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Effects of nilotinib on platelet function in patients with chronic myeloid leukemia in chronic phase

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ABSTRACT

Background: Tyrosine kinases are highly expressed in platelets and play an important role in their activation process. Some studies have reported the blocking effects of tyrosine kinase inhibitors on different platelet functions.

Objectives: Evaluate the effects of nilotinib on platelets aggregation in 42 patients with chronic phase of CML and correlate the results with clinical and hematological parameters: age, complete blood count and presentation.

Patients and methods: This study was conducted on 42 patients diagnosed as Chronic Phase of Chronic Myeloid Leukemia based on clinical, morphological and cytogenetic study. All patients were on Nilotinib treatment and were attending the National Center of Hematology in Baghdad. About 9 mL of venous blood sample were collected from each patient and control subjects, samples divided into 3 parts for complete blood count, platelet aggregation test and PT and aPTT.

Results: The mean age was 41.3 ± 1.7 (mean ± SEM) years old. M:F ratio of 1.2:1. Mean duration of nilotinib therapy(1.4 years). All patients had normal PT and aPTT.

Only 16 (38%) patients had abnormal aggregation response to epinephrine, but there was no statistically significant differences with control group.

Conclusion: Nilotinib had no adverse effect on platelet function nor patients clotting tests.

1. Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm, which results from the neoplastic transformation of hematopoietic stem cells [1].

Chronic myeloid leukemia represents about 14% of all leukemias and 20% of adult leukemias worldwide. The annual incidence is approximately 1.6 cases per 100,000 adults with a slight male preponderance [2]. The median age of onset is 50–60 years [3].

Tyrosine kinase inhibitors (TKIs) are orally administered agents that compete with adenosine triphosphate (ATP) for its binding site on ABL, leading to abolishing tyrosine phosphorylation of the proteins involved in BCR-ABL signal transduction and finally resulting in apoptosis of the cancer cell [4].

Nilotinib is a new, orally active, tyrosine kinase inhibitor that is more potent than is imatinib. Nilotinib functions through competitive inhibition at the ATP-binding [5,6].

Some studies proved that tyrosine kinase inhibitors, such as imatinib and ponatinib, are associated with several complications, including bleeding diathesis as well as thrombosis [7]. While the third-generation inhibitor ponatinib can inhibit primary hemostasis even in the absence of thrombocytopenia the second-generation inhibitor nilotinib is not commonly associated with bleeding diathesis [7,8,9]. In contrast, a high prevalence of platelet dysfunction was demonstrated in CML patients receiving all types of TKIs [10].

A stepwise tests are required when investigating platelet function disorders. Some of the tests are: complete blood count and blood film, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), bleeding time or platelet function analysis with the PFA-100 and tests of platelet aggregation [11].

2. Platelet aggregometry

Light transmission aggregometry is still the most used test for the

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identification and diagnosis of platelet function disorders [12].

3. Patients and methods

This case control study was conducted from June 2014 to December 2014 on 42 patients diagnosed as chronic phase of Chronic Myeloid Leukemia based on clinical, morphological and cytogenetic study. All patients were on nilotinib therapy as a second line treatment after a failure of imatinib therapy, and patients' compliance with therapy were ensured.

The patients were attending the National Center of Hematology in Baghdad. Clinico-hematological parameters including the age and gender of the patients, stage of disease, duration of treatment of imatinib and nilotinib and clinical presentation were obtained from the available case sheets of the patients.

The control group consists of 30 age and gender matched healthy persons. The work was approved by ethical council of Iraqi Committee for Medical Specialization in accordance with Hilsinki declaration. Verbal consent was obtained from each patient. The work was conducted at the Laboratory of National Center of Hematology and Al-Yarmouk Teaching Hospital Laboratory in Baghdad.

