Diagnostic Value of Urine Deoxypyridinoline for Detecting Bone Metastases in Breast Cancer Patients

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Abstract. Deoxypyridinoline (Dpd), a crosslink product of collagen molecules found in bone and excreted in urine during bone degradation, has been described as a marker of bone turnover in metastatic breast cancer. In this study, the urine deoxypyridinoline/creatinine (Dpd/Cre) ratio was determined by enzyme immunoassay in urine samples from 116 women with breast cancer. Bone metastases were confirmed by x-ray or CT scan, with follow-up >6 mo. The urine Dpd/Cre ratio was significantly higher in patients with bone metastasis, compared to those without bone metastasis (p <0.05). In patients with bone metastasis, ratios of urine Dpd/Cre were higher in those with multiple lesions, compared to those with a solitary lesion, and the values also reflected therapeutic response (p <0.05). Serial monitoring of urine Dpd/Cre revealed that an elevation was correlated with disease progression. Patients with stable bone disease under effective therapy had significant diminution of the urine Dpd/Cre ratios, compared to those with progression of bone disease (p <0.05). In conclusion, the urine Dpd/Cre ratio may be a useful marker for detecting bone metastases and evaluating their response to therapy. (received 18 June 2002; accepted 16 July 2002)

Keywords: breast cancer, bone metastasis, deoxypyridinoline, tumor marker

Introduction

Bone metastasis is present in 69% of terminal breast cancer patients [1]. It is often difficult to detect early bone metastases and to assess their therapeutic responses solely on the basis of bone scans [2] and bone x-rays [3]. Bone scans are much more sensitive, but relatively non-specific, while bone x-rays are specific, but less sensitive [4,5]. Owing to the slow changes seen in bone during therapeutic response, bone scans should be re-checked after 3 mo [2]. It is important to find other methods, such as biochemical markers, for detecting bone metastases and monitoring their therapeutic responses.

Pyridinoline cross-links contribute to the structural rigidity and integrity of collagen fibrils in bone. When bone resorption occurs, osteoclastic degradation of bone matrix releases pyridinoline and deoxypyridinoline (Dpd) into the circulation; these compounds are then excreted in urine [6]. Measurements of urinary elimination of these compounds may provide useful information on the change of bone resorption [7-9]. Dpd is a more sensitive marker, since it is found predominantly in bone, and it is well suited for diagnosis and monitoring of the breakdown of bone matrix by cancer cells [10,11]. Studies have shown that urine Dpd elimination is significantly increased in patients with bone metastasis, compared to normal subjects [12-14]. Monitoring urine Dpd elimination has revealed significant diminution after bisphosphonate therapy, according to previous reports [15,16].
In this study, we measured urine Dpd in patients with metastatic breast cancer and examined the role of Dpd as a biochemical marker of bone metastasis. Sequential examinations were performed to monitor the response of urine Dpd during treatment and to evaluate its correlation with bone metastasis activity.

**Materials and Methods**

**Patients and study design.** The study population comprised 116 women (average age 48 ± 11.4 yr) with breast cancer who were treated at Kaohsiung Medical University Hospital from July 1995 to December 2001. Based on clinical, radiological, and scintigraphic evidence, patients with metastatic breast cancer, as well as those without it, were selected for this study. Patients with skeletal, endocrine, or hepatic diseases, or with previous medical conditions or drug therapies that affect bone metabolism, were excluded from this study.

All patients were referred for 99mTc-polyporphosphate bone scan for evaluation of skeletal metastases. Bone metastases were confirmed by bone scintigraphy, x-ray, or CT scan. Radiological examinations were performed on metastatic bone lesions every 1 to 2 mo to evaluate the therapeutic response. The criteria for therapeutic response were defined according to WHO criteria, which were used to classify patients into stable and progressive bone disease groups. The category of “stable disease” (SD) included patients with partial response or lack of change that was sustained for at least 2 mo; the remaining cases, which included newly diagnosed, untreated cases, were assigned to the category of “progressive disease” (PD).

