

Original Research Article

Detection of New Marker in Prostate Cancer Patients with Advanced Bone Metastasis

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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. In Iraq prostate cancer is the first most common cancer before all cancer, 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 bone, this led to lose of bone mineral density (BMD) and cause osteoporosis; therefore we measured urinary deoxy pyridinoline (DPD) as a marker of collagen degradation activity, in metastasis prostate cancer patients, in addition we measured prostate specific antigen (PSA) serum total-alkaline phosphatase activity, and calcium, and phosphate as minerals of bone. This study included 50 patients with prostate tumor and 30 healthy subjects as control. Patients were classified according to stage of tumor. Patients with benign prostate hyperplasia (B.P.H), patients with metastasis prostate cancer (M.P.C), and patients with localized prostate cancer (L.P.C). The results showed a highly significant ($P < 0.000$) increase in the level of PSA, DPD, Ca^{2+} and PO_4^{3-} and there was a highly significant ($P < 0.01$) increase in the activity of T-ALP in patients with prostate tumor compared with the healthy subject. In addition the results revealed a high significant ($P < 0.000$) increase in the levels of DPD, 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 levels of Ca^{2+} and PO_4^{3-} in patients with M.P.C compared with L.P.C and B.P.H patients. The data suggest that serial monitoring of deoxy pyridinoline (DPD) could be clinically useful as marker of metastatic bone tumors and for treatment monitoring.

Keywords: Prostate cancer, Deoxy pyridinoline, Bone markers, Prostate specific antigen, Bone metastasis.

Introduction

Prostate cancer is more common among male's cancers (lung, prostate, and colon cancer) in any population (Fletcher, 2007). It is leading morbidity and mortality worldwide (Routh, and Leibovich, 2005). Prostate cancer is increasing significantly in the developed countries and most common cause of cancer death in the men (Kolawole, 2011) (Garnick, and Fair, 1996). Prostate cells can begin to mutate and can metastasis into surrounding tissue, such as bone (Carroll, *et al*, 2005) When the bone matrix is resorbed, the cross-link residues, pyridinoline and deoxy pyridinoline (DPD) released from the collagen molecules and eventually excreted in urine (Seibel, *et al*, 1992). Several reports suggest that the assay of these collagen cross-link residues may provide valuable markers of bone metastasis in patients with prostate cancer or breast cancer (Ikeda, *et al*, 1996) (Paterson, *et al*, 1991)

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 deoxy pyridinoline (DPD) crosslinks (Seyedin, *et al*, 1993). Prostate specific antigen (PSA) is widely accepted as the most important marker for detecting prostate cancer and for monitoring treatment¹⁰ (Takayama, *et al*, 1994). However, it has a low positive predictive value for bone metastases¹¹ (Ommen, *et al*, 1994). PSA is prostate specific but not prostate cancer specific and is measured most commonly by radioimmunoassay (Scher, 2001). 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 (Tähtelä, 2004). Elevated skeletal alkaline phosphatase levels may indicate the presence of bony metastasis in 70% of affected patients (Wolff, *et al*, 1999). When the cancer cells dissolve bone, calcium is released this lead to high levels of calcium in the blood (Smith, *et al*, 2005). 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 (Wilson, *et al*, 2011)

The aim of this study was to investigate the role of DPD as a biochemical marker of bone metastasis and

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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, fifty patients with prostate tumor with ages ranged between (50-80) years were taken from Al-Hussein Hospital / Kerbala and diagnosed by urologists and oncologists in the same Hospital

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 (Refotron Plus system, Germany) used (Alkaline phosphatase strips, Roche diagnostics GmbH, Sandhofer, Strasse 116 D-68305, Mannheim, Germany).

Determination of Ca^{2+} and PO_4^{3-}

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 Immunosorbent Assay (ELISA) using (Human deoxyypyridinoline 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 \pm standard error (Sd.E). P-value \leq 0.05 was considered significant.

