Original Research Article

The Egyptian Journal of Immunology
Volume 30 (2), 2023: 59–72.
www.Ejimmunology.org

Kinetic of liver enzymes and serum electrolytes in HCV, HBV, and HIV negative chronic phase chronic myeloid leukemia patients treated with imatinib or nilotinib

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Abstract

The outcome for chronic phase (CP) chronic myeloid leukemia (CML) patients was changed dramatically since the introduction of tyrosine kinase inhibitor (TKI) therapy. This study intended to evaluate side effects of TK Imatinib or Nilotinib on liver enzymes and serum electrolytes in relation to hematologic and molecular response in HCV-, HBV-, and HIV-, CP-CML patients. The study was a quasi-experimental pre-post single group design, included 38 HCV-, HBV-, and HIV-newly diagnosed Philadelphia positive CP-CML patients with normal hepatic and renal function. They were divided equally into two groups, 19 received Nilotinib, and 19 received Imatinib. Hematologic, BCR-ABL gene expression by RT-PCR, electrolytes and liver enzymes were measured at baseline and after 6 months of treatment. Patients age ranged between 20 and 62 years. Anemic manifestations represented the highest rate (n=23, 60.5%). The mean WBCs count was significantly reduced after treatment (p<0.001). The WBCs count was significantly reduced in the Nilotinib group than the Imatinib group (97% and 94%, respectively, p=0.049). The mean hemoglobin level was significantly increased after treatment (p=0.010). The mean platelet level did not change over the treatment period. The mean AST, ALT, and ALP levels were significantly increased after treatment, (p=0.014, p=0.002, and p=0.047, respectively). The ALP level was significantly increased in both groups (p=0.001). The mean sodium potassium, phosphorous, and calcium level was not changed over the treatment period. The mean BCR-ABL gene expression was sharply decreased after treatment (p<0.001). A higher reduction was observed in the Nilotinib group (99%) than the Imatinib group (91.5%) (p=0.025). Imatinib resulted in rise of AST and ALP levels than Nilotinib, while both had the same effect on the ALT level. Higher reduction in BCR-ABL gene expression was achieved by Nilotinib. Nilotinib and Imatinib did not affect serum levels of sodium, potassium, phosphorous, or calcium.

Keywords: CML, TKI, Imatinib, Nilotinib, ALT, AST, ALP, Phosphorus, calcium, Potassium, sodium.

Date received: 19 December 2022; **accepted:** 22 February 2023

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder with clonal expansion of differentiated myeloid cells and accounts for 15% of adult leukemias.[1] CML is characterized by increased proliferation and accumulation of the granulocytic cell line, without loss of differentiation.[2] BCR-ABL protein, a cancer-causing protein, has an active tyrosine kinase region of ABL, resulting in continuous cell growth and proliferation.[3]

Imatinib was the first Food and Drug Administration (FDA, USA) approved tyrosine kinase inhibitors (TKIs) drug used as first-line treatment for newly diagnosed CML patients and those who were resistant or intolerant to interferon.[4] Second generation TKIs as dasatinib, nilotinib, and bosutinib were primarily approved in CML patients who were resistant or intolerant to imatinib. Later, these second generation TKIs were approved in the first line setting.[5] Life expectancy for CML patients has substantially improved since the advent of TKIs.[6]

Molecular monitoring of BCR-ABL1 by real-time quantitative polymerase chain reaction (RT-qPCR) every 3 months is recommended to assess response to TKIs.[7] Plasma levels of soluble vascular cell adhesion molecule 1 (sVCAM-1), and transforming growth factor beta 1 (TGFβ1) are considered as predictors of hematological and molecular responses to TKIs in CML patients.[8] Also, interleukin 10 (IL-10) is a predictors of hematological and molecular responses to in CML patients and also, associated with advanced stage of multiple myeloma.[9,10] Tuberculosis (TB) and hepatitis C virus (HCV) coinfected patients showed higher serum levels of IL-10.[11] Lower programmed death-ligand 1 (PD-L1) expression promotes sustained deep molecular response (DMR) in CML patients,[12] and plays a role in maintaining complete hematologic response (CHR).[13] Also, lower PD-L1 expression promotes good response to induction therapy in acute myeloid leukemia (AML) patients.[14]

The incidence of non-HIV-associated hematologic malignancies, including chronic myeloproliferative disorders, is increasing in HIV-infected (HIV+) patients.[15] Nearly 50% of oral drugs on the market are associated with hepatotoxicity.[16] Unfortunately, TKIs are no exception.[17] To date, more than 25 clinical cases of imatinib induced hepatitis have been reported.[18] Formation of reactive metabolites play a key role in TKI-induced hepatotoxicity.[19]

Tyrosine kinase inhibitor drugs including imatinib, dasatinib, nilotinib, bosutinib and axitinib had dose-dependent hyponatremia through induction of syndrome of inappropriate antidiuretic hormone secretion (SIADH) syndrome.[20] Imatinib can induce hypophosphatemia and inappropriate phosphaturia through tubular damage and secondary hyperparathyroidism due to decreased calcium levels.[21]

The aim of the current study was to evaluate side effects of tyrosine kinase inhibitors Imatinib (GLEEVEC ®) or Nilotinib (TASIGNA®) on liver enzymes and serum electrolytes in relation to hematologic response in HCV-, HBV-, and HIV-chronic phase CML patients.

Patients and Methods

Ethical considerations

The protocol of the study was reviewed and approved by the Medical Ethics Committee of Faculty of Medicine, Assiut University, Egypt, (approval No. 17101136, dated July 2020). All lines of treatment for CML were explained to each patient before enrollment in the study with explanations of the advantages and disadvantages of each line. The right to withdraw from the study at any time was emphasized. An informed written consent was obtained from each patient before enrollment.

The current study was a quasi-experimental pre-post single group design. The study included 38 patients with newly diagnosed, HCV-, HBV-, and HIV-, Philadelphia positive chronic phase (CP) CML patients with normal hepatic and renal function tests. They were referred to the Hematology Unit, Internal Medicine Department, Assiut University Hospital, Assiut, Egypt from January 2020 till February 2022. Their ages ranged from 20 to 62 years. They included 22 males and 16 females.

The exclusion criteria included: blast or accelerated CML phase, patients with renal or hepatic impairment, CML patients who have previously been treated with TKI, HCV and/or HBV positive patients, HIV positive patients, those with end organ failure, and patients with comorbidities to interfere with TKI treatment.

Assessment of patients' response to treatment

These included complete hematologic response (CHR), complete normalization of peripheral blood counts with leukocyte count <10 x 10^9/L; platelet count <450 x 10^9/L; no immature cells, such as myelocytes, promyelocytes, or blasts in peripheral blood; and no signs and symptoms of disease with resolution of palpable splenomegaly.

