Evaluation of relationship between some biochemical parameters and osteodystrophy in patients with chronic renal failure

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Osteodystrophy has become a frequent complication in chronic renal failure. The present study has been aimed to evaluate the role of tartrate-resistant acid phosphatase (TRACP) in patients with end stage renal disease (ESRD). Forty patients with (ESRD) who underwent hemodialysis and 37 age- and gender-matched healthy individuals were included in the study. Several biochemical parameters, such as the activities of total acid phosphatase (ACP), tartrate-resistant acid phosphatase (TRACP), prostatic acid phosphatase (PAP) and alkaline phosphatase (ALP), as well as testosterone, calcium and phosphorus concentrations were measured to evaluate the bone complication in ESRD patients. The results indicated presence of a significant increase ($P < 0.001$) in TRACP and ACP activities with non-significant difference in PAP activity ($P > 0.05$) in sera of 25% of the studied patients. A significant increase ($P < 0.01$) in ALP activity and phosphorus concentration ($P < 0.01$) was also detected in the patients group. Meanwhile, a significant decrease in calcium concentrations ($P < 0.001$) and testosterone level ($P < 0.01$) were observed in the sera of these patients, in comparison with that of the control group. In conclusion, the overall results of this study enable us to classify the included ESRD patients into two groups with respect to bone remodeling: the first group with high turnover rate and the second group with a change in their remodeling rate. Furthermore, the increased phosphate concentration together with the decrease in calcium and testosterone levels in the sera of ESRD patients seems to be the main factors that cause metabolic disturbances and lead to development of renal osteodystrophy.

Keywords: Acid phosphatase (ACP), Alkaline phosphatase (ALP), Calcium concentration, End stage renal disease, Osteodystrophy, Phosphorus concentration, Prostatic acid phosphatase, Tartrate-resistant acid phosphatase, Testosterone

Uremic syndrome represents the end stage of chronic renal failure (CRF). It is associated with metabolic events and complications, such as osteodystrophy$^1$. Acid phosphatases (EC 3.1.3.2; orthophosphoric-monoester phosphohydrolyase) are a family of enzymes that are widespread in nature and are found in many animal and plant species. But, the precise functional role of these molecular facilitators, despite much research is not fully understood. However, human acid phosphatases (ACPs) have shown significant impact in clinical investigation and intervention. For example, tartrate resistant acid phosphatase (TRACP) is detected in the serum in raised amounts accompanying pathological bone resorption$^2$. It is known that serum alkaline phosphatase (ALP) measurements are of particular interest in the investigation of two groups of conditions: hepatobiliary disease and bone disease associated with increased osteoplastic activity$^3$. Slightly to moderate elevation of ALP activity has been reported in bone disease with CRF$^4,5$.

Bone growth and remodeling are normal physiological events that occur at a high rate throughout childhood and adolescence and to a much lesser extent adult years$^2$. These occur as events net result of the activity of two types of bone cells, which have opposing actions: those synthesizing new bone material (known as osteoblastic)$^2$ and those responsible for resorbing or breaking down existing bone material (known as osteoclasts)$^2,6$. This synergistic, ongoing process is under the control of various hormones, such as estrogens, testosterone, parathormone and calcitonin, as well as vitamin D

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Abbreviations: ACP, acid phosphatase; ALP, alkaline phosphatase; CRF, chronic renal failure; ESRD, end stage renal disease; PAP, prostatic acid phosphatase; TRACP, tartrate resistant acid phosphatase; PTH, parathyroid hormone.
metabolites and prostaglandins. An exaggerated rate of bone resorption underlies the pathophysiology of many human diseases. For example, Paget's disease, malignant hypercalcaemia, renal osteodystrophy, hyperthyroidism, hyperparathyroidism, post-menopausal osteoporosis and breast cancer.

A pathological increase in bone resorption is observed when osteoclasts are stimulated into resorption activity at an increased rate. This upsets the normal balance between bone resorption and bone synthesis. Osteoclast resorbs bone by setting on bone surfaces and secreting acid, enzymes and free radicals into the space between the bone and the cell. The secreted enzymes include proteolytic enzymes, in addition to ACP. The increase in osteoclast activity is accompanied by an increase in the synthesis and secretion of TRACP. The active form of the enzyme is suggested to play a part in bone resorption both inside and outside the osteoclast cell.

