Is Imatinib Protective Against to Osteoporosis? - A Comparative Study in Terms of Bone Densitometry on CML Patients and Healthy Volunteers
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ABSTRACTS

Objective: Chronic Myeloid Leukaemia (CML) is a clonal disorder in which cells of the myeloid serie undergo excessive proliferation. The characteristic genetic abnormality of the disease, the Philadelphia (Ph) chromosome, results from a reciprocal translocation between the long arms of chromosomes 9 and 22. The molecular consequence of this translocation is the production of fusion protein bcr-abl. Imatinib is an effective agent in treating CML by targeting the constitutively active tyrosine-kinase domain of bcr-abl. The aim of our study is to determine possible effect of use of imatinib on osteoporosis via comparing dual-energy X-ray absorptiometry (DXA) results of CML group and control group.

Subjects and Methods: This study was performed on 20 patients with CML and 20 people in control group who visited Turgut Ozal Medical Center. Both blood and urine analyses in addition to dual-energy X-ray absorptiometry (DXA) measurements were performed at Turgut Ozal Medical Center, Faculty of Medicine, Inonu University. Mann–Whitney U test were used to compare CML patients and controls. A p < 0.05 was considered statistically significant.

Results: The mean-serum level of phosphate in CML group was found significantly lower than the control group (p < 0.05). The mean-urine calcium level of the patients with hypophosphatemia was found significantly higher (p < 0.05) than the patients with normophosphatemia.

Conclusion: The significant correlation between hypophosphatemia and the use of imatinib which has been determined in our study was harmonious with outcomes of previous studies. However, we did not observe a statistically significant correlation between the use of imatinib and osteoporosis in this study.

Keywords: Hyperparathyroidism, hypophosphatemia, imatinib, osteoporosis

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INTRODUCTION

Chronic Myeloid Leukaemia (CML) is a clonal hematopoietic stem-cell disorder that characterized with reciprocal translocation between nine and 22 chromosomes. Studies conducted in 1980s have revealed that the "bcr-abl" fusion gene formed by the fusion of "abl" proto-oncogene on chromosome nine and "bcr" gene on chromosome 22 based on translocation, encodes a protein stimulating stem-cell growth and having tyrosine kinase activity inhibiting apoptosis in patients with CML. Based on this information providing an insight to the molecular basis of the disease, drugs aiming at tyrosine kinase inhibition have been developed since the midst of 1990s. Currently imatinib, a target-specific tyrosine kinase inhibitor, is the first choice for treatment of chronic phase CML.

Imatinib was designed as an inhibitor targeting the bcr-abl tyrosine kinase (1). The inhibition of abl-specific tyrosine kinase by imatinib prevents signal conduction in cells carrying bcr-abl gene and induces apoptosis, selectively suppressing proliferation of CML cells. In addition, imatinib inhibits several other tyrosine kinases, eg, PDGFR α/β, c-Kit and c-Fms (2). Since these tyrosine kinases play an important role in osteoblast and osteoclast functions, anabolic or catabolic changes may occur in bone metabolism in patients on long-term imatinib therapy.

Changes in bone metabolism were first reported in patients receiving long-term imatinib therapy who were noted to have laboratory evidence of hypophosphatemia with concomitant phosphaturia, and decreased biochemical markers of both bone formation and resorption (3). It is conceivable that reduced levels of calcium and phosphate seen in imatinib-treated patients could result from one or a combination of the following mechanisms: decreased intestinal absorption of phosphate and calcium, increased urinary loss of phosphate and calcium, decreased dissolution of calcium and phosphate from bone, or sequestration of
phosphate and calcium from extracellular fluid into the bone (4). Francois et al described a patient with haematuria who commenced treatment with imatinib and subsequently developed proximal tubular renal dysfunction (partial Fanconi syndrome) and hypophosphatemia (5).

Jönsson et al have shown that CML patients treated with imatinib have signs of suppressed bone turnover, ie, low levels of serum calcium and bone markers, and an increase in PTH level while increased BMD (6). Even, a retrospective analysis of iliac crest trephine biopsies suggested increased trabecular bone volume in CML patients taking imatinib (7). Thereafter, with prospective study of imatinib a transient increase in bone formation markers and a decrease in bone resorption markers were noted (8). Recent data suggest that imatinib targets cells of the skeleton, stimulating the retention and sequestration of calcium and phosphate to bone, leading to decreased circulating levels of these minerals.

