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Original Research

Skeletal effects of nilotinib on prepubertal and pubertal rats

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Abstract

Background: Nilotinib belongs to a group of anti-cancer drugs called tyrosine kinase inhibitors (TKIs), which are currently the mainstay treatment of chronic myeloid leukemia (CML). **Objectives:** In this study, we aimed to investigate the effects of this drug on bones around puberty in vivo. **Methods:** Juvenile male Wistar rats were divided to three groups and were treated orally once a day. Group 1 was administered vehicle; group 2 was administered nilotinib at a low-dose (30.85 mg/kg) and group 3 was treated with a high-dose (41.13 mg/kg). The treatment continued from week 4 until week 7 of age (pre-pubertal and pubertal life). Methods: Serum calcium, insulin-like growth factor-1 (IGF-1) and procollagen type I (PINP) were measured by enzyme-linked immunosorbent assay (ELISA). Femur bone was collected for histopathological evaluation using Hematoxylin & Eosin stain, and Masson's trichrome (MTC) staining. Immunohistochemistry was performed, using the bone cellular markers antiosteopontin (OPN) and antitartrate-resistant acid phosphatase (TRAP). **Results:** Serum Calcium, IGF-1 and PINP declined in a dose-dependent manner ($p < 0.05$). Histopathological evaluation revealed dose-dependent degeneration of bone lamellae and disruption and disorganization of cellular components. Moreover, the study revealed a decrease in collagen, decrease in OPN, and increase in TRAP immunostaining. **Conclusion:** Nilotinib had deleterious skeletal effects around puberty, possibly requiring long-term monitoring of bone growth and mineralization.

Keywords: Nilotinib, Bone, Calcification, Puberty

INTRODUCTION

The prognosis of patients who suffer from Philadelphia (Ph*)/ BCR-ABL1-positive chronic myeloid leukemia (CML) has profoundly improved over the past years with the introduction of effective antineoplastic drugs which result in complete remission. Due to their high efficacy in inducing remission, tyrosine kinase inhibitors (TKIs) are considered the standard of care for this type of hematologic cancer. Currently, most patients with this disease have a normal life expectancy, thanks to the use of those effective medications. Indeed, the observed improvement in the prognosis of patients was due to the introduction of imatinib, which was the parent first-generation (TKI) to be discovered.[1] Following the use of imatinib, resistance to this drug emerged, due to which several other second- and third- generation TKIs were introduced including bosutinib, nilotinib, and dasatinib. Those newer TKIs are described to be more effective, albeit with lower adverse effects profile compared to imatinib.[2,3] The choice of a specific TKI for a newly diagnosed CML patient depends on several factors and individual characteristics. Those include TKI drug compliance, lifestyle preferences, comorbidities, distinct adverse effects profile, and physician-clinical center experience.[4] In addition to their efficacy in CML, TKIs have been reported to be effective for inducing remission in other types of cancer including lung cancers, gastrointestinal stromal tumors, and human epidermal growth factor receptor 2 (HER2)-positive breast cancers.[5]

To maintain remission, TKI therapy may be continued for decades or even lifelong, because despite treatment, CML cells remain in the bone marrow.[6] Due to the long-term therapy with TKIs, a lot of attention was given to obtain more detailed knowledge of their potential adverse effects. Although potentially life-threatening congestive cardiac failure remains the most serious adverse effect of TKIs, other unfavorable toxic effects that affect several body systems have been reported.[7] Those include skin, hair, hematological, endocrine, and gastrointestinal adverse outcomes. Skeletal adverse effects were commonly observed in pediatric patients receiving long-term treatment with imatinib.[8] Similarly, dasatinib, a second generation TKI, has also been reported to cause significant skeletal problems by causing dysregulated bone remodeling.[9]

Nilotinib is a second-generation, FDA-approved TKI, that

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is indicated for cases of resistance to or intolerance to imatinib in patients with CML.[10] Despite its superior efficacy compared to first-generation TKIs, a recent systematic review and meta-analysis compared the cardiovascular adverse events (CAE) of both imatinib and nilotinib and has revealed that the former results in higher risk of CAE compared to the latter.[11] Investigations of the impact of nilotinib on bone health, however, have been scarce. A recent study *in vitro* showed that this drug causes negative impact on bone cells. In this investigation, the research team exposed a human osteoblastic cell line to this drug and assessed its effects on bone mineralization capacity as well as on mRNA expression of genes that regulate osteoclastogenesis.[12] Their results revealed that nilotinib interfered with mineralization and resulted in a decline in certain osteoblast marker genes, including alkaline phosphatase, osteocalcin, and osterix. In addition, they reported that this TKI increased the RANKL/OPG ratio, which is known to stimulate osteoclastogenesis. The authors concluded that imatinib directly inhibited osteoblast differentiation and promoted a pro-osteoclastogenic environment.

