

RESEARCH ARTICLE

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Profiling Tyrosine Kinase Inhibitor-Specific Signatures of Homocysteine Metabolism and Mineral Homeostasis in Chronic Myeloid Leukemia

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Abstract

Background: The application of tyrosine kinase inhibitors (TKIs) has revolutionized the management of chronic myeloid leukemia (CML), rendering it a controllable disease. However, long-term administration is associated with adverse metabolic effects, particularly disruptions in homocysteine metabolism and mineral balance. A comprehensive comparison across TKI types remains absent. **Objective:** This study systematically characterized and compared the metabolic signatures elicited by first- and second-generation TKIs Imatinib, Nilotinib, and Dasatinib on homocysteine and mineral parameters in chronic myeloid leukemia. **Methods:** This retrospective cross-sectional study involved 90 stable CML patients treated with TKI therapy for at least six months at Baghdad Oncology Teaching Hospital between March 2024 and April 2025. Patients were divided into three equal groups based on their medication: Imatinib, Nilotinib, or Dasatinib. Fasting blood samples were collected and analyzed for homocysteine, PTH, calcium, potassium, creatinine, vitamin B12, and ALP. Statistical methods, including ANOVA and correlation analyses, were used to compare groups and explore drug-specific effects. **Results:** The three TKI groups demonstrated distinct metabolic disturbance patterns. Nilotinib was associated with the highest homocysteine levels and showed strong positive correlations with PTH and ALP. Dasatinib caused a moderate elevation in homocysteine and uniquely correlated positively with potassium. Imatinib showed the most favorable homocysteine profile, which was primarily inversely correlated with calcium. Multivariate analysis confirmed that these metabolic profiles effectively distinguished the three TKI groups. **Conclusion:** The present study demonstrates that Imatinib, Nilotinib, and Dasatinib elicit distinct and discernible metabolic signatures. These results support the adoption of personalized, TKI-specific monitoring approaches in the management of CML, aiming to proactively reduce long-term cardiovascular and skeletal adverse effects and thereby improve alignment between the quality and duration of survival.

Keywords: Chronic Myeloid Leukemia, Tyrosine Kinase Inhibitors, Homocysteine, Personalized Monitoring

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Introduction

Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative neoplasm that arises from a pluripotent hematopoietic stem cell and is characterized by the unchecked proliferation and accumulation of mature granulocytes and their precursors. The defining feature of CML, identified more than sixty years ago, is the presence of the Philadelphia (Ph) chromosome, which results from a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11) [1, 2]. This genetic aberration results in the formation of the **BCR-ABL1** fusion gene, which encodes a constitutively active *BCR-ABL* tyrosine kinase [3]. This oncoprotein serves as the primary driver of leukemogenesis, aberrantly stimulating downstream

signaling pathways such as *RAS/MAPK*, *JAK/STAT*, and *PI3K/AKT*. This dysregulation results in unchecked cellular proliferation, diminished apoptosis, and impaired adhesion to the bone marrow stroma [4].

Before the introduction of targeted therapy, the prognosis for chronic myelogenous leukemia (CML) was unfavorable, characterized by median survival durations of five to seven years and a nearly certain progression to a fatal blast crisis [5]. The therapeutic landscape for chronic myeloid leukemia (CML) experienced a significant transformation at the beginning of the 21st century with the advent of Tyrosine Kinase Inhibitors (TKIs). Imatinib mesylate, the inaugural *BCR-ABL1* TKI, exhibited remarkable efficacy in eliciting sustained cytogenetic and molecular responses, thereby redefining CML from

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a fatal condition to a manageable chronic disease for the majority of patients [6]. The success of imatinib prompted the development of second and third-generation tyrosine kinase inhibitors, including nilotinib and dasatinib. These drugs were engineered to address resistance and enhance potency. Currently, they are commonly employed as initial treatment options, thereby advancing the depth and speed of molecular responses [7, 8].

The primary focus of CML management has transitioned from attaining short-term remission to promoting long-term survival with an improved quality of life, which requires continuous TKI therapy over decades. Nonetheless, prolonged TKI use presents considerable clinical challenges due to a range of chronic, non-hematological adverse effects. These off-target toxicities result from the inhibition of kinases other than BCR-ABL1, such as c-KIT, PDGFR, and SRC family kinases, which are essential to various physiological functions [9, 10]. Common adverse effects encompass cardiovascular events, metabolic disturbances, and organ toxicities, which together contribute to long-term morbidity [11]. Among these, two interconnected areas of increasing significance are disruptions in homocysteine metabolism and mineral homeostasis. Hyperhomocysteinemia, characterized by elevated plasma homocysteine levels, has been increasingly observed in patients undergoing TKI therapy [12].