Molecular response, included early molecular response (EMR): BCR-ABL1 (IS) <10% at 3 and 6 months; major molecular response (MMR): BCR-ABL1 (IS) <0.1% or >3-log reduction in BCR-ABL1 transcripts from the standardized baseline, if qPCR (IS) was not available; and deep molecular response (DMR): MR4.0: BCR-ABL1 (IS) <0.01% MR4.5: BCR-ABL1 (IS) <0.0032%

Relapse was defined as any sign of loss of hematologic response; and log increase in BCR-ABL1 transcript levels with loss of MMR.

Methods

All study patients were diagnosed through history taking, clinical examination, complete blood count, bone marrow examination (aspiration), and genetic study by the polymerase chain reaction (RT-PCR) for BCR-ABL gene. They were treated by administration of tyrosine kinase inhibitors (Imatinib 400mg/day or Nilotinib 600mg/day.

Blood samples

At the time of diagnosis, venous blood samples (about 7 ml) were collected from each study subject under aseptic conditions. Each sample was divided into 2 parts. The first part (3 ml) was collected into one tube containing anticoagulant (K3 EDTA) and used for complete blood count (CBC) and reticulocyte count and differential picture. The second part (4 ml) was allowed to clot in Wassermann tubes. Sera were obtained by centrifugation and used for biochemical and immunological testing.

Complete blood count was carried out using an automated hematology system (ADVIA 2120i Hematology System, Siemens Healthcare Diagnostics Inc. Tarrytown, NY 10591, USA) according to the manufacturer's instructions, at the Hematology Laboratory, Clinical Pathology Department, Assiut University Hospitals.

Bone marrow aspiration and examination was done for diagnosis and cytogenetic studies, under complete aseptic conditions according to the method described by Paiva et al., 2018.[22.]

Biochemical investigations included liver function tests, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum alkaline phosphatase. Serum electrolytes included sodium, potassium, calcium, phosphorus. These investigations were performed using an automated blood chemistry analyzer (Siemens ADVIA instruments, Germany), according to the manufacturer's instructions.

Immunological markers included antinuclear antibodies (ANA), antimitochondrial antibodies (AMA) and anti-smooth muscle antibodies (ASMA) were done on an immune diagnostic system (Orgentec Diagnostic GmbH, Germany), according to the manufacturer's instructions, to exclude all immune mediated increase in liver enzymes. To exclude change in hepatitis induced liver enzymes, anti-HCV Ab, anti-HIV Ab, and HBS Ag assay were done using an immunoassay analyzer (ARCHITECT i1000sr immunology analyzer, Abbott, Germany), according to the manufacturer's instructions.

Molecular analysis

- RNA extraction and cDNA synthesis

Extraction of total RNA was carried from whole blood by TRIzol® reagent (TRI Reagent L.S). The extracted RNA was reverse-transcribed to cDNA by using gene-specific primer (ABL-1).

- RT-PCR conditions

The primers sequences used for qualitative RT-PCR were BCR-ABL1; Forward primer: 5'-ACTCCAGACTGTCCACAGCA-3', and Reverce

primer: 5'-TTGGGGTCATTTTCACTGG-3'. DNA amplification was done in 30 µL reaction mixture, included 15µL PCR Mix (Invitrogen PCR Super Mix includes PCR buffer, 22 mM Tris HCL, pH 8.4), 1.65mM MgCL2, 220µM dNTPs, and 22 U recombinant TaqDNA polymerase/mL), 1 µL of 10 pM Primer Mix (forward and reverse primers), 0.25µL enzyme RT (SuperScript™ III Reverse Transcriptase 200 U/µL Invitrogen, USA), 5µL template RNA, and 9 µL nuclease-free water. The thermal cycling conditions were reverse transcription at 50 C for 40min for one cycle, then 30 cycles each of denaturation at 94 °C for 15s, annealing at 63 °C for 30s, and extension at 72 C for 45s.

Statistical analysis

Data were verified, coded by the researcher, and analyzed using IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA). Descriptive statistics: Means, standard deviations, medians, ranges, frequency, and percentages were calculated. Normality of continuous variables was tested using Kolmogorov–Smirnov test/Shapiro–Wilk test as appropriate. For continuous variables with more than two interval measurements; one-way repeated measure ANOVA (RM-ANOVA) test was calculated to test the mean differences of the data that follow normal distribution and had repeated measures, post-hoc test was calculated using Bonferroni corrections for pairwise comparisons between the study intervals. Significance was considered at p <0.05.

Results

The baseline characteristics of the studied cohort are presented in Table 1. The age of the participating ranged between 20 and 62 years with a median of 42 and a mean of 41.7 ± 11.5 years. Also, 22 (58%) were males. Anemic manifestations represented the highest rate (n=23, 60.5%), followed by abdominal discomfort (n=15, 39.5%), hepato-splenomegaly (n=11, 29%), splenomegaly (n=10, 26%), bleeding tendency (n=5, 13%) and others (n=9, 24%). Contrarily, only 10.5% (n=4) was discovered accidentally. Regarding drug treatment, half of the cases (n=19) started Imatinib at dose of 400 mg/day, about 44.7% (n=17) started Nilotinib at dose of 600 mg/day and only two cases (5.3%) started Nilotinib at dose of 800 mg/day.

Table 1. Baseline Characteristics of the 38 studied Cohort.

Variable	Category	
Age/years	Mean ± SD	41.66 ± 11.5
	Median (Range)	42 (20 – 62)
Sex	Female	16 (42.1%)
	Male	22 (57.9%)
Clinical Manifestations	No Complaint Accidentally Discovered	4 (10.5%)
	Anemic Manifestation	23 (60.5%)
	Abdominal Discomfort	15 (39.5%)
	Hepato-Splenomegaly	11 (28.9%)
	Splenomegaly	10 (26.3%)
	Bleeding Tendency	5 (13.2%)
	Others	9 (23.7%)
Treatment Type	Imatinib	19 (50%)
	Nilotinib	19 (50%)
Starting Dose	Imatinib 400 mg/day	19 (50%)
	Nilotinib 600 mg/day	17 (44.7%)
	Nilotinib 800 mg/day	2 (5.3%)

The mean WBCs count showed significantly steady reduction after treatment (p<0.001), Figure 1. The mean hemoglobin level showed significantly (p=0.010) increase after treatment (Table 2). The mean platelet level did not change over the treatment period (p=0.285), Table 3.