To the best of our knowledge, no *in vivo* work has been performed on nilotinib's potential adverse effects on bones. In this study, we aimed to explore the effect of this TKI on bone growth, mineralization biochemical and histopathological bone parameters in juvenile rats following exposure to two different doses of nilotinib during the prepubertal and pubertal periods. This stage of development was intentionally selected because peak bone growth takes place during this period.

METHODS

Ethical Approval

Ethical approval was obtained from the Research and Ethics Committee at the Arabian Gulf University (Approval number G3-AGU-12-22). All animal studies were conducted in compliance with the specifications outlined in the Arabian Gulf University Guide for the care and use of animals and in accordance with National Institutes of Health guide for the care and use of laboratory animals.

Animals

Juvenile male Wistar rats (n=24) weighing 180–220 grams were kept under standardized laboratory conditions. Male rats were selected for the study, rather than females, to eliminate the potential influence of hormonal fluctuations associated with the estrous cycle in females, which could impact the study's outcomes.[13] The animals were kept in spacious and wide-mesh cages, in a pathogen-free animal facility. Prior to their inclusion in the experiments, the rats were acclimated for two weeks in the animal house under ambient temperature of 25 ºC, 12-hour light-dark cycles, and *ad libitum* access to standard rodent chow and tap water. Following acclimatization, the rats were randomly assigned into three experimental groups: control group (n=8) were administered an equivalent volume of vehicle, 5% dimethyl sulfoxide (DMSO), 10% polyethylene glycol (PEG) and 20% Tween 80 in deionized water, low-dose nilotinib 2 (n=8) received (30.85 mg/kg) nilotinib (Novartis Pharmaceuticals, New Jersy, USA) and high-dose nilotinib 3 (n=8) was treated with (41.13 mg/kg) nilotinib. All animals were treated once daily between 9–10 am via oral gavage. The drug was administered over the prepubertal and pubertal periods.[14] That is, treatment was undertaken from the first day of week 4 of age until the last day of week 7 of age; total duration of treatment was 28 days. Doses were selected based on the clinically approved doses in humans, which are 300 mg/kg and 400 mg/kg. Those human doses were converted to animal equivalent ones by dividing the human dose over 60 and multiplying them by a factor of 6.17.[15] At the end of the treatment regimen (day 29), the rats were humanely euthanized by exposing them to CO2 inhalation and blood samples were collected by cardiac puncture and immediately kept under -80 degrees Celsius until further analysis.

Assessment of Blood Parameters

Serum calcium, insulin-like growth factor-1 (IGF-1) and procollagen type I (PINP) levels in serum were analyzed by using enzyme-linked immunosorbent assay (ELISA). Samples were analyzed in duplicate, randomized, and blinded manner to avoid bias. Markers concentrations in serum were measured by using ELISA kit according to the manufacturer's instructions. The kit which was used for measuring calcium levels was Rat calcium (Ca) ELISA Kit MBS3807893 (Abcam, Cambridge, UK). The PINP levels were measured by using the kit ab285314 – Rat Collagen Type I (Abcam, Cambridge, UK). However, the kit which we employed to measure IGF-1 concentrations was Rat IGF-1 ELISA Kit Catalog Number ERIGF1 (Thermo-Fischer Scientific, Waltham, MA USA).

Histopathological Evaluation

Following animal sacrificing, femur bones were dissected, washed with saline, fixed in 10% neutral buffered formalin, and then decalcified using ethylene diamine tetra acetic acid (EDTA) for four weeks. Specimens from the upper third of femur were trimmed, and longitudinal sections were processed and embedded into paraffin blocks. This part of bone was selected to obtain more trabecular bone. The blocks were cut into sections of 5 µm thickness by a rotatory microtome. The sections were stained with Hematoxylin and Eosin (H&E) for general morphology and Masson's trichrome for collagen fibers.[16, 17] Trabecular bone was the area of interest in this study.

Immunohistochemistry

The paraffin sections of the upper third of femur bone were mounted on positively charged slides and processed for immunohistochemical (IHC) processing by using an Avidin-Biotin detection system (Vectastain Elite ABC Kit Universal, Vector laboratories, UK), according to the manufacturer's instructions. Sections were incubated separately with the following primary antibodies: anti osteopontin (OPN) antibody (osteoblast marker); dilution 10 µg/ml, rabbit polyclonal antibody (ab34686, Abcam, UK), anti-Tartrate-resistant acid phosphatase (TRAP) antibody (osteoclast marker); dilution 1: 5000, rabbit monoclonal antibody ab218011 (Abcam, UK).[18, 19] The primary antibody was incubated for 10 minutes at room temperature. The negative control was nonimmune goat

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serum. The reaction was visualized using 3,3-diaminobenzidine tetrahydrochloride (DAB). Consequently, the sections were counterstained with Harris hematoxylin. The slides were examined and photographed using a light microscope (Axio Scope A1, Carl Zeiss Microscopy, Germany) fitted with a digital camera.

