

## **Biochemical Bone Markers in Prostate Cancer Patients with Advanced Bone Metastasis**

**الدالات الكيموحيوية للعظم لمرضى سرطان البروستات المنتشرالى العظم**

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### **Abstract**

Prostate cancer is now recognized as one of the most important medical problems facing the male population and it is one of the most common cancer types. For this reason led us to investigate the prostate tumor markers for detection prostate cancer. In advanced stages, the prostate cancer is metastasis and arrived to the bone this led to lose of bone mineral density (BMD) and cause osteoporosis, therefor we measured urinary deoxypyridinoline (DPD) as a marker of collagen degradation activity, serum total-alkaline phosphatase activity, and measurement of calcium and phosphate as minerals of bone. This study included 46 patients with prostate tumor their ages ranging (50-80) year and 30 healthy subjects, matched in age with patients as control. Patients were classified according to stage of tumor. 7 patients with benign prostate hyperplasia (B.P.H) , 39 patients with carcinoma, 24 patients with metastasis prostate cancer (M.P.C), and 15 patients with localized prostate cancer (L.P.C). The results showed a significant ( $p < 0.0001$ ) increase in levels of PSA, DPD, T-ALP,  $Ca^{+2}$  and  $PO_4^{-3}$  in prostate tumor compared with the control group. In addition the results revealed a high significant ( $p < 0.000$ ) increase in the level of urine DPD , serum PSA in patients with M.P.C compared with L.P.C and B.P.H patients, also there was a high significant ( $p < 0.01$ ) increase in the level of  $Ca^{+2}$  and  $PO_4^{-3}$  in patients with MPC compared with L.P.C and BPC patients. The data suggest that serial monitoring of deoxypyridinoline (DPD) could be clinically useful as marker of metastatic bone tumors and for treatment monitoring.

**Keywords:** Prostate cancer, Deoxypyridinoline, Bone markers, Prostate specific antigen, Bone metastasis.

### **الخلاصة**

يعد سرطان البروستات من احد المشاكل الطبية المهمة التي تواجه الرجال وهو اكثر انواع السرطان انتشارا. وهذا قادنا الى التحري عن علامات دالة لتشخيص سرطان البروستات. ينتشر سرطان البروستات في مراحله المتقدمة ويصل الى العظم ويؤدي الى فقدان وفرة المعادن في العظم مسببا هشاشة العظام ، لذا تم قياس مادة الديوكسي بايريدونيلين في الادراكعامة لهدم فعالية الكولاجين، وفعالية انزيم الفوسفاتيز القاعدي وايضا تم قياس الكالسيوم والفوسفات كمعادن موجودة في العظم. تضمنت هذه الدراسة 46 مريضا مصابا بورم البروستات، تراوحت اعمارهم من (50-80) سنة و 30 شخصا من الاصحاء تتطابق اعمارهم مع مجموعة المرضى كمجموعة سيطرة. قسموا المرضى وفقا الى درجة الورم. 7 مرضى كان لديهم ورم بروتات حميد، 39 مريضا كانوا مصابين بسرطان البروستات، 24 منهم كانوا بمرحلة سرطان البروستات المنتشر و 15 مريضا مصاب بسرطان البروستات الغير منتشر. اظهرت النتائج زيادة معنوية ( $p < 0.0001$ ) في مستوى (PSA, DPD, T-ALP,  $Ca^{+2}$  and  $PO_4^{-3}$  عند مرضى ورم البروستات مقارنة بمجموعة الاصحاء. بالاضافة الى ذلك اظهرت النتائج زيادة معنوية عالية ( $P < 0.000$ ) في تركيز PSA و DPD في مرضى سرطان البروستات المنتشر مقارنة بمجموعة السرطان الغير منشر ومجموعة الورم الحميد، كذلك كان هناك زيادة معنوية عالية ( $p < 0.01$ ) في مستوى كل من  $Ca^{+2}$  و  $PO_4^{-3}$  في مرضى سرطان البروستات المنتشر مقارنة مع سرطان البروستات الغير منتشر ومجموعة الورم الحميد.

## **Introduction**

Prostate cancer is more common among male`s cancers (lung, prostate, and colon cancer) in any population<sup>1</sup>. It is leading morbidity and mortality worldwide<sup>2</sup>. Prostate cancer is increasing significantly in the developed countries and most common cause of cancer death in the men<sup>3, 4</sup>. Prostate cells can begin to mutate and can metastasis into surrounding tissue, such as bone<sup>5</sup>. When the bone matrix is resorbed, the cross-link residues, pyridinoline and deoxypyridinoline (DPD) released from the collagen molecules and eventually excreted in urine<sup>6</sup>. Several reports suggest that the assay of these collagen cross-link residues may provide valuable markers of bone metastasis in patients with prostate cancer<sup>7</sup> or breast cancer<sup>8</sup>.