In vitro, imatinib has complex effects on skeletal tissue. It may be due to that imatinib has direct effects on osteoclasts and osteoblasts through inhibition of stem-cell factor receptor (c-Kit), macrophage colony-stimulating factor receptor (c-Fms) and the platelet-derived growth factor receptors (PDGFR α and β). Such complex skeletal actions make it difficult to predict the effect of imatinib on the skeleton in vivo. With the development of subsequent tyrosine kinase inhibitors such as dasatinib and nilotinib, hypophosphatemia has been considered as a drug class effect on phosphate metabolism due to off target effects on osteoblasts and osteoclasts.

SUBJECTS AND METHODS

This study was performed as a case-control study on 20 patients with CML and 20 people in control group who visited Turgut Ozal Medical Center. The procedures approved by the regional ethics committee before the beginning of the study. Informed consent was obtained in accordance with the Declaration of Helsinki.
Both CML group and the control group were evaluated for osteoporosis. The patients who had a history of osteoporosis and/or urolithiasis were removed from the study. Furthermore, the people with other conditions that may lead to disorder of bone and mineral metabolism (eg hyperparathyroidism, hyperthyroidism, Cushing’s syndrome, chronic renal failure, users of some drugs such as containing calcium or phosphate, diuretics, phosphate-binding agents, bisphosphonates, calcitonin, teriparatide, vitamin D, glucocorticoid, sex hormones and thyroid hormones) were excluded too.

All of the blood and urine analyses were performed at the Laboratory of Hematology and Laboratory of Biochemistry, Turgut Ozal Medical Center, Faculty of Medicine, Inonu University. Complete blood counts were determined in Beckman Coulter LH–780 analysers by spectrophotometric method. The serum levels of blood urea nitrogen (BUN), creatinine, calcium, phosphate, magnesium and the urine levels of calcium and phosphate were measured with photometry using the Aeroset–500 instrument. The serum levels of hormones (free T3, free T4, TSH, cortisol and intact parathyroid hormon) were determined using a chemiluminescent immunometric assay with an Immulite–2000 Analyser. Dual-energy X-ray absorptiometry (DXA) results of the cases were obtained from measurements using Hologic QDR 4500 W (S/N 49584) device at Department of Nuclear Medicine, Turgut Ozal Medical Center, Faculty of Medicine, Inonu University. According to the results of lumbar spine (L1-L4) and proximal femur DXA, T-score less than -2.5 as osteoporosis, between -2.5 and -1 as osteopenia, -1 and above as normal were considered.

The Cockcroft-Gault formula was used while calculating glomerular filtration rates (GFR) of the cases.

Mann-Whitney U test were performed to compare CML patients and controls. A $p < 0.05$ was considered statistically significant.
RESULTS

There were 20 patients (9 women, 11 men; median age, 46.95 years; range, 25–70 years) in CML group. All of the patients had chronic phase of CML and they have been receiving imatinib mesylate therapy (400 mg per day) for nine to 84 (median, 32.5) months. The control group also consisted of 20 healthy volunteers (9 women, 11 men; median age, 46.75 years; range, 30–75 years) who do not use any medicines Table 1.

Table. 1: The mean age of the cases and the distribution of the cases by gender

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender</th>
<th>Case number</th>
<th>Median age</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>Male</td>
<td>11</td>
<td>50.64</td>
<td>11.99</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>42.44</td>
<td>16.02</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>Control</td>
<td>Male</td>
<td>11</td>
<td>50.18</td>
<td>12.55</td>
<td>36</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>42.56</td>
<td>6.98</td>
<td>30</td>
<td>49</td>
</tr>
</tbody>
</table>

CML: chronic myeloid leukaemia; Min: minimum; Max: maximum

The groups were compared according to haematological parameters including the value of haemoglobin and the numbers of leukocytes and platelets. The number of leukocytes (WBC) in CML group was found significantly lower than the control group ($p < 0.05$). The averages of leukocyte numbers were detected $6.10 \pm 2.21$ (x 1000/mm$^3$) and $7.53 \pm 1.65$ (x 1000/mm$^3$) in CML group and in the control group, respectively. On the other hand, there were no significant differences between the groups in terms of haemoglobin values and platelet numbers.