Statistical Analysis

Collected data was analyzed by using the SPSS-26. The p value < 0.05 was considered statistically significant. To measure the statistical differences among the different experimental groups, one way ANOVA test followed by Tukey's *post-hoc* test was used.

RESULTS

Blood Parameters

Among the control and treatment groups, we reported no rat mortality. Regarding the data on skeletal biochemical parameters, we observed dose-dependent decline in serum calcium, IGF-1 and procollagen type I ($p < 0.05$) (Table 1). The p value in the results of the biochemical was significant when comparing both treatment groups to the control group as well as when comparing high-dose to low-dose nilotinib groups.

Histopathological Evaluation

When histopathological evaluation was performed by using H&E staining, we reported a dose-dependent degeneration of bone lamellae, and disruption and disorganization of the cellular components (Figure 1). As for the MTC staining data, we observed that this staining diminished in the treatment groups compared to the control group. The decline in MTC staining followed a dose-dependent manner. That is, the staining was significantly lower with increasing nilotinib dose (Figure 2). The p value was < 0.05 for both the low-dose and high-dose nilotinib groups compared to the control group as well as the high-dose compared to the low-dose nilotinib groups.

Immunohistochemistry

Immunohistochemistry data on the osteoblast and osteoclast markers were also evaluated in this study. The osteoblast marker (OPN) revealed a dose-dependent decrease. Indeed, the observed decline in this staining was more marked when nilotinib-treated groups were compared to the control group ($p < 0.05$) and when the high-dose nilotinib group was contrasted to the low-dose one ($p < 0.05$) (Figure 3). Lastly, the osteoclast marker (TRAP), was observed to exhibit an upregulation in

Table 1. Effect of nilotinib administration on bone biochemical parameters in rats (n=24)

Parameter Group	Serum calcium (mg/dl)	Serum IGF-1 (ng/ml)	Serum Collagen type-1 (ng/ml)
Control	10.03±0.26	767.53±23.28	66.19 ±3.16
Low dose nilotinib	8.54±0.54*	633.60±10.55*	55.42 ±3.57*
High dose nilotinib	6.52±0.51*,#	392.71±13.35*,#	43.59 ±3.57*,#

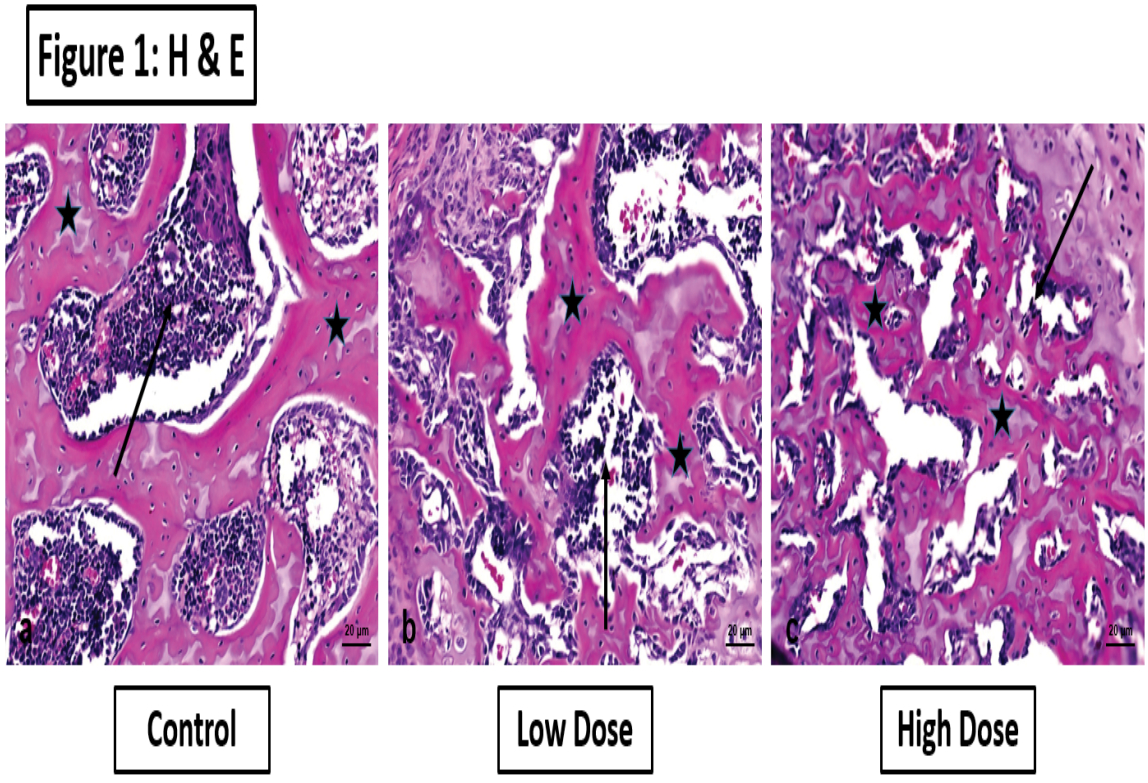

Figure 1. Light microscopic photomicrographs of the femur trabecular bone, showing different bone cells within the bone (a): Control group (b): Low dose nilotinib-treated group (c): High dose nilotinib-treated group. Note the trabecular bone (asterisks), and the bone marrow in-between (arrows). H& E stain. X 200.