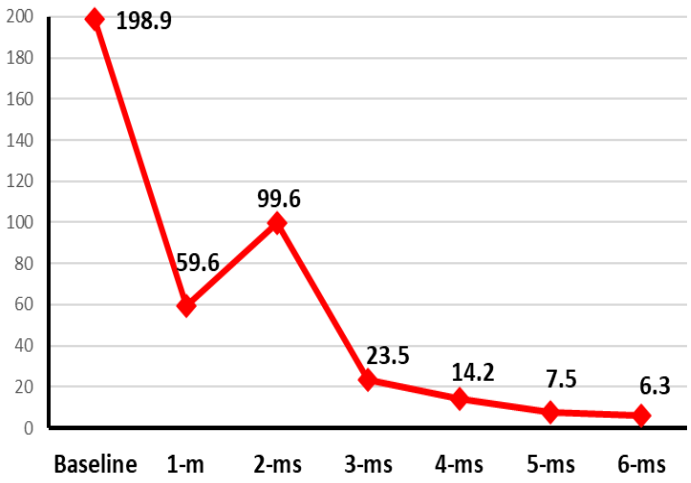

Figure 1. Mean WBCs Level of the studied Cohort over Time

Table 2. Effect of Treatment on the Hemoglobin Level.

HGB (g/dl)	Mean± SD	*p*-value**		
Baseline (1)	9.63 ± 1.7	1 vs. 2=0.974 (NS)	2 vs. 4=0.040	3 vs. 7=0.006
1-month (2)	9.61 ± 1.4	1 vs. 3=0.957 (NS)	2 vs. 5=0.059 (NS)	4 vs. 5=0.777 (NS)
2-months (3)	9.60 ± 1.6	1 vs. 4=0.191 (NS)	2 vs. 6=0.007	4 vs. 6=0.199 (NS)
3-months (4)	10.31 ± 1.4	1 vs. 5=0.212 (NS)	2 vs. 7=0.005	4 vs. 7=0.058 (NS)
4-months (5)	10.27 ± 1.4	1 vs. 6=0.070 (NS)	3 vs. 4=0.073 (NS)	5 vs. 6=0.062 (NS)
5-months (6)	10.52 ± 1.3	1 vs. 7=0.035	3 vs. 5=0.079 (NS)	5 vs. 7=0.030
6-months (7)	10.77 ± 1.5	2 vs. 3=0.971 (NS)	3 vs. 6=0.021	6 vs. 7=0.108 (NS)
p-value*	0.010			

*Repeated Measure ANOVA test was used to compare the mean difference between groups over time. **Pairwise comparison on single time interval (Mann-Whitney U-test). *P* > 0.05 is not significant (NS).

Table 3. Effect of Treatment on the Platelet Count

Platelet Count $\times 10^3$ /µl	Mean± SD	*p*-value**		
Baseline (1)	411.25 ± 68.1	1 vs. 2=0.279 (NS)	2 vs. 4=0.241 (NS)	3 vs. 7=0.509 (NS)
1-month (2)	356.55 ± 21.8	1 vs. 3=0.383 (NS)	2 vs. 5=0.635 (NS)	4 vs. 5=0.390 (NS)
2-months (3)	383.75 ± 77.1	1 vs. 4=0.161 (NS)	2 vs. 6=0.432 (NS)	4 vs. 6=0.248 (NS)
3-months (4)	300.05 ± 54.2	1 vs. 5=0.830 (NS)	2 vs. 7=0.956 (NS)	4 vs. 7=0.211 (NS)
4-months (5)	389.85 ± 51.8	1 vs. 6=0.859 (NS)	3 vs. 4=0.084 (NS)	5 vs. 6=0.783 (NS)
5-months (6)	379.05 ± 35.1	1 vs. 7=0.199 (NS)	3 vs. 5=0.949 (NS)	5 vs. 7=0.607 (NS)
6-months (7)	355.10 ± 17.8	2 vs. 3=0.501 (NS)	3 vs. 6=0.857 (NS)	6 vs. 7=0.362 (NS)
p-value*	0.285 (NS)			

*Repeated Measure ANOVA test was used to compare the mean difference between groups over time. **Pairwise comparison on single time interval (Mann-Whitney U-test). *P* > 0.05 is not significant (NS).

The mean AST level was significantly increased after treatment (*p*=0.014), Table 4. The mean ALT level was significantly steady increased after treatment (*p*=0.002), Table 5. The mean ALP level was significantly steadily increased after treatment (*p*=0.047), Table 6. The mean sodium, potassium, calcium, and phosphorous levels did not change over the treatment period.

Table 4. Effect of Treatment on the AST Level.

AST (U/L)	Mean± SD	*p*-value**		
Baseline (1)	23.17 ± 8.4	1 vs. 2=0.396 (NS)	2 vs. 4=0.175 (NS)	3 vs. 7=0.003
1-month (2)	24.44 ± 9.1	1 vs. 3=0.334	2 vs. 5=0.126 (NS)	4 vs. 5=0.499 (NS)
2-months (3)	24.28 ± 8.3	1 vs. 4=0.038	2 vs. 6=0.006	4 vs. 6=0.008
3-months (4)	26.58 ± 8.3	1 vs. 5=0.056	2 vs. 7=0.001	4 vs. 7=0.003
4-months (5)	27.44 ± 9.2	1 vs. 6=0.004	3 vs. 4=0.104 (NS)	5 vs. 6=0.002
5-months (6)	29.86 ± 9.1	1 vs. 7=0.002	3 vs. 5=0.132 (NS)	5 vs. 7=0.001
6-months (7)	31.08 ± 9.1	2 vs. 3=0.911	3 vs. 6=0.010	6 vs. 7=0.091 (NS)
p-value*			0.014	

*Repeated Measure ANOVA test was used to compare the mean difference between groups over time. **Pairwise comparison on single time interval (Mann-Whitney U-test). *P* > 0.05 is not significant (NS).

Table 5. Effect of Treatment on the ALT Level.

AST (U/L)	Mean± SD	*p*-value**		
Baseline (1)	20.11 ± 7.4	1 vs. 2=0.001	2 vs. 4=0.076 (NS)	3 vs. 7<0.001
1-month (2)	23.53 ± 8.3	1 vs. 3=0.146 (NS)	2 vs. 5=0.004	4 vs. 5=0.026
2-months (3)	22.33 ± 9.1	1 vs. 4=0.003	2 vs. 6<0.001	4 vs. 6<0.001
3-months (4)	26.91 ± 9.3	1 vs. 5<0.001	2 vs. 7<0.001	4 vs. 7<0.001
4-months (5)	29.83 ± 9.6	1 vs. 6<0.001	3 vs. 4=0.031	5 vs. 6=0.005
5-months (6)	32.33 ± 9.8	1 vs. 7<0.001	3 vs. 5=0.004	5 vs. 7=0.037
6-months (7)	33.99 ± 9.9	2 vs. 3=0.342 (NS)	3 vs. 6<0.001	6 vs. 7=0.246 (NS)
p-value*			0.002	

*Repeated Measure ANOVA test was used to compare the mean difference between groups over time. **Pairwise comparison on single time interval (Mann-Whitney U-test). *P* > 0.05 is not significant (NS).