During the last twenty years several biochemical markers of both bone formation and resorption have been introduced. Most of these markers are derived from type I collagen. Assays for measuring urinary excretion of smaller breakdown products of type I collagen were introduced, first, enzyme-linked immunosorbent assay (ELISA) method which measured free pyridinoline (Pyr) and deoxypyridinoline (DPD) crosslinks<sup>9</sup>. Prostate specific antigen (PSA) is widely accepted as the most important marker for detecting prostate cancer and for monitoring treatment<sup>10</sup>. However, it has a low positive predictive value for bone metastases<sup>11</sup>. PSA is prostate specific but not prostate cancer specific and is measured most commonly by radioimmunoassay<sup>12</sup>. Also the alkaline phosphatase (ALP) activity was found to be elevated in bone diseases, and for decades it was the only laboratory parameter reflecting bone formation<sup>13</sup>. Elevated skeletal alkaline phosphatase levels may indicate the presence of bony metastasis in 70% of affected patients<sup>14</sup>. When the cancer cells dissolve bone, calcium is released this lead to high levels of calcium in the blood<sup>15</sup>. In addition, phosphate is a rise over twice in blood of patients with greater risk of overall prostate cancer and lethal and high grade cancers, compared to patients without cancer; this is due to tumor growth or tumorigenesis, and bone losing<sup>16</sup>.

The aim of this study was to investigate the role of DPD as a biochemical marker of bone metastasis and relationship of other associated parameters (total-alkaline phosphatase, serum phosphate, and serum calcium) in patients with prostate cancer.

## **Materials and methods**

### **Patients and control**

During the period from October 2013 to April 2014, forty six patients with prostate tumor with ages ranged between (50-80) years were taken from Al-Hussein Hospital / Kerbala and diagnosed by urologists and oncologists.

Control group consisted of 30 healthy people who were free from signs and symptoms of cancer, matched in age with patients, and had not history for prostate problem.

### **Collection of samples**

Five milliliters of venous blood were drawn from patients and control in the early morning after an overnight fast, and 5 ml of urine specimens were taken for measurement of creatinine and DPD. The samples were centrifuged at 3000 xg for 15 minute, and then serum was separated and stored at -70 °C until analysis.

### **Determination of PSA**

PSA was determined by analytical immune assay (AIA) -360 TOSOH, Japan and used prostate specific antigen (ST AIA-PACK PSALL),3-8-2 Shiba,Minato-Ku, Tokyo 105-8623, Japan.

### **Determination of T-ALP**

Bone formation marker serum total-alkaline phosphatase (T-ALP)] was detected by strips method used (Reflotron Plus system, Germany) used (Alkaline phosphatase strips, Roche diagnostics GmbH, Sandhofer, Strasse 116 D-68305, Mannheim, Germany).

### **Determination of Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup>**

Serum calcium and phosphate concentrations were detected by spectrophotometric method by using (Spinreact 7 E-17176 Sant Estevede bas (GI), Spain) kit

### **Determination of creatinine**

Urine creatinine was determined spectrophotometric method by using Randox kit from UK. Creatinine was used as a correction factor.

### **Determination of DPD**

Urine DPD was detected by Enzyme- linked Immuno Sorbent Assay (ELISA) using (Human deoxyridinoline ELISA kit, CSB-E08399h, CUSABIO, China).

### **Statistical analysis**

Statistical Package for Social Sciences (SPSS) version 19 was used for statistical analysis. Student t-test was used to analyze the results. All of the data are expressed as mean ± standard error (Sd.E). P-value ≤ 0.05 was considered significant.

### **Results and Discussion**

The results showed a highly significant (p<0.001) increase in the level of PSA, DPD, Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> and there was a highly significant (p<0.01) increase in the level of T-ALP in patients with prostate tumor compared with the healthy subject (Table 1). Evaluating the blood for prostate-specific antigen (PSA) levels and conducting a digital rectal exam (DRE) are two ways to screen for prostate cancer<sup>17</sup>.

The level of PSA as an independent variable is a better predictor of prostate cancer than suspicious findings on digital rectal exam (DRE) or trans rectal ultrasonography (TRUS)<sup>18</sup>. In previous study of Cheryl, *et al.* (2006) documented the prostate specific antigen (PSA) as the optimal tumor marker for prostate cancer, and effective for early detection, staging and monitoring patients after definitive treatment. PSA as a tumor marker would have a high sensitivity, specificity and positive predictive value for distinguishing men with BPH from men with prostate cancer<sup>19</sup>.