Figure 2: MTC

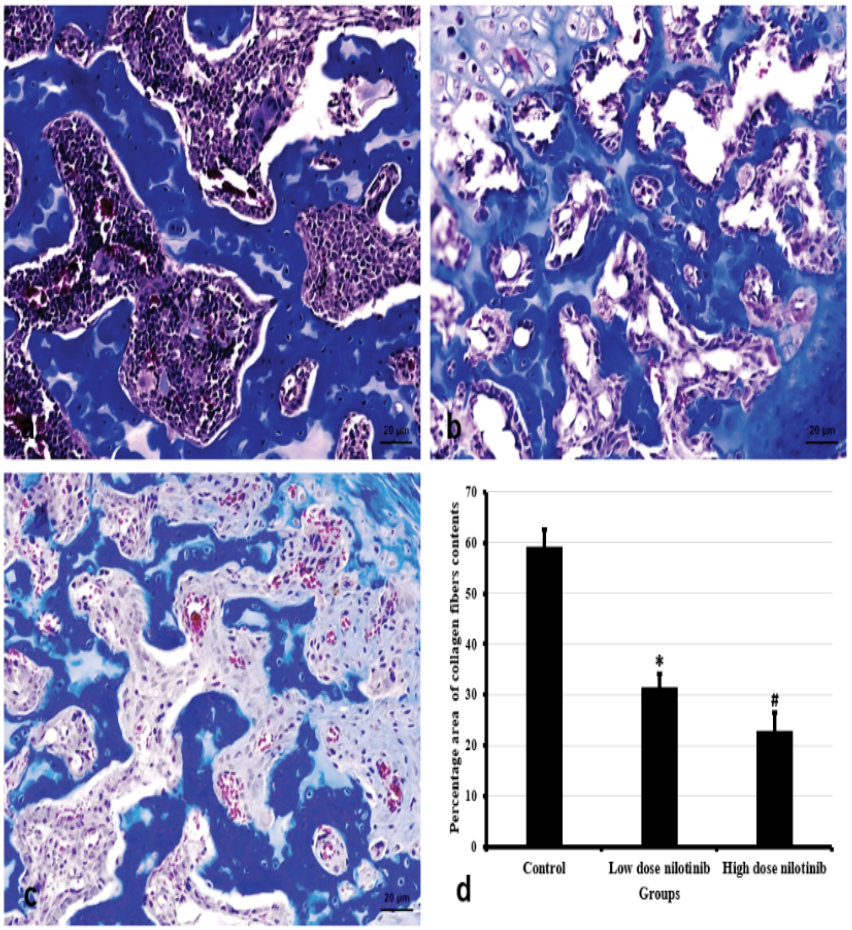

Figure 2. Photomicrographs of the femur trabecular bone illustrating the collagen fibers. (a): Control section reveals strong blue staining of collagen fibers. (b): Low dose nilotinib-treated group shows a mild decrease in collagen fiber content. (c) High dose nilotinib-treated group is showing a marked decrease in the amount of collagen fibers. Masson trichrome stain, X 200. (d): Quantification of the Percentage area of collagen fiber contents. Values are mean±SD. *P<0.05 vs control, high dose nilotinib groups and #P<0.05 vs control and low dose nilotinib groups, as determined by a one-way ANOVA followed by Tukey's post-hoc test.