Homocysteine, a sulfur-containing amino acid, relies heavily on B vitamin metabolism, including B12, B6, and folate.

Elevated homocysteine levels are an established, independent risk factor for endothelial dysfunction, atherosclerosis, and thromboembolic events, thereby raising concerns about the long-term cardiovascular risk in survivors of chronic myelogenous leukemia (CML) [13, 14]. Concurrently, tyrosine kinase inhibitors (TKIs) have been implicated in interfering with mineral and bone metabolism. Notably, imatinib has been associated with hypophosphatemia and alterations in bone turnover, potentially through inhibition of PDGFR and other kinases involved in osteoclast regulation [15, 16]. Parameters such as Parathyroid Hormone (PTH), calcium, and Alkaline Phosphatase (ALP) serve as vital biomarkers in regulating homeostatic mechanisms. Disruptions within this axis may contribute to the development of metabolic bone disease, thereby elevating the long-term risk of osteoporosis and fracture [17, 18]. The relationship between homocysteine metabolism and bone health remains an active area of research, with evidence indicating that hyperhomocysteinemia may adversely affect bone quality and potentially contribute to a cycle of metabolic and skeletal deterioration in long-term recipients of tyrosine kinase inhibitors [19, 20].

Despite the well-documented individual toxicities associated with various tyrosine kinase inhibitors (TKIs), a significant gap remains in the comprehensive and comparative analysis of their specific effects on the interaction between homocysteine metabolism and mineral homeostasis. Although it is acknowledged that different TKIs exhibit distinct off-target kinase inhibition profiles, a systematic, direct comparison that delineates

their unique metabolic signatures is currently lacking. Most existing studies have focused on single agents or have not thoroughly examined the correlations between homocysteine levels and key mineral parameters, such as parathyroid hormone (PTH), calcium, potassium, and alkaline phosphatase (ALP), across diverse TKI treatment regimens. Understanding whether imatinib, nilotinib, and dasatinib induce distinct patterns of metabolic disruption is essential for developing tailored monitoring and management strategies.

This study aims to systematically examine and compare the metabolic signatures induced by first- and second-generation TKIs specifically Imatinib, Nilotinib, and Dasatinib focusing on homocysteine metabolism and mineral balance in a well-defined cohort of CML patients. Using multivariate analysis, the research intends to identify drug-specific disruption patterns, helping to establish distinct risk profiles for each TKI. The ultimate goal is to move beyond a one-size-fits-all monitoring approach towards personalized, TKI-specific follow-up care. This would enable clinicians to proactively address long-term cardiovascular and skeletal risks, ensuring that survival quality matches its duration in modern CML management.

Materials and Methods

Study Design

This study employed a retrospective, cross-sectional design to delineate the metabolic profiles linked to Tyrosine Kinase Inhibitors (TKIs). The group included 90 adults who had been diagnosed with Chronic Myeloid Leukemia (CML) and had been on a stable TKI treatment for at least six months. Clinical and laboratory data were retrospectively extracted from medical records at a prominent oncology teaching hospital in Baghdad and subsequently anonymized. Data collection transpired from March 2024 to April 2025, yielding a contemporary and pertinent sample. The study design facilitated a comprehensive analysis of empirical data to investigate the specific impacts of TKI therapies on homocysteine metabolism and mineral homeostasis.

Study Population

The study involved 90 adult patients diagnosed with Chronic Myeloid Leukemia (CML) who were receiving active management at a prominent oncology teaching hospital in Baghdad.

Inclusion criteria

The study included adult patients, aged 18 years or older, with a verified diagnosis of chronic myeloid leukemia (CML). Participants were required to have undergone uninterrupted treatment with a single tyrosine kinase inhibitor (TKI), such as Imatinib, Nilotinib, or Dasatinib, for at least 6 months before data collection. Also, all of the laboratory results for homocysteine, parathyroid hormone (PTH), calcium, potassium, and alkaline phosphatase had to be available during the study period.