4. Specimen collection

About 9 mL of venous blood sample were collected from each patient and control subjects and was processed as follows:

1. About 5 ml of blood was anticoagulated with buffered trisodium citrate for platelet function test and the anticoagulant volume had been calculated using special formula [13].
2. Two ml blood was put in ethylenediaminetetra-acetic acid (EDTA) to perform complete blood count.
3. About 1.8 ml venous blood was put into a seperated plastic tube containing 0.2 ml trisodium citrate dehydrate, then centrifuged without delay at 2500 g for 15 min to prepare platelet poor plasma for measurement PT and aPTT.

4.1. Platelet function test

In this study the platelet function test was performed using light transmission aggregometer of BIO/DATA corporation, the platelet aggregation profiler 8E (PAP-8E), which is PC-controlled. The work done according to manufacturer instructions [13].

4.2. Interpretation and analysis

The primary method currently used to analyze and interpret aggregation results is pattern recognition (visual comparison). The comparison is between the patterns from the patient sample and the known donor sample. Minor numeric differences in curve parameters are not clinically significant [14].

4.3. Statistical analysis

Data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2010. Numeric data were expressed as mean ± SD. Student *t*-test and ANOVA were used to analyze numeric data while Chi-square was used to analyze discrete data. Pearson correlation was used to determine relation between maximum aggregation and duration of treatments. *P*- Value of less than 0.05 was considered significant.

5. Results

The mean age of patients in this study was 41.3 ± 10.9 years old (Mean ± SD). There was slight male predominance with M: F ratio 1.2:1. Other patients and control characteristics are shown in table 1. The frequency of cytopenia showed in Fig. 1.

Table 1
Clinical parameters of patients and control cases.

	Patients	Controls	*P* value (*t*-test)
Age (years)	Mean 41.3 ± 10.8 Range 18–65	36.8 ± 8.9	0.070
Gender: **M**	23(54.8%)	12(40%)	0.217
F	19 (45.2%)	18 (60%)	
Imatinib duration (years)	Mean 5.9 ± 2.8 Range(1–10)	-	-
Nilotinib duration (years)	Mean 1.4 ± 0.8 Range (0.5–3.5)	-	-
WBC ($\times 10^9$/L)	Mean 6.7 ± 1.8	6.9 ± 1.9	0.608
HCT %	Mean 41.2 ± 4.8	40 ± 4.7	0.332
Platelets ($\times 10^9$/L)	Mean 217.9 ± 47.4	258 ± 71.0	0.005*
PT (second)	Mean 13.4 ± 1.3	13.5 ± 1.3	0.633
PTT (second)	Mean 33.5 ± 2.5	33.3 ± 2.7	0.810
Presentation		-	-
splenomegaly	42(45.3%)		
Anemia	9(21.4%)		
Fever, weight loss	5(11.9%)		
Bleeding	3(7.1%)		
Non-specific	6(14.3%)		

Table 2
The relation between platelet aggregation response and hematological parameters of cases (Platelets count and hematocrit).

	Count	**Platelets** Mean ± SD	*P* value (*t*-test)	**HCT%** Mean ± SD	*P* value (*t*-test)
	Low	72. 7 ± 4.9		88.3 ± 6.3	
ADP %			0.031*		0.005*
	Normal	82.9 ± 7.8		80.3 ± 7.6	
	Low	77. 7 ± 4.1		86.7 ± 5.1	
Collagen %			0.294		0.007*
	Normal	82.0 ± 6.9		80.2 ± 6.6	
	Low	29.0 ± 44.1		56.6 ± 32.1	
Epinephrine %			0.143		0.925
	Normal	57.8 ± 31.4		55.5 ± 33.3	
	Low	1.0 ± 1.7		1.9 ± 2.3	
RIPA (0.5 mg) %			0.513		0.706
	Normal	2.4 ± 3.5		2.4 ± 3.7	
	Low	81. 7 ± 3.2		87.7 ± 2.4	
RIPA (1.2 mg) %			0.403		0.019*
	Normal	84.4 ± 5.6		83.2 ± 5.7	

In this study all 42 patients had normal platelet aggregation response upon stimulation with ADP, collagen, RIPA 0.5 mg and RIPA 1.2 mg. Whereas 16 (38%) out of 42 patients had abnormal platelet aggregation on stimulation with epinephrine, but there was no statistically significant differences in comparison with control group $p = 0.341$.