TRACP is reported to occur at much higher concentrations in the serum of patients with skeletal disease than their corresponding control. A direct relation has been suggested between excessive osteoclast facilitated bone resorption and the secreted amount of TRACP in the circulation. Therefore, serum TRACP has been indicated as a disease associated marker for the clinical diagnosis of excessive bone resorption and for quantitatively monitoring the rate and progression of metabolic bone disorders.

Renal osteodystrophy in its broadest context encompasses all the disorders of bone and mineral metabolism caused by chronic renal failure. Bone serves the reservoir for calcium and phosphorus, the major mineral components of the body. These minerals occur in combination with organic and inorganic compounds and as free ions. Their two major roles are structural components of bone and regulatory agents in body fluids.

The present study has been aimed to evaluate the role of TRACP in the process of bone mass changes in patients with end stage renal disease (ESRD).

Materials and Methods
Patients and samples
A total of 40 patients with chronic renal failure patients at end stage renal disease (ESRD), aged 14-67 yrs attending Al-Karama and Specialist Surgeries Hospitals in Baghdad city were included in the study. They were all undergoing hemodialysis treatment for 2-24 months at the time of the study. Patients diagnosed as having hepatitis were excluded from the study. As a control, 37 age- and gender-matched healthy individuals were included in the study.

Six milliliters (mL) of venous blood were collected from the healthy donors and the patients (before hemodialysis). Blood samples were centrifuged at 2000 × g for 10 min after blood coagulation and serum was separated into two test tubes, in which one of them was mixed with 20% glacial acetic acid in proportion of 10 µL: 1 mL serum to stabilize acid phosphatase activity. All sera were stored at -20°C until being used.

Determination of protein concentration
Total serum protein concentration was determined by Lowry's method using bovine serum albumin as standard.

Determination of serum acid phosphatase activity (ACP), tartrate resistant acid phosphatase (TRACP) and prostatic acid phosphatase (PAP) activities
The activities of serum ACP, TRACP and PAP were determined using 4-nitrophenyl phosphate (4-NPP) as a substrate according to the microplate assay method of Lau et al., with some modification. The enzyme activity (in U/L) was estimated from the calibration curve and the specific activity of enzyme was expressed in U/mg of protein. The calibration curve (Fig. 1) was plotted between the absorbance of different solutions of 4-nitrophenol vs. their concentrations, which was converted into U/L (0.16, 0.6, 1, 4, 7, 10, 15 and 20) according to the following equation:

\[ \text{U/L} = \frac{\text{Conc.} \times \text{Vt}}{\text{min} \times \text{Vs}} \times \varepsilon \times \text{Vsl} \]

where Vt is the total volume of the reaction mixture and Vs volume of the sample used in the assay.

Determination of serum ALP activity
The activity of serum ALP was determined using colorimetric assay according as described previously. The activity was expressed in U/L according to the following equation:

\[ \text{U/L} = \frac{\Delta A}{\text{min} \times \text{Vt} \times 10^6 \text{µmol/mol} \times V_s \times 1} \]

Where Vt is the total volume of the reaction mixture; ε is the molar absorption coefficient; Vs represents volume of the sample used in the assay and l is the
length of the optical path in cm. The specific activity of enzyme was expressed in U per mg of protein.

**Determination of serum testosterone concentration**

Testosterone concentrations in the samples were determined using VIDAS testosterone kit.

**Determination of calcium and phosphorus**

Determination of calcium was done based on colorimetric determination of total calcium without deproteinization in human urine and serum. Inorganic phosphorus was determined based on colorimetric determination, without deproteinization of serum phosphorus using a single reagent which formed a phosphomolybdate complex in the presence of a reducing agent (ferrous sulphate).

**Statistical analysis**

The results were expressed as the mean ± standard deviation. Statistical and correlation analysis were undertaken using student t-test and Pearson's correlation coefficients. A \( P < 0.05 \) was accepted as statistically significant, highly significant when \( P < 0.001 \), not significant when \( P > 0.05 \). SPSS (for windows, version 11.0) was used for the statistical analysis.

**Results**

The mean values of both activities and specific activities of ACP, TRACP, PAP and ALP in the sera of patients with ESRD were compared with that of the control group. The results showed presence of a non-significant increase (\( P > 0.05 \)) of ACP, TRACP and PAP and a highly significant increase (\( P < 0.001 \)) in both activity and specific activity of ALP (Table 1).

