Relationship Between Serum Levels of Interleukin-6, Tumor Necrosis Factor-α and Bone Turnover Markers in Prostate Cancer Patients

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Abstract. To determine the relationship between the serum levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and those of bone formation and resorption markers in prostate cancer patients, we measured the serum levels of the cytokines and examined their relationship to biochemical markers of bone turnover in 46 untreated patients with prostate cancer. The carboxy-terminal propeptide of type I procollagen (PICP) levels were used as a parameter of bone formation, and the carboxy-terminal telopeptide of type I collagen (ICTP) levels were used as a marker of bone resorption. The relationship of these markers to the degree of bone metastasis was also examined. The serum levels of IL-6, PICP, ICTP, and prostate-specific antigen (PSA) were significantly higher in the patients with prostate cancer with bone metastasis (n=23) than in the patients without bone metastasis (n=23). The serum levels of TNF-α in approximately 85% of the patients were under the detectable limit (5 pg/ml). The serum levels of IL-6 were not correlated with those of PICP or ICTP, but were related to the extent of bone metastasis. These results indicate that among patients with prostate cancer, IL-6 and TNF-α may not play major roles in the increased bone resorption in the patients with metastatic spread to bone. Our study thus demonstrated that the serum levels of IL-6 are closely related to the metastatic burden to osseous tissue in prostate cancer patients.

Key words: Interleukin-6, Tumor necrosis factor-α, Carboxy-terminal propeptide of type I procollagen (PICP), Carboxy-terminal telopeptide of type I collagen (ICTP), Prostate cancer

INTERLEUKIN-6 (IL-6) was originally identified as a T-cell-derived cytokine that induces the final maturation of B cells into antibody-producing cells, and later studies disclosed multiple biological activities of IL-6 that differ widely among various types of tissues and cells [1, 2]. The expressions of IL-6 and its receptor have been observed in several human prostate cancer cell lines [3]. It was recently reported that IL-6 functions as a paracrine growth factor for an androgen-sensitive human prostate cancer cell line and as an autocrine growth factor for two androgen-insensitive human prostate cancer cell lines [4]. IL-6 has also been reported to play an important role in the mediation of the imbalance of bone remodeling that leads to bone loss. IL-6 stimulates osteoclastogenesis and bone resorption and has been implicated in the pathogenesis of the bone loss that accompanies estrogen deficiency [5–7]. Tumor necrosis factor-α (TNF-α) also possesses a wide variety of physiological activities, notably a potent antitumor action [8]. TNF-α as well as IL-6 increases bone resorption [9].

Biochemical markers of bone turnover have been developed [10]. During the formation of type I collagen, the carboxyterminal propeptide of type I
procollagen (PICP) is cleaved from procollagen molecules. This propeptide is released into circulation only during the synthesis of collagen [11, 12]. At the time of bone resorption, the carboxyterminal telopeptide of type I collagen (ICTP) is excreted through collagen degradation, and this excretion rate is considered a parameter of the rate of bone degradation [13]. The measurement of serum PICP and ICTP levels has been reported to be useful in evaluating bone metabolism in various diseases [14–17].

The circulating levels of IL-6 and TNF-α were recently reported to be high in patients with primary hyperparathyroidism, and also closely correlated with those of bone resorption markers [18]. The aim of the present study was to clarify the relationship between the serum levels of IL-6 and TNF-α and those of bone turnover markers present or not in prostate cancer patients as well as patients with primary hyperparathyroidism.

Patients and Methods

Patients

A total of 46 untreated individuals were examined and put into the following two groups: prostate cancer patients with bone metastasis (n=23) and prostate cancer patients without bone metastasis (n=23). All of the subjects were inpatients of Toyama Medical and Pharmaceutical University Hospital. The diagnosis of each patient was confirmed histologically.

No therapeutic intervention, rectal examination, or endoscopic procedure was performed in any of these patients immediately before the blood collection. Since the serum levels of IL-6 and TNF-α were reported to be affected by tissue injury and hemorrhage under aseptic conditions, samples from patients who had undergone a biopsy of the prostate were excluded [19, 20]. Samples from patients who had other malignancies or who were considered to have any inflammation region including that caused by an indwelling urethral catheter were also excluded. None of the patients had impaired renal function, as evidenced by normal serum creatinine and urea nitrogen levels.

All serum samples were collected in the morning after an overnight fast and were stored at −30 °C until assayed.