Figure 3: OPN

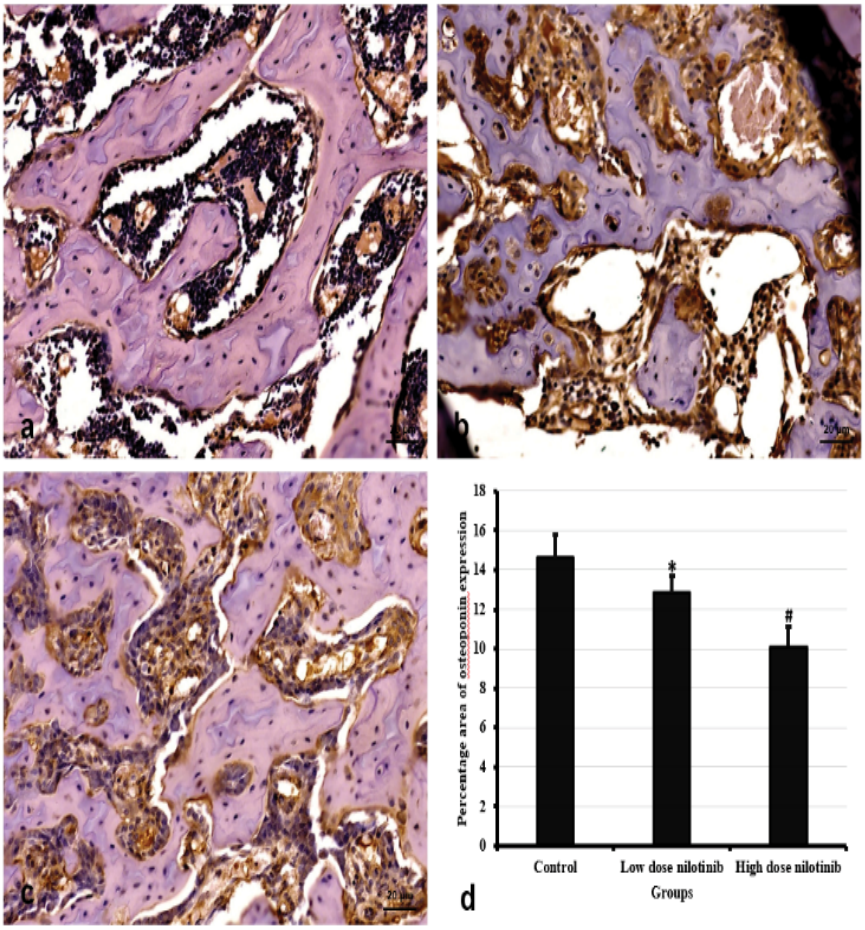

Figure 3. Light microscopic photomicrographs of the femur trabecular bone immunostained with anti osteopontin antibody (osteoblast marker). (a): Control, (b): Low dose nilotinib, showing a decrease in immunostaining (c): High dose nilotinib showing a more decrease in immunostaining. X 200. (d): Percentage area of anti-OPN immunoreactivity. Values are mean±SD. *P<0.05 vs control, high dose nilotinib groups and #P<0.05 vs control and low dose nilotinib groups, as determined by a one-way ANOVA followed by Tukey's post-hoc test.

staining when examining the treatment groups and comparing them to the control group ($p < 0.05$). Moreover, the enhanced staining was more marked in the high-dose nilotinib group when compared to the low-dose nilotinib group indicating that the upregulation of TRAP was dose-dependent ($p < 0.05$) (Figure 4).

DISCUSSION

TKIs are considered a forefront targeted therapy for several types of solid tumors.[20] Particularly, the use of TKIs has revolutionized the treatment of CML, because they allowed patients who suffer from this hematological malignancy to enjoy a life expectancy like normal people.[21] Because TKIs fail to eradicate CML cells from the bone marrow, following successful induction therapy, long-term use of those drugs must be continued to maintain remission, prevent relapse, and prolong survival.[22] Following the clinical use of the first TKI imatinib, the skeletal adverse effects of this class of drugs were observed. Indeed, this medication was found to cause hypophosphatemia, hypocalcemia, and dysregulation of bone remodeling.[23] Those initial findings guided other investigations that focused on the effects of other TKIs on bone health, which eventually reported impairment of bone resorption by all TKIs.[24] Our study was conducted to explore the impact of the second-generation TKI nilotinib on bones. Overall, by exposing peri-pubertal juvenile rats around to different doses of nilotinib, we reported significant skeletal effects of this drug including adverse effects on skeletal growth, bone mineralization, and histopathological alterations.

In this research, and for the first time, we have conducted an *in vivo* study by using a rat model to investigate the skeletal effects of nilotinib administration around the time of puberty. We chose the peripubertal period because this stage of life plays a major role in bone growth and development. Indeed, by the end of the adolescent age, bone mass doubles.[25] Our findings revealed downregulation in several mineralization parameters including a decline in serum calcium, pro-collagen type I, collagen staining and osteoblastic activity, whereas we observed overexpression of osteoclastic activity. In line