Table 6. Effect of Treatment on the ALP Level.

ALP (U/L)	Mean± SD	*p*-value**		
Baseline (1)	86.28 ± 8.1	1 vs. 2=0.011	2 vs. 4=0.031	3 vs. 7=0.002
1-month (2)	98.83 ± 7.1	1 vs. 3=0.004	2 vs. 5=0.018	4 vs. 5=0.423 (NS)
2-months (3)	96.50 ± 11.5	1 vs. 4=0.004	2 vs. 6=0.006	4 vs. 6=0.102 (NS)
3-months (4)	107.27 ± 13.6	1 vs. 5=0.003	2 vs. 7=0.003	4 vs. 7=0.048
4-months (5)	109.97 ± 14.7	1 vs. 6<0.001	3 vs. 4=0.030	5 vs. 6=0.086 (NS)
5-months (6)	115.66 ± 18.8	1 vs. 7<0.001	3 vs. 5=0.026	5 vs. 7=0.039
6-months (7)	120.35 ± 14.3	2 vs. 3=0.495	3 vs. 6=0.006	6 vs. 7=0.026
p-value*			0.047	

*Repeated Measure ANOVA test was used to compare the mean difference between groups over time. **Pairwise comparison on single time interval (Mann-Whitney U-test). *P* > 0.05 is not significant (NS).

The mean BCR-ABL gene expression showed significantly sharp decrease after treatment (*p*<0.001) Table 7. The mean BCR-ABL gene expression at baseline (61.4 ± 7.1) was significantly higher compared with 3-months level (10.3 ± 2.4, *p*<0.001) and 6-months (3.1 ± 0.7, <0.001). Likewise, the mean BCR-ABL gene expression at 6-months was significantly lower than 3-months (<0.001).

Table 7. Effect of Treatment on the BCR-ABL Gene Expression.

BCR-ABL Gene Expression%	Mean± SD	*p*-value**
Baseline (1)	61.42 ± 7.1	1 vs. 2<0.001
3-months (2)	10.27 ± 2.4	2 vs. 3<0.001
6-months (3)	3.05 ± 0.7	1 vs. 3<0.001
p-value*	< 0.001	

*Repeated Measure ANOVA test was used to compare the mean difference between groups over time. **Pairwise comparison on single time interval (Mann-Whitney U-test). *P* > 0.05 is not significant (NS).

The mean AST level was comparable in the two groups over the study period from baseline till 6-months with no difference (*p*> 0.05). Moreover, there was significant rise in the AST level in Imatinib group when compared to the level at 6-months with the baseline value (*p*=0.038). However, there was no rise in the Nilotinib group (*p*=0.321). Also, significant difference was found for the interaction between time and treatment type (p=0.024) i.e., Imatinib group had more effect on the rise of AST level (Figure 2).

The mean ALT level was comparable in the two groups over the study period from baseline till 6-months with no difference (*p*> 0.05). Moreover, there was significant rise in the ALT level in Nilotinib group when compared to the level at 6-months with the baseline value (*p*=0.008). However, there was no rise in the Imatinib group (*p*=0.138). Further, no difference was found for the interaction between time and treatment type (*p*=0.238) i.e., the rise of ALT level was equal in both groups (Figure 3).

The mean ALP level was comparable in the two groups over the study period from baseline till 6-months with no difference (*p*> 0.05). Moreover, there was significant rise in the ALP level in both groups when compared to the level at 6-months with the baseline value (*p*=0.001 and =0.038). As well, significant difference was found for the interaction between time and treatment type (*p*=0.044) i.e., the rise of ALP level was more significant in the Imatinib group (Figure 4).

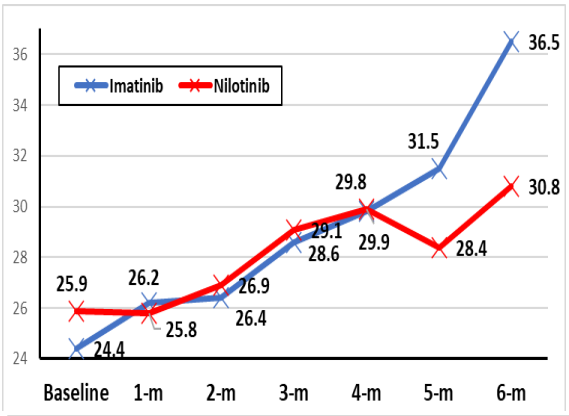

Figure 2. Mean AST in Imatinib vs. Nilotinib Treatment Modality

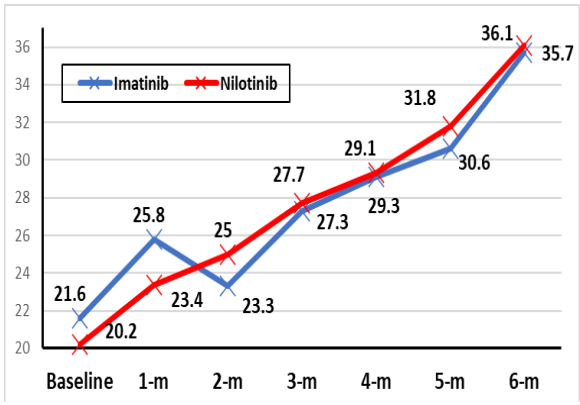

Figure 3. Mean ALT in Imatinib vs. Nilotinib Treatment Modality.

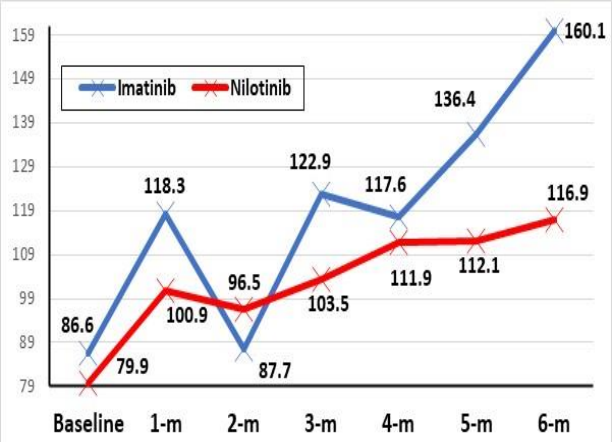

Figure 4. Mean ALP in Imatinib vs. Nilotinib Treatment Modality

The mean serum electrolyte parameters levels (Na, K, Ca, P) were within the normal range and comparable in the two groups over the study period from baseline till 6-months with no difference (p>0.05). Moreover, no change was observed when comparing the level at 6-months with the baseline value in both groups (p>0.05). Also, the change of serum electrolyte parameters over time in both groups was similar (Table 8).