Exclusion criteria

The study excluded individuals with preexisting conditions that notably influence homocysteine or mineral metabolism, including stage 4 or 5 chronic kidney disease, primary hyperparathyroidism, and active liver cirrhosis. Participants receiving medications known to affect homocysteine levels, such as methotrexate or anticonvulsants, as well as those consuming high doses of B vitamins, were also omitted. Pregnant or breastfeeding women were excluded, as were patients without complete medical records or essential laboratory data required for comprehensive analysis.

TKI Therapy Stratification and Dosing

The cohort consisted of 90 patients, evenly divided into three groups based on their targeted tyrosine kinase inhibitor (TKI) therapy, with 30 patients per group. Patients in the Imatinib group received a consistent dosage of 400 mg once daily. In the Nilotinib group, the majority 28 out of 30 patients (93.3%) were administered 300 mg twice daily. Similarly, in the Dasatinib group, 29 patients (96.7%) received 100 mg once daily. This stratification enabled a direct comparison of therapies' metabolic effects, with a consistent average treatment duration of approximately 3 years across all groups.

Data Collection and Laboratory Measurements

Blood samples were collected from participants through standard venipuncture after an overnight fast. Serum and plasma were separated by centrifugation and analyzed in the hospital's central laboratory using routine automated analyzers and commercially available reagent kits. Detailed information on the analytical methods and equipment for each parameter is provided below.

Homocysteine (Hcy)

Plasma homocysteine levels were measured using an automated clinical chemistry analyzer (Mindray BS-200 model). The enzymatic cycling method was employed for analysis, in which homocysteine is continuously converted to S-adenosyl-L-homocysteine, which is subsequently converted to adenosine, producing a chromogenic product. The resulting color intensity, as assessed photometrically, directly correlates with the homocysteine concentration in the sample. The typical reference range for plasma homocysteine is 5-15 $\mu\text{mol/L}$ [21].

Parathyroid Hormone (PTH)

An electrochemiluminescence immunoassay (ECLIA) on a Roche Cobas e411 analyzer was used to measure intact PTH levels in serum. This method uses a sandwich principle, in which PTH in the sample binds to both a biotinylated antibody and a ruthenium-labeled antibody. Chemiluminescence is then used to measure the complex formed. The normal range for intact PTH is typically 15-65 pg/mL [22].

Calcium (Ca)

A clinical chemistry analyzer (Abbott Architect c4000) was used to measure total serum calcium by the

colorimetric method. Arsenazo III dye is used in the test. It makes a purple complex with calcium ions. The analyzer measures the amount of light this complex absorbs, which is directly proportional to the total calcium in the serum. The normal range for serum calcium is 8.6 to 10.2 mg/dL [23].

Potassium (K)

A clinical chemistry analyzer (Medica EasyLyte) with an ion-selective electrode (ISE) module was used to measure serum potassium levels. The ISE directly measures potassium concentration by detecting the electrical potential generated by potassium ions as they cross a membrane. The normal range for potassium in the blood is 3.5 to 5.1 mmol/L [24].

Alkaline Phosphatase (ALP)

A clinical chemistry analyzer was used to measure serum ALP activity using a kinetic rate method. The test uses p-nitrophenyl phosphate (pNPP) as a base. The ALP enzyme in the sample cleaves pNPP to p-nitrophenol, a yellow product. The analyzer monitors the rate of increase in absorbance at 405 nm, which is directly related to ALP activity. The normal range for ALP in adults is usually 40 to 129 U/L [25].

Creatinine (Cr)

Serum creatinine levels were determined using a clinical chemistry analyzer (Abbott Architect c4000) employing the Jaffe method, a kinetic colorimetric assay. In an alkaline picrate solution, creatinine forms a red-orange complex. The rate of color development, measured photometrically at 510 nm, is directly proportional to the serum creatinine concentration. The established reference interval for serum creatinine in adults generally ranges from 0.6 to 1.2 mg/dL [26].

Vitamin B12

Serum vitamin B12 levels were measured using an electrochemiluminescence immunoassay (ECLIA) performed on a Roche Cobas e411 analyzer. This sandwich assay utilizes ruthenium-labeled intrinsic factor to bind vitamin B12 in the sample, with the resulting chemiluminescent signal being directly proportional to vitamin B12 concentration. The normal reference range for serum vitamin B12 is 200-900 pg/mL [27].

Ethical Considerations

The study protocol was approved by the Institutional Review Board (IRB) of the Iraqi National Cancer Research Center at the University of Baghdad (Approval Number: 917), ensuring adherence to the ethical standards outlined in the Declaration of Helsinki. Due to its retrospective nature and reliance on previously collected, anonymized data, the IRB waived the requirement for informed consent from individual participants.

