Assessment of Interleukin 1 β Serum Level in Different Responder Groups and Stages of Chronic Myeloid Leukemia Patients on Imatinib Mesylate Therapy

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Received: 12 November 2013 / Accepted: 15 January 2014 / Published online: 7 February 2014
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Abstract Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of an acquired mutation which affects the hematopoietic stem cell, leading to a striking overproduction of immature granulocytes. The first important clue to its pathogenesis the Philadelphia chromosome created by a reciprocal translocation between chromosomes 9 and 22 (t [9; 22] [q34; q11]). The development of the BCR–ABL-targeted imatinib mesylate represents a paradigm shift in the treatment of CML. Imatinib displays inhibitory activity against other kinase(s) that play a role in monocyte/macrophage development. Accordingly many studies revealed the role of cytokines in pathophysiology of myeloid neoplasia including participation of IL-1 β in the pathogenesis of CML. This study designed to assess the behavior of IL-1 β through newly diagnosed patients, different responders groups (optimal, suboptimal and failure cytogenetic response) and advanced stages (acceleration and crisis groups) of CML Iraqi patients whom receiving Imatinib mesylate (tyrosine kinase inhibitor), trying to elucidate the role of immunity in pathophysiology of CML disease development and treatments. In this study 96 Iraqi CML patients under imatinib mesylate treatment categorized by complete blood picture and fluorescent

in situ hybridization analysis into different response groups and stages, then used an enzyme linked immunosorbent assay technique to assess serum level of IL-1 β in each response group and advance stage (acceleration and transformed) of CML patients, in comparison to level in 32 healthy control subjects and 32 newly diagnosed CML. Out of 128 patients the mean serum of interleukin 1 β level (pg/ml) for the newly diagnosed, optimal responded, suboptimal responded, failure cytogenetic and advance stage of CML were 6.53 ± 3.81 , 18.47 ± 4.29 , 18.69 ± 3.03 , 5.73 ± 2.44 , and 18.10 ± 3.10 , respectively. While healthy was 12.17 ± 3.44 . The measurement of IL-1 β before and during treatment of CML patients may contribute to the early identification of responder and non responder patients, and help in the earlier choice and/or design of alternative therapeutic strategies.

Keywords Chronic myeloid leukemia · Interleukin-1 β · Imatinib mesylate

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of primitive hemopoietic progenitor cells [1, 2]. It accounts for 15 % of all adult leukemias [3]. The cytogenetic hallmark of CML is the Philadelphia chromosome (Ph), created by a reciprocal translocation between chromosomes 9 and 22 (t [9; 22] [q34; q11]) [4–6].

The course of the CML is characteristically triphasic: a chronic phase (CP) lasting 3–6 years is followed by transformation to an accelerated phase (AP) and then a terminal blast phase of short duration [7]; the latter of which resembles an acute myeloid state [8]. The mechanisms behind CML disease progression are not fully

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understood, increasing evidence suggests that Src family kinases may be involved in CML disease progression through the induction of cytokine independence and apoptotic protection [9].

Based on international randomized study of interferon and STI571 (IRIS) trial results, imatinib mesylate (Gleevec; Novartis Pharma) was approved the first-line treatment of CML [10]. Imatinib mesylate, a selective inhibitor of BCR–ABL kinase activity, selectively inhibits downstream signaling and the growth of BCR–ABL-positive cells, inducing apoptosis of these cells [11]. Although the majority of patients respond well to imatinib, a relevant proportion does not show an optimal response [12].

In CML, altered interactions between leukemia stem cell and the bone marrow microenvironment can occur as a result of direct intercellular contact; secreted factors such as cytokines or chemokines; or through micro-environmental matrix; Specific signals from the surrounding stromal cells; proteins that regulate cellular adhesion and migration; growth factor receptor and integrin signals [13]. The deregulation of the hematopoietic processes leads to abnormal hematopoiesis and is associated with the development of cancer, including leukemia [14].

