTHrP in SCC of the head and neck is unknown, because the behavior of the circulating form of PTHrP in patients has not been determined. In the present study, the PTHrP concentrations in serum samples from 54 patients (37 with SCC and 17 with benign tumors) were measured by a recently developed radioimmunoassay directed toward the C-terminal region of PTHrP, and the laboratory data including those calcium levels in corresponding samples were reviewed retrospectively. Results showed hypercalcemia in four patients with advanced cancer and in whom elevation of the serum PTHrP concentration was observed simultaneously. The regression analysis also revealed the linear relationship of the calcium level to the PTHrP concentration, but not to the concentration of phosphorus or creatinine, suggesting that monitoring of serum PTHrP level is useful for prediction of hypercalcemia associated with head and neck cancer.

Key words: hypercalcemia; PTHrP; serum PTHrP concentration; squamous cell carcinoma; head and neck cancer.

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Patients with neoplastic diseases including squamous cell carcinoma (SCC) of the head and neck often suffer from various complications, of which humoral hypercalcemia of malignancy (HHM) is a common and serious syndrome. Parathyroid hormone-related protein (PTHrP) has been isolated and cloned from hypercalcemic tumor cells[7,8,11,14] and is considered to be the predominant cause of HHM[9]. Recently, assay systems for detecting circulating PTHrP in patients have been developed and have assisted the diagnosis of hypercalcemia[2,13]. However, with these assays, it is difficult to determine the serum levels of PTHrP in normocalcemic patients or normal subjects. KASAHARA et al.[5] developed a novel radioimmunoassay directed toward a C-terminal region of PTHrP, consisting of the amino acid sequence 109 to 141, PTHrP (109–141), and reported measurement of the serum level of PTHrP as the concentration of PTHrP (109–141) in normal subjects. If hypercalcemia associated with head and neck cancer is actually caused by an increase of circulating PTHrP, its serum level should increase before the appearance of hypercalcemia or with progress of dis-

ease. To determine whether PTHrP is involved in hypercalcemia and to assess the significance of measurement of serum PTHrP levels in patients with SCC of the head and neck, we used this assay method to measure the concentrations of PTHrP of stored serum samples obtained at various times during the clinical course of the disease, and we also reviewed the calcium levels of the same samples retrospectively.

Material and methods

Patients

The subjects studied were 37 patients (22 men and 15 women 43–82 years old (mean 61.7 years) with SCC of the head and neck (33 SCC of the oral cavity and four of the maxillary sinus) and 17 patients with benign tumors who registered and received treatment at the Dental Hospital of Tokushima University (Japan) during the 5-year period from 1987 to 1991 inclusive. The serum samples taken at some points during the clinical course of disease, e.g., at the first examination, under treatment, and sometimes at the terminal stage, were stored at −20°C for less than 1 week or at −80°C for a longer period until use for the assay, and the laboratory data of the same samples, including the concentrations of calcium, phosphorus, and creatinine, were reviewed retrospectively. The serum calcium concentration was corrected by the following equation: corrected calcium (mg/100 ml)=measured calcium (mg/100 ml)+4.0 – albumin (g/100 ml).

Hypercalcemia was defined as the presence of a corrected serum calcium level of more than 11.0 mg/100 ml, on at least two occasions, during the clinical course.

Measurement of serum PTHrP

Serum PTHrP concentration was measured by a radioimmunoassay using a D-0102 kit for the C-terminus of PTHrP (Daiichi Radioisotope Institute, Tokyo, Japan)[5]. Briefly, the kit was composed of antibody raised against human PTHrP (109–141), ^{125}I-labeled [Tyr^{108}]-PTHrP (108–141), and synthetic PTHrP (109–141) as a standard. A mixture of 100 μl of ^{125}I-labeled PTHrP (108–141) and 100 μl of antibody was incubated with 200 μl of standard solution or sample for 22 h. Then the second antibody was added and incubation was continued for 30 min. The mixture was then centrifuged, and the radioactivity associated with the precipitated immune complex was counted in a gamma counter, model ARC-301 (Aloka, Japan). It has been reported that with this assay the detectable standard range of PTHrP (109–141) is 10–1000 pmol/l[5], and that the concentration of PTHrP in the serum of normal subjects is in the range of 20.2–50.8 pmol/l (mean±2 SD)[12].

