

# Clinical Significance of Urinary Hydroxyproline in Bone Metastases

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**Abstract Objective** To detect the significance of urinary hydroxyproline(uHOP) in prognosis and response to therapy of bone metastases. **Methods** uHOP, activity of serum alkaline phosphatases(AKP) and calcium concentration were tested in 35 healthy adults (group A), 20 primary (group B) tumor patients and 20 bone metastases (group C) with the photometer. **Result** In three groups, the mean values of uHOP were  $24.16 \pm 9.91$ ,  $34.97 \pm 18.54$  and  $67.58 \pm 26.36$ ; the activity of AKP were  $104.31 \pm 30.03$ ,  $114.20 \pm 54.75$  and  $154.15 \pm 89.28$ ; The calcium concentration were  $2.25 \pm 0.21$ ,  $2.33 \pm 0.42$  and  $2.61 \pm 0.45$ , respectively. There was no significant difference in uHOP between healthy adults and primary tumor patients ( $p > 0.05$ ), and uHOP in bone metastases was higher than that in healthy adult people and primary tumor patients ( $p < 0.01$ ). **Conclusions** uHOP is a specifically sensitive maker of bone collagen turnover, and a valuable indicator in determining and monitoring bone metastases, so it can be used as one of early objective maker in prognosis and response to therapy of bone metastases.

**Key Words** Bone metastases; Urinary hydroxyproline(uHOP); Bone collagen

Bone metastases are common in advanced malignant tumors, which destroy bone and break-down bone matrix, lead to changes of biochemical makers. Hydroxyproline (HOP) is a special biochemical maker of collagen turnover. Platt(1964) reported that urinary hydroxyproline (uHOP) which was a indicator in assessment of bone metastases and its therapy, clearly increased in the patients with bone metastases<sup>[1-3]</sup>. None has reported that at china. We determined urinary hydroxyproline excretion in patients with bone metastases, contrasted with healthy adults and patients with primary tumor, as to discuss its value.

## MATERIALS AND METHODS

### Clinical materials

Researched subjects were divided into three groups. Group A: Thirty-five healthy adults (20 males and 15 females) were ambulatory, from 21 to 64 (average 43.89) years old. All of them had no metabolism disorders, diabetes mellitus and liver or kidney function disorders. Group B: twenty patients with primary tumor (12 males and 8 females)

were from 35 to 81 (average 57.70) years old. Among them 4 cases were lung cancers (combined liver or brain metastases or acromegaly), 3 breast cancers, 2 prostatic cancers, 2 giant cell carcinomas of bone, 1 cardiac cancer, 1 kidney cancer (complicated lung metastases), 1 esophagus cancer, 1 nasopharynx cancer, 1 liver cancer, 1 rectum cancer, 1 uterus intermembraneous cancer, 1 malignant lymph tumor and 1 maliginated chondroma. All had evidences of neoplasm through pathology, without clinical bone metastases and symbol of bone metastases in X-ray or ECT. Group C: Twenty patients (11 males and 9 females) with bone metastases were from 21 to 78 (average, 56.55) years old. Among which 2 cases were lung cancers, 2 esophagus cancers, 4 breast cancers, 4 metastases of unknown origin, 3 prostatic cancers, 1 liver cancer, 1 small round cell cancer of forearm, 1 thyroid gland cancer, 1 multiple myeloma, 1 rectum cancer. All had been proved by pathohistology, with clinical bone metastases and symbol of X-ray or ECT. In them 12 cases were osteolytic lesions (60.0%), 4 osteoplastic lesions (20.0%), 3 mixed lesion (15.0%), 1 identified lesion. Seven were single site bone metastases (35.0%) and thirteen were multiple site bone metastases (65.0%)

### Apparatus

Model 722 photometer was produced by Shang-

hai third analysis instrument CO. Reagent of HOP (provided by Nanjing jiangcheng bioengineering institute) includes A, B, C, D. Reagent A, B and C are liquid (60 ml×1 amp), stored in 4°C. Reagent D is HOP standard (5 mg×3 amp), stored in cool. Reagent D (5 mg/amp) was dissolved with distilled water(50 ml) into the solution 100 μg/ml, and than distilled into the solution with concentration of 5 μg/ml before used. Reagent of serum alkaline phosphatases(AKP) and serum calcium were produced by DADE BE HRING RXL CO.