The results of enzymes activities in the sera of studied patients (Figs 1 & 2) showed that 25% of the patients (Group A) had a significant increase (\( P < 0.001 \)) in both activities and specific activities of both ACP and TRACP, in comparison with that of the control group. Whereas non-significant differences were observed in these values of PAP (\( P > 0.05 \)). This indicated that the observed increase in total ACP activity in the sera of these patients was due to the increase in TRACP activity, rather than to PAP activity.

On the other hand, 5% of patients with ESRD were found to have a significant increase (\( P < 0.001 \)) (Group B) in both activities and specific activities of ACP, TRACP and PAP, in comparison with that of their corresponding control values. Meanwhile among the studied patients, 35% (Group C) were found to have a significant decrease (\( P < 0.001 \)) in both activities and specific activities of all of the three enzymes (ACP, TRACP and PAP) in comparison with that of control group. While the results of remaining 35% of the patients (Group D) showed non-significant differences in both the activities and

<table>
<thead>
<tr>
<th>Group</th>
<th>Enzyme</th>
<th>Activity U/L</th>
<th>Specific activity U/mg</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range × 10^3</th>
<th>Mean × 10^3</th>
<th>± SD × 10^3</th>
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<tr>
<td>Control</td>
<td>ACP</td>
<td>5.84-1.5</td>
<td>8.74</td>
<td>1.42</td>
<td>(0.07-0.16)</td>
<td>0.12</td>
<td>0.02</td>
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<td></td>
<td>TRACP</td>
<td>4.89-0.8</td>
<td>7.67</td>
<td>1.44</td>
<td>(0.07-0.16)</td>
<td>0.10</td>
<td>0.02</td>
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<tr>
<td></td>
<td>PAP</td>
<td>0.04-2.9</td>
<td>1.01</td>
<td>0.83</td>
<td>(0.00-0.04)</td>
<td>0.014</td>
<td>0.01</td>
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<tr>
<td></td>
<td>ALP</td>
<td>25.5-97.5</td>
<td>62.4</td>
<td>15.68</td>
<td>(0.35-1.36)</td>
<td>0.855</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>ACP</td>
<td>2.4-18.0</td>
<td>9.21</td>
<td>4.56</td>
<td>(0.03-0.31)</td>
<td>0.13</td>
<td>0.067</td>
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<tr>
<td></td>
<td>TRACP</td>
<td>2.3-16.0</td>
<td>8.0</td>
<td>3.79</td>
<td>(0.03-0.28)</td>
<td>0.11</td>
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<td>PAP</td>
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<td>1.16</td>
<td>1.32</td>
<td>(0.0-0.1)</td>
<td>0.01</td>
<td>0.019</td>
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<td></td>
<td>ALP</td>
<td>27.8-393</td>
<td>112.8</td>
<td>80.36</td>
<td>(0.45-3.68)</td>
<td>1.648</td>
<td>1.23</td>
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</tr>
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</table>

* n = samples number

![Fig. 1](image-url)
the specific activities of ACP, TRACP and PAP in comparison with that of control group.

When the correlation between TRACP activity (bone resorption marker) and ALP activity (bone formation marker) was studied in sera of the patients with ESRD, a non-significant correlation ($r = 0.23, P > 0.05$) was found (Fig. 3).

Table 2 shows the mean values of protein concentration in the sera of control and patients with ESRD. The results clearly revealed non-significant differences ($P > 0.05$) in protein concentration between the control and their corresponding patients groups.

The mean values of testosterone concentration in sera of both genders (male and female) of control and
patients with ESRD are shown in Table 3. The results showed a significant decrease in this hormone concentration in sera of both male \((P < 0.05)\) and female \((P < 0.01)\) patients with ESRD in comparison with that of control group.