Fig. 2 shows impaired aggregation response to epinephrine with normal aggregation response to other agonists.

Mean maximum aggregation response in patients to ADP, collagen, epinephrine, RIPA 0.5 mg and RIPA 1.2 mg were (Mean ± SD): 82.2 ± 8.0, 81.7 ± 6.8, 55.7 ± 32.6, 2.3 ± 3.4 and 84.2 ± 5.4 respectively. Whilst the mean MA for control group in response to ADP, collagen, epinephrin, RIPA0.5 mg and 1.2 mg were: 83.4 ± 7.0, 77.7 ± 8.7, 62.9 ± 27.8, 2.0 ± 1.8 and 84.6 ± 6.1 respectively as shown in Fig. 3. There were no statistically significant differences.

By applying Pearson correlation, there was positive correlation between duration of nilotinib treatment and the platelet aggregation with epinephrine and negative correlation with ristocetin 1.2 mg, this association was statistically significant in regard to epinephrine but statistically insignificant regarding response to ristocetin (Fig. 4 and 5). Furthermore, no correlation observed with other clinical parameters.

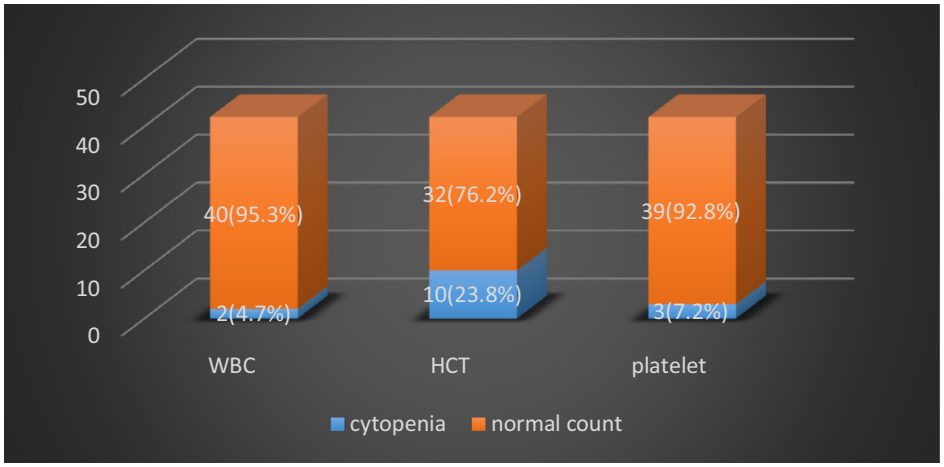

Fig. 1. Frequency of cytopenias The figure shows the percentage of cases who had a cytopenia compared to other cases who had a normal WBC, Hct and Platelet counts.

By applying *t*-test there was statistically significant difference between low and normal HCT with platelet aggregation response on stimulation with ADP, collagen and ristocetin 1.2 mg, while it was not significant regarding stimulation with epinephrine and low concentration of ristocetin. Additionally, there was statistically significant difference between low and normal platelets count with platelet aggregation response on stimulation with ADP, while it was not significant regarding stimulation with epinephrine, collagen and both low and high dose ristocetin as shown in Table 2.

6. Discussion

The incidence of leukemia in Iraq about 4.43/100 000 population per year, and it is occupying the third rank of the commonest ten cancers in Iraq according to Iraqi cancer registry in 2011 [15].

In the current study the mean age and M: F ratio were in concordance to registered data of chronic myeloid leukemia cases that were obtained from Baghdad teaching hospital from 2003–2015, the number of all patients receiving Imatinib (2618 case), about 405 of them were receiving Nilotinib as a second line of treatment, and also to data of National Center of Hematology (280 registered CML cases on imatinib) and approximately 55 patients were shifted to nilotinib as a second line of treatment.