Tyrosine phosphorylation of these BCR–ABL substrates results in the constitutive activation of multiple cytoplasmic and nuclear signalling cascades, which are shared with cytokines known to regulate the proliferation, differentiation and survival of hemopoietic cells [15].

Participation of IL-1 β in the pathogenesis of CML is supported by several studies. Analysis of adherent layers from CML patients showed constitutive expression of IL-1 β mRNA and biologically active protein [16].

From this era, the present study conducted to assess the behavior of IL-1 β through different responders groups (optimal, suboptimal and failure cytogenetic response) and advanced stage of CML patients whom receiving imatinib (tyrosine kinase inhibitor, TKI), trying to elucidate the role of immunity in pathophysiology of CML disease development and treatments.

Patients and Methods

Patients

The cross sectional study was conducted on 128 Iraqi cases of chronic myeloid leukemia with different stages and different therapy responses, receiving imatinib mesylate 400–800 mg per day for at least 18 months were evaluated. Patients were free of fever and any chronic illness such as diabetes mellitus, hypertension and infection; also they had no history of smoking or alcohol drinking. We used leftover samples to avoid the need for patients consent [17].

Laboratory tests including complete blood picture (CBC) and fluorescent in situ hybridization (FISH) results were considered in our evaluation also. The patients were grouped to those responding, failure of response to therapy and those with progression disease advance stage : according to the European leukemia net (ELN) classification [18] CML patients on imatinib 400 mg/day for more than 18 months with FISH analysis results of optimal response (normal CBC and 0 % of FISH result), CML patients on imatinib 400 mg/day or more for more than 18 months of therapy with FISH analysis results of suboptimal and failure response (normal CBC and FISH analysis of 1–35 % and >35 % consequently) and CML patients on imatinib 400 mg/day or more for more than 18 months of therapy with FISH analysis results of advance stage (up normal CBC and FISH analysis >35 %). Of all cases, 32 patients were optimal response while another 22, 26, 16 patients were suboptimal response, failure cytogenetic and advance stage of CML subsequently. Also 32 newly diagnosed CML not receiving any therapy and another 32 healthy volunteers were included as comparative groups.

Sample Collection

Five ml of venepuncture blood sample was taken from 128 CML patients and 32 healthy control groups, and dispensed in plain tube and the serum specimens for quantitative determination of IL-1 β concentrations were separated immediately after collection of venous blood and stored at –20 °C freezer until analysis.

Determination of IL-1 β Level

Serum IL-1 β level was determined by ELISA using a quantitative sandwich enzyme immunoassay technique (Abcam, UK). All tests were carried out by following manufacturer instructions. Serum IL-1 β level was calculated by interpolation from a standard curve that was performed in the same assay as that for the sample.

Statistical Analysis

The data were processed using the Statistical Packages for Social Sciences-Version 20 (SPSS-20 “PASW” Statistics). Serum level of IL-1 β was presented as mean $\pm$ SD and differences between means were assessed by using independent Student *t* test.

Results

In present study, 128 cases of CML at different stages were included with male: female ratio 1.2:1 and mean age

Table 1 Distribution of chronic myeloid leukaemia cases according to the imatinib therapy doses

Imatinib (dose/mg)	Newly		Failure		Advanced		Suboptimal		Optimal	
	No.	%	No.	%	No.	%	No.	%	No.	%
400	–	–	7	26.9	4	25	4	63.6	32	100
≥600	–	–	19	73.1	12	75	8	36.4	–	–

Table 2 Distribution of chronic myeloid leukaemia cases according to the mean $\pm$ SD of disease duration, WBC count and FISH results