Table 8. Comparison of Mean Serum Electrolyte Parameters between Groups.

(Mean ± SD)	Imatinib (n=19)	Nilotinib (n=19)	*p*-value*
Na			
Baseline	137.84 ± 3.9	138.37 ± 3.3	= 0.657 (NS)
1-month	138.38 ± 4.3	138.62 ± 3.5	= 0.663 (NS)
2-months	136.78 ± 2.9	137.94 ± 4.3	= 0.472 (NS)
3-months	138.44 ± 2.3	138.50 ± 3.5	= 0.953 (NS)
4-months	138.80 ± 3.2	137.79 ± 3.3	= 0.410 (NS)
5-months	138.18 ± 2.9	138.69 ± 3.4	= 0.670 (NS)
6-months	138.47 ± 2.5	137.94 ± 3.2	= 0.588 (NS)
P-value**	= 0.416	= 0.602	*p****=0.249 (NS)
K			
Baseline	3.86 ± 0.4	3.94 ± 0.7	= 0.683 (NS)
1-month	3.81 ± 0.3	3.82 ± 0.6	= 0.485 (NS)
2-months	3.62 ± 0.3	3.71 ± 0.7	= 0.664 (NS)
3-months	3.86 ± 0.2	3.91 ± 0.6	= 0.202 (NS)
4-months	3.67 ± 0.3	3.77 ± 0.7	= 0.931 (NS)
5-months	3.65 ± 0.3	3.96 ± 0.6	= 0.114 (NS)
6-months	3.66 ± 0.5	3.84 ± 0.6	= 0.332 (NS)
P-value**	= 0.307	= 0.688	*p****=0.526 (NS)
Ca			
Baseline	8.67 ± 0.5	8.73 ± 0.4	= 0.472 (NS)
1-month	8.74 ± 0.3	8.65 ± 0.5	= 0.550 (NS)
2-months	8.73 ± 0.5	8.62 ± 0.4	= 0.524 (NS)
3-months	8.66 ± 0.3	8.72 ± 0.5	= 0.690 (NS)
4-months	8.64 ± 0.4	8.64 ± 0.3	= 0.985 (NS)
5-months	8.59 ± 0.4	8.78 ± 0.5	= 0.292 (NS)
6-months	8.68 ± 0.6	8.71 ± 0.6	= 0.918 (NS)
P-value**	= 0.401	= 0.073	*p****=0.391 (NS)

Table 8. Continued.

(Mean ± SD)	Imatinib (n=19)	Nilotinib (n=19)	*p*-value*
Phosphorous			
Baseline	3.62 ± 0.6	3.58 ± 0.6	= 0.822 (NS)
1-month	3.44 ± 0.5	3.58 ± 0.5	= 0.459 (NS)
2-months	3.58 ± 0.5	3.57 ± 0.6	= 0.968 (NS)
3-months	3.48 ± 0.4	3.39 ± 0.6	= 0.612 (NS)
4-months	3.50 ± 0.5	3.24 ± 0.4	= 0.159 (NS)
5-months	3.41 ± 0.6	3.22 ± 0.4	= 0.114 (NS)
6-months	3.34 ± 0.4	3.33 ± 0.6	= 0.268 (NS)
P-value**	= 0.279	= 0.180	*p****=0.236 (NS)

*Independent t-test was used to compare the mean differences. ** One-way Repeated Measure ANOVA was used to compare the mean differences over time. ***Two-way Repeated Measure ANOVA was used to test interaction between group and time. *P* > 0.05 is not significant (NS).

Figure 5 illustrates the differences in the mean percent change of the CBC parameters and the BCR-ABL Gene expression. There was significantly higher reduction in the WBCs count in the Nilotinib group (97%) than the Imatinib group (94%) (p=0.049). Likewise, there was significantly higher reduction in the BCR-ABL Gene expression in the Nilotinib group (99%) than the Imatinib group (91.5%) (p=0.025). However, there was no difference in the HB level and platelet count in Nilotinib group (21% and 11%) than the Imatinib group (14% and 5.5%) (*p*=0.106 and =0.559).

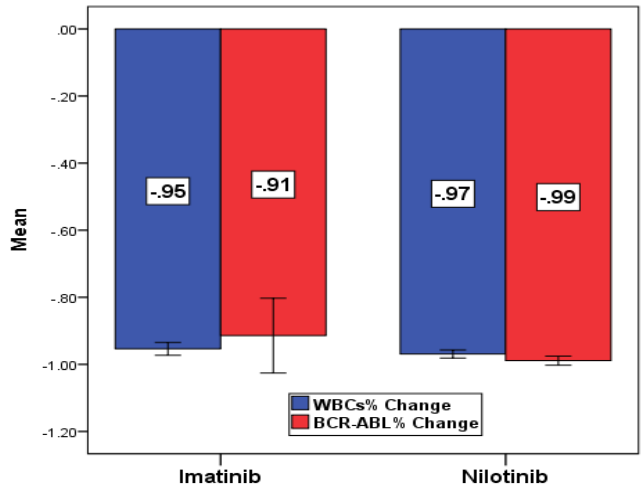

Figure 5. Differences in % change of WBCs count and BCR-ABL between group.

Discussion

The current study aimed to evaluate side effects of tyrosine kinase inhibitors (TKI) Imatinib and Nilotinib on liver enzymes and serum electrolytes in relation to hematologic response in HCV-, HBV-, and HIV-chronic phase CML patients. This study compared two important TKI and shedding a light on possible side effects in such longer period of treatment to reach complete remission.

The demographic data of the current cohort indicated a mean age of 41.7 ± 11.5 years, and 58% were males. This come in agreement with Chang et al., (2015) who showed that the males were affected more than the females and chronic phase of CML was common in the younger age group.[23] A study by Mendizabal et al., (2013) in low- and middle-income countries showed that the mean age of diagnosis was 38.5 years. Asians were youngest (38.3 years) patients, Africans (39.5 years), then Southern/Eastern Europeans (41.1 years) and Latin Americans were the oldest with (41.3) years.[24]

In the current study, anemic manifestations represented the highest rate (60.5%), followed by abdominal discomfort (39.5%), hepato-splenomegaly (29%), splenomegaly (26%), bleeding tendency (13%) and others (24%). Contrarily, only 10.5% was discovered accidentally.