Statistical Analysis

To investigate the effects of various TKI treatments on patient metabolism, a series of statistical analyses was employed. Initially, patient cohorts were characterized by

their mean and standard deviation. To compare critical metabolic indicators such as homocysteine and mineral concentrations across the three TKI groups, a one-way ANOVA was utilized. When significant differences were identified, post hoc tests were conducted to determine which groups exhibited disparities.

Furthermore, Pearson's correlation analysis was conducted to examine the specific relationships between homocysteine levels and other parameters within each TKI group. Subsequently, a multivariate analysis of variance (MANOVA) was performed to assess whether the overall metabolic profile could differentiate among the treatment groups. A p-value of less than 0.05 was regarded as indicative of statistical significance across all tests. Moreover, Figure 1 was generated by employing RStudio [28, 29].

Results

Patient Characteristics and TKI Stratification

The baseline characteristics of the 90 patients with chronic myeloid leukemia (CML), categorized by their tyrosine kinase inhibitor (TKI) treatment regimen, are summarized in Table 1. The three groups Imatinib (n=30), Nilotinib (n=30), and Dasatinib (n=30) showed similar demographics, with no statistically significant differences in age (p=0.551) or gender distribution (p=0.902). All subjects received standard, stable doses of their respective TKIs. Treatment durations, creatinine, and vitamin B12 levels did not differ significantly among groups (p=0.184). Table 1.

Comparative Analysis of Metabolic Parameters Across TKI Groups

Analysis of key metabolic parameters indicated notable, drug-specific modifications. Plasma homocysteine (Hcy) concentrations demonstrated significant variation across groups (p<0.001), with the Nilotinib-treated cohort showing the highest mean level at $16.8 \pm 5.9 \mu\text{mol/L}$, followed by the Dasatinib group at $12.5 \pm 3.8 \mu\text{mol/L}$,

and the Imatinib group at $9.2 \pm 2.1 \mu\text{mol/L}$.

Serum parathyroid hormone (PTH) levels exhibited a significant gradient (p<0.001), with the highest concentrations observed in the Dasatinib group ($46.7 \pm 10.2 \text{ pg/mL}$), intermediate levels in the Nilotinib group ($35.1 \pm 11.5 \text{ pg/mL}$), and the lowest in the Imatinib group ($25.8 \pm 8.9 \text{ pg/mL}$). Additionally, a significant difference in serum potassium levels was observed (p=0.005), with the lowest levels in the Dasatinib cohort ($5.2 \pm 0.8 \text{ mmol/L}$). No statistically significant differences were identified for serum calcium (p=0.065) or alkaline phosphatase (ALP, p=0.221) (Table 2).

TKI-Specific Correlation and Clustering Profiles

To examine the relationship between homocysteine and mineral homeostasis, correlation analyses were performed within each TKI group, revealing distinct, statistically significant signatures unique to each drug. Imatinib demonstrated a moderate, inverse correlation between homocysteine and serum calcium (r = -0.41, p = 0.025). Nilotinib showed strong positive correlations between homocysteine and parathyroid hormone (PTH) (r = 0.72, p < 0.001) and between homocysteine and alkaline phosphatase (ALP) (r = 0.55, p = 0.002). Dasatinib presented a unique profile, with a significant positive correlation between homocysteine and potassium (r = 0.47, p = 0.009) (Table 3).

Multivariate Analysis of TKI-Specific Metabolic Signatures

The Multivariate Analysis of Variance (MANOVA) was employed to evaluate whether the combined set of measured metabolic parameters such as homocysteine, PTH, calcium, and others differed significantly across the three TKI groups. This approach offers the advantage of identifying collective variations that may not be apparent when analyzing each parameter individually. Subsequently, Linear Discriminant Analysis (LDA) was applied to visually delineate these distinct, TKI-specific metabolic profiles.

Table 1. Baseline Characteristics of CML Patients Stratified by TKI Regimen

Characteristic	Imatinib (n=30)	Nilotinib (n=30)	Dasatinib (n=30)	p-value
Age (Years)	45.1 ± 12.5	47.3 ± 11.8	43.8 ± 13.1	0.551 ¹
Gender (Male/Female)	16 / 14	17 / 13	15 / 15	0.902 ²
Treatment Duration (Months)	38.5 ± 15.2	34.1 ± 12.8	35.8 ± 14.5	0.184 ¹
Creatinine (mg/dL)	0.79 ± 0.16	0.81 ± 0.14	0.76 ± 0.15	0.432 ¹
Vitamin B12 (pg/mL)	584.2 ± 198.5	623.4 ± 235.1	551.7 ± 241.3	0.458 ¹

Data presented as Mean ± Standard Deviation. ¹One-way ANOVA; ²Chi-square test.