**Methods**

All subjects were asked to a diet in collagen-containing products for 12 hours and to collect fast urine (1 ml) on the second day. After mixing urine specimen with hydrochloric acid (6 mol/L) in the covered tube, we put the tube in the toast chest (125°C) to hydrolysis for 5 hours. Then add natrium oxyhydrogen (4 mol/L) to the tube to make it Ph6+distilled water→10 ml; take 3 ml or 4 ml off the tube +1% active carbon (mixing)→centrifugalizing (3500 r/min) for 10 minutes. Upper clear liquid (2 ml) taken off the tube was used as tested sample. Three tube were demarcated tube A, tube B and tube C, respectively added distilled water(2 ml), HOP standard (5 μg/ml, 2 ml) and the tested sample (2 ml). Reagent A was added to the three tubes and mixed for 10 minutes. Reagent B was added to the three tubes and mixed for 5 minutes. Reagent C was added to the three tubes and mixed to hydrolysis for 15 minutes in the temperature

of 60°C.

$$\text{HOP(mg/L)} = \frac{\text{TubeC}-\text{TubeA}}{\text{TubeB}-\text{TubeA}} \times \text{concentrate of TubeB} \times \text{diluting multiple}$$

Three tubes were measured with model 722 chomphoter in the water temperature of 0°C and 1cm diaphragm (550 nm). To calculate value of uHOP by the following formula.

Serum AKP and calcium were measured with automatic chemical analysis instrument. Normal referring value of uHOP, serum AKP and calcium is (13.8~42.7) mg/L, (50~136) U/L, (2.0~2.7) mmol/L respectively. All data were managed under softwork of SPSS10.0, based on t-test and analysis of variance.

**RESULTS**

The mean value of uHOP, activity of AKP and calcium concentration in three group see table 1. There was no significant difference in uHOP between Group A and Group B (*p*>0.05). uHOP in bone metastases was much higher than that in healthy adults and primary tumors (*p*<0.01).

There was no significance in serum AKP during groups. Serum calcium in bone metastases is higher than that in healthy adults (*p*<0.05), but not than that in primary tumors (*p*>0.05). There was no significance in serum calcium between primary tumors

**Table 1.** The value of uHOP, AKP and Ca<sup>2+</sup> in 3 Groups (mg/L)( $\bar{x} \pm s$ )

Groups	n	uHOP	AKP	Ca <sup>2+</sup>
A	35	24.16±9.91	104.31±30.03	2.25±0.21
B	20	34.97±18.54 <sup>▲</sup>	114.20±54.75	2.33±0.42
C	20	67.58±26.36 <sup>**</sup>	154.15±89.26	2.61±0.45 <sup>*</sup>

<sup>▲</sup> *p*>0.05 compared to group A; <sup>\*</sup> *P*<0.05 compared to group A;

<sup>\*\*</sup> *P*<0.01 compared to group A and B

and healthy adults.

**DISCUSSION**

The amino acid hydroxyproline occurs almost exclusive in collagen, where it accounts for 13% of the total amino acids. Two-thirds of body collagen is in bone, where it forms 90% of bone matrix or

30% of bone weight. Except that elastic protein contains 1% of hydroxyproline, others do not contain it<sup>[4]</sup>. HOP is a special amino acid in collagen and a special chemical maker of collagen-turnover<sup>[5]</sup>. Degradation of collagen releases HOP back into the blood circulation, about 5%~10% of which is excreted in the urine. Total excretory output of hydroxyproline by urine in a healthy adult is about 25 mg/day. uHOP is largely partly from bone, few

from skins and complements etc. Its change is consistent with bone collagen turnover rate, which reflects bone turnover<sup>[7]</sup>.