Statistical analysis

In order to verify the linearity of the relationship between serum levels of calcium and those of PTHrP, phosphorus, or creatinine, a simple regression analysis was used. Statistical significance for differences between means was evaluated by the unpaired t-test. Statistical analysis was done using Stat View software on an Apple Macintosh computer. P values of <0.0001 were considered significant.

Results

The serum concentrations of PTHrP at 290 points in 37 patients with SCC and at 31 points in 17 nonmalignantly affected patients were measured, and the laboratory data for the serum calcium levels of the corresponding samples were reviewed. No hypercalcemia was detected at the time of the first medical examination in any patient. Four patients with SCC subsequently developed hypercalcemia with progression of the disease, indicating that hypercalcemia is a late manifestation of SCC of the head and neck. As shown in Table 1, a serum calcium level of more than 11.0 mg/100 ml was found in 30 samples from four patients with advanced SCC who had originally been normocalcemic and who had not responded to treatment. Moreover, the serum PTHrP concentrations in these patients were significantly elevated compared with those with normocalcemic SCC (unpaired t-test, $t=14.829$; df=288; $P<0.0001$) and patients with benign tumors (unpaired t-test, $t=5.240$; df=59; $P<0.0001$), respectively. On the other hand, there was no significant difference (unpaired t-test, $t=0.840$; df=289; $P=0.4019$) between the PTHrP levels of patients with SCC and with benign tumors who were normocalcemic (Table 1).

Since hypercalcemia was observed in only advanced stages of cancer, the PTHrP levels and the laboratory data of advanced cases were compared. Treatment of eight of the 37 SCC patients was unsuccessful and their disease progressed. Table 2 shows data on these eight patients at the time when their serum calcium levels were highest during their clinical course. In the group that developed hypercalcemia, the levels of phosphorus remained within, or slightly below, the normal range and the creatinine level was slightly increased in two cases, but the serum levels of PTHrP and calcium were elevated simultaneously to more than 300 pmol/l and 14.0 mg/100 ml, respectively. The PTHrP concentration of the normocalcemic group was low (Table 2). Fig. 1 shows the interrelationship between the serum levels of calcium (y-axis, mg/100 ml) and PTHrP (x-axis pmol/l) of these eight patients. A simple regression analysis revealed that there was a linear relationship between the concentrations of calcium and PTHrP ($P<0.0001$) where the parameters of the analysis were computed with the regression equation, $Y=9.259+0.011X$; regression coefficient (R)=0.783; $R^2=0.614$.

The same analysis was also carried out between the calcium levels and the levels of phosphorus (mg/100 ml) or creatinine (mg/100 ml), where no obvious relationship was observed. The parameters of each analysis were as follows: the regression equation, Y(calcium)=9.224+0.241X (phosphorus); regression coefficient (R)=0.106; $R^2=0.011$, and the regression equation, Y(calcium)=9.132+0.96X (creatinine); regression coefficient (R)=0.298; $R^2=0.089$, respectively.

These results indicate that calcium level increased in association with PTHrP concentration. In addition, the value of serum PTHrP tended to vary more sensitively with therapeutic effects or progression of the disease than that of serum calcium. For example, as shown in Fig. 2, in case 1 (Table 2), the PTHrP concentration decreased on surgical, radiotherapeutic, or chemotherapeutic treatment and then rapidly increased with advance of disease.

Discussion

In this study, of 37 cases of SCC of the oral cavity and maxillary sinus, four advanced cases developed hypercalcemia, defined here as the presence of a corrected serum calcium level of more than 11.0 mg/100 ml, during the clinical course. Thus, the overall incidence of hypercalcemia associated with SCC of the head and neck was 10.8%. Vassilopoulou-Sellin et al.[15] reported a rather low prevalence of hypercalcemia in newly registered patients with various cancers including that of the head and neck. In our study, hypercalcemia was also not detected at the first examination. Hypercalcemia associated with head and neck cancer is likely to be manifested only in advanced patients. In our study, four of eight cases that did not respond to treatment and progressed to an advanced stage finally developed hypercalcemia. Thus, the occurrence of

Table 1. Summary of calcium levels and serum PTHrP concentrations in patients examined

Tumor		No. of patients	No. of samples	Ca* (mg/100 ml)	PTHrP* (pmol/l)
SCC	Hypercalcemic	4[a]	30	13.1±1.6[b]	243.5±223.2[c]
	Normocalcemic	37	260	9.4±0.5	35.9±16.8
Benign		17	31	9.4±0.3	33.3±9.3

* Values are presented as means±SD.

[a] Patients originally included among 37 normocalcemic patients who subsequently developed hypercalcemia.

