Urinary Bone Resorption Markers (Deoxypyridinoline and C-Terminal Telopeptide of Type I Collagen) in Healthy Persons, Postmenopausal Osteoporosis and Patients with Type I Diabetes

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ABSTRACT

Purpose: Deoxypyridinoline (DPD) is a derivative of hydroxyypyridinium, which is released during bone resorption into the bloodstream and is eliminated unmodified with urine. A further collagen-derived marker of bone resorption is the C-terminal telopeptide of type I collagen (β-CTX-I, here abbreviated as CTX), which is released in bone resorption and almost entirely excreted by the kidneys. The aim of our study was to investigate different well-described patient groups as well as normal probands in view of differences and expected correlations of these two parameters: patients with insulin-dependent diabetes mellitus, postmenopausal osteoporosis and healthy control persons.

Materials and Methods: We used a solid-phase chemiluminescence enzyme immunoassay (Pyrilinks D-IMMULITE) for urinary DPD measurement and for the assessment of urinary CTX we used a quantitative ELISA (Osteometer Biotec A-S, CrossLaps® ELISA).

Results: We found a highly significant correlation between both parameters in the group of healthy persons (r = 0.75, p < 0.05, n = 28) as well as in the group of patients with diabetes mellitus type I (r = 0.79, p < 0.05, n = 65). Also, a significant correlation was observed between DPD and CTX (r = 0.583, p < 0.05, n = 88) in the group of female osteoporotic patients.

Conclusions: Despite good correlations between DPD and CTX in all of the investigated groups, these urinary markers were of limited diagnostic significance in the group of postmenopausal osteoporosis due to a wide spread (few patients showed concentrations above the range of healthy persons) in this newly diagnosed drug-naïve patient collective.

Key words: Biochemical markers of bone metabolism in urine, deoxypyridinoline, DPD, carboxyterminal peptide of type I collagen, CTX, chemiluminescence enzyme immunoassay, ELISA

INTRODUCTION

The organic bone matrix consists of 90% of type I collagen in a triple-helical protein structure. Type I collagen of bone is cross-linked by collagen-specific compounds. In humans these „cross-links“ are derivatives of hydroxyypyridinium: pyridinoline (PYD) and DPD (DPD). DPD is generated by enzymatic splitting of amino acid lysine by lysyl-oxidase. In the process of bone resorption, DPD is released and undergoes renal elimination in an unmodified form. In contrast to formerly used parameters such as hydroxyproline, the elimination of DPD is independent of nutrition and therefore a specific parameter of bone resorption [1,2]. During the regeneration process of bone, small peptide fragments of type I collagen are generated by bone resorption. These are aminotelopeptides (NTX) or carboxyterminal telopeptides of type I collagen (CTX), which enter the blood stream and subsequently undergo renal elimination. Bone metabolism is a permanent...
process of remodelling: formation and resorption of bone is a coupled process. Therefore, the measurement of biochemical parameters of bone turnover may show an imbalance of the osteoblastic formation or osteoclastic resorption processes with a high specificity and sensitivity. Numerous studies have demonstrated the association between the elimination rate of collagen degradation products and the bone turnover rate [3,4].

In the diagnostic work-up of patients with osteoporosis, the determination of osteocalcin in serum or plasma is often used as a fairly specific parameter of bone formation. However, due to rapid degradation in serum or plasma, the pre-analytical handling of osteocalcin is difficult [5], and some osteocalcin may derive from bone resorption [6].

In addition, in patients with osteoporosis, e.g. after menopause, bone resorption may be stronger than bone formation. Therefore, markers of collagen resorption are now used more often [7-9].

The advantage of these parameters is the uncomplicated measurement in the urine due to their largely intact renal elimination without modification.

There are enzyme immunoassays and ELISAs commercially available for the measurement of free DPD and peptide-bound derivatives of hydroxypyridinium, e.g. for the N-terminal peptide of type I collagen (NTX) and for the C-terminal peptide (CTX) [1,2,8].

The aim of our work was to compare a solid phase chemiluminescence enzyme immunoassay for analysis of DPD with an ELISA for the measurement of CTX. We will demonstrate our results in well-described different groups: a control group of healthy persons, a group of patients with insulin-dependent diabetes mellitus (type I), known to be at a higher risk for osteoporosis and female (postmenopausal) osteoporosis patients, who were diagnosed according to the DVO guideline with DXA measurement and T-score values below -2.5.