It may seem an expected result due to imatinib effect that the average of WBC numbers in the patients with CML was lower than the cases in the control group, because none of the patients with CML had splenomegaly, myelofibrosis and accelerated or blastic transformation.
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The mean-serum level of phosphate in CML group was found significantly lower than the control group ($p < 0.05$). Whereas, there were no significant differences between the groups in terms of the other parameters (serum levels of parathyroid hormon and calcium, calcium and phosphate in spot urine, T-scores of lumbar spine and proximal femur, GFR). The findings are summarized in Table 2 and 3.

Table 2: The parameters compared between the groups

<table>
<thead>
<tr>
<th></th>
<th>CML</th>
<th>Control</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case number (N)</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>9/11</td>
<td>9/11</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>46.95 ± 14.19</td>
<td>46.75 ± 10.89</td>
<td>N.S.</td>
</tr>
<tr>
<td>WBC (x 1000/mm³)</td>
<td>6.10 ± 2.21</td>
<td>7.53 ± 1.65</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.21 ± 0.85</td>
<td>13.9 ± 1.47</td>
<td>N.S.</td>
</tr>
<tr>
<td>Plt (x 1000/mm³)</td>
<td>246.25 ± 117.15</td>
<td>275.55 ± 64.41</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum Ca (mg/dL)</td>
<td>9.46 ± 0.52</td>
<td>9.55 ± 0.39</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum P (mg/dL)</td>
<td>2.84 ± 0.65</td>
<td>3.48 ± 0.76</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Parathyroid hormon (mg/dL)</td>
<td>66.91 ± 39.83</td>
<td>53.88 ± 18.31</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ca in spot urine (mg/dL)</td>
<td>11.38 ± 9.51</td>
<td>7.13 ± 5.91</td>
<td>N.S.</td>
</tr>
<tr>
<td>P in spot urine (mg/dL)</td>
<td>50.35 ± 41.19</td>
<td>37.92 ± 25.93</td>
<td>N.S.</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>103.95 ± 11.06</td>
<td>113.30 ± 26.47</td>
<td>N.S.</td>
</tr>
<tr>
<td>T-score of lumbar spine</td>
<td>-1.09 ± 1.26</td>
<td>-0.49 ± 1.02</td>
<td>N.S.</td>
</tr>
<tr>
<td>T-score of proximal femur</td>
<td>-0.13 ± 1.48</td>
<td>0.06 ± 0.77</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 3. The comparison of T-scores of lumbar spine and proximal femur between the groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>n</th>
<th>Mean ± Standard deviation</th>
<th>Min–Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>T-score of lumbar spine</td>
<td>20</td>
<td>-1.09 ± 1.26</td>
<td>-3.5 – 1.5</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>T-score of proximal femur</td>
<td>20</td>
<td>-0.13 ± 1.48</td>
<td>-2.3 – 4.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Control</td>
<td>T-score of lumbar spine</td>
<td>20</td>
<td>-0.49 ± 1.02</td>
<td>-2.4 – 1.6</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>T-score of proximal femur</td>
<td>20</td>
<td>0.06 ± 0.77</td>
<td>-1.1 – 1.7</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N: case number   Min: minimum   Max: maximum   CML: chronic myeloid leukaemia   N.S.: not significance

The patients in CML group were divided to two subgroups according to mean-serum levels of phosphate. Six patients with hypophosphatemia (≤ 2.5 mg/dL) and 14 patients with normal serum level of phosphate (2.5 – 4.5 mg/dL) were determined. The patients with hypophosphatemia were compared with the normophosphatemic patients in terms of age, gender, duration of imatinib treatment, serum levels of parathyroid hormone and calcium, calcium and phosphate in spot urine, T-scores of lumbar spine and proximal femur and GFR.

There were no significant differences between the subgroups in terms of all these parameters except calcium level in spot urine. The mean-urine calcium level of the patients with hypophosphatemia was found significantly higher ($p < 0.05$) than the patients with normophosphatemia.