Many literatures showed that about 50% of CML patients were asymptomatic and the remaining presented with anemia, splenomegaly, fever, bleeding tendency, hepatomegaly, lymphadenopathy and complications such as renal failure, hearing loss and priapism.[23,25,26] During the current study, the mean WBCs count showed significantly steady reduction after treatment (p<0.001). This can be supported by Jbireal et al., (2019) who reported that treatment of CML patients with Imatinib mesylate resulted in significant WBCs reduction to the normal range (p< 0.0001).[27]

Also, our results agreed with those of Jain et al., (2013) who reported that after 12 months of treatment with Imatinib, 95% of patients achieved complete hematological response in Imatinib treated patients when compared with untreated patients. WBC counts showed a significantly better profile in the Imatinib group (p<0.0001).[28] Moshfeghi et al., (2015) reported that treatment with Imatinib resulted in decreasing counts of white blood cells.[29] Furthermore, the study by Nakahara et al., (2019) reported that Nilotinib treatment of CML cases resulted in decreasing white blood cell counts. [30]

The present study showed significant increase in the mean hemoglobin level after treatment (p=0.010). These data agreed with those of Jbireal et al., (2019) who revealed that hemoglobin concentration was increased significantly in patients treated with Imatinib mesylate (p=0.003). They reported increase of HB after 4 doses (11.91g/dl), 8 doses (12.12g/dl), 12 doses (11.27g/dl), and 16 doses (11.91g/dl) post-treatment, when compared with untreated patients (10.16g/dl).[27] The current study showed that HG levels did not change in the Nilotinib treated group (21%) than the Imatinib group (14%). However, Moshfeghi et al., (2015) reported that Imatinib treatment resulted in increased hemoglobin concentration.[29]

The current study showed that the mean platelets level was insignificantly changed over the treatment period (p=0.285). There was no increase in platelet count in Nilotinib group (11%) than the Imatinib group (5.5%) (p=0.106 and p=0.559). This agreed with those reported by Alqasim et al., (2018) who showed that Nilotinib treatment had no adverse effect on platelets.[31] However, Jbireal et al., (2019) reported that blood platelets count decreased significantly after 4,8,12, and 16 doses post–treatment, (232 $x10^9$/L, 244.3 $x10^9$/L, 237.1 $x10^9$/L, and 284.4 $x10^9$/L, respectively) as compared to the untreated patients (336.6 $x10^9$/L) (p=0.008).[27]

The present study showed that the mean AST level was significantly increased after Imatinib treatment (p=0.014). Moreover, there was significant rise in the AST level in the Imatinib treated group when comparing the level at 6-months with the baseline value (p=0.038). However, there was no change in the AST level as a result in the Nilotinib treated group (p=0.321). Also, significant difference was found for the interaction between time and treatment type (p=0.024) i.e., Imatinib treatment had more effect on the rise of AST level.

The current study showed significantly steady increase in the mean ALT level after treatment (p=0.002). The mean ALT level was comparable in the two treatment groups over the study period from baseline till 6-months (p> 0.05). Moreover, there was significant rise in the ALT level in Nilotinib group when comparing the level at 6-months with the baseline value (p=0.008). However, there was no rise in the Imatinib group (p=0.138). Furthermore, no difference was found for the interaction between time and treatment type (p=0.238) i.e., the rise of the mean ALT level was similar in both groups.

As well, the mean ALP level showed significantly steady increase after treatment (p=0.047). The mean ALP level was comparable in the two groups over the study period from baseline till 6-months with no difference (p > 0.05). Moreover, there was significant rise in

the ALP level in both treatment groups when compared the level at 6-months with the baseline value (p=0.001 and =0.038). As well, significant difference was found for the interaction between time and treatment type (p=0.044) i.e., the rise of the mean ALP level was more significant in the Imatinib group.

However, Moshfeghi et al., (2015) reported that changes in liver enzymes (ALT and AST) were not clinically significant however, the changes in these enzymes were statistically significant.[29] A previous study reported a low-grade elevation in serum ALT and/or AST in 25% to 30% and a high-grade elevation in approximately 2% of patients treated with TKIs.[32] Also, Khelifa et al., (2022) reported that elevated hepatic enzymes (grade 3 or 4) were seen in 10% to 15% of patients treated with Nilotinib but rarely progress to hepatitis.[33]

The systematic review and meta-analysis by *Wang et al., (20121)* which included 9 trials involving 3475 patients showed that patients received new-generation TKIs were more likely to experience all grades of ALT and AST elevation compared with those received Imatinib (p<0.001). New-generation TKIs drugs were associated with a significantly higher rate of MMR at 1 year compared with Imatinib (p< 0.001). Also, a significant increase in the risk of hepatotoxicity was associated with the use of Bosutinib, Nilotinib, and Ponatinib compared with Imatinib.[34] Also, the meta-analysis by Teo et al., (2013) found elevation in alanine transaminase (Pooled OR 5.22, 95% CI 2.88–9.46), aspartate transaminase (Pooled OR 6.15, 95% CI 3.09–12.25) and total bilirubin (Pooled OR 1.76, 95% CI 0.59–5.24) was higher with the use of TKI drugs than compared to the controls.[35]

In the current study, the mean sodium, potassium, calcium, and phosphorus serum levels showed no change over the treatment period. Also, in the current study the mean serum electrolyte levels (Na, K, Ca, P) showed no difference between Imatinib and Nilotinib groups (p>0.05). Moreover, no change was observed when comparing the level at 6-months with the baseline value in both groups (p>0.05). Also, no difference was found for the interaction between time and treatment type (p=0.024) i.e., the change of serum electrolyte parameters over time in both groups was similar. In agreement with our results a study by Moshfeghi et al., (2015) reported that Imatinib treatment resulted in no change in all serum electrolytes.[29]

A previous study indicated that Imatinib and Nilotinib acted on both the BCR-ABL1 gene and the platelet derived growth factor which may cause kidney disorders and affect blood potassium levels.[36] A retrospective study by Marcolino et al., (2011), included 100 CML in chronic phase (CML-CP) patients, and another study by *Yilmaz et al., (2015)* included 253 patients with CML-CP treated with Imatinib, reported that the patient's blood potassium level was not increased.[37,38]