Table 2. Metabolic Parameters Stratified by TKI Regimen

Parameter	Imatinib (n=30)	Nilotinib (n=30)	Dasatinib (n=30)	p-value
Homocysteine (Hcy, $\mu\text{mol/L}$)	9.2 ± 2.1	16.8 ± 5.9	12.5 ± 3.8	<0.001
PTH (pg/mL)	25.8 ± 8.9	35.1 ± 11.5	46.7 ± 10.2	<0.001
Calcium (Ca, mg/dL)	9.3 ± 0.5	9.4 ± 0.4	9.1 ± 0.6	0.065
Potassium (K, mmol/L)	5.7 ± 0.5	5.6 ± 0.6	5.2 ± 0.8	0.005
ALP (U/L)	92.5 ± 13.2	90.1 ± 15.8	96.8 ± 17.1	0.221

Data presented as Mean ± Standard Deviation. P-values from one-way ANOVA.

Table 3. TKI-Specific Correlation Coefficients (r) between Homocysteine and Mineral Parameters

TKI Group	PTH	Calcium (Ca)	Potassium (K)	ALP
Imatinib	0.14	-0.41*	0.22	0.19
Nilotinib	0.72*	-0.21	0.18	0.55*
Dasatinib	0.25	-0.31	0.47*	0.30

Correlation coefficients (r) range from -1 to +1, reflecting the strength and direction of relationships. A positive r indicates a direct association: as homocysteine levels rise, the related parameter also tends to increase. Conversely, a negative r suggests an inverse association: higher homocysteine is associated with a decrease in the other parameter. The absolute value of r determines the strength: values above 0.5 are strong, 0.3 to 0.5 moderate, and below 0.3 weak. Hierarchical clustering of the correlation matrices further emphasized the differences in metabolic network profiles. Imatinib was found to primarily influence a simplified Hcy-Ca axis, while Nilotinib demonstrated a closely interconnected Hcy-PTH-ALP complex. In contrast, Dasatinib exhibited a distinct Hcy-K relationship.* $p < 0.05$

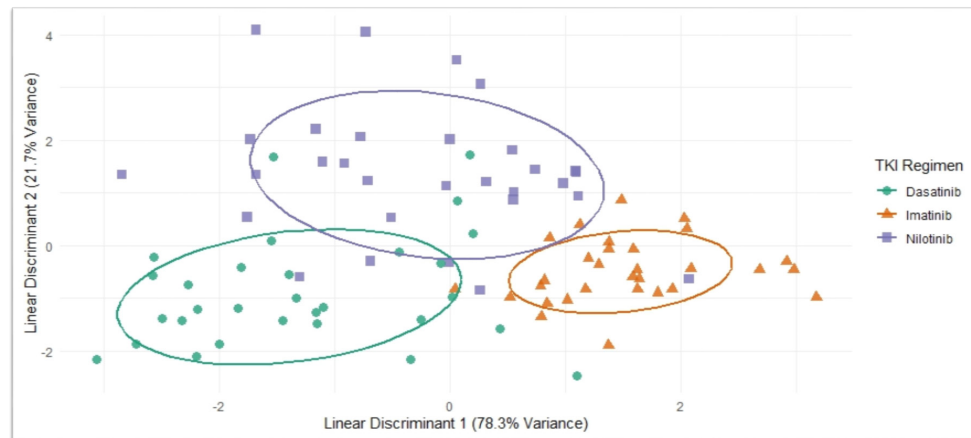


Figure 1. Linear Discriminant Analysis of Metabolic Profiles by TKI Regimen

The findings revealed a highly significant overall effect (Pillai's Trace = 0.674, $p < 0.001$), indicating that the metabolic profiles are clearly segregated based on TKI treatment. To illustrate this clustering, a Linear Discriminant Analysis (LDA) was conducted. The first linear discriminant (LD1), which explained 78.3% of the variance and was primarily influenced by homocysteine (Hcy) and parathyroid hormone (PTH), effectively differentiated the three TKI groups. The second discriminant (LD2), accounting for 21.7% of the variance and affected by potassium and calcium levels, further distinguished the Dasatinib group (Figure 1).