Disease status	Disease duration(years)		FISH result (%)		WBC count ($\times 10^9$)	
	Mean $\pm$ SD (range)		Mean $\pm$ SD(range)		Mean $\pm$ SD (range)	
Newly	–		92.94 $\pm$ 8.69 (50–98)		224.9 $\pm$ 96.51(61.6–450)	
Optimal	3.53 $\pm$ 1.67 (1–8)		0		5.9 $\pm$ 2.26(2.2–11.7)	
Suboptimal	4.23 $\pm$ 1.63 (2–8)		12.91 $\pm$ 11.94 (1–33)		6.6 $\pm$ 2.43(3.4–12.2)	
Failure	5.31 $\pm$ 2.56 (1–11)		61.54 $\pm$ 17.46 (9–88)		6.2 $\pm$ 2.98(2–11.7)	
Advanced	6.63 $\pm$ 4.11 (1–13)		78.19 $\pm$ 13.14(58–99)		61.2 $\pm$ 52.54(6.3–159)	

41.06 $\pm$ 12.42 years ranging 20–76 years. By FISH BCR–ABL analysis for 80 (62.5 %) of CML patients, 25, 17.18, and 20.31 % had optimal, suboptimal cytogenetic response and failure cytogenetic result, respectively. While 25 % of the studied groups were newly diagnosed CML and 12.5 % were in advance stage of CML patients.

All CML patients except of newly diagnosed CML included in this study were on imatinib mesylate. Of these patients, 100, 63.6, 26.9, and 25.0 % (optimal, suboptimal, failure, advance stage, respectively) were on 400 mg/day while 36.4, 73.1, and 75.0 % (suboptimal, failure, advance stage, respectively) were on 600–800 mg/day of imatinib dose (Table 1).

Patients characteristics according to their disease duration, CBC indices and FISH analysis results are shown in (Table 2).

The mean serum $\pm$ SD (pg/ml) of interleukin 1 β level for the newly diagnosed, optimal responded, suboptimal responded, failure cytogenetic CML and advance stage of CML was 6.53 $\pm$ 3.81, 18.47 $\pm$ 4.29, 18.69 $\pm$ 3.03, 5.73 $\pm$ 2.44, and 18.10 $\pm$ 3.10, respectively (Fig. 1), while the mean level of interleukin 1 β for healthy control group was 12.17 $\pm$ 3.44 (Fig. 1).

The statistical analysis of IL-1 β mean level through different stages of CML patients by using Student *t* test for two independent means at 0.05 level of significance is shown in (Table 3).

Discussion

The progress made in the understanding of chronic myeloid leukemia since the recognition of a common chromosomal abnormality to the introduction of evermore effective

Fig. 1 Distribution of mean IL-1 β through different groups of CML patients

tyrosine kinase inhibitors is unprecedented in cancer [19]. Recent mathematical models have been developed to study the dynamics of CML under imatinib treatment. None of these models incorporates the immune response through different stages of CML [20].

Novel molecular targeted therapies, such as imatinib for CML, represent the first agents that inhibit cancer cells more than other dividing cells such as immune cells [21].

In our study, 128 cases of CML at different stages the mean $\pm$ SD (pg/ml) of serum interleukin 1 β level for the newly diagnosed, optimal responded, suboptimal responded, failure cytogenetic and advance stage of CML were 6.53 $\pm$ 3.81, 18.47 $\pm$ 4.29, 18.69 $\pm$ 3.03, 5.73 $\pm$ 2.44, and 18.10 $\pm$ 3.10, respectively, while the mean level for healthy control group was 12.17 $\pm$ 3.44.

Regarding newly diagnosed CML patients, our study analysis showed a significant decrease in serum level of IL-1 β (*P* value 0.0001), when compared to the results of healthy control group. This may be contributed; to the abnormal microenvironment and abnormalities in

Table 3 Shows *P* value of each studied group of CML patients according to the IL-1 β mean level

IL-1 β (pg/ml)	Newly	Failure	Advanced	Suboptimal	Optimal	Healthy
Mean	6.53	5.73	18.1	18.69	18.47	12.17
<i>P</i> value compared to healthy	0.0001*	–	–	–	–	–
<i>P</i> value compared to optimal	0.0001*	0.0001*	–	0.836	–	–
<i>P</i> value compared to advanced	–	0.0001*	–	–	–	–

* Significant using *t* test for two independent means at 0.05 level of significance

progenitors themselves of CML patients, as a presence of malignant stromal macrophages may secrete a low level of IL-1 β which lead to decrease in the level of apoptosis.