In China, a study by Wang et al., (2015) reported that the decrease in blood potassium levels of all grades was more prevalent in CML-CP patients treated with Imatinib (50%) compared to Nilotinib (21.1%). The decrease in blood calcium levels of all grades was more common in patients taking Imatinib compared to Nilotinib (50.8% vs 34.6%).[17]

A study by Berman et al., (2006), included 63 CML patients who were treated with Imatinib reported that these patients had low-normal blood calcium levels and low blood phosphate levels when compared to the study control group.[39] A prospective study conducted by Hasan et al., (2015), included 30 Nilotinib-treated CML patients showed a significant decrease in blood calcium levels when compared to the study control group; whereas the comparison of blood calcium levels before and after taking Nilotinib also showed a decrease, but not significantly.[40]

The current study also showed that the mean BCR-ABL gene expression was sharply decrease after treatment (p<0.001). The mean BCR-ABL gene expression at baseline (61.4 ± 7.1) was significantly higher compared with 3-months level (10.3 ± 2.4, p<0.001) and 6-months (3.1 ± 0.7, <0.001). Likewise, the mean BCR-ABL gene expression at 3-months was significantly higher than 6-months (<0.001). There was significantly higher reduction in the BCR-ABL gene expression in the Nilotinib

treated group (99%) than the Imatinib treated group (91.5%) (p=0.025).

Our results agreed with those reported by Hochhaus et al., (2013) who revealed that deeper molecular responses and more selective BCR-ABL inhibition was achieved with Nilotinib in comparison to Imatinib.[41] Also, in agreement with our findings, a study by Kantarjian et al., (2011) revealed that significantly more patients in the Nilotinib treated groups achieved a complete molecular response than did those in the Imatinib group. The study concluded that Nilotinib continues to show better efficacy than Imatinib for the treatment of patients with newly diagnosed CML in chronic phase.[42] As well, Saglio et al., (2010) concluded that Nilotinib was shown to be a more potent inhibitor of BCR-ABL than Imatinib.[43]

In conclusion, Imatinib treatment resulted in rise of AST and ALP levels than Nilotinib, while both had the same effect on the rise of ALT level. Higher reduction in BCR-ABL gene expression was achieved by Nilotinib. Neither Nilotinib nor Imatinib had effect on serum levels of sodium, potassium, phosphorous, or calcium.

Author Contributions

MRA, HAN, ASA and conceived and designed the research. NMM and ASA recruited patients. ASA carried out the clinical investigations. MRA, NMM, ASA and HAN collected clinical data. MRA, HAN, ASA, NMM contributed to the interpretation of data for the work. MRA prepared the original draft of the manuscript. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Medical Ethics Committee of Faculty of Medicine, Assiut University, Assiut, Egypt, (approval No. 17101136, dated July 2020).

Informed consent

An informed written consent in Arabic language (the native language of the study participants) was obtained from each subject involved in the current study before enrollment.

References

1. Maher M, Samra M, Abdelfatah R et al. (2019). Imatinib Generic in Newly Diagnosed Patients with Chronic Myeloid Leukemia in Chronic Phase: Egyptian National Cancer Institute Experience. Clinical Lymphoma, Myeloma and Leukemia. Sep 1; 19: S296-7.

2. Khair HE, Elderdery AY, Nour BY et al. (2021). BCR-ABL KINASE DOMAIN MUTATIONS-E255K, Y253 H AND M351T AMONG SUDANESE POPULATION WITH CML. 12, 112-118.

3. Schiffer CA. (2002). BCR-ABL tyrosine kinase inhibitors for chronic myelogenous leukemia. New England Journal of Medicine. Jul 19; 357(3):258-65.

4. Kantarjian H, Sawyers C, Hochhaus A, et al. (2002). Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. New England Journal of Medicine. Feb 28;346(9):645-52.

5. García-Gutiérrez V, Hernández-Boluda JC. (2019). Tyrosine kinase inhibitors available for chronic myeloid leukemia: efficacy and safety. Frontiers in oncology. Jul 3; 9:603.

6. Lin Q, Mao L, Shao L, et al. (2020). Global, Regional, and National Burden of Chronic Myeloid Leukemia, 1990-2017: A Systematic Analysis for the Global Burden of Disease Study 2017. Front Oncol. 10:2789.

7. Radich JP, Deininger M, Abboud CN, et al. (2018). Chronic myeloid leukemia, version 1.2019, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. Sep 1;16(9):1108-35.

8. Abdel Hammed MR, Ahmed YA, Adam EN, et al. (2022). sVCAM-1, and TGFβ1 in chronic phase, chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. Egypt J Immunol. Oct;29(4):163-173. PMID: 36208045.

9. Mohammed, D., Khallaf, S., El-Naggar, M., et al, (2021). Interleukin-10: A Potential Prognostic Marker in Patients with Newly Diagnosed Multiple

Myeloma. Research in Oncology, 17(1), 38-41. doi: 10.21608/resoncol.2021.51503.1127

10. María Jazmín Toloza, Yesica Bestach, Marco Lincango-Yupanki, et al, (2022). Expression dynamics of the immune mediators ARG1, TBET, CIITA, IL10 and TGFB1 in chronic myeloid leukaemia patients during the first year of imatinib therapy. Gene; 813 146110. https: //doi.org/10.1016/j.gene.2021.146110

11. El-Mokhtar MA, Elgendy SG, Eldin AS. et al. (2020). Hepatitis C Virus Affects Tuberculosis Specific T Cells in HIV-Negative Patients. Viruses. 15; 12(1):101. doi: 10.3390/v12010101. PMID: 31952232; PMCID: PMC7019953.

12. Amy Hughes, Jade Clarson, Carine Tang, et al. (2016). CML patients with deep molecular responses to TKI have restored immune effectors and decreased PD-1 and immune suppressors, Blood, Volume 129, Issue 9,2017, Pages 1166-1176, ISSN 0006-4971, https://doi.org/10.1182/blood--10-745992.

13. Lee MY, Park CJ, Cho YU. et al. (2020). Differences in PD-1 expression on CD8+ T-cells in chronic myeloid leukemia patients according to disease phase and TKI medication. Cancer Immunol Immunother 69, 2223–2232. https://doi.org/10.1007/s00262-020-02617-5

14. Abdel Hafeez LA, Mansor SG, Zahran AM et al. (2021). Expression of programmed death ligand-1 (PDL-1) in Acute Myeloid Leukemia Patients and its relation to post induction Response. SECI Oncology Journal, 9(2): 106-111. doi: 10.21608/secioj.2021.170554

15. Robert Schlaberg, Julie G., Michael J. et al. (2008). Chronic myeloid leukemia and HIV-infection, Leukemia & Lymphoma, 49:6, 1155-1160, DOI: 10.1080/10428190802074601

16. Weng Z, Wang K, Li H, et al. (2015). A comprehensive study of the association between drug hepatotoxicity and daily dose, liver metabolism, and lipophilicity using 975 oral medications. Oncotarget. Jul 7;6(19):17031.