TKI Regimen as a Primary Predictor of Homocysteine Levels

To identify the principal determinants of homocysteine concentrations, a multiple linear regression analysis was employed. This statistical approach effectively isolates the impact of a specific variable in this context, the type

of tyrosine kinase inhibitor (TKI) while accounting for confounding factors such as age, gender, and renal function indicated by creatinine levels.

The results demonstrated that the tyrosine kinase inhibitor (TKI) regimen was the most significant predictor among the analyzed variables. Specifically, patients treated with Nilotinib exhibited homocysteine levels approximately 7.42 $\mu\text{mol/L}$ higher than those receiving Imatinib, after adjusting for confounding factors. Additionally, Dasatinib treatment was associated with a 3.18 $\mu\text{mol/L}$ increase in homocysteine levels. Conversely, variables such as age, gender, and creatinine did not display statistically significant associations with homocysteine concentrations, underscoring the predominant influence of targeted TKI therapy on this metabolic parameter. Table 4,

TKI Treatment Duration and Dosage Profiles

To contextualize the observed metabolic differences,

Table 4. Multiple Linear Regression Model for Predictors of Plasma Homocysteine Levels

Predictor	β Coefficient	Standard Error	Standardized β	t-value	p-value
(Intercept)	8.15	2.10	-	3.88	<0.001
TKI [Nilotinib]	7.42	1.21	0.63	6.13	<0.001
TKI [Dasatinib]	3.18	0.98	0.31	3.24	0.002
Age	0.03	0.03	0.08	1.01	0.315
Gender [Male]	0.45	0.65	0.06	0.69	0.491
Creatinine	0.92	1.01	0.08	0.91	0.366

The reference category for the TKI regimen is Imatinib. The model ($F(4, 85) = 15.32$, $p < 0.001$) explains 41.8% of the variance in homocysteine levels (Adjusted $R^2 = 0.418$). β Coefficient represents the unstandardized coefficient

Table 5. TKI Treatment Duration and Dosage Stratified by Regimen

TKI Regimen	Imatinib (n=30)	Nilotinib (n=30)	Dasatinib (n=30)	p-value
Treatment Duration (Months)	38.5 ± 15.2	34.1 ± 12.8	35.8 ± 14.5	0.184 ¹
Dosage (Most Common)	400 mg OD (100%)	300 mg BID (93.3%)	100 mg OD (96.7%)	-

Data presented as Mean ± Standard Deviation. ¹One-way ANOVA. Abbreviations: OD, Once Daily; BID, Twice Daily

Table 6. Correlation Coefficients (r) between Treatment Duration and Metabolic Parameters by TKI Group

TKI Group	Homocysteine (Hcy)	PTH	Calcium (Ca)	Potassium (K)	ALP
All Patients	0.11	0.08	-0.09	-0.14	0.05
Imatinib	-0.02	0.18	-0.22	0.10	0.13
Nilotinib	0.21	0.15	0.07	-0.18	-0.05
Dasatinib	0.41*	0.28	-0.19	-0.24	0.22

* $p < 0.05$ was considered statistically significant. Correlation analysis was performed using Pearson's correlation test. Correlation coefficients (r) range from -1 to +1, reflecting the strength and direction of relationships. A positive r indicates a direct association: as homocysteine levels rise, the related parameter also tends to increase. Conversely, a negative r suggests an inverse association: higher homocysteine is associated with a decrease in the other parameter. The absolute value of r determines the strength: values above 0.5 are strong, 0.3 to 0.5 moderate, and below 0.3 weak.

the treatment duration and standard dosing regimens across the three TKI groups were analyzed. The mean duration of therapy was similar among patients receiving Imatinib (38.5 ± 15.2 months), Nilotinib (34.1 ± 12.8 months), and Dasatinib (35.8 ± 14.5 months), with no statistically significant difference ($p=0.184$). This consistency in treatment exposure supports the conclusion that the differing metabolic profiles are more likely a direct result of the specific TKI agent rather than variations in therapy duration.

The analysis of dosing patterns showed that patients mainly received standard, clinically approved regimens. All patients in the Imatinib group were on a once-daily 400 mg dose. In the Nilotinib group, most (93.3%) received 300 mg twice daily. Similarly, almost all patients (96.7%) in the Dasatinib group received a once-daily 100 mg dose. This consistent dosing reduces the risk of confounding from varying drug intensity and supports the generalizability of our results to typical clinical settings (Table 5).