In the imatinib therapy era, the assessment of IL-1 β level through different responder groups and stages of CML patients, our results showed, CML patients who had achieved optimal (FISH analysis <1 %) cytogenetic response, their IL-1 β serum level was significantly increased with *P* value 0.0001, when compared it with the newly diagnosed CML patients; suggesting that an imatinib inhibit the BCR–ABL cells proliferation and this might give chance of normal macrophage to proliferate and to produce IL-1 β . Beside improvement of bone marrow microenvironment by imatinib, increasing serum level of IL-1 β lead to an increasing in apoptosis of leukemic cells and this was shown in [22]. Engler et al. [23], also speculate that rapid depletion of mature CML cells by imatinib may deprive CD34+ cells of essential cytokines normally produced by the mature leukemic population. This cytokine-dependent environment may remove the proliferative advantage of leukemic hematopoiesis, facilitating the regrowth of the residual nonleukemic hematopoietic cells that will be reflected in deeper molecular responses.

From another view, those CML patients who had progression in their disease and they lost their cytogenetic response (suboptimal cytogenetic response i.e. FISH analysis 1–35 % and failure cytogenetic response i.e. FISH analysis >35 %), IL-1 β serum level showed non-significant change with *P* value 0.836 for those with suboptimal responders while it's showed a statistical significant decreased in serum level for those patients with failure cytogenetic result when compared them to the optimal responders.

While patients in advance disease stage (acceleration and blast transformation), our data showed a significant increase in serum level of IL-1 β when compared with the failure group, this is may be provide evidence that:

1. Leukocytes from patients with CML have high levels of biologically active IL-1 β ;
2. IL-1 β levels are significantly higher in patients in accelerated and blast crisis phases of the disease, that is also shown in Wetzler et al. [16].

The bone marrow microenvironment supports and regulates the proliferation and differentiation of hematopoietic cells. Clinically, CML is characterized by an abnormal expansion of malignant BCR–ABL positive myeloid cells. The abnormal haematopoiesis in CML is related, in part, to abnormalities in the hematopoietic cells themselves leading to abnormal interactions between CML progenitors and stroma [24].

From our data, imatinib seems to be capable to remodel the bone marrow microenvironment leading to conditions favourable to immune function restore and activate cells of the immune system. Thus microenvironment of the bone marrow gradually improves till optimal cytogenetic response is achieving where BCR–ABL then will be at a very low level. So the IL-1 β will be rise to increase the apoptosis of cloned cells by increasing normal macrophage and monocytes levels This idea is similar to the conclude by Poggi and Zocchi [25] were found patients who are responder to imatinib mesylate, the production of the stromal-derived factor-1 (SDF-1) and of the B lymphocyte activating factor of the tumour necrosis factor family (BAFF), both involved in normal B cell development and maturation is induced by imatinib, at variance with non responder patients. Also another study which showed that antileukemia T-cell responses develop in the majority of analyzed CML patients in hematologic and cytogenetic remission under imatinib treatment [21].

In our study, those responders CML patients were loss their imatinib efficacy and their complete cytogenetic response (i.e. failure cytogenetic group), the IL-1 β level return to decrease as it may be due to increasing of BCR–ABL progenitors which will lead to produce abnormal macrophages and monocytes, accordingly there will be decreasing in apoptosis capability which may cause more progression of the disease.

Regarding suboptimal responders we suggesting that patients started to lose their cytogenetic response by increasing leukemic cloned cells in spite of still no change and affect the microenvironment of the bone marrow.

It is also possible that activated RAS promotes the proliferation of leukemic blasts by stimulating the expression of cytokines such as IL-1 β , which can then serve as

autocrine growth factors, and simultaneously activating components of the cell cycle machinery or by inducing other pathways that function independently of cytokine production. So it may imply that activation of RAS by mutation is responsible, at least in some cases, for autocrine IL-1 β production in leukemias [26].

