Original Article

Comparison of Serum and Urine Assays for Biochemical Markers of Bone Resorption in Postmenopausal Women with and without Hormone Replacement Therapy and in Men

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Abstract. Biochemical markers of bone resorption have been used to characterize metabolic bone disease and assess therapeutic response. Most studies have used the urinary measurement of collagen crosslinks, but serum assays have recently been developed that may have less analytic and biologic variability. In the present study, we measured urine and serum N- and C-terminal crosslinked telopeptides of type I collagen (NTX and CTX) and serum bone sialoprotein (BSP) in postmenopausal women with or without hormone replacement therapy (HRT) and in men of similar age. In these populations, the variability of serum and urine markers was similar, except that serum NTX showed somewhat lower variability in postmenopausal women. Urine and serum assays correlated well with one another and were significantly lower in postmenopausal women on HRT compared with untreated women. The difference in women on HRT was similar for sNTX, uNTX and BSP (35–40%) and greater for sCTX and uCTX (52–53%). There was an inverse correlation between markers and bone mineral density, largely attributable to the high correlation in women not on HRT. Fractional excretion of NTX and CTX were estimated at 0.20 ± 0.07 and 0.44 ± 0.11, respectively. These values were independent of the concentration of the marker or of creatinine in the urine. We conclude that serum markers are useful measures of bone resorption in these populations, in whom the use of such markers is likely to be helpful in the management of osteoporosis.

Keywords: Bone mineral density; Bone sialoprotein; C-terminal telopeptide crosslinks; N-terminal telopeptide crosslinks; Osteoporosis

Introduction

Biochemical markers of bone resorption have proven useful for characterizing metabolic bone disease and assessing therapeutic response in many clinical studies [1,2]. Most of these studies have relied on measurements of the urinary excretion of collagen crosslinks [3]. However, serum assays which can assess bone resorption have recently been developed [4–6]. These include the N-terminal (NTX) and C-terminal (CTX) crosslinked telopeptides of type I collagen and bone sialoprotein (BSP). One potential advantage of serum assays is that they may be less subject to both analytic and biologic variability. Analytic variability in urine assays is increased by the need for measuring urine creatinine and calculating excretion as a ratio. Biologic variability may be increased by differences in renal clearance, particularly for peptides which could be reabsorbed and/or degraded in the kidney.

The present study was undertaken to compare the serum markers (sNTX, sCTX and BSP) with urine markers (uNTX and uCTX) in postmenopausal women age 44–65 years who were or were not on hormone replacement therapy (HRT) and in men age 54–70 years, and to assess the renal fractional excretion rates of these markers. These are groups in which the use of resorption markers for assessment of bone turnover is likely to be of prognostic value. The results support the utility of the
currently available immunoassays for serum markers, show that both urine and serum assays correlate well with one another and provide evidence that there is tubular reabsorption and/or degradation of NTX and CTX.

**Subjects and Methods**

**Subjects**

The characteristics of the study subjects are indicated in Table 1. They were healthy volunteers who were recruited for a longitudinal study of bone loss rates. They were not receiving drugs which affect bone metabolism, had no recent fractures, and had no evidence of metabolic bone disease other than primary osteoporosis. All women were at least 3 years postmenopausal. Those on HRT had been taking 0.625 mg of conjugated equine estrogen daily or its equivalent for at least 3 years. A fasting blood sample and a second voided morning urine sample were obtained between 0800 and 1000 hours from each subject after appropriate informed consent. The study was approved by the Institutional Review Board at the University of Connecticut Health Center.

**Crosslink Assays**

The crosslink assays were carried out in the General Clinical Research Center (GCRC) Core Laboratory. Urine and serum levels of crosslinked N-telopeptides of type I collagen (NTX) were measured by competitive-inhibition enzyme-linked immunosorbent assays (ELISA), kindly provided by Ostex International, Seattle, WA. Both assays utilize the same monoclonal antibody: Mab 1H11. Serum NTX levels are expressed in nanomoles bone collagen equivalents per liter (nM BCE) and urine NTX values are reported as nM BCE per millimole creatinine (nM Creat) to correct for urinary dilution. The intra-assay variabilities for duplicate measures from individual subjects for serum and urine NTX for the assays in this study were 5.2 ± 4.1% and 4.9 ± 4.0%, respectively. The inter-assay variabilities reported by the manufacturer for serum and urine NTX are 6.9% and 4.0%, respectively. Multiple samples from these subjects were not obtained. In a previous study, serum NTX was measured in a control group at 0, 12 and 24 weeks and showed a coefficient of variation of 7.9 ± 4.9 [7].

Using the Crosslaps ELISA and the Crosslaps one-step ELISA kindly provided by Osteometer Biotech, Denmark, degradation products of C-terminal telopeptides of type I collagen (CTX) were measured in urine and serum respectively. The urine Crosslaps ELISA uses polyclonal antibodies against the amino acid sequence of EKAHD-β-GGR, whereas the serum one-step ELISA utilizes two monoclonal antibodies highly specific for the same amino acid sequence and requires the two chains to be crosslinked for a specific signal.

Urine CTX levels are express in µg/mM Creat and serum CTX results are given in picomoles/liter (pM). The intra-assay variability for duplicate samples for our serum CTX data was 5.9 ± 5.0% and for the urine CTX 7.1 ± 7.2%. The inter-assay variabilities reported by the manufacturer for serum and urine CTX are 5.1% and 4.4%, respectively.

Fractional excretion (FE) was calculated as

$$FE = \frac{U_x/P_x}{U_{crea}/P_{crea}}$$

where x is either NTX or CTX.

To obtain this value, second-voided morning urine samples were compared with serum values for 5 women on HRT and 5 not on HRT selected for a range of 8- to 10-fold for values of uNTX. Because different antibodies and units of measures are used in the uCTX and sCTX assays, urine samples were serially diluted 1:5, 1:25 and 1:125 in Crosslaps standard diluent, a phosphate-buffered saline solution, and re-assayed by the serum Crosslaps one-step ELISA. The urine dilutions and corresponding serum samples were assayed in duplicate at the same time. The serum ELISA method could successfully analyze the urine samples and, following correction for dilution, the coefficient of variation between the three dilutions (or two dilutions if one serial dilution was out of range) was 5.6 ± 3.6%.

All assays were run in duplicate and any pair of samples with a coefficient of variation over 10% was reassayed, and the values in closest agreement were averaged. Five of 49 serum CTX samples (10%) were repeated as compared with 11 urine CTX specimens (22%). Likewise, 4 serum NTX samples (8%) were repeated compared with 7 urine NTX samples (14%).

**Determination of Bone Sialoprotein in Serum**

The assays to determine bone sialoprotein in serum were carried out at the University of Heidelberg. Serum immunoreactive bone sialoprotein (BSP) was quantified by a new radioimmunoassay [6,8]. In brief, 100 µl of 125I-labelled purified human BSP (1.5 ng/ml) and 100 µl of a chicken anti-human BSP antibody (1:200 dilution) were added to an equal volume of serum or standard and incubated for 24 h at 4°C. Following the addition of 100 µl of a donkey anti-chicken IgG (1:150 dilution) and a second incubation step (2 h, 4°C), antibody-bound radioactivity was centrifuged for 10 min at 2000 g and the supernatant was discarded. The radioactive pellet was washed with 250 µl of an aqueous buffer and centrifuged for 10 min, after which the supernatant was discarded and radioactivity measured. The coefficients of variation were 7.0 ± 4.9 for intra-assay variability and 9.1 ± 6.8 for inter-assay variability.