Furthermore, in order to evaluate the metastatic calcification state in sera of the patients with ESRD, serum calcium and phosphorus concentrations were measured using colorimetric methods and the product of serum calcium concentration multiplying by serum phosphorus concentration \([\text{Ca} \times \text{Pi}]\) was estimated in the studied groups, as described previously\(^{32}\) and the result are shown in Table 4. The mean values reflected a highly significant decrease in calcium concentration \((P < 0.001)\) and a highly significant increase in phosphorus concentration \((P < 0.001)\) and \([\text{Ca} \times \text{Pi}]\) value in the sera of the patients with ESRD, in comparison with that of control group. Among the studied patients group, 50% of patients had a value of \([\text{Ca} \times \text{Pi}]\) greater than 72 mg\(^2/dL^2\).

**Discussion**

The non-significant elevation in the TRACP activity (Table 1) in sera of patients was in agreement with the previous study, wherein using two immunoassays methods it is reported that TRACP activity is not significantly increased in patients with ESRD requiring hemodialysis\(^{12}\). Similarly, a non-significant elevation in the mean value of PAP activity in sera of patients with ESRD observed in the present study in comparison with the control group was in accordance with the findings of earlier study showing that the concentration of PAP is not significantly different between control and hemodialysis patients\(^{33}\).

In the present study, 25% of the studied patients (Group A) had a significant increase in TRACP activity (Fig. 1), indicating the presence of bone resorption among this group, since TRACP activity is reported to increase with the increase of bone resorption rate as a result of orthoclastic secretion of TRACP during bone resorption\(^{2,13,27}\).

Earlier, a significant increase in TRACP activity has also been reported in the sera of 26% of the studied patients with ESRD\(^{28}\), while in another study a significant increase in TRACP activity is reported in only 12.5% of studied patients with chronic renal failure\(^{34}\).

In our studied patients, one male and one female, who constituted 5% of the present studied patients (Group B), a significant increase was observed in both activities and specific activities of TRACP and PAP. This indicated that in addition to bone resorption, the increase in the PAP activity in these patients might be due to osteomyelitis, metastatic, non-prostatic carcinoma, myeloproliferative syndrome, prostatic carcinoma and tuberculosis with a concurrent lupus-like syndrome\(^{35}\).

In the present study, 35% of the studied patients (Group C) showed a decrease in both activities and specific activities of total ACP, TRACP and PAP. Earlier, it has been suggested that decreased activity of ACP in uremic patients is as a result of progressive
impairment of phagocyte ability of neutrophils. It is also reported that measurement of total ALP may provide an index of osteoblastic bone formation, because increased total ALP in uremic patients is often the result of increase in the bone-specific isoform activity.

Several studies have been carried on ALP activity as a bone formation marker in patients with chronic renal failure. A significant increase in ALP activity is reported in sera of patients with variable degrees of predialysis CRF and in sera of patients with chronic kidney disease under conservative management, while in another study, ALP activity is found to be not significantly different between control and patients with CRF. In the present study, a significant increase of ALP activity was observed in sera of patients with ESRD undergoing hemodialysis treatment.

Although TRACP activity has been found to correlate with bone resorption rate, but only few studies have been done on uremic patients. A correlation has been found between serum TRACP activity and bone ALP level using electrophoretic methods. On the other hand, using an enzymatic method, another group has found a correlation between serum TRACP activity and the number of osteoclasts and the percentage of eroded bone surface. Similarly, serum TRACP appears to correlate with total ALP and parathyroid hormone. In the present study, a non-significant correlation was observed between TRACP activity and ALP activity in sera of patients with ESRD (Fig. 3).

Bone remodeling, also called bone turnover, is an essential part of bone health. It begins with resorption of old bone by osteoclasts, followed by the formation of a new bone by osteoblasts. Remodeling is often referred to as being coupled (formation is linked to resorption). This process is under the control of various hormones, such as testosterone. A significant decrease in testosterone concentration in sera of patients with ESRD in comparison with that of the control group in the present study was due to a decrease in its production as a result to impaired hypothalamic pituitary function and increased testosterone metabolism. Such decrease in testosterone concentration was found to be associated with an increase in ALP activity in sera of patients with ESRD.

Using type I procollagen amino terminal peptide (PINP), total osteocalcin (TOC) and carboxylated osteocalcin as a bone formation marker and tartrate-resistant acid phosphatase 5b (TRACP 5b) as a bone resorption marker. Testosterone has been reported to contribute to bone turnover rate, since bone formation increases with increase in serum testosterone levels. Another study using TRACP 5b activity as a bone resorption marker and serum osteocalcin concentration as a bone formation marker has suggested that serum sex hormone concentrations may induce changes in bone metabolism in middle-aged men, while serum estradiol and testosterone concentrations are inversely correlated with both bone turnover markers. On the other hand, using human peripheral blood CD14(+) osteoclast precursors, it is shown that testosterone directly inhibits osteoclast formation and bone resorption at physiological concentrations.