Malignant stromal macrophages appear to be responsible for the defects in stromal function and so cytokine may work dependently or independently on the cloned progenitors of the disease.

IL-1 β may play a strong role in the pathogenesis of CML. This may be indicating that, cytokines in the malignant cloned disease working independently.

In this study, serum level of IL-1 β during activity of the disease was low and this returned to increase after initiation of the TKI as it inhibit the cloned cells and modulate the microenvironment of the bone marrow, so TKI give a chance for normal macrophage to work properly and secrete IL-1 β which may increase and re-establish the apoptosis in the treated CML patients. After patients loss their disease control by TKI, IL-1 β level return to decrease again before patients transform to acceleration stage and this change in the IL-1 β level may give us an early idea about the disease transformation from CP to advance stage.

Our results may differed and/or agree with some reports, although we tested a CML patients in different cytogenetic responses which was a new era to understand what's happening and to open a new view regarding role the of TKI in arrangement of the microenvironment of bone marrow in CML patients through analysis of IL-1 β and it may give us an idea regarding using IL-1 β as a test in detection disease progression before the transformation to advance stage.

Conclusion

This study showed, IL-1 β may have a role in the pathogenesis of newly diagnosed CML.

Imatinib therapy might not only work through suppression of BCR–ABL myeloid leukemia cells but it may be open a window for the native immunity to work against proliferating leukemic cells, through re-arrangement of the microenvironment of the bone marrow and reestablishing the apoptosis by increase IL-1 β level that is secreted from regeneration of macrophage and monocyte cells.

Decreasing IL-1 β level during treatment with imatinib may give us an early idea about the disease status and progression before transformation to another stage (advance stage), beside of loosing disease control by imatinib therapy.

References

1. Sawyers CL (1999) Chronic myeloid leukemia. *N Engl J Med* 340(17):1330–1340
2. Monroy RH, Viveros PV, Franco JC (2007) Chronic myeloid leukemia current concepts in physiopathology and treatment. *Cancerologia* 2:137–147
3. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM (1999) The biology of chronic myeloid leukemia. *N Engl J Med* 341(3):164–172
4. Warmuth M, Danhauser-Riedl S, Hallek M (1999) Molecular pathogenesis of chronic myeloid leukemia: implications for new therapeutic strategies. *Ann Hematol* 78:49–64
5. Reinhold U, Hennig E, Leiblein S, Niederwieser D, Deininger MWN (2003) FISH for BCR–ABL on interphases of peripheral blood neutrophils but not of unselected white cells correlates with bone marrow cytogenetics in CML patients treated with imatinib. *Leukemia* 17:1925–1929
6. Bennour A, Bellâaj H, Youssef YB, Elloumi M, Khelif A, Saad A, Sennana H (2011) Molecular cytogenetic characterization of Philadelphia-negative rearrangements in chronic myeloid leukemia patients. *J Cancer Res Clin Oncol* 137:1329–1336
7. Sessions J (2007) Chronic myeloid leukemia in 2007. *JMCP* 13(8):S4–S7
8. Goldman JM, Mughal TI (2011) Chronic myeloid leukemia. In: Hoffbrand AV, Catovsky D, Tuddenham EGD, Green AR (eds) *Postgraduate haematology*, 6th edn. Wiley-Blackwell Publishing Ltd, Hoboken, pp 483–502
9. Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, Talpaz M (2003) BCR–ABL independence and LYN kinase overexpression in chronic myelogenous leukaemia cells selected for resistance to STI571. *Blood* 101(2):690–698
10. O'Brien SG, Francois GF, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 348(11):994–1004
11. Schiffer CA (2007) BCR–ABL tyrosine kinase inhibitors for chronic myelogenous leukemia. *N Engl J Med* 357(3):258–265
12. Furukawa T, Narita M, Koike T, Takai K, Nagai K, Kobayashi M, Koyama S, Seki Y, Takahashi H, Fujiwara M, Kishi K, Nikkuni K, Isahai N, Higuchi W, Nomoto N, Maruyama S, Masuko M, Kuroha T, Abe T, Toba K, Takahashi M, Aizawa Y, Shibata A (2011) Clinical value of assessing the response to imatinib monitored by interphase FISH and RQ-PCR for BCR–ABL in peripheral blood for long-term survival of chronic phase CML patients: results of the Niigata CML-multi-institutional cooperative clinical study. *Int J Hematol* 93:336–343
13. Crews LA, Jamieson CHM (2012) Chronic myeloid leukemia stem cell biology. *Curr Hematol Malig Rep* 7:125–132
14. Banerjee P, Crawford L, Samuelson E, Gerold Feuer G (2010) Hematopoietic stem cells and retroviral infection. *Retrovirology* 7(8):1–17
15. Hazlehurst LA, Bewry NN, Nair RR, Pinillalbarz J (2009) Signaling networks associated with BCR–ABL-dependent transformation. *Cancer Control* 16(2):100–107
16. Wetzler M, Kurzrock R, Estrov Z, Kantarjian H, Gisslinger H, Underbrink MP, Talpaz M (1994) Altered levels of interleukin-1 beta and interleukin-1 receptor antagonist in chronic myelogenous leukemia: clinical and prognostic correlates. *Blood* 84(9):3142–3147