The PD cases were categorized according to their responses to chemotherapy, endocrine treatment, and/or bisphosphonates. Bisphosphonate therapy consisted of two regimens: pamidronate (90 mg, iv, every 3-4 wk), or clodronate (1600 mg, po, daily). The two subsets (SD and PD) were analyzed for differences in urine Dpd levels.

**Sample collection and analysis.** Non-fasting samples of urine (10 ml) and venous blood (5 ml) were collected between 9 and 11 am. Borate (1 g/L) was added to urine samples to prevent bacterial growth. The samples were stored at -20°C until analyzed. Prolonged exposure of the samples to light, especially direct sunlight, was avoided. The blood was allowed to clot and serum was separated immediately. Aliquots were taken for standard biochemical profiles. The biochemical profiles were performed within 24 hr after collection.

Urine and serum creatinine (Cre) concentrations were assayed by standard laboratory methods using a Hitachi 736-40 autoanalyzer with purchased reagents (Wako, Japan). Urine Dpd was measured by enzyme immunoassay on microtitre plates using reagents (Pyrilin R5 TM-D) supplied by the manufacturer (Metra Biosystems, France). Standards were included on each plate for calibration and results were calculated from the calibration curve using Softmax software and an IBM-compatible computer. High and low control samples were included in each assay. Each standard, control, and urine sample was assayed in duplicate. Urine Dpd results were expressed as urine Dpd/Cre ratio (nmol/mmol). The cutoff value for urine Dpd/Cre ratio was 8 nmol/ mmol, according to our previous study in healthy control subjects [14].

**Statistics.** Results are reported as mean ± SD. The significance of differences of urine Dpd/Cre ratios in the stable disease group vs the progressive disease group was analyzed by t-test. For sequential monitoring data, paired t-tests were used to compare urine Dpd/Cre ratios before and after treatment. The criterion for statistical significance was p <0.05.

**Results**

Intra-assay and inter-assay data for quality control of Dpd and Cre analyses are shown in Table 1.

<table>
<thead>
<tr>
<th>Assay (units)</th>
<th>QC samples assayed (n = 20)</th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dpd (nmol/L)</td>
<td>low level</td>
<td>2.40</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>high level</td>
<td>6.00</td>
<td>8.1</td>
</tr>
<tr>
<td>Cre (mmol/L)</td>
<td>low level</td>
<td>0.93</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>high level</td>
<td>1.25</td>
<td>0.9</td>
</tr>
</tbody>
</table>
As summarized in Table 2, the patients were 23 to 76 yr old, with an average of 48 yr. Seventy of the patients had bone metastasis and 46 patients had extraskeletal metastasis. Among the 70 patients with bone metastasis, 50 had ≥2 skeletal lesions and 20 cases had solitary bone lesions. In 46 patients with extraskeletal metastasis, 12 cases had metastasis to liver, 25 to lung and 17 to soft tissues. Forty-nine patients had received hormone therapy and 62 had received chemotherapy. Of 53 cases with bisphosphonate therapy, 27 were treated with pamidronate and 26 with clodronate. Thirty-nine patients showed progressive disease (PD) and 31 showed stable disease (SD).

The mean urine Dpd/Cre ratio in all patients was 10.2 ± 4.5 nmol/mmol (Table 3). When the patients were categorized according to bone metastasis, Dpd/Cre ratios were higher in patients with bone metastases (12.5 ± 7.5 nmol/mmol) than in those without (7.6 ± 3.2 nmol/mmol) (p <0.05). Menopausal status was unlikely to be correlated with Dpd/Cre ratio, since the ratios in premenopausal patients (11.5 ± 8.1 nmol/mmol, n = 66) were comparable to those in postmenopausal patients (12.0 ± 7.9 nmol/mmol, n = 50).

Urine Dpd/Cre ratios of 70 patients with bone metastases were further analyzed. When the 70 patients were subdivided into groups according to the extent of the disease in the bone, higher Dpd/Cre ratios were observed in patients with ≥2 bone lesions (15.4 ± 7.2 nmol/mmol) compared to those with a solitary bone lesion (9.7 ± 3.4 nmol/mmol) (p <0.05).