Prostate cancer with bone metastasis: Twenty-three patients aged 54–93 yr with metastatic prostate cancer before treatment comprised this group. The assessment of metastases to the bone was based on plain X-ray skeletal surveys and radioisotopic bone imaging. Bone scans were performed with 99mTc-methylenediphosphonate. Computed tomography and magnetic resonance imaging were also performed in some patients.

The extent of bone metastases (extent of disease, EOD grade) in each patient was classified by the method of Soloway et al. [21] as follows. EOD 1, the number of bony metastases is less than six, each of which is less than 50% of the size of a vertebral body (one lesion about the size of a vertebral body would be counted as two lesions); EOD 2, the number of bone metastases is between six and 20, with the size of lesions as described above; EOD 3, the number of metastases is more than 20 but less than a “super scan"; and EOD 4, “super scan" or its equivalent, i.e., more than 75% of the ribs, vertebrae and pelvic bones have lesions. The EOD grading of the 23 patients with bone metastasis as shown by bone scan was determined; five patients were EOD 1, 11 were EOD 2, five were EOD 3, and two were EOD4.

Prostate cancer without bone metastasis: This group consisted of 23 patients aged 59–90 yr, with untreated prostate cancer. Four patients were T1; six were T2; 11 were T3, and two were T4, according to the TNM classification of the Union Internationale Contre le Cancer (UICC).

Measurements

Cytokines: The serum IL-6 levels were determined with a human IL-6 two-step sandwich chemiluminescent enzyme assay (CLEIA) kit (Fujirebio Inc., Tokyo). The intra-and inter-assay coefficients of variation were 2.1–2.7% and 5.3–7.3%, respectively. The detection limit was 0.2 pg/ml.

The serum TNF-α levels were determined with a human TNF-α two-step sandwich enzyme-linked immunosorbent assay (ELISA) kit (Otsuka Pharmaceutical Co., Tokushima). The intra- and inter-assay coefficients of variation were 1.6–7.8% and 1.8–6.2%, respectively. The detection limit was 5 pg/ml.
Markers of bone turnover: The serum PICP concentrations were measured by a radioimmunoassay (RIA) based on the two-antibody method, with a PICP RIA kit (Orion Diagnostica, Espoo, Finland) [11]. The intra- and inter-assay coefficients of variation were 1.9–8.0% and 3.0–3.9%, respectively. The detection limit and the normal range were 1.2 and 26–222 ng/ml, respectively.

The serum ICTP concentrations were assayed by an RIA (Telopeptide ICTP, Orion Diagnostica) based on the two-antibody method [13]. The intra- and inter-assay coefficients of variation were 3.6–5.0% and 4.0–8.7%, respectively. The detection limit and the normal range were 0.34 and 1.8–5.0 ng/ml, respectively.

Markers of prostate cancer: The serum prostate-specific antigen (PSA) levels were determined with the Tandem-R PSA Assay (Hybritech, San Diego, CA, U.S.A.) [22]. The intra- and inter-assay coefficients of variation were 1.4–5.8% and 3.3–6.3%, respectively. The detection limit and the normal range were 0.13 and 0–4.0 ng/ml, respectively.

**Statistical analysis**

Differences in the IL-6, TNF-α, PICP, ICTP and PSA levels between the two patient groups were determined by the Wilcoxon-Mann-Whitney test. Correlations of the IL-6, TNF-α, PICP, ICTP and PSA levels were evaluated by Pearson’s correlation test. Differences in serum IL-6, PICP, ICTP and PSA levels within the four grades of EOD were examined by Kruskal-Wallis test, as were the differences in serum IL-6 levels within the primary sites (T classification).

**Results**

**IL-6, TNF-α, PICP, ICTP and PSA in prostate cancer patients**

The serum levels of IL-6, PICP, ICTP and PSA were significantly higher in the prostate cancer patients with bone metastasis than in the prostate cancer patients without bone metastasis (Table 1). The ages of the patients with bone metastasis were similar to those of the patients without bone metastasis. The relationships between IL-6 and PICP, and between IL-6 and ICTP in the patients with and without bone metastasis are depicted separately in Fig. 1. No relationship was apparent between IL-6 and the two bone turnover markers in the patients with bone metastasis (Fig. 1A) or in the patients without bone metastasis (Fig. 1B). The