These results comparable to other Iraqi studies done in 2013 and 2012 [16,17]. Similar results were also reported in Saudi Arabia study [18].

The mean age of CML patients was slightly higher in Turkish study (46 years old) [19]. But it is lower in Iran (34 years old) [20] and Pakistan [21]. These differences may be attributable to differences in sample size in each study and type of patients or may be because of different reported ethnic groups.

There was slight male predominance in this study which was concordant with the results of other Iraqi studies [16,17,22], and with Turkish results [19].

At time of aggregation test patients had different types of cytopenias as shown in Fig. 1. Worldwide studies proved that: the most common adverse events associated with nilotinib therapy were thrombocytopenia (20 − 33%) and neutropenia (13 − 31%) [4,5]. This also agreed with Tasigna prescribing information by (Novartis,2014) [23]. Similarly study by Kantarjian et al showed that 11% have anemia, 31% neutropenia and 30% have thrombocytopenia [24]. In spite that current study agreed with others but these data may not be statistically representative because of small sample size and differences in patients selection (patients with platelets count less than 100 were excluded), but they still give simple idea about the side effect of nilotinib.

The PT and aPTT in this study were normal in all patients, this agreed with Cardama et al study, [7] suggesting that all patients in this study have an intact secondary heamostasis at time of test performance.

All 42 patients enrolled in this study had normal platelet aggregation response upon stimulation with ADP, collagen and ristocetin in low

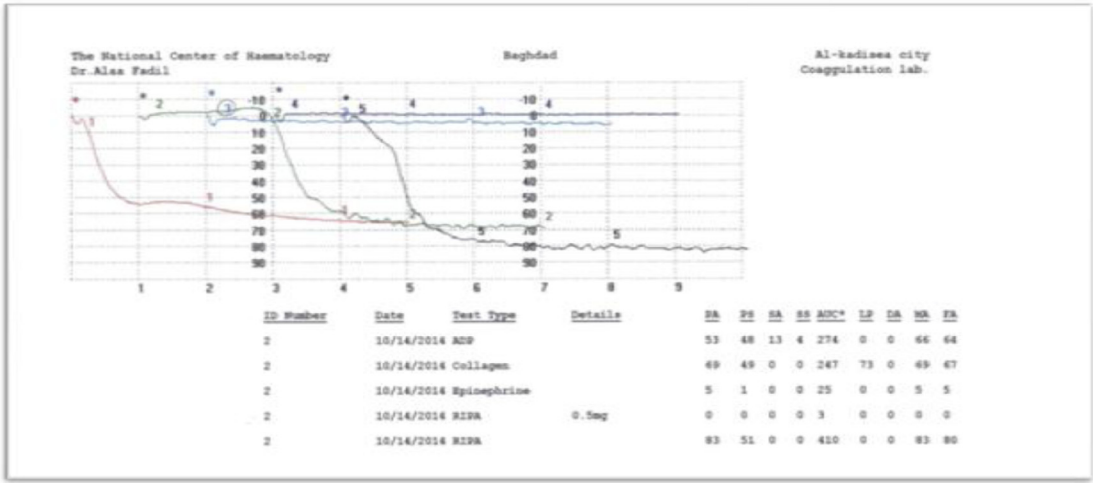

Fig. 2. Impaired aggregation response to epinephrine with normal aggregation response to other agonists.