Figure 4: TRAP

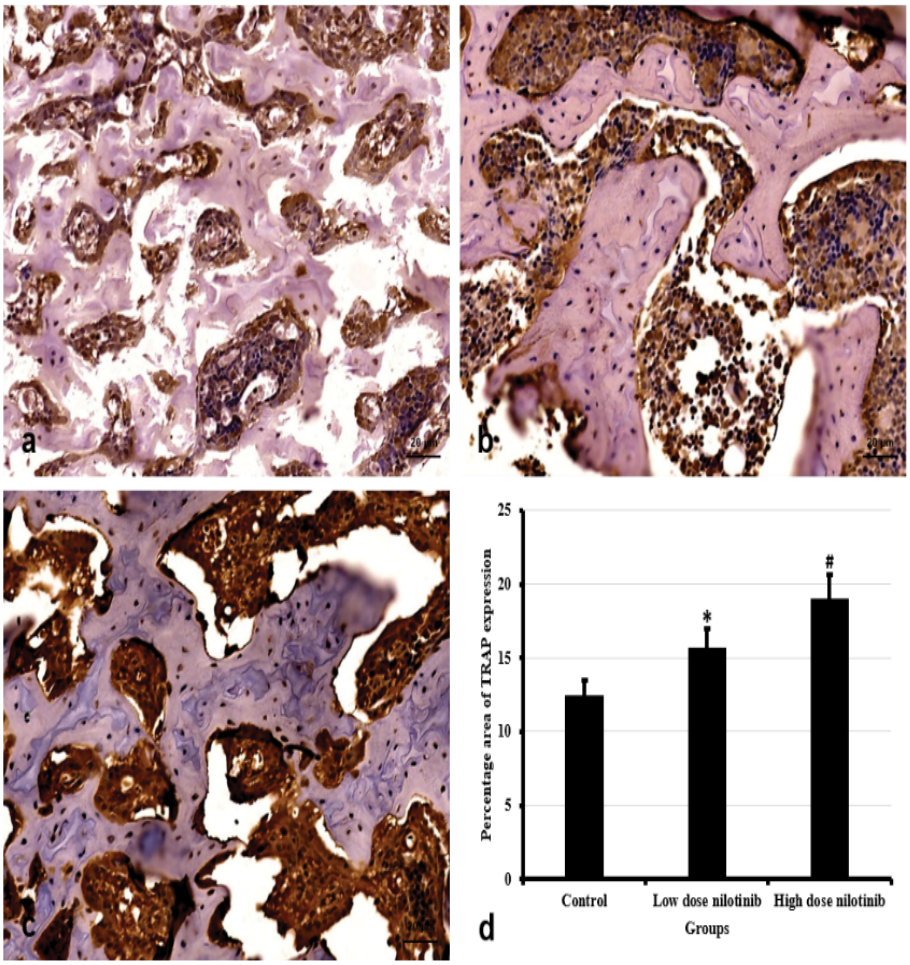

Figure 4. Light microscopic photomicrographs of the femur trabecular bone immunostained with anti-TRAP antibody (osteoclast marker). (a): Control, (b): Low dose nilotinib, showing an increase in immunostaining (c): High dose nilotinib showing a more increase in immunostaining. Mag: X 200. (d): Percentage area of anti-TRAP immunoreactivity. Values are mean±SD. *P<0.05 vs control, high dose nilotinib groups and #P<0.05 vs control and low dose nilotinib groups, as determined by a one-way ANOVA followed by Tukey's post-hoc test.

with our findings, *in vitro* evidence has reported that nilotinib induced unfavorable effects on bone cells. Kroschwald and his colleagues investigated the effect of both imatinib and nilotinib on bone cells *in vitro*. The investigators exposed the osteoblastic cell line SaOS-2 to both agents. The results of this study reported that both TKIs interfered with mineralization, downregulated osteoblast marker genes and stimulated osteoclastogenesis. Nilotinib had more marked adverse effects on bones compared to imatinib.[26] In a related study, O'Sullivan et al. investigated the effects of nilotinib on bone cells *in vitro*.[27] In agreement with our data, the investigators found that this drug inhibited osteoblast proliferation and differentiation. Interestingly, however, they reported an increase in osteoclastic cells proliferation in one type of cells but a decrease in another. In another study *in vivo*, Tauer et al. conducted a study on juvenile rats where animals were exposed to different doses of the first-generation TKI imatinib. The investigators reported a decrease in bone mass density and femoral breaking strength compared to normal animals. Like our data, the authors also revealed lower bone growth, lower pro-collagen type-1 and increase in the osteoclast marker TRAP in a dose-dependent manner.[28] In a recent study, Indarwulan et al. investigated the parameters that could accurately assess bone conditions in patients receiving imatinib for CML. The authors concluded that type I collagen could be used as an accurate measure of bone mineral density in those patients.[29] Our data and the findings of other suggest that nilotinib administration may have an important effect on bone metabolism and turnover.

One of the findings in this study was the decline in IGF-1, indicating diminishing growth hormone in the treated rats. This observation was in agreement with previous report by Ulmer and co investigators who examined the effect of two TKIs on the growth hormone *in vivo*. This study revealed that exposure of rats to those two medications results in a significant drop in IGF-1 and insulin-like growth factor binding protein-1 indicating decline in growth hormone in those animals.[30] In another related study, Cai et al conducted a human investigation where the impact of TKIs on bone growth was examined in children who suffered from Philadelphia chromosome-positive CML.