**MATERIALS AND METHODS**

**IMMULITE-assay for quantitative analysis of urinary DPD**

The DPD-IMMULITE-system (distributed in Germany by DPC Biermann, Bad Nauheim) is an automated chemiluminescence system. The methodology of this test procedure is a competitive solid-phase immunoassay principle. The assay uses monoclonal anti-DPD-mouse antibodies, which are bound to a solid phase (coated sphere). DPD and alkaline phosphatase (AP)-conjugated DPD compete for binding sites at the monoclonal anti-DPD antibody. The detection of DPD is made possible by the AP-conjugated DPD. A two-point adjustment of the calibration curve is achieved with two calibration standards (a high and a low concentration of DPD) within a matrix of phosphoric acid. The measurement range of this assay ranges from 7 – 300 nmol/L with an analytic sensitivity of about 4.4 nmol/L [10].

**Analysis of CTX in urine (Urine CrossLaps® ELISA)**

The Urine CrossLaps® ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) used in this study is an enzyme immunoassorbent assay employing polyclonal antibodies (rabbit) which react with the amino acid sequence of EKAHD-ß-GGR in which the aspartic-acid (D) exists in a ß-isomeric form. The underlying method is a competitive binding assay. The CTX in the standards, controls or samples is bound to the antibodies of the CrossLaps assay and the remaining free antibodies are bound to a solid phase available in excess (immunostrips). After incubation of this reaction and a wash step, an incubation with peroxidise-conjugated antibodies is performed for 60 minutes. After a washing step, a further incubation with chromogenic substrate is done for 15 minutes. Then the reaction is stopped and the absorption measured at 450 nm. The lower limit of detection is about 50 µg/l. The standard curve is generated on the basis of six standards of rising concentrations.

**Statistical analysis**

A descriptive statistical analysis (arithmetic mean ± standard deviation and median) was performed for all measured parameters and samples. A paired and an unpaired Student’s t-test were used to compare the variables and correlations. The regression analysis of the variables was done with the software Excel 7.0 and SPSS [11]. With the SPSS software Mean values, 95% confidence interval of mean, median, standard deviation of mean and arithmetic mean, were calculated.

**Patients and healthy controls**

A total of 181 persons were investigated. Fasting samples were taken by venipuncture before 10 a.m. The second morning urine was taken for the urinary investigation. All samples were directly converted and stored at minus 80°C before measurement.

Renal function was in the normal range in all subjects investigated. All serum creatinine values measured were below the level of 1.2 mg/dl. Healthy individuals and the different patient groups investigated are shown in Tab. 1.

The group of healthy controls consisted of 11 men and 17 women aged 19 – 89 years, mean age 56.5. The group of patients with type I diabetes consisted of 28 female and 37 male patients, aged 35 – 67 years, mean age 53.6. The third group consisted of 88 patients with osteoporosis (postmenopausal women), aged 55 – 91, mean age 65.8 years.

The female patient group with osteoporosis was diagnosed by bone mineral densitometry (following the criteria for diagnosis of osteoporosis of the DVO 2006 guideline, DXA). All female osteoporotic patients received calcium and vitamin D supplementation. The sample collection was performed before a specific anti-osteoporotic treatment (e.g. bisphosphonates) was started.
RESULTS

The comparison of the parameters DPD and CTX in the entire group (n = 181) we showed a significant correlation of r=0.52; p < 0.05 as shown in Fig. 1. The 95% confidence interval for r was between 0.251 and 0.529.

Fig. 2 shows the correlation of these two parameters in the group of healthy subjects (n = 28, r = 0.75, p < 0.05). In Fig. 3 the correlation of the parameters compared in the patient group with diabetes mellitus type I is shown (n = 65, r = 0.79, p < 0.05).

The results of the female osteoporotic patients are depicted in Fig. 4 (n = 88, r = 0.583, p < 0.05).

Fig. 5 illustrates, the urinary excretion of DPD in all investigated groups in a box-plot and whiskers diagram regardless of the potential therapies (e.g. insulin, calcium, vitamin D). The arithmetic mean of the group of healthy individuals was 15.4 nM DPD/mM creatinine. The 95% confidence interval of the mean value ranged from 11.1 nM DPD/mM creatinine to 19.7 nM DPD/mM creatinine.

In the group of patients with diabetes mellitus type I, the arithmetic mean was 21.0 nM DPD/mM creatinine. The 95% confidence interval of the mean value ranged from 11.3 nM DPD/mM creatinine to 30.8 nM DPD/mM creatinine (Tab. 2).

The group of female patients with osteoporosis showed an arithmetic mean of 38.5 nM DPD/mM creatinine. The 95% confidence interval of the mean value ranged from 28.3 nM DPD/mM creatinine to 48.7 nM DPD/mM creatinine (Tab. 2).