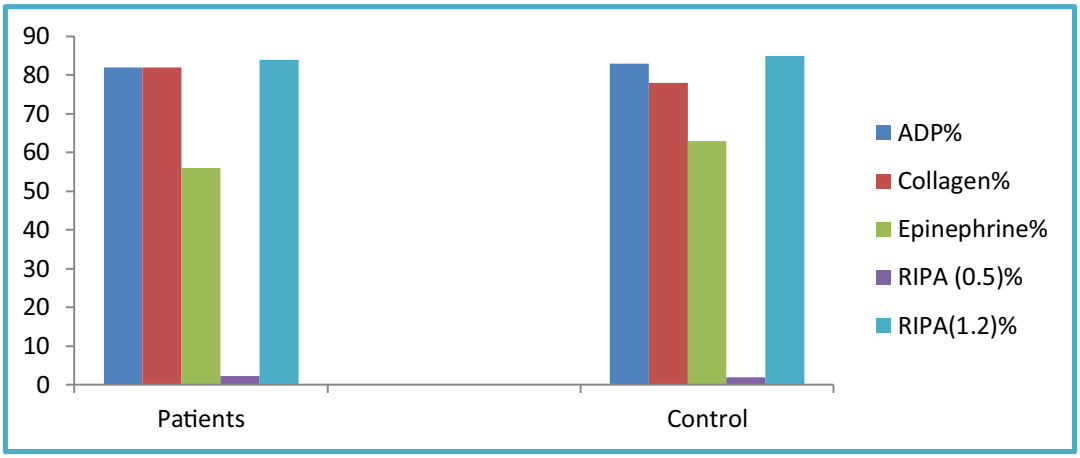

Fig. 3. Mean maximal aggregation response to various agonists.

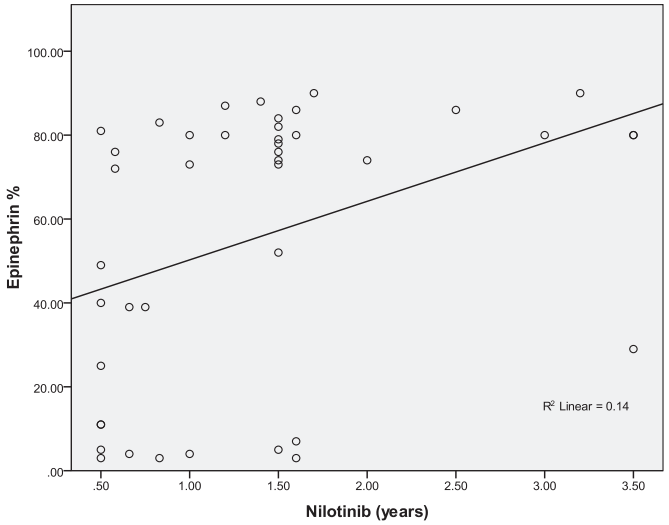

Fig. 4. Correlation between maximum platelet aggregation response to epinephrine and duration of nilotinib treatment.

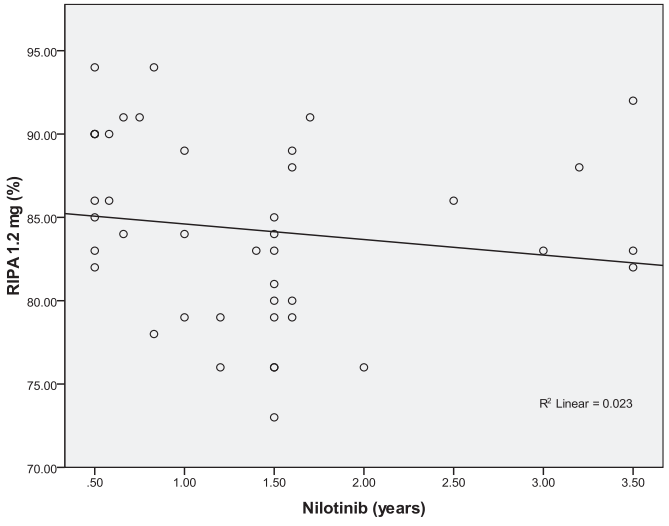

Fig. 5. Correlation between maximum platelet aggregation response to high dose ristocetin and duration of nilotinib treatment.

and high dose, whereas 16/42 (38%) patients had abnormal aggregation with epinephrine. The abnormal response to epinephrine alone is clinically insignificant since some normal people have severely reduced responses to epinephrine [25]. Accordingly all patients had no risk of bleeding.