17. Apperley JF. (2015). Chronic myeloid leukaemia. The Lancet. Apr 11;385(9976):1447-59.

18. Lopina, N., Dmytrenko, I., Hamov, et al. (2019). Novel Score-based Decision Approach in Chronic Myeloid Leukemia Patients After Acute Toxic Imatinib-induced Liver Injury. Cureus 11(4):e4411.

19. Teo Y L, Ho H K, Chan A, et al. (2015). Formation of reactive metabolites and management of tyrosine kinase inhibitor-induced hepatotoxicity: a literature review. Expert Opin Drug Metab Toxicol 11(2):231-42.

20. Hill J, Shields J, Passero V et al. (2016). Tyrosine kinase inhibitor-associated syndrome of inappropriate secretion of anti-diuretic hormone 22(5):729-732.

21. François H, Coppo P, Hayman JP, et al. (2008). Partial Fanconi Syndrome Induced by Imatinib Therapy: A Novel Cause of Urinary Phosphate Loss. American Journal of Kidney Diseases 51(2):298-301.

22. Paiva A S, Paiva H, Cavalcanti G B, et al. (2018). Detection of the BCR-ABL Gene By the Real-Time PCR Method in Patients with Chronic Myeloid Leukemia in Rio Grande Do Norte, Brazil. Blood132(Supplement 1):5432-5432

23. Chang F, Qazi RA, Khan M, et al. (2015). Clinico hematological profile and phase distribution of chronic myeloid leukemia. Biology and Medicine.;7(5):1.

24. Mendizabal AM, Garcia-Gonzalez P, Levine PH. et al. (2013). Regional variations in age at diagnosis and overall survival among patients with chronic myeloid leukemia from low- and middle-income countries. Cancer Epidemiology. Jun 1;37(3):247-54.

25. Gamal AH. (2013). The clinical and laboratory presentation of CML. Clinical hemotology 5th edition springer publication.:126-39.

26. Sharma P, Singh T. (2013). Bone Marrow Histology in CML: A Continuing Relevance. Journal of Bone Marrow Research. Mar 27:1-2.

27. Jbireal JM, Azab AE, Alzahani S, et al, (2019). Haematological and cytogenetic changes in CML patients treated with imatinib mesylate in Western Libya. Hematol Transfus Int J.;7(3):50-7.

28. Jain P, Das VN, Ranjan A, et al, (2013). Comparative study for the efficacy, safety and quality of life in patients of chronic myeloid leukemia treated with Imatinib or Hydroxyurea. Journal of Research in Pharmacy Practice. Oct;2(4):156.

29. Moshfeghi K, Nazemizadeh N, Mehrzad V, et al, (2015). Hematologic, liver enzymes and electrolytes changes in chronic myeloid leukemia after Imatinib medication. Indian Journal of Cancer. Jul 1;52(3):305.

30. Nakahara R, Sumimoto T, Ogata M, et al, (2019). Successful determination of nilotinib dosage by therapeutic drug monitoring in a patient with chronic myeloid leukemia developing hepatic dysfunction: A case report. Clin Case Rep. Jun 14; 7(7):1419-1421. doi: 10.1002/ccr3.2191. PMID: 31360502; PMCID: PMC6637326.

31. Alqasim AMZ, Obaid GM, Yaseen YG, et al, (2018). Effects of nilotinib on platelet function in patients with chronic myeloid leukemia in chronic phase. Leuk Res Rep. Jun 19; 11:46-50.

32-Shah RR, Morganroth J, Shah DR. (2013). Hepatotoxicity of tyrosine kinase inhibitors: clinical

and regulatory perspectives. Drug safety. Jul;36(7):491-503.

33. Khelifa L, Ben Amor I, Kassar O, et al, (2023). Life threatening hepatotoxicity induced by Nilotinib. J Oncol Pharm Pract. Mar; 29(2):465-468. doi: 10.1177/10781552221102588. Epub 2022 Jun 1. PMID: 35651314.,

34. Wang Z, Wang X, Wang Z, et al, (2021). Comparison of Hepatotoxicity Associated with New BCR-ABL Tyrosine Kinase Inhibitors vs Imatinib Among Patients with Chronic Myeloid Leukemia: A Systematic Review and Meta-analysis. JAMA Network Open. Jul 1;4(7): e2120165.

35. Teo Y L, Ho HK, Chan A et al. (2013). Risk of tyrosine kinase inhibitors-induced hepatotoxicity in cancer patients: a meta-analysis. Cancer Treat Rev 39(2):199-206.

36. Prenggono MD, Yasmina A, Ariyah M, et al. (2021). The effect of imatinib and nilotinib on blood calcium and blood potassium levels in chronic myeloid leukemia patients: a literature review. Oncol Rev. Nov 26;15(2):547. doi: 10.4081/oncol.2021.547. PMID: 34976304; PMCID: PMC8649642.

37. Marcolino MS, Boersma E, Clementino NCD, et al. (2011). Imatinib treatment duration is related to decreased estimated glomerular filtration rate in chronic myeloid leukemia patients. Ann Oncol; 22:2073-9

38. Yilmaz M, Lahoti A, O'Brien S, et al. (2015). Estimated glomerular filtration rate changes in patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. Cancer 121:3894-904

39. Berman E, Nicolaides M, Maki RG, et al. (2006). Altered bone and mineral metabolism in patients receiving imatinib mesylate. N Engl J Med; 354:2006-13

40. Hasan BF, Matti BF, Hameed RY, et al. (2015). Evaluation of electrolytes disturbances in Iraqi chronic myeloid leukemia patients treated with nilotinib with monitoring of response by FISH study. Baghdad Sci J 12:110-8.

41. Hochhaus A, Saglio G, Larson RA, et al. (2013). Nilotinib is associated with a reduced incidence of BCR-ABL mutations vs imatinib in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Blood. May 2;121 (18):3703-8.

42. Kantarjian HM, Hochhaus A, Saglio G, et al. (2011). Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. Lancet Oncol. Sep;12(9):841-51. doi: 10.1016/S1470-2045(11)70201-7. Epub 2011 Aug 17. Erratum in: Lancet Oncol. 2011 Oct;12(11):989.

43. Saglio G, Kim DW, Issaragrisil S, et al. (2010). ENESTnd Investigators. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. Jun 17;362(24):2251-9. doi: 10.1056/NEJMoa0912614. Epub 2010 Jun 5.