Ziff (1956) reported that uHOP increased in rheumatoid arthritis and acromagly. Then Platt reported uHOP levels highly increased in prostatic cancer, breast cancer, lung cancer, kidney cancer, Hodgkin's disease, multiple myeloma with bone metastases or latent bone metastases. Its degree of increase has correlation with bone metastases activity. While extensive bone destruction, uHOP can be up to 100mg/L or more<sup>[6]</sup>, Urine hydroxyproline levels of two patients with extensive bone metastases of this study were up to 101.7mg/L and 110.2mg/L. When bone metastases site increase and develop, urine hydroxyproline level keeps on increase. When condition is stable and improving, urine hydroxyproline level falls. uHOP of 17 patients with bone metastases in this study were more than 45mg/L(85%), which was consistent with references<sup>[7]</sup>. During 3 bone metastases with normal uHOP, one with rectum cancer developed new bone metastases in pelvis after followed-up for 1 month, uHOP up to 89.7mg/L. The other two had still normal levels of uHOP after followed-up for 1month. Some reported that in liver metastases without bone metastases, AKP was high while uHOP normal<sup>[9]</sup>. One liver cancer with scapular metastases was normal in uHOP. Maybe because liver cancer cells excessively brokedown free uHOP, which didn't reflect bone metastases itself. One thyroid gland cancer with extensive bone metastases didn't have change in uHOP in that osteoclast induced-osteosis overwhelmed. uHOP can keep stable under such certain condition, and increase with progressive stage.

Latent bone metastases occurring, uHOP levels increase<sup>[1-3]</sup>. Urine hydroxyproline of 6 primary tumors was more than 45mg/L. One prostatic cancer, followed-up 3 months later, had been discovered spinal metastases (T12, L1), whose uHOP was up to 90.8mg/L. One breast cancer infiltrating soft tissue of chest wall, whose uHOP was 56.0mg/L, had no clinical bone metastases after 12 weeks followed-up, perhaps with dormant bone metastases. Urine hydroxyproline increase suggests that bone metastases occur, then clinical symptom and symbol of X-ray or ECT appear.

Serum AKP reflects osteoblast activity. Serum calcium reflects balance of bone mineral and absorption. Both are not able to reflect bone and col-

lagen breakdown. It was reported that in patients with bone metastases, the increasing rate of serum AKP and calcium were, 40% and 30% respectively; while increasing rate of uHOP was 70%~80%<sup>[3,9]</sup>. In liver metastases, serum AKP is markedly elevated, with normal uHOP. Therefore, scholarships agreed that uHOP was an important maker in distinguishing liver metastases from bone metastases<sup>[8]</sup>.

In bone metastases the increasing rate of urine hydroxyproline was 85.0% (17/20), of AKP was 40.0%(8/20), and of Calcium was 35.0%(7/20). Average increase values of AKP, calcium and uHOP respectively, were 13.2%, 9.4% and 40.1%. This findings demonstrated uHOP was more sensitive than serum AKP and calcium as a chemical maker. They were positive correlation, so measure of them together had an important meanings clinically.

Measure of uHOP is disturbed by collagen food. Traditional determination methods need collecting urine for 24 hours, no eating collagen food for 4 days. High pressure liquid chromatography (HPLC) determine uHOP is more sensitive<sup>[10]</sup>, less interference, collecting fast early morning urine (2ml). It cut short operating time, suitable for the aged and outpatients.

Early measure of uHOP in the intimating cancers of the breast, prostate, thyroid gland, kidney etc. is advantageous to discover earlier bone metastases or latent bone metastases. Only single osteolysis lesion occur in the spinal and in the rib, clinically difficult distinguishing whether to be malignant or benign; if uHOP increases, that has differentiating significance. Measure of uHOP not only discovers early bone metastases, but also monitors bone metastases progress and assesses therapy of tumors as well<sup>[11]</sup>. If the therapy is effective or bone lesions shrink, uHOP consistently falls; conversely, uHOP elevates<sup>[5,6]</sup>. The results of this study suggested that uHOP is a valuable indicator in determining and monitoring bone metastases and can be used as one of early objective maker in prognosis and response to therapy of bone metastases.

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