Renal osteodystrophy in its broadest context encompasses all the disorders of bone and mineral metabolism caused by chronic renal failure. Prominent among these are disturbances in calcium and phosphorus metabolism that ultimately lead to alterations in parathyroid gland function and to various types of renal diseases. Several reports have reemphasized the critical role of this biochemical disturbance in the development of soft tissue and vascular calcification.

In the present study, hypocalcemia was observed in sera of patients with ESRD (Table 4). This may be explained as follows: the increase in serum phosphorus concentration leads to the formation of calcium-phosphorus complexes and impairs calcitriol synthesis, resulting in decreased intestinal absorption of calcium and skeletal resistance to the actions of parathyroid hormone and calcitriol. Other factors related to the uremic environment might lead to decreased ionized calcium, as advanced uremia is associated with the retention of various anions, which causes an increased complex formation with calcium and, therefore, decreases ionized fraction of total calcium. It is worth mentioning here that hemoglobin concentration that occurs after the hemodialysis procedure is also suggested to result into an increase in the calcium protein-bound fraction.

The hypocalcemia stimulates parathyroid hormone (PTH), releasing directly by inactivating the calcium-sensing receptor, leading to increase in plasma PTH concentration within minutes, as the concentration of blood ionized calcium declines. Thus, PTH mobilizes calcium from bone, decreases renal tubular
excretion and by increasing the synthesis of calcitriol, increase intestinal calcium absorption. Phosphate retention and hyperphosphatemia are extremely common in patients with ESRD. These result from the decreased ability of the failing kidney to excrete phosphorus in the urine as a consequence of the decrease in the glomerular filtration rate, which would lead to the retention of phosphorus in blood, causing serum phosphorus to rise. This transient episode of hyperphosphatemia gives rise to a decrease in the levels of ionized calcium in the blood, triggering an increase in the secretion of PTH. On the other hand, hyperphosphatemia is reported to promote the development of parathyroid gland hyperplasia and high ambient phosphorus concentration facilitates PTH synthesis by stabilizing PTH mRNA and facilitating message translation. Such increase in PTH secretion will lead to increase osteoclastic bone resorption, releasing calcium and phosphate from bone into the extracellular fluid, increasing renal tubular reabsorption of calcium as well as inhibiting phosphate reabsorption and stimulating synthesis of 1,25-dihydroxyvitamin D, which further increases absorption of calcium and phosphate from the gut.

Apart from its role as a contributor to hyperparathyroidism, hyperphosphatemia represents an independent risk factor for death in patients treated with hemodialysis, even after adjusting for other co-morbid conditions. The mechanism responsible for death is unknown, but may be related to the abnormally high (Ca × P) product (greater than 72 mg²/dL²). In addition, a predisposition to metastatic calcification has been observed in patients with chronic renal failure, when the product of (Ca × P) is elevated. In the present study, 50% of patients with ESRD had a value of (Ca × P) greater than 72 mg²/dL² (Table 4), suggesting that these patients had a metastatic calcification and a risk factor of death.

The observed increase in [P] together with the decrease in [Ca] and testosterone level in the sera of the present study ESRD patients seemed to be the main factors that caused metabolic disturbances and led to the development of renal osteodystrophy. It is previously reported that in a typical remodeling cycle, bone resorption takes 7-10 days, whereas its formation requires 2-3 months and when there is a change in the rate of remodeling, bone resorption markers fall faster than its formation markers, since a shorter time is needed for the resorption than for the formation (2-12 weeks for resorption markers, 12-24 weeks for formation markers). Thus, based on this information, as well as on the overall results of current study, the renal osteodystrophy among our studied patients can be classified into two groups with respect to bone remodeling: The first group included patients with high bone turnover rate, since they showed an increase in both bone resorption rate (TRACP activity), and bone formation rate (ALP activity) and the second group included patients with mixed bone turnover, since they showed normal or decreased bone resorption rate (TRACP activity) and an increase in bone formation rate (ALP activity).


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