[b] Serum calcium level in hypercalcemic SCC patients was significantly higher than those in normocalcemic SCC (unpaired *t*-test, $t=28.961$; df=288; $P<0.0001$) and benignly affected patients (unpaired *t*-test, $t=12.862$; df=59; $P<0.0001$), respectively. There was no significant difference between calcium levels in normocalcemic SCC and benignly affected patients (unpaired *t*-test, $t=0.190$; df=289; $P=0.8498$).

[c] PTHrP concentration in hypercalcemic SCC patients was significantly higher than those in normocalcemic SCC (unpaired *t*-test, $t=14.829$; df=288; $P<0.0001$) and benignly affected patients (unpaired *t*-test, $t=5.240$; df=59; $P<0.0001$), respectively. No significant difference was observed between PTHrP concentrations in normocalcemic SCC and benignly affected patients (unpaired *t*-test, $t=0.840$; df=289; $P=0.4019$).

Table 2. Laboratory data and serum PTHrP concentrations of advanced cancer patients*

Case no.	Ca (mg/100 ml)	P (mg/100 ml)	Cr (mg/100 ml)	PTHrP (pmol/l)
Hypercalcemic				
1	15.3	2.8	2.5	860.7
2	14.3	2.1	0.5	328.0
3	15.4	3.5	1.4	331.4
4	15.0	2.5	0.9	411.5
Normocalcemic				
5	9.1	3.2	0.6	45.6
6	9.8	5.2	2.4	63.2
7	9.7	4.1	0	35.0
8	9.9	2.6	0.9	26.7

* Patients who did not respond to treatments and progressed into advanced stages of disease. Ca: calcium; P: phosphorus; Cr.: creatinine.

All data are for same samples obtained at time calcium concentration was highest in each case.

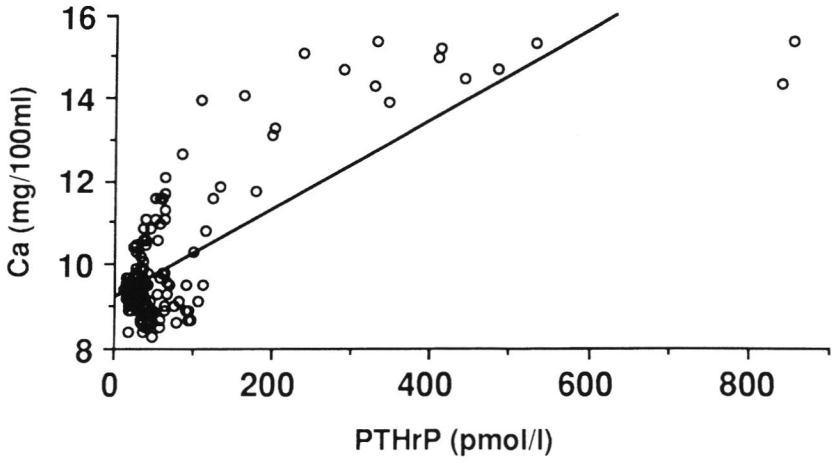

Fig. 1. Correlation between calcium and PTHrP concentrations in serum samples from eight patients who did not respond to treatment. PTHrP concentrations were measured by radioimmunoassay for C-terminal region of PTHrP. Regression curve is shown. Parameters of regression analysis were computed as follows: regression equation, $Y=9.259+0.011X$; regression coefficient (R)=0.783; $R^2=0.614$.

hypercalcemia in patients with SCC of the head and neck may be considerable (50%) when the disease is not cured successfully. These results are consistent with a previous observation that HHM is a common complication of SCC[11].

We have measured the concentration of serum PTHrP by a radioimmunoassay and found that the concentration of PTHrP was greatly elevated in all patients who developed hypercalcemia, but not in those wiht normocalcemia (Tables 1 and 2), suggesting a close relationship of elevation of the serum PTHrP concentration with hypercalcemia associated with SCC of the head and neck. This correlation was also supported by a regression analysis to verify the relationship between the concentrations of serum calcium and PTHrP in these patients (Fig. 1), where the R and R^2 were calculated as 0.783 and 0.614, respectively. On the other hand, there was no obvious relationship of the calcium levels to the concentrations of phosphorus or creatinine. Thus, although multiple factors may be linked to HHM[16], hypercalcemia associated with squamous cell carcinoma of the head and neck is mainly caused by elevation of circulating PTHrP, which may be overproduced and secreted by carcinoma cells[6]. These results may reflect the observations that keratinocytes, from which SCC of the oral cavity, as well as the skin, originate, produce PTHrP[10], and that all sections from SCC of various sites, including the oral cavity, react strongly with an anti-PTHrP antibody[3]. In an immunohistochemical study, we observed that the sections from the hypercalcemic patients with SCC of the head and neck were strongly stained by an antiserum raised against PTHrP (Matsumoto & Rikimaru, unpublished data).