Excretion of deoxyridinoline expressed as ratio to creatinine excretion (Deoxyridinoline/Creatinine), creatinine is a correction factor. Increases of between two and three times the upper limits of normal have been reported in people with osteoporosis, primary hyperparathyroidism, Osteomalacia, thyrotoxicosis and several inflammatory conditions, though the biggest increases (four or more times upper limit of normal) are seen in immobilization, Paget's disease of bone and metastatic cancer. A decrease in the pretreatment value of > 30% has been considered indicative of a good response in osteoporosis<sup>20</sup>. Garnero, *et al.* (2000) suggested the levels of bone resorption markers (such as DPD levels) mainly reflect the overall skeletal change of bone resorption, which can be altered by various factors besides abnormalities of the subchondral bone turnover<sup>21</sup>.

The T-ALP and B-ALP can be used in diagnosing advanced prostate cancer. The prostate cancer related increase in serum ALP activity is considered to reflect accelerated bone turnover after bone metastatic prostate cancer<sup>22</sup>. In previous study, of Nishizawa, *et al.* (2012) is observed the bone formation markers are substances directly or indirectly produced by osteoblasts at each stage of osteoblast differentiation. They reflect various aspects of osteoblast function and bone formation, and most are measured in the blood. One of these markers is alkaline phosphatase (ALP)<sup>23</sup>.

Bone turnover is the process of resorption followed by replacement by new bone with little change in shape, and it occurs throughout a person's life. Osteoclasts break down bone (bone resorption), releasing the minerals, resulting in a transfer of calcium from bone fluid to the blood. The osteoclast attaches to the osteon (layers of compact bone tissue surrounding a central canal), and secretes collagenase and other enzymes. Calcium (comprises over 40% of bone mass), magnesium, phosphate and products of collagen are released into the extracellular fluid as the

osteoclasts tunnel into the mineralized bone<sup>24</sup>. In recent study of Heaney, *et al.* (2012) is showed the phosphate is an essential mineral that is required by every cell in the body for normal function. Approximately 85% of the body's phosphate is found in bones and teeth, and it's a major structural component of bone in the form of a calcium-phosphate salt called hydroxyapatite<sup>25</sup>.

(Table 1) The level of parameters under study in patients with prostate cancer and control group

Parameter	Patients n=46 Mean±Sd.E	Control n=30 Mean±Sd.E	P-value
PSA (ng/ml)	83.67±15.67	2.10±0.141	0.000
DPD (nmole/mmolCr.)	269.03±69.08	5.06±0.27	0.000
T-ALP (IU/L)	301.34±62.60	85.82±3.62	0.008
Ca <sup>2+</sup> (mg/dl)	9.41±0.09	8.46±0.05	0.000
PO <sub>4</sub> <sup>3-</sup> (mg/dl)	4.95±0.176	3.71±0.09	0.000

Patients were classified according to the stage of tumor. 7 patients with benign prostate hyperplasia (B.P.H), 39 patients with carcinoma, 24 patients with metastasis prostate cancer (M.P.C), and 15 patients with localized prostate cancer (L.P.C). The results revealed a high significant ( $p < 0.001$ ) increase in the level of urine DPD , serum PSA in patients with MPC compared with LPC and BPC patients, also there was a high significant ( $p < 0.01$ ) increase in the level of Ca<sup>+2</sup> and PO<sub>4</sub><sup>-3</sup> in patients with MPC compared with LPC and BPC patients (Table 2). The patients with bone metastasis had greatly significant levels of DPD in urine than had patients with L.P.C or with BPH ( $P < 0.001$ ). The serum PSA level greater than 100 ng /ml has been found to be the single most important indicator of metastatic disease, with a positive predictive value of 100%<sup>26</sup>. The study of Aldemir, *et al.* (2010) revealed the PSA value is found to be highly significant in the metastatic group, as expected<sup>27</sup>. However, the results of ALP and DPD appeared to be the most powerful predictor of bone metastasis. This result refocuses attention on serum ALP as an important marker of metastatic disease. Urinary DPD may provide a useful marker to supplement ALP and PSA in evaluating bone scan results and the response to hormonal therapy<sup>28</sup>.

Heaney, *et al.* (2012) is showed the most serious adverse effect of abnormally elevated blood levels of phosphate (hyperphosphatemia) is calcification of non-skeletal tissue. Calcium-phosphate deposition can lead to organ damage, especially kidney damage, because the kidneys are very efficient at eliminating excess phosphate from the circulation<sup>25</sup>. Therefore, other study of Funck-Brentano, *et al.* (2011) is revealed an increase in bone turnover where resorption exceeds formation is not only inversely correlated with bone mineral density (BMD), but may also alter bone architecture and porosity, increasing the risk of fracture beyond that due to reduced BMD, and can therefore be an independent predictor of fracture risk<sup>29</sup>.