Thirty-one patients with bone metastases were classified as SD, and the remaining 39 cases were classified as PD at the beginning of sample collection. In 12 of these cases (3 SD cases and 9 PD cases), repeat samples were not collected, owing to loss to follow-up (n = 6) or death (n = 6). The initial urine Dpd/Cre ratios were significantly higher in the PD group (14.8 ± 7.8 nmol/mmol) than in the SD group (13.0 ± 6.5 nmol/mmol). After therapy, the Dpd/Cre ratios of patients reached lower levels (9.5 ± 7.8 nmol/mmol) than the initial levels in the SD group (p <0.05).

The 17 patients treated with bisphosphonate in the SD group had lower urine Dpd/Cre ratios (8.0 ± 6.3 nmol/mmol) than the 11 patients without bisphosphonate (10.1 ± 5.8 nmol/mmol) and lower than the initial levels (12.8 ± 6.4 nmol/mmol). In contrast, the urine Dpd/Cre ratios of the PD group reached much higher levels (19.2 ± 8.0 nmol/mmol) than the initial levels (p <0.05). Even in PD patients treated with bisphosphonate (n = 22), the urine Dpd/Cre ratios were higher (18.9 ± 8.2 nmol/mmol) than the initial levels (15.2 ± 7.7 nmol/mmol). In the 46 patients without bone metastasis, 10 cases (22%) had elevated initial ratios of urine Dpd/Cre, and 7 of these cases developed bone metastasis within 12 mo.

In patients with bone metastasis, urine Dpd/Cre ratios did not differ in premenopausal (12.6 ± 12.5 nmol/mmol, n = 39) and postmenopausal patients (12.0 ± 7.9 nmol/mmol, n = 31) (p >0.05).
Sequential monitoring of Dpd/Cre ratios after initiation of bisphosphonate therapy (pamidronate in 22 cases, clodronate in 21 cases) was performed in 43 patients with bone metastases. When the second urine samples were collected, significant elevation of Dpd/Cre ratio (18.9 ± 8.2 nmol/mmol, n = 22) was observed in the PD group when compared with their initial levels (p < 0.05). The Dpd/Cre ratios of the SD group were lower (8.0 ± 6.3 nmol/mmol, n = 17) than their initial levels (12.8 ± 6.4 nmol/mmol, n = 18) (p < 0.05). This observation indicates that Dpd/Cre ratios reached nearly normal values under effective bisphosphonate treatment in the SD group, and that Dpd/Cre ratios reached much higher levels in the PD group. There were no significant differences in the Dpd/Cre ratios after pamidronate vs clodronate therapies in the SD or PD groups. This suggests that the Dpd change reflects the therapeutic response, rather than the different anti-resorptive therapeutic agents.

**Discussion**

Since bone destruction in most patients with metastatic breast cancer is caused by osteolysis followed by bone formation, urine Dpd may be a suitable marker for breast cancer metastasis, as was suggested in our previous study [14]. In the present study, urine Dpd levels were significantly higher in patients with bone metastases than in those without bone metastasis, suggesting that Dpd may be a useful marker for bone metastasis of breast cancer. However Dpd is unlikely to be an early tumor marker, for higher Dpd/Cre ratios were not observed in patients with a solitary bone metastasis. The extent of bone metastasis was previously reported to be correlated with Dpd/Cre ratios in breast cancer and other cancers [14]. In addition, a higher level of urine Dpd/Cre ratio was also found in patients with severe bone metastases, but was not correlated with more bone lesions, as was discussed in our previous report, which suggested that the initial bone destruction was followed by bone formation during bone metastatic status. Therefore, urine Dpd/Cre ratio seems to be an appropriate marker for multiple bone metastases, through imaging studies are needed to identify bone metastasis in its early stages.