These results comparable to Cardama et al results that nilotinib showed normal aggregation response to ADP, collagen and ristocetin in low and high dose and even epinephrine [7]. Additionally study by Loren et al proved that, treatment with nilotinib had little effect on platelet aggregation and lag time in response to collagen [8]. In contrast, Thailand study showed significant platelet dysfunction in patients who received nilotinib; impaired platelet aggregation response (<60%) to collagen, arachidonic acid and epinephrine was observed in 53, 33 and 47%, respectively [10].

This disagreement may be due to the difference in the duration of nilotinib treatment among patients and differences in sample size and ethnic group may also relevant, since the frequencies of different fusion oncogenes associated with leukemia can vary in different ethnic groups [26].

In comparison to other studies conducted on different types of TKIs in CML patients: Wangsuekul and his team concluded that there was significant platelet dysfunction among patients who received all type of TKIs including nilotinib, imatinib and dasatinib [9].

While Cardama, et al stated that nilotinib had normal aggregation response to all agonists in contrast to other TKIs like: Dasatinib in which (85%) exhibited reduced epinephrine induced platelet aggregation, and normal platelet aggregation with other agonists. On the other hand, (33%) of patients on Imatinib had normal platelet aggregation, and (66%) had impairment in arachidonic acid, including (13%) with impaired epinephrine aggregation while all have normal aggregation with ADP, collagen and ristocetin [7].

Furthermore Loren et al examined the effects of BCR-ABL inhibitors on platelet aggregation in response to the GPVI agonist collagen-related peptide, and showed that nilotinib and imatinib had minimal effects on collagen induced platelet aggregation [8].

The possible causes for these variations is the difference in mechanisms of action of each TKI since most of the tyrosine kinase inhibitors used were nonselective, and it is difficult to ascertain a role for the different tyrosine kinases present in platelet.

Interestingly, like imatinib, nilotinib inhibits Bcr-Abl by binding to an inactive, DFG-out conformation of the ABL kinase domain, thus blocking the tyrosine phosphorylation of proteins involved in Bcr-Abl signal transduction [5], adding to that nilotinib has selectivity to the c-KIT and PDGF receptor kinases and has no activity against targets such as the Src-family of tyrosine kinases [24].

To our knowledge, this study is the first regional and local study regarding platelets dysfunction in CML patients so unfortunately there

is no data presented here from other clinical studies to be correlated with our data in order to be verified. However, some positive findings may be observed. For example; there was positive correlation between duration of nilotinib treatment and the platelet aggregation with epinephrine. This correlation may be explained by a possible inhibitory effect of BCR-ABL transcripts on aggregation with epinephrine. The longer duration of treatment with nilitinib will decrease the number of BCR-ABL transcripts. Thus abolishing their inhibitory effect on platelet aggregation.

This suggestion may be explained based on the knowledge that: newly diagnosed MPN patients have impaired aggregation response to epinephrine; the most common finding among the cases with absence of platelet aggregation response was complete loss of both first and secondary waves of epinephrine [27].

7. Conclusions

1. Nilotinib had no adverse effect on platelet function; this is reflected by normal platelets aggregation results. Thus the patients had no risk of bleeding.
2. Nilotinib had no effect on patients clotting tests indicating that the patients have normal secondary hemostasis.
3. A significant relation was found between the platelet aggregation in response to added ADP and the platelet count of the patients. And significant relation also observed with aggregation response to ADP, collagen and ristocetin and patients' hematocrits.

Conflict of interests

The authors declare that they have no conflict of interests. They did not receive funding for this research project.

Authors contribution

AMZ Alqasim and AF Alwan designed the study and helped in interpretation of results and writing the manuscript. GM Obaid collected patients' data and blood samples and helped in writing the manuscript. YG Yaseen performed platelet function tests and helped in interpretation of results.

All authors read and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lrr.2018.05.003.

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