| Table 1. Serum IL-6, TNF-α, PICP, ICTP and PSA levels in prostate cancer patients without bone metastasis, and patients with bone metastasis |
|-----------------|-------------|-----------------|-----------------|-----------------|-----------------|
| Disease         | All patients | prostate cancer without bone metastasis | prostate cancer with bone metastasis | P* |
| Age (yrs)       | (n=46)       | (n=23)          | (n=23)          | P=0.7088        |
| IL-6 (pg/ml)    | 12.2 ± 28.2  | 2.5 ± 2.7       | 21.8 ± 37.7     | P<0.0001        |
| TNF-α (pg/ml)   | 35.3 ± 24.7  | 6.0 (n=1)       | 41.2 ± 22.5 (n=5) | P=0.1432        |
| PICP (ng/ml)    | 185.0 ± 183.5| 95.2 ± 30.0 (90.0) | 274.8 ± 226.2 (198.0) | P<0.0001        |
| ICTP (ng/ml)    | 5.4 ± 4.8    | 3.3 ± 1.3 (2.8) | 7.4 ± 6.1 (5.9) | P=0.0002        |
| PSA (ng/ml)     | 519.2 ± 1254.1| 39.3 ± 60.0 (17.5) | 978.2 ± 1640.6 (360) | P<0.0001        |

Values are the mean ± SD (median). a) P values are between the patients with bone metastasis and the patients without bone metastasis. b) The patients whose serum TNF-α levels were within detectable ranges (>5 pg/ml). IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; PICP, carboxy-terminal propeptide of type I procollagen; ICTP, carboxy-terminal telopeptide of type I collagen; PSA, prostate-specific antigen.
relationship between IL-6 and PSA in the patients with and without bone metastasis was also examined. No relationship was observed in the former ($r = -0.133$) or latter ($r = -0.115$) group. Only five (22%) of the patients with bone metastasis and one (4%) of the patients without bone metastasis had detectable serum levels of TNF-α. Among these six patients, the relationship between their serum levels of IL-6 and TNF-α was examined. No significant relationship was apparent ($r = 0.376$, $P = 0.4929$). The serum levels of IL-6, PICP, ICTP and PSA in the patients whose TNF-α levels were under the limit of detection and those whose TNF-α levels were within the detectable range were compared. The mean ± SD levels of IL-6 in the patients whose TNF-α levels were under the limit of detection and in those whose TNF-α levels were within the detectable range were 11.1 ± 15.3 pg/ml and 60.5 ± 66.8 pg/ml, respectively ($P = 0.0209$). The serum levels of the remaining markers in the groups divided by TNF-α detectability were not significantly different.

Fig. 1. Relationships between the serum levels of PICP, ICTP and IL-6 in prostate cancer patients with (A) or without (B) bone metastasis.
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Table 2. Serum IL-6, PICP, ICTP and PSA levels of prostate cancer patients with bone metastasis as a function of EOD grading by the method of Soloway

<table>
<thead>
<tr>
<th>EOD</th>
<th>1 (n=5)</th>
<th>2 (n=11)</th>
<th>3 (n=5)</th>
<th>4 (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>5.1 ± 4.9</td>
<td>9.3 ± 12.8</td>
<td>51.6 ± 57.8</td>
<td>58.1 ± 77.7</td>
</tr>
<tr>
<td>PICP (ng/ml)</td>
<td>114.8 ± 28.9</td>
<td>208.9 ± 91.9</td>
<td>373.6 ± 226.9</td>
<td>790.0 ± 275.8</td>
</tr>
<tr>
<td>ICTP (ng/ml)</td>
<td>4.4 ± 2.5</td>
<td>6.0 ± 2.8</td>
<td>8.3 ± 5.1</td>
<td>21.0 ± 13.1</td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td>168.9 ± 177.5</td>
<td>1269.6 ± 1930.6</td>
<td>435.6 ± 393.7</td>
<td>2755 ± 3033.5</td>
</tr>
</tbody>
</table>

Values are the mean ± SD (median). EOD, extent of disease; IL-6, interleukin-6; PICP, carboxy-terminal propeptide of type I procollagen; ICTP, carboxy-terminal telopeptide of type I collagen; PSA, prostate-specific antigen.