Table 4: The comparison of calcium and phosphate levels in spot urine between the subgroups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Case number (N)</th>
<th>Parameters</th>
<th>Mean ± Standard Deviation (mg/dL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>P ≤ 2.5 mg/dL</td>
<td>6</td>
<td>Calcium in spot urine</td>
<td>19.17 ± 10.55</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Phosphate in spot urine</td>
<td>37.57 ± 32.93</td>
<td>N.S.</td>
</tr>
<tr>
<td>P: Normal</td>
<td>14</td>
<td>Calcium in spot urine</td>
<td>8.05 ± 7.03</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(2.5 – 4.5 mg/dL)</td>
<td>14</td>
<td>Phosphate in spot urine</td>
<td>55.83 ± 44.21</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S.: not significance
DISCUSSION

Previous studies have shown that occur a series of changes associated with bone and mineral metabolism as well as hypophosphatemia in some of imatinib-treated patients.

Berman and colleagues first reported in 2006 the occurrence of imatinib-treated patients with hypophosphatemia compared to healthy controls (3). In this study, hypophosphatemia (serum level of phosphate < 2.5 mg/dL) was detected in 25 of 49 patients receiving imatinib. Furthermore, fractional phosphate excretion in urine of imatinib-treated patients was significantly high compared to control group. In the same study, mean serum level of parathyroid hormone in patients with hypophosphatemia was higher than normophosphatemic patients.

In our study, hypophosphatemia (serum level of phosphate < 2.5 mg/dL) was detected in six of 20 patients with CML. There was a significant difference when compared with control group. However, there was no significant difference in terms of serum level of parathyroid hormone and phosphate level in spot urine between hypophosphatemic patients and normophosphatemic patients. Whereas, mean calcium level in spot urine of hypophosphatemic patients was significantly higher than normophosphatemic patients (19.16 and 8.05 mg/dL, respectively). Glomerular filtration rates and serum parathyroid hormone levels of the cases in both CML group and control group were normal, so urinary calcium loss may be related renal tubular dysfunction. Likewise, François et al (5) described a case with hypophosphatemia due to partial Fanconi syndrome after imatinib treatment.

Berman et al also reported increased phosphate excretion in a subgroup of patients with normal serum phosphate levels and no increase in parathyroid hormone levels, which could suggest proximal tubular dysfunction. Although we detected hypophosphatemia in CML group compared to healthy controls, there was no significant difference in terms of both calcium and phosphate level in spot urine between these groups. But, we also found increased
calcium excretion in a subgroup of CML patients that they had low serum phosphate levels and normal parathyroid hormone levels.

Additionally, we compared both groups (CML and control) and subgroups (CML patients with low serum phosphate level and normal serum phosphate level) in terms of osteoporosis by T-scores of lumbar spine and proximal femur. As a result, neither between the groups nor between the subgroups was found significant differences.

The formation of osteoclasts from monocyte-macrophage precursor cells in vitro is largely dependent on 2 factors: receptor activator of nuclear factor-KB ligand (RANKL), which drives osteoclast fusion, activity, and survival, (9) and macrophage colony-stimulating factor (M-CSF), which is essential for the proliferation and survival of the osteoclast precursor cells and for survival of the mature osteoclast (10). The activities of osteoblasts and osteoclasts are tightly coupled because of the interaction of the receptor for the activation of nuclear factor-κB (RANK) ligand on osteoblasts and its receptor, RANK, on osteoclast precursors (11). Furthermore, M-CSF which provides differentiation of macrophages into mature osteoclasts is secreted by stromal cells and osteoblasts. In addition to supporting osteoclast differentiation and proliferation, studies by Arai et al have shown that M-CSF via its receptor, c-Fms, regulates the expression of RANK by osteoclast precursors (12). In vitro, imatinib inhibits both osteoclast differentiation and function. The inhibition of osteoclast differentiation is likely to result from an inhibition of c-fms signal transduction, whereas inhibition of osteoclast function occurred indirectly via a decrease in RANK expression.

Platelet-derived growth factor is a potent mitogen for osteoblasts and is locally synthesised by skeletal cells. While it increases the proliferation of cells of the osteoblast lineage, PDGF inhibits both bone matrix formation and the differentiation of the osteoblast precursors (13). The PDGF isoforms PDGF-AA and PDGF-BB have been shown to stimulate replication (14) and migration (15) of rat osteoblasts in vitro. PDGF-BB has been shown to
increase the number of rat osteoblasts *in vivo* (16) and the survival of osteoblasts may be stimulated by PDGF-BB. *In vivo*, localized administration of PDGF accelerates fracture healing (17) and systemic administration increases bone density in ovariectomized rats.

Otherwise, in cultures of iliac crest-derived primary human bone cells, PDGF-BB treatment significantly increased osteoclastic bone resorption *in vitro* (18).