Influence of Treatment Duration on Metabolic Parameters

Correlation analysis examining the relationship between treatment duration and metabolic parameters across the entire cohort (N=90) yielded no significant associations, including homocysteine levels ($r = 0.11$, $p = 0.302$). Notably, when stratified by TKI, a significant correlation was observed only in the Dasatinib cohort, in which longer treatment duration correlated with a gradual increase in homocysteine concentrations ($r = 0.41$, $p = 0.025$). No comparable associations reached statistical significance in the Imatinib or Nilotinib groups (Table 6).

Discussion

The introduction of Tyrosine Kinase Inhibitors (TKIs) has significantly altered the prognosis for individuals with Chronic Myeloid Leukemia (CML), establishing it as a manageable chronic illness for most patients. Nonetheless, this achievement has highlighted a new clinical challenge: the long-term management of metabolic toxicities

associated with treatment. Our research aimed to explore this complex issue by moving beyond a simple catalog of side effects to identify the distinct metabolic signatures associated with different TKI agents. The most notable finding of our study is that first-line and second-generation TKIs do not produce identical off-target metabolic effects; each induces a distinct pattern of disruption, particularly regarding the intricate relationship between homocysteine metabolism and mineral balance.

Our findings clearly indicate that second-generation tyrosine kinase inhibitors, particularly nilotinib, are significant contributors to elevated plasma homocysteine levels. Patients undergoing nilotinib therapy demonstrated notably higher homocysteine concentrations compared to those receiving imatinib, with a considerable subset surpassing the established cardiovascular risk threshold. This observation aligns with an accumulating body of recent scholarly research. A recent investigation identified significant hyperhomocysteinemia in patients treated with nilotinib, linking this biochemical imbalance to increased oxidative stress and endothelial damage [30]. The underlying mechanism is presumably due to unintended interactions between targeted pharmacological agents and fundamental cellular biochemical processes. It is hypothesized that tyrosine kinase inhibitors (TKIs), particularly the more potent second-generation TKIs, may interfere with the metabolism or cellular absorption of B vitamins, such as B12 and B6, which serve as essential cofactors for enzymes involved in homocysteine clearance [31].

This process results in a biochemical bottleneck, causing an accumulation of the deleterious amino acid homocysteine. Notably, beyond absolute homocysteine levels, the distinct correlation patterns observed for each tyrosine kinase inhibitor (TKI) offer significant insights. In particular, nilotinib exhibited a remarkably strong positive correlation between homocysteine and parathyroid hormone (PTH). This indicates a potential self-perpetuating cycle unique to this medication, in which elevated homocysteine levels may directly or indirectly stimulate parathyroid gland function. Conversely, the

drug's direct influence on bone and mineral metabolism could indirectly affect homocysteine regulation. The notable correlation between homocysteine concentrations and alkaline phosphatase, a well-established marker of bone turnover, further supports this hypothesis.

This triad of interrelationships delineates a distinctly interconnected "bone-mineral-homocysteine axis" that is specifically disrupted by nilotinib. This observation corroborates findings from a separate investigation indicating that TKI-induced alterations in bone metabolism are drug-specific and may have enduring consequences for skeletal integrity in CML survivors [32-34].

Our results indicate that cardiovascular risk assessment should be integrated with bone health monitoring in patients receiving nilotinib. The cohort treated with dasatinib exhibited a distinct metabolic profile. Although homocysteine levels were elevated relative to imatinib, this increase was less pronounced than that observed with nilotinib. Notably, a significant positive correlation between homocysteine and potassium was identified as a defining characteristic of this group. The physiological underpinnings of this association are not immediately apparent, but may be attributable to dasatinib's well-documented effects on fluid retention and renal tubular dysfunction [35]. It is conceivable that shared mechanisms of transport or renal processing may connect potassium and homocysteine homeostasis [36-38]. Additionally, the positive correlation between treatment duration and homocysteine levels observed solely in the dasatinib group suggests a cumulative, time-dependent toxicity that clinicians should monitor during extended follow-up.

Furthermore, compared with others, the imatinib profile was notably subdued yet retained its unique signature. It was associated with the most favorable homocysteine levels among the three groups. Its main metabolic feature was an inverse relationship between homocysteine and serum calcium. This finding supports existing research on imatinib's effects on bone metabolism, including cases of hypocalcemia linked to the inhibition of off-target kinases, such as (Platelet-Derived Growth Factor Receptor) PDGFR, which are involved in bone remodeling [39].