Relationship of IL-6, PICP, ICTP and PSA to the extent of bone metastasis

We compared IL-6, PICP, ICTP and PSA as a function of metastatic burden in bone estimated by the method of Soloway (Table 2). The mean and median values for IL-6, PICP and ICTP increased with the extent of bone metastasis. The serum levels of IL-6 and PICP were significantly increased according to EOD upgrading (IL-6; P=0.0247, PICP; P=0.0062), whereas the serum levels of ICTP and PSA were not related to the EOD grade (ICTP; P=0.0848, PSA; P=0.0722).

Relationship of IL-6 to the tumor extension of the primary site in patients without metastasis

Three of the patients without bone metastasis had lymph node metastasis. The remaining 20 patients were clinically considered to have no metastatic tumor. The tumor extension of the primary site (T classification) thus reflected the tumor burden of the whole body. The serum levels of IL-6 were not related to the T classification (P=0.9145).

Discussion

It is now thought that bone-derived cytokines are the local effectors of the bone resorption induced by systemic calciotropic hormones such as PTH. It was recently reported that the serum levels of IL-6 were increased in 38 patients with untreated primary hyperparathyroidism and strongly correlated with the serum levels of ICTP (r=0.87) [18]. The high level of IL-6 is consistent with experimental evidence suggesting that PTH increases the production of this cytokine by osteoblasts [23, 24]. In the present study, the mean IL-6 levels in the patients with bone metastasis were almost identical to those reported in patients with hyperparathyroidism, but no such high correlation between the serum levels of IL-6 and ICTP was observed in the present study. The results suggested that activated bone turnover accomplished by bone metastasis was not directly associated with IL-6. IL-6 may not play a major role in the increased bone resorption in prostate cancer patients with metastatic spread to bone. The levels of both the bone formation and the bone resorption markers were previously shown to be significantly higher in prostate cancer patients with bone metastasis than in benign prostatic hypertrophy patients and prostate cancer patients without bone metastasis[25]. Nevertheless, the exact process underlying the overproductions of PICP (osteoblastic synthesis of bone matrix) and ICTP (degradation product from osteoclastic activity) accompanying bone metastasis of prostate cancer remains to be elucidated.

In the present study, the serum levels of IL-6 were related to the extent of bone metastasis and

"Relationship of... to the extent of bone metastasis"
were not related to the T classification in the patients without metastasis. These results suggested that the serum levels of IL-6 were closely related to the metastatic burden to the osseous tissue in the prostate cancer patients. IL-6 is produced by many cells in the bone microenvironment, including marrow stromal cells, monocyte-macrophages, osteoclasts, and osteoblasts [26]. In addition, it was also suggested that metastatic prostate cancer cells produce IL-6 [3, 4]. In metastatic lesions, activation to release IL-6 from some of these cells may take place, but whether the mode of action is due to mechanical tissue damage or humoral factor(s) remains to be clarified. We speculate that the activation may influence the effect only on small surrounding adjacent bone tissues. A trabecular bone has a high surface-to-volume ratio, and 70 to 85% of the surface of the bone is in contact with the bone marrow [7]. In accordance with the extent of bone metastasis, the possibility for the metastatic lesion to be exposed to the bone marrow would be increased. This may explain the IL-6 efflux to the circulation.

Only one study has been reported regarding the serum levels of IL-6 in prostate cancer patients [27]. In that report, the serum levels of IL-6 were determined in hormone-refractory prostate cancer patients by means of an ELISA different from that used in the present study. A bimodal distribution was observed and the upper quartile of IL-6 levels from 9 to 61 pg/ml; the median IL-6 level as a whole was 2.3 pg/ml. This value was similar to that observed in the present study. In the previous report, there was no clear correlation between the serum levels of PSA and IL-6 (r=0.08). This result was identical to that of the present study.

IL-6 potentiates the effects of other hormones such as PTH-related protein (PTHrP) on calcium homeostasis and osteoclast bone resorption in vivo [28]. It was reported that PTHrP was demonstrated in clinically localized prostate cancer [29], but hypercalcemia occurs in less than 2% of prostate cancer patients [30]. The hypercalcemic effect of PTHrP may be attenuated by other unknown factors.

It was reported that the serum levels of TNF-α were increased in patients with untreated primary hyperparathyroidism and correlated with the serum levels of ICTP [18]. In the present study, the serum levels of TNF-α in approximately 85% of the patients were less than 5 pg/ml. Because there is no evidence that TNF-α functions as a paracrine/autocrine growth factor in human prostate cancer cell lines, TNF-α may not play any role in prostate cancer, regardless of metastatic spread to bone.

References

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