17. Food and Drug Administration (2006) Guidance for sponsors, institutional review boards, clinical investigators and FDA staff, guidance on informed consent for in vitro diagnostic device studies using leftover human specimens that are not individually identifiable. FDA, Silver Spring
18. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, Cervantes F, Deininger M, Gratwohl A, Guilhot F, Hochhaus A, Horowitz M, Hughes T, Kantarjian H, Larson R, Radich J, Simonsson B, Silver RT, Goldman J, Hehlmann R (2009) Chronic myeloid leukemia: an update of concepts and management recommendations of European leukemia net. *Am Soc Clin Oncol* 27(35):6041–6051
19. Cortes J, Kantarjian H (2012) How I treat newly diagnosed chronic phase CML. *Blood* 120(7):1390–1397
20. Kim PS, Lee PP, Levy D (2008) Dynamics and potential impact of the immune response to chronic myelogenous leukemia. *PLoS Comput Biol* 4(6):e1000095
21. Chen CI-U, Maecker HT, Lee PP (2008) Development and dynamics of robust T cell responses to CML under imatinib treatment. *Blood*. doi:[10.1182/blood-2007-12-128397](https://doi.org/10.1182/blood-2007-12-128397)
22. Taburee P, Siripin D, Wongjindanon N, Fucharoen S, Tanyong DI (2011) Interleukin-1 beta and tumor necrosis factor-alpha induced apoptosis via caspase in leukemic K 562 and HL-60 cell lines. *Songklanakarin J Sci Technol* 33(5):487–492
23. Engler JR, Frede A, Saunders V, Zannettino A, White DL, Hughes TP (2010) The poor response to imatinib observed in CML patients with low OCT-1 activity is not attributable to lower uptake of imatinib into their CD34+ cells. *Blood* 116(15):2776–2778
24. Bhatia R, McGlave PB, Dewald GW, Blazar BR, Verfaillie CM (1995) Abnormal function of the bone marrow microenvironment in chronic myelogenous leukemia: role of malignant stromal macrophages. *Blood* 85(12):3636–3645
25. Poggi A, Zocchi MR (2012) Imatinib mesylate can help to direct natural immunity toward an anti-leukemic reactivity by acting on the bone marrow microenvironment. *Oncoimmunology* 1(2):214–216
26. Beaupre DM, Talpaz M, Marini FC 3rd, Cristiano RJ, Roth JA, Estrov Z, Albitar M, Freedman MH, Kurzrock R (1999) Auto-crine interleukin-1b production in leukemia: evidence for the involvement of mutated RAS. *Cancer Res* 59:2971–2980