Imatinib inhibits tyrosine kinases that important in osteoblast (PDGF-R) and osteoclast (c-Fms) function, suggesting that long-term exposure to imatinib may alter bone homeostasis. In a study by Jönsson *et al* (6), regional bone mineral density (BMD) was examined in CML patients treated with imatinib for 24 to 73 months. In this study, imatinib-treated patients had significantly higher areal lumbar spine and total hip BMD compared with normal aged-matched controls, as determined by DXA. But these results were incompatible with the changes O'Sullivan *et al* (19) observed by DXA. In the study conducted by Fitter *et al* (7), trabecular bone volumes from bone biopsy samples taken during the time of diagnosis and after the imatinib therapy were measured, and the results were compared with those of CML patients receiving recombinant interferon α (rIFN-α). As a result, it was found that eight of 17 patients in the imatinib group had a significant increase in trabecular bone volume than baseline and when compared to patients who received rIFN-α therapy, the difference was found statistically significant.

It was shown that the alteration of bone and mineral metabolism (*eg* hypophosphatemia and hyperparathyroidism) occur after imatinib treatment. One possible explanation for the abnormalities in bone and mineral metabolism seen in these patients is that imatinib, by inhibiting the PDGF receptor, affects the formation and resorption of bone. The *in vivo* consequences of the dual effects of imatinib on osteoblasts, inhibition of proliferation and stimulation of differentiation, are difficult to predict, but in the initial stages of imatinib therapy, the dominant effect is to increase bone formation (20). Studies of humans
treated with imatinib suggest a biphasic effect of the drug on bone turnover, such that in the early phase of treatment bone formation is increased but with continuation of therapy both components of bone remodeling are decreased (8).

Some authors suggest that physiological bone loss might be decelerated or even inhibited in CML patients treated with imatinib, possibly through drug-mediated inhibition of osteoclast formation and activity (21). Even though imatinib has an initial positive net effect on bone mass it stabilizes over time (22). The authors speculate that there is a concomitant imatinib-mediated inhibition of bone formation, due to a decrease in mesenchymal stem-cell proliferation and terminal differentiation of osteoblasts (23, 24), which would explain why bone mineral density remains stable and does not increase.

Despite the fact that the results of Fitter et al suggesting a significant increase in trabecular bone volume in patients using imatinib, we found no significant difference between the imatinib group and control subjects with respect to lumbar vertebra T-score and proximal femur T-score. Albeit imatinib inhibits both osteoclast differentiation and function, in the study of Dewar et al (25) could not be excluded the possibility that imatinib may inhibit the function of other unidentified targets involved in the osteoclastogenic process.

In light of their findings and those of previous studies, Fitter et al proposed a model to explain the action of imatinib on bone remodeling. According to this explanation; imatinib restrains bone resorption (by inhibiting c-Fms on osteoclasts) and stimulates bone formation (by inhibiting PDGF-R on osteoblasts), resulting in sequestration of calcium and phosphate to bone. The decrease in serum calcium stimulates parathyroid hormone secretion, which decreases renal phosphate absorption, leading to hypophosphatemia. Similarly, Vandyke et al (4) suggested that decreased dissolution of calcium and phosphate from the bone, or increased deposition of calcium and phosphate in newly formed bone, may result in decreased serum calcium and phosphate levels in imatinib-treated patients.
Hypophosphatemia, as well as hyperparathyroidism and inhibition of osteoclast activity, after imatinib therapy have also been observed in therapies with other tyrosine kinase inhibitors that are used in imatinib-resistant CML, such as nilotinib (26, 27) and dasatinib (28, 29), and in renal cancer, such as sunitinib (30), suggesting that these effects on bone metabolism may be a common property of tyrosine kinase inhibitors.

CONCLUSION
In conclusion, the significant correlation between hypophosphatemia and the use of imatinib which has been determined in our study was harmonious with outcomes of previous studies. Since urinary P excretion was similar in CML and control groups, hypophosphatemia was attributed to the action of imatinib on bone metabolism, mediated by potential changes in functions of osteoblasts and osteoclasts caused by long-term imatinib use.

Consequently, we did not observe a statistically significant correlation between the use of imatinib and osteoporosis in our study. More comprehensive researches are needed to show the effects of tyrosine kinase inhibitors on bone remodelling.
REFERENCES