They aimed to compare the effect of the first-generation and second-generation TKIs on bone growth by exposing those children to imatinib or dasatinib, respectively. The results of that study revealed a similar adverse outcome on the height of the two groups of children indicating that both TKIs caused similar adverse outcomes on bone growth.[31]

The adverse effects of nilotinib on rat's bone histomorphometry was also confirmed in this study. We reported histopathological changes indicating unfavorable effects of this drug on bone lamella and cellular components. A previous study conducted by Nurmio et al. investigated the effects of imatinib on the growing bones in peripubertal rats. The investigators revealed that this drug resulted in significant effects on bones as manifested by abolishing osteoclasts in the metaphyseal osteochondral junction, augmenting bone cohesion, inducing local osteopetrosis and local osteopetrosis at the osteochondral junction. The observed undesirable effects on bones continued in the experimental rats until adulthood.[32]

The impact of TKIs on osteoclast formation and resultant bone resorption has been subjected to a major debate. Imatinib and nilotinib were reported to decrease osteoclast formation and actions by direct and indirect mechanisms.[33] O'Sullivan et al. demonstrated that the inhibition of osteoclastogenesis is mediated by indirect mechanisms. Specifically, the investigators found that nilotinib inhibits the platelet-derived growth factor receptor beta (PDGFRβ) signaling through which they stimulate an increase in the synthesis of osteoprotegerin. The latter is known to diminish osteoclast function. In our study, however, we demonstrated that nilotinib increased TRAP, a marker for osteoclasts. Compared to the debate on the impact of TKIs on osteoclasts, our findings on the effect of nilotinib on osteoblasts was in agreement with a previous study conducted by O'Sullivan et al. who showed that this drug interfere with osteoblast proliferation and differentiation.[27] By and large, our findings and the data reported by previous studies indicate that nilotinib, similar to other TKIs, induce unfavorable effects on bone growth and mineralization parameters.

CONCLUSIONS

Our findings indicated that nilotinib caused detrimental effects on bone mineralization and bone growth in prepubertal and pubertal rats. Although human studies may be required to confirm the deleterious effects of nilotinib on bones, those significant findings are of clinical importance due to the long-term use of this drug in children who are diagnosed with CML. Those children might require continuous monitoring of their growth and bone mineral density throughout treatment.

AUTHORS CONTRIBUTION

Yasin I. Tayem*: Conceptualization, data curation, formal analysis, funding acquisition, methodology, project administration, supervision, validation, writing- original draft.

Wael A. Nasr El-Din: Conceptualization, data curation, formal analysis, funding acquisition, methodology, validation, writing-revieing and editing.

Aisha N. Rashid: Conceptualization, data curation, formal analysis, methodology, validation.

Sindhan Veeramuthu: Conceptualization, data curation, formal analysis, methodology, validation.

Manal A. Othman: Conceptualization, data curation, formal analysis, funding acquisition, methodology, validation, writing-revieing and editing.

CONFLICT OF INTEREST

The authors certify that they have no conflicts of interest pertinent to this article.

References

1. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001 Apr 5;344(14):1031-7. https://doi.org/10.1056/NEJM200104053441401
2. Vener C, Banzi R, Ambrogi F, Ferrero A, Saglio G, Pravettoni G, Sant M. First-line imatinib vs second- and third-generation TKIs for chronic-phase CML: a systematic review and meta-analysis. Blood Adv. 2020 Jun 23;4(12):2723-2735. https://doi.org/10.1182/bloodadvances.2019001329
3. Heim D, Ebnöther M, Favre G. Chronische Myeloische Leukämie – Update 2020, [Chronic myeloid leukemia - update 2020]. Ther Umsch. 2019;76(9):503-509. https://doi.org/10.1024/0040-5930/a001124
4. Ciftciler R, Haznedaroglu IC. Tailored tyrosine kinase inhibitor (TKI) treatment of chronic myeloid leukemia (CML) based on current evidence. Eur Rev Med Pharmacol Sci. 2021 Dec;25(24):7787-7798. https://doi.org/10.26355/eurrev_202112_27625
5. Shyam Sunder S, Sharma UC, Pokharel S. Adverse effects of tyrosine kinase inhibitors in cancer therapy: pathophysiology, mechanisms and clinical management. Signal Transduct Target Ther. 2023 Jul 7;8(1):262. https://doi.org/10.1038/s41392-023-01469-6
6. Leak S, Horne GA, Copland M. Targeting BCR-ABL1-positive leukaemias: a review article. Camb Prism Precis Med. 2023 Mar 6;1:e21. https://doi.org/10.1017/pcm.2023.9
7. Hartmann JT, Haap M, Kopp HG, Lipp HP. Tyrosine kinase inhibitors - a review on pharmacology, metabolism and side effects. Curr Drug Metab. 2009. Jun;10(5):470-81. https://doi.org/10.2174/138920009788897975
8. Tauer JT, Hofbauer LC, Jung R, Gerdes S, Glauche I, Erben RG, Suttorp M. Impact of long-term exposure to the tyrosine kinase