Results and Discussion

The results showed a highly significant ($P < 0.000$) increase in the level of PSA, DPD, Ca^{2+} and PO_4^{3-} and there was a highly significant ($P < 0.01$) increase in the activity 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 (Internet1).

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) (Catalona, *et al*, 1994). In previous study, 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.

Excretion of deoxyypyridinoline expressed as ratio to creatinine excretion (Deoxyypyridinoline/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 (Internet 2). 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 sub-chondral bone turnover.

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 (Westerhuis, *et al*, 1997). In previous study, Nishizawa, *et al*. (2012) 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).

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 (Jane, *et al*, 2014) In recent study, Heaney, *et al*. (2012) are 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.

Table 1 The levels of parameters under study in patients with prostate cancer and control group

| Parameter | Patients n=50 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 ²⁺ (mg/dl) | 9.41±0.09 | 8.46±0.05 | 0.000 |
| PO ₄ ³⁻ (mg/dl) | 4.95±0.176 | 3.71±0.09 | 0.000 |

Patients were classified into three groups according to the stage of tumor. Group 1 patients with benign prostate hyperplasia (B.P.H), group 2 patients with metastasis prostate cancer (M.P.C), and group 3 patients with localized prostate cancer (L.P.C). The results revealed a high significant (P<0.000) increase in the level of urine DPD, serum Ca²⁺ and PO₄³⁻ 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 serum PSA in patients with M.P.C compared with B.P.H patients (Table 2). 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% (Rana, *et al*, 1992). The study of (Aldemir, *et al*. 2010) revealed the PSA value is found to be highly significant in the metastatic group, as expected. The patients with bone metastasis had greatly significant levels of DPD in urine than those patients with L.P.C or with B.P.H (P<0.000). However, the results of DPD and ALP appeared to be the most powerful predictor of bone metastasis. This result refocuses attention on serum DPD 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.

Heaney, *et al*. (2012) are 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. Therefore, other study by 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 (Funck-Brentano, *et al*, 2011).

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=10 Mean±Sd.E | L.P.C n=15 Mean±Sd.E | M.P.C n=25 Mean±Sd.E |
|--|----------------------------|----------------------------|----------------------------|
| PSA (ng/ml) | 18.37±3.35 | 62.47±23.04 | 115.97±24.64 ** |
| DPD (nmole/mmol eCr.) | 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 ²⁺ (mg/dl) | 8.80±0.113 | 9.09±0.12 | 9.80±0.11* |
| PO ₄ ³⁻ (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 Ca²⁺ (r = 0.52) and DPD with PO₄³⁻ (r = 0.49), in addition there was a high positive correlation between Ca²⁺ and PO₄³⁻ (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 (Wymenga, *et al*, 2001). Additional studies with more patients and information during the follow-up are needed.

In addition, this study revealed that 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, DPD is an analogue of pyridinoline and has a greater specificity for bone than does pyridinoline (Aksoy, *et al*, 2001).

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

| Parameter1 | Parameter2 | n | (r) | P-value |
|------------------|-------------------------------|----|---------|---------|
| PSA | DPD | 25 | - 0.077 | 0.720 |
| PSA | T-ALP | 25 | 0.577** | 0.003 |
| PSA | Ca ²⁺ | 25 | 0.133 | 0.536 |
| PSA | PO ₄ ³⁻ | 25 | 0.187 | 0.381 |
| DPD | T-ALP | 25 | - 0.199 | 0.351 |
| DPD | Ca ²⁺ | 25 | 0.520** | 0.009 |
| DPD | PO ₄ ³⁻ | 25 | 0.499* | 0.013 |
| T-ALP | Ca ²⁺ | 25 | - 0.031 | 0.886 |
| T-ALP | PO ₄ ³⁻ | 25 | - 0.600 | 0.781 |
| Ca ²⁺ | PO ₄ ³⁻ | 25 | 0.721** | 0.000 |

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

In our study, patients with prostate tumor were classified into two groups, group 1 patients were treated with

chemotherapy drug, and group 2 patients were not treat by chemotherapy drug. The results, revealed a highly significant ($P<0.000$) increase in PSA and DPD levels, also there was a highly significant ($P<0.01$) increase in Ca^{2+} and PO_4^{3-} levels in patients who are treated with chemotherapy compared with those patients not treat with chemotherapy, whereas there isn't any significant difference ($P>0.05$) in the activity of T-ALP in patients who are treated with chemotherapy (Table 4).