Fig. 6 demonstrates the values of CTX in a box-plot and whiskers diagram. In the healthy control group, the arithmetic

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**Table 1. Distribution of patient groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>181</td>
</tr>
<tr>
<td>Healthy</td>
<td>28</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>65</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>88</td>
</tr>
<tr>
<td>&lt; 50 years of age</td>
<td>26</td>
</tr>
<tr>
<td>&gt; 50 years of age</td>
<td>62</td>
</tr>
</tbody>
</table>

Mean age

Healthy: 57 years (56.50)
IDDM: 54 years (53.2)
Osteoporosis: 66 years (65.79)

**Figure 1.** Correlation of parameters - total group.

**Figure 2.** Correlation of parameters in healthy persons.

**Figure 3.** Correlation of parameters in type I diabetes mellitus patients.

**Figure 4.** Correlation of parameters in osteoporosis patients.
The results in the group of diabetes type I patients showed an arithmetic mean of 568.0 µg/L. The 95% confidence interval of the mean value ranged from 291.8 µg/L to 844.1 µg/L.

The patients with osteoporosis showed an arithmetic mean of 748.9 µg/L. The 95% confidence interval of the mean value ranged from 388.7 µg/L to 1109.1 µg/L (Tab. 2).

**DISCUSSION**

The data presented here indicate a good correlation of both analysed parameters of collagen degradation. As expected, we measured the lowest values in the control group of healthy persons, due to the lowest bone turnover in this group. Higher values are found in the different patient groups and also significantly positive correlations could be observed. We found consistently increased markers of bone resorption within the group of patients with insulin-dependent diabetes mellitus [12]. Diabetes mellitus type II (NIDDM) is not associated with an increased risk for osteoporosis. In contrast, these patients
show rather high bone mineral density and extraosseous calcifications (e.g. Dish-syndrome), but diabetes type I is known to constitute a common risk factor for osteoporosis. For both degradation parameters higher values were found in this group. This fact reflects the elevated bone metabolism with emphasis on the resorptive component.

The observed values of DPD and CTX of the female patients with diagnosed osteoporosis showed a good correlation also, but the spread of individual values was significantly higher. Within this female patient group, the diagnosis of osteoporosis was verified by bone mineral densitometry (following the criteria for diagnosis of osteoporosis of the DVO 2006 guideline). All female osteoporotic patients received calcium and vitamin D supplementation; however, they did not receive specific anti-osteoporotic drug therapy [13]. It is noticeable that all values obtained in the osteoporosis group before the initiation of a specific therapy show an enormous spread. This could point to the limitation of the diagnostic significance of these pre-therapy values. However, the diagnostic value of collagen degradation parameters under anti-resorptive anti-osteoporotic therapy is widely undisputed [7,14].

One reason for a limited diagnostic significance of pre-therapy values in our patients with osteoporosis might be that urinary markers could be less reliable than serum or plasma markers. Furthermore, free DPD may be less reliable than total DPD, which however, is usually determined by HPLC after acid hydrolysis, which needs special equipment and training [15]. The imprecision of these urinary tests is also increased by the variable urine dilution and by the correction for creatinine [16,17]. Despite limited diagnostic significance, Garnero et al. showed in 1999, in a four-year-follow-up study, that women with high bone turnover (bone marker levels initially 2 SD above the premenopausal mean level) had a 2-6 fold higher loss of bone mineral density (BMD) than women with low turnover [18]. In addition, three prospective studies, EPIDOS [19], Rotterdam [20], and OFELY [21] show that high resorption markers predict an elevated risk for fractures in postmenopausal women. From these studies it may be concluded that postmenopausal women in the highest quartile are at an elevated risk for future fractures. In addition, high pretreatment levels of markers of bone turnover resulted in a better anti-fracture efficacy of the bisphosphonate alendronate [22].

CONCLUSIONS

In our investigation a good correlation of urinary free DPD and CTX in all three groups was found with a higher spread of the values of DPD. In the patient group of postmenopausal osteoporosis, we observed a wider spread of both parameters at the chosen pre-treatment time-point. A limitation of our study is that we have no longitudinal observation of subsequent measurements under therapy. Like noted before, it will be of greater diagnostic importance to document therapy-induced changes of these parameters for monitoring therapy control in patients under long-term anti-resorptive therapy [18], e.g. to assess reduction of bone turnover under antiresorptive therapy also in order to prevent over suppression of bone remodelling. In addition, serum or plasma markers with automated assays and standardized sampling (e.g. EDTA plasma samples taken in fasting patients in the morning before 08:30 h) may have a better potential for pre-therapeutic estimation of bone resorption [23].

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REFERENCES


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