This indicates that the primary metabolic pathway of imatinib involves a simplified homocysteine-calcium axis, highlighting its unique off-target kinase inhibition profile in comparison to second-generation agents. The effectiveness of a multivariate approach was validated by our advanced statistical models, which distinguished the three TKI groups solely on the basis of their combined metabolic parameters. This study provides strong, evidence-based support for the conclusion that the metabolic profiles identified are not incidental but are closely associated with specific TKI regimens. It underscores the idea that selecting a particular TKI influences the patient's metabolic pathway and the associated risks. The translation of these findings into clinical practice is critical. The paradigm for managing chronic myeloid leukemia (CML) should shift from an exclusive emphasis on molecular responses to a more personalized, preemptive strategy addressing metabolic health. For patients beginning nilotinib therapy, there may be an increased risk

for hyperhomocysteinemia and its related cardiovascular and skeletal complications. This warrants baseline and periodic assessment of homocysteine levels, parathyroid hormone (PTH), and alkaline phosphatase (ALP), in addition to standard lipid and glucose measurements. Additionally, this underscores the need to investigate the potential of B-vitamin supplementation (such as B12 and folic acid) as a preventive intervention, an area currently under clinical investigation [40].

For patients treated with dasatinib, it is essential to recognize the potential for time-dependent elevations in homocysteine levels and the intriguing association with potassium homeostasis. This underscores the importance of implementing tailored monitoring protocols and maintaining a low threshold for evaluating renal electrolyte regulation in symptomatic cases. The comparatively benign homocysteine profile observed with imatinib offers reassurance; however, continued vigilance regarding calcium metabolism and bone health remains advisable.

This study's limitations are acknowledged. Its cross-sectional design permits identification of associations but does not allow for definitive conclusions regarding causality. The absence of detailed dietary data, especially concerning B vitamin intake, introduces a confounding factor, given the diet's substantial influence on homocysteine levels. Future research should involve prospective, longitudinal studies that include comprehensive dietary assessments, precise treatment duration, and vitamin level measurements to validate and extend these findings. Such studies are also needed to explore direct clinical outcomes, such as fracture incidence and cardiovascular events, associated with these metabolic profiles.

In conclusion, this study offers a detailed, TKI-specific characterization of the metabolic disturbances associated with prolonged CML therapy. It is demonstrated that nilotinib, dasatinib, and imatinib possess distinct off-target profiles, each exerting a unique influence on homocysteine metabolism and its interactions with essential minerals and hormones. These findings compellingly support a shift in clinical approach towards personalized follow-up care, in which the selection of a TKI guides a customized monitoring strategy. By systematically identifying and addressing metabolic side effects, clinicians can aim not only for durable leukemia remission but also for the preservation of long-term cardiovascular, metabolic, and skeletal health, thereby aligning survival quality with its duration.

Author Contribution Statement

Design and development: Kawakeb N Abdulla1, Istikrar M. Hade. Gathering and organizing data: Kawakeb N Abdulla, Azal Hamoody Jumaa. Data analysis/interpretation: Azal Hamoody Jumaa, Shallal Murad Hussein. Article composition: Istikrar M. Hade, Azal Hamoody Jumaa. Critique the essay for significant ideas: Istikrar M. Hade, Shallal Murad Hussein. Statistical analysis expertise: Ahmed Hazem Abdulkareem , Youssef Shakuri Yasin. Ultimate article endorsement

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Declaration of Generative AI and AI-assisted technologies in the writing process

The authors state that they did not use any generative AI or AI-assisted tools in this work.

Conflicts of interest

The authors declare they have no conflicts of interest.

Abbreviations

ALP: Alkaline Phosphatase
ANOVA: Analysis of Variance
Ca: Calcium
CML: Chronic Myeloid Leukemia
ECLIA: Electrochemiluminescence Immunoassay
Hcy: Homocysteine
IRB: Institutional Review Board
ISE: Ion-Selective Electrode
LDA: Linear Discriminant Analysis
LD1: First Linear Discriminant
LD2: Second Linear Discriminant
MANOVA: Multivariate Analysis of Variance
OD: Once Daily
BID: Twice Daily
pNPP: p-Nitrophenyl Phosphate
PTH: Parathyroid Hormone
TKI: Tyrosine Kinase Inhibitor

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