inhibitor imatinib on the skeleton of growing rats. PLoS One. 2015 Jun 24;10(6):e0131192. https://doi.org/0.1371/journal.pone.0131192
9. Vandyke K, Dewar AL, Diamond P, Fitter S, Schultz CG, Sims NA, Zannettino AC. The tyrosine kinase inhibitor dasatinib dysregulates bone remodeling through inhibition of osteoclasts in vivo. J Bone Miner Res. 2010 Aug;25(8):1759-70. https://doi.org/10.1002/jbmr.85
10. Kantarjian HM, Hughes TP, Larson RA, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Boquimpani C, Pasquini R, Clark RE, Dubruille V, Flinn IW, Kyrcz-Krzemien S, Medras E, Zanichelli M, Bendit I, Cacciatore S, Titorenko K, Aimone P, Saglio G, Hochhaus A. Long-term outcomes with frontline nilotinib versus imatinib in newly diagnosed chronic myeloid leukemia in chronic phase: ENESTnd 10-year analysis. Leukemia. 2021 Feb;35(2):440-453. https://doi.org/10.1038/s41375-020-01111-2
11. Li S, He J, Zhang X, Cai Y, Liu J, Nie X, Shi L. Cardiovascular adverse events in chronic myeloid leukemia patients treated with nilotinib or imatinib: A systematic review, meta-analysis and integrative bioinformatics analysis. Front Cardiovasc Med. 2022 Nov 8;9:966182. https://doi.org/10.3389/fcvm.2022.966182
12. Kroschwald LM, Tauer JT, Kroschwald SI, Suttorp M, Wiedenfeld A, Beissert S, Bauer A, Rauner M. Imatinib mesylate and nilotinib decrease synthesis of bone matrix in vitro. Oncol Lett. 2019 Aug;18(2):2102-2108. https://doi.org/10.3892/ol.2019.10518
13. Nasr El-Din WA, Abdel Fattah IO. L-arginine mitigates choroid plexus changes in Alzheimer's disease rat model via oxidative/inflammatory burden and behavioral modulation. Tissue Cell. 2024 Dec;91:102572. https://doi.org/10.1016/j.tice.2024.102572
14. H W Aledani T, N Al-Hayder M, S Al-Mayyahi R. Severe deterioration in sperm parameters and testes of rats administered naproxen and diclofenac at pre-puberty: An experimental study. Int J Reprod Biomed. 2022 Nov 2;20(10):831-840. https://doi.org/10.18502/ijrm.v20i10.12267
15. Shin JW, Seol IC. Interpretation of animal dose and human equivalent dose for drug development. J Korean Orient Med. 2010;31:1–7.
16. Liu H, Zhu R, Liu C, Ma R, Wang L, Chen B, Li L, Niu J, Zhao D, Mo F, Fu M, Brömme D, Zhang D, Gao S. Evaluation of Decalcification Techniques for Rat Femurs Using HE and Immunohistochemical Staining. Biomed Res Int. 2017;2017:9050754. https://doi.org/10.1155/2017/9050754
17. Ren S, Jiao G, Zhang L, You Y, Chen Y. Bionic Tiger-Bone Powder Improves Bone Microstructure and Bone Biomechanical Strength of Ovariectomized Rats. Orthop Surg. 2021 May;13(3):1111-1118. https://doi.org/10.1111/os.12954
18. Si J, Wang C, Zhang D, Wang B, Zhou Y. Osteopontin in Bone Metabolism and Bone Diseases. Med Sci Monit. 2020 Jan 30;26:e919159. https://doi.org/10.12659/MSM.919159
19. Hayman AR. Tartrate-resistant acid phosphatase (TRAP) and the osteoclast/immune cell dichotomy. Autoimmunity. 2008 Apr;41(3):218-23. https://doi.org/0.1080/08916930701694667
20. Esteban-Villarrubia J, Soto-Castillo JJ, Pozas J, San Román-Gil M, Orejana-Martín I, Torres-Jiménez J, Carrato A, Alonso-Gordoa T, Molina-Cerrillo J. Tyrosine Kinase Receptors in Oncology. Int J Mol Sci. 2020 Nov 12;21(22):8529. https://doi.org/10.3390/ijms21228529
21. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, Janssen JJWM, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saußele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020 Apr;34(4):966-984. https://doi.org/10.1038/s41375-020-0776-2
22. Bhatia R. Novel approaches to therapy in CML. Hematology Am Soc Hematol Educ Program. 2017 Dec 8;2017(1):115-120. https://doi.org/10.1182/asheducation-2017.1.115