(Table 2) The levels of parameters under study in three groups of prostate patients; metastasis prostate cancer (M.P.C), localized prostate cancer (L.P.C), and benign prostate hyperplasia (B.P.H)

Parameter	B.P.H n=7 Mean±Sd.E	L.P.C n=15 Mean±Sd.E	M.P.C n=24 Mean±Sd.E
PSA (ng/ml)	18.37±3.35	62.47±23.04	115.97±24.64 **
DPD (nmole/mmolCr.)	5.92±0.45	5.48±0.35	510.48±112.22 *
T-ALP (IU/L)	201.7±18.63	204.99±47.81	390.74±114.37
Ca <sup>2+</sup> (mg/dl)	8.80±0.113	9.09±0.12	9.80±0.11*
PO <sub>4</sub> <sup>3-</sup> (mg/dl)	3.78±0.28	4.06±0.10	5.85±0.18*

\* P<0.000 : M.P.C versus L.P.C and B.P.H; \*\* P<0.01 : M.P.C versus B.P.H

By using person's correlation coefficient, the results revealed positive correlation between PSA and T-ALP ( $r = 0.57$ ) in metastasis prostate cancer patients, also there was positive correlation between DPD and Ca<sup>2+</sup> ( $r = 0.52$ ) and DPD with PO<sub>4</sub><sup>3-</sup> ( $r = 0.49$ ), in addition there was high positive correlation between Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> ( $r = 0.72$ ) (Table 3). The possible bone metastasis is essential in the treatment of patients with prostate cancer. Serum ALP and PSA results with the assay of urinary DPD may provide valuable additional indicators of metastases to the bone in untreated patients, and in monitoring the efficacy of therapy<sup>30</sup>. Additional studies with more patients and information during the follow-up are needed.

(Table 3) The correlation between parameters in metastasis prostate cancer (M.P.C) patients

Parameter1	Parameter2	n	(r)	P-value
PSA	DPD	24	- 0.077	0.720
PSA	T-ALP	24	0.577**	0.003
PSA	Ca <sup>2+</sup>	24	0.133	0.536
PSA	PO <sub>4</sub> <sup>3-</sup>	24	0.187	0.381
DPD	T-ALP	24	- 0.199	0.351
DPD	Ca <sup>2+</sup>	24	0.520**	0.009
DPD	PO <sub>4</sub> <sup>3-</sup>	24	0.499*	0.013
T-ALP	Ca <sup>2+</sup>	24	- 0.031	0.886
T-ALP	PO <sub>4</sub> <sup>3-</sup>	24	- 0.600	0.781
Ca <sup>2+</sup>	PO <sub>4</sub> <sup>3-</sup>	24	0.721**	0.000

\*\*Correlation is significant at the 0.01 level

\*Correlation is significant at the 0.05 level

Urine DPD was a greater level in bone metastasis prostate cancer patients (MPC) group than those patients with LPC and BPH group (Fig 1). This result indicates that DPD is a good clinical marker for bone metastasis in patients with prostate cancer. In a previous study, Aksoy.*et al.* (2001) was found that patients with bone metastases had highly significant mean concentrations of urinary DPD than those of cancer patients without clinical evidence of bone involvement; the results demonstrate that urinary DPD may be a clinical marker of bone metastasis in prostate cancer. DPD is an analogue of pyridinoline and has a greater specificity for bone than does pyridinoline<sup>31</sup>.

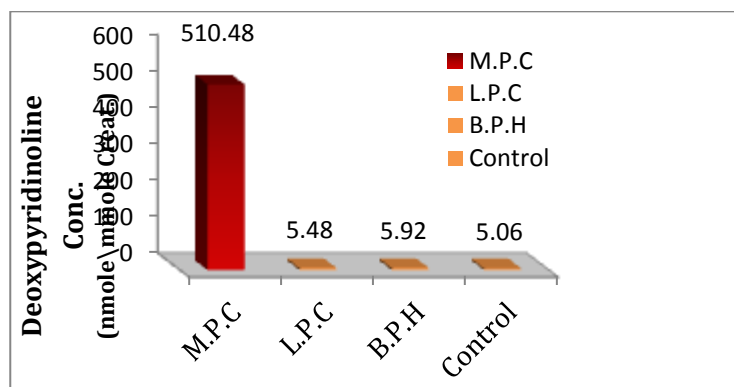


Figure 1. Deoxyypyridinoline (DPD) values in the different groups (M.P.C, L.P.C, B.P.H) of patients and control group

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