Molecular[8,14] and amino acid sequence studies[7] revealed that three types of PTHrP, consisting of 139, 141, and 173 amino acids, could be produced and showed that they may undergo post-transcriptional processing, indicating that some species of PTHrP molecules may circulate in body fluids[1]. In fact, the circulating behavior in the plasma of the N- and C-terminus of PTHrP[2] is reported to be different. Moreover, the C-terminal fragment was shown to act as an osteoclast inhibitor, as opposed to an osteoclast activator[4]. Since the radioimmunoassay used in the present study detected the C-terminal region of PTHrP, we are uncertain

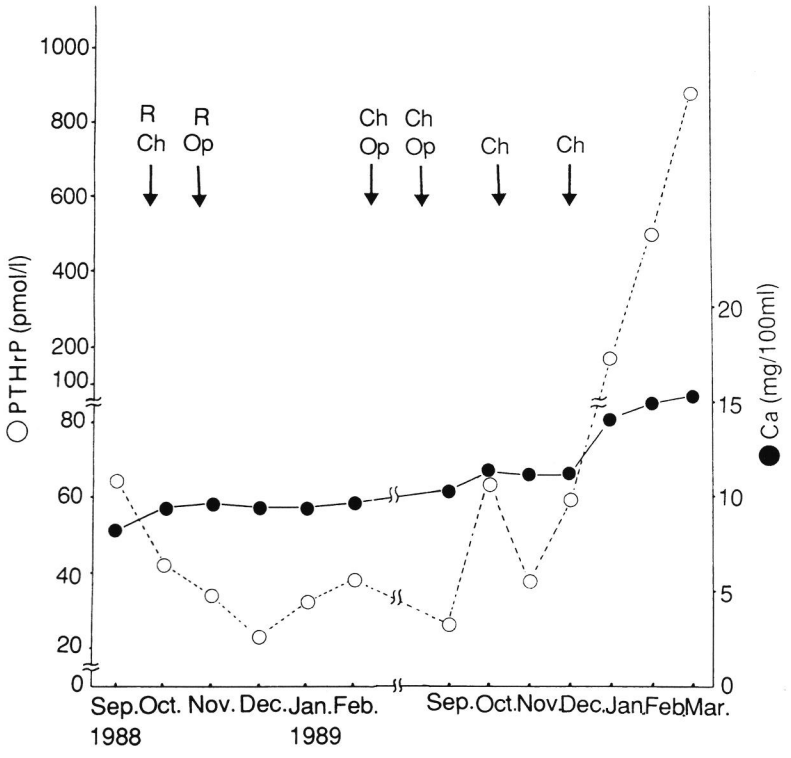

Fig. 2. Changes of serum levels of PTHrP (○) and calcium (●) during clinical course of case 1 in Table 2. Patient was treated by chemotherapy (Ch), radiotherapy (R), and/or surgical operation (Op) as indicated.

whether we measured the concentration of all biologically significant PTHrP molecules. However, Fig. 1 shows the strong relationship between the serum calcium level and the serum PTHrP level measured by this radioimmunoassay for its C-terminal fragment, suggesting that this assay was an effective for measuring circulating PTHrP as the N-terminal assay[13]. Interestingly, with the radioimmunoassay used here, the concentrations of PTHrP in the serum of not only hypercalcemic, but also normocalcemic, patients[12] can be measured, whereas other assays cannot detect plasma PTHrP in most normal or normocalcemic subjects[2,13]. Moreover, the serum PTHrP level was found to change sensitively with the progress or regression of disease (Fig. 2).

Our observations, mainly based on eight patients with advanced SCC, of whom four were hypercalcemic and four were normocalcemic, demonstrate a significant role of increase in the serum PTHrP level in the development of hypercalcemia. They also indicate that monitoring of serum PTHrP levels is useful for diagnosis and prediction of HHM associated with SCC of the head and neck and for assessment of treatment. The serum PTHrP level does not seem to be a useful parameter for prognosis because its elevation was observed only in advanced stages of disease.

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