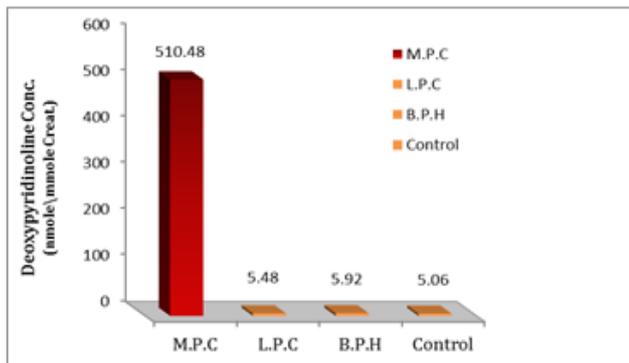


Figure 1: Deoxyypyridinoline (DPD) level in patients with prostate tumor (B.P.H, L.P.C, M.P.C) and control group.

Table 4 The levels of parameters under study in patients of prostate tumor with and without chemotherapy drug

| Parameter | Without Chemotherapy drug n=10 | With Chemotherapy drug n=40 | P-value |
|--|---|--------------------------------------|---------|
| | Mean±Sd.E | Mean±Sd.E | |
| PSA (ng/ml) | 18.37±3.35 | 95.40±17.86 | 0.000 |
| DPD (nmole/mmole e Cr.) | 5.92±0.45 | 316.25±79.24 | 0.000 |
| T-ALP (IU/L) | 201.71±18.63 | 319.30±73.56 | 0.129 |
| Ca ²⁺ (mg/dl) | 8.80±0.11 | 9.52±0.097 | 0.004 |
| PO ₄ ³⁻ (mg/dl) | 3.78±0.28 | 5.16±0.18 | 0.004 |

Although most of patients were taking chemotherapy, but it did not contribute to the return of the normal level, of PSA and DPD, that's mean chemotherapy did not show any interest in the treatment of cancer. The primary target of chemotherapy is to stop the cancer cells from dividing and the cancer cells from growing, but when we look at whether a drug is working. A rising of PSA during hormonal therapy doesn't mean the patient out of options. It means the patient need to consider the use of other systemic therapies such as chemotherapy, alternative hormonal medications, or agents that target the spread of prostate cancer (metastasis) (Prostate Cancer Foundation). In a previous study, Banfi, et al. (2001) measured bone mineral density (BMD) in 26 patients and revealed a high-dose chemotherapy caused a 10% loss in cortical bone and 20% in trabecular bone and increase in bone resorption rate (pyridinoline (PYD), deoxyypyridinoline (DPD), Ca^{2+} , PO_4^{3-} and other bone contents). This has been attributed to chemotherapy without a dose-dependent of irradiation are

shows toxicity to bone marrow stromal osteoprogenitors and can cause osteopenia by direct damage of the osteoblastic compartment, as a mechanism distinct from and summable to hypogonadism .

Conclusion

Deoxyypyridinoline (DPD) could be clinically useful as a marker of metastatic bone tumors and for treatment monitoring. Prostate cancer is associated with elevated deoxyypyridinoline (DPD), PSA, T-ALP, calcium, and phosphate values. DPD gives us conclusive evidence as to whether the cancer reached to the bone or not. Chemotherapy is not enough drugs to treat the cancer. Therefore must use dependent-dose of radiotherapy with it to save the bone.

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