In this study, urine deoxypyridinoline was measured by an enzyme-linked immunosorbent assay (ELISA), which correlated well with the results obtained by high performance liquid chromatography with fluorometric detection [17,18]. In our previous study [14], urine Dpd/Cre ratios were around 5.6 nmol/mmol in a healthy group of Taiwanese women, similar to the findings in a study by Ohishi et al [19]. Although age-related changes of urinary Dpd/Cre ratios were noted in some reports [19,20], which showed significant increases in children and postmenopausal women, there has been no systematic study that indicates that the
changes in Dpd excretion are caused by the menopause. In this study, there was no significant relationship between urine Dpd/Cre ratios and the menopausal status. This suggests that the bone destruction induced by the menopause is less than that caused by cancer metastases.

In this study, 22% of cases with extraskeletal metastasis had elevated urinary Dpd/Cre ratios in the initial test, and developed bone metastasis during the 12 mo follow-up. Furthermore, those patients continuously had much higher urinary Dpd/Cre ratios. This reveals that bone metabolism is affected by breast cancer micrometastasis, which cannot be detected in clinical images, and that increased osteoclastic activity is frequently caused by cancer cells [21]. It may also be caused by humoral mediators that influence bone homeostasis via parathyroid hormone related protein, cytokines, prostaglandin, and transforming growth factors [22]. These findings may be correlated to the 25% of cases of stage III cancers that had elevated levels of Dpd/Cre ratios in our previous study [14]. Bone micro-metastasis was therefore detected by a bone turnover marker before the metastatic bone destruction could be detected by imaging studies.

Radiography and bone scans have been generally accepted as routine examinations for bone metastasis [2,3]. Bone scans are more sensitive than bone radiography, whereas radiography is more specific, suggesting that diagnosis of bone metastasis generally needs a combination of the two images [4]. In this study, we found that urine Dpd/Cre ratios showed positive correlation with therapeutic response of bone metastatic lesions, and also that the elevation of urine Dpd/Cre ratios correlated with progression of bone metastasis. These results are consistent with the report by Lüftner et al [15] in a smaller number of cases, and other bone turnover markers which have been reported in other studies [23-25]. Imaging technics combined with monitoring of urine Dpd/Cre ratios appears to offer additional information for the detection and evaluation of bone metastasis.

Bone metabolism is characterized by both bone resorption and bone formation. Most metabolic bone diseases, including cancer metastasis, disrupt these two activities. Various bone resorption markers, such as the carboxyterminal propeptide of type I collagen, the carboxyterminal telopeptide of type I collagen [25-27], and bone formation markers such as bone-specific alkaline phosphatase, and osteoclaeine, have been tested for detecting bone metastasis [14, 28]. Bone resorption markers were reported to be superior to bone formation markers for detection of bone metastasis [29]. Based on the findings of this study, although other bone turnover markers may be useful in detection and evaluation of bone metastasis, urine Dpd can be recommended for use in combination with imaging examinations.

Patients in this study received various modalities of therapy, including several chemotherapeutic regimens, endocrine drugs, and different dosages of radiation. This shows that the therapeutic responses were derived from a heterogenous population. For example, cytoxic chemotherapy with drugs such as cyclophosphamide and methotrexate has been reported to affect mineralized tissue metabolism. Analysis of data regarding the responses to different modalities of therapy needs further evaluation and a greater number of cases. Lüftner et al [15] reported that patients with stable bone disease during iv pamidromate treatment had a significant fall of Dpd-crosslink, compared to those with progressive disease, and concluded that the net bone turnover is not increased at Dpd-crosslink elimination <8 nmol/mmol. In our study, the level of Dpd/Cre ratio decreased to normal in the SD group after therapy, especially after bisphosphonate therapy. These results indicate that bisphosphonate has an important role in inhibiting the destruction of bone, as has also been confirmed by other bone resorption markers. Otherwise, the two bisphosphonate therapies seem to have similar effects on bone destruction.

In conclusion, this study revealed that the urine Dpd/Cre ratio is a useful marker for bone metastasis caused by breast cancer. Furthermore, Dpd can be useful in evaluating therapeutic response, especially in patients with progressive disease.

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References


