

Serum level of TNF-alpha in patients with chronic myeloid leukemia on Imatinib therapy

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Abstract

A new chemotherapy Imatinib have a potent antitumor effect specially in chronic myeloid leukemia. Various proinflammatory cytokines are involved in the pathogenesis of chronic myeloid leukemia but at different extends. Tumor necrosis factor alpha (TNF- α) is a major regulatory cytokine which stimulates proliferation of dividing cells while inducing apoptosis . The aim of this study is to determine the effect of Imatinib chemotherapy on serum levels of TNF-alpha as atrial to understand the mechanism by which Imatinib induce its antitumor effect. 52 CML patients from those who were attending to Oncology and Nuclear Hospital in Mosul / Iraq and 30 healthy control were participated in this study. Serum TNF-alpha concentrations were determined using the commercially available enzyme-linked immuno-sorbent assay kit. TNF- α serum levels were significantly higher in patients compared to control also the the TNF- α serum levels were significantly lower in patients who were receiving Imatinib therapy than patients who did not. we have demonstrated that imatinib strongly inhibits TNF- α production as form of its antitumor activity also confirm that TNF- α could be used as a good indicator for assessment of therapeutic response of Imatinib in CML patients.

Introduction

Chronic myelogenous leukemia (CML), also known as chronic myeloid leukemia, is a myeloproliferative disorder characterized by increased proliferation of the granulocytic cell line without the loss of their capacity to differentiate. CML accounts for 20% of all adult leukemias worldwide⁽¹⁾. CML is begins in the chronic phase, and over the course of several years progresses to an accelerated phase and ultimately to a blast crisis. Blast crisis is the terminal phase of CML and clinically behaves like

an acute leukemia . Approximately 85% of patients with CML are in the chronic phase at the time of diagnosis ⁽²⁾.

Imatinib

Imatinib mesylate, also known Gleevec (Novartis), has been approved for the treatment of chronic myeloid leukemia. Imatinib binds reversibly to several target kinases, among which c-kit, c-abl, and platelet-derived growth factor receptor are most sensitive to drug-induced inhibition of kinase activity⁽³⁾. These kinases are known to be causally involved in the pathogenesis of many diseases. However, the same

kinases similarly regulate key functions of immune cells, particularly macrophages, T cells, and dendritic cells (DCs) ⁽⁴⁾. imatinib impaired the function and maturation of DCs from CD34 progenitors. previously has been confirm that the inhibitory role of imatinib on T cell activation with reduced phosphorylation of Lck and ERK12 and subsequent inhibition of a delayed-type hypersensitivity reaction in a murine model of inflammation ⁽⁵⁾.

Many pleiotropic proinflammatory cytokines, play a role in the activation, growth and apoptosis of leukemic B-cells, which are able to produce TNF, leading to B-CLL aggressiveness and disease progression⁽⁶⁾. TNF- α is a major effector and regulatory cytokine that stimulate the acute phase reaction and systemic inflammation. It has a pleiotropic role in the pathogenesis of several immuneregulated diseases and hematologic malignancies ^(7,8).

TNF- α was initially thought to be a product only of macrophages, T-cells, B-cells and monocytes. Recently TNF- α has now been shown to be produced by a wide variety of tumor cells, including those of CML. It induces other inflammatory mediators and proteases that regulate inflammatory responses. It is also produced by tumors cells and can act as an endogenous tumor promoter. The role of TNF- α has been linked to all steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis ^(9,10). Inhibition of TNF- by means of antibodies or soluble decoy receptors has proven to have a clinical benefit in patients suffering from immune-mediated diseases. The hypothesis that

imatinib primarily inhibits macrophages is further supported by the observation that imatinib also inhibits the expression of the inducible nitric oxide synthase (iNOS), which regulates TNF- production upon Concanavalin A (Con A) application even though it is still widely unknown to what extent nitric oxide participates in the execution of TNF- α induced apoptosis ⁽¹¹⁾.

Aim of study

The aim of this study is to determine the effect of Imatinib chemotherapy on serum levels of TNF-alpha as a trial to understand the mechanism by which Imatinib induce its antitumor effect

Material and methods

This study include 52 Patients (30 men and 22 women), from those who were attending the Oncology and Nuclear Hospital in Mosul / Iraq from the period of 1st of March 2013 and 1st of May 2013, complaining of chronic myeloid leukemia leukemia that diagnosed by bone marrow inspiration. Patients were divided into two groups first one include those who had not receive any treatment (N=18) and the second include those who had taken Imatinib for at least six months (N=34) an addition to 30 apparently normal healthy control (17 men and 13 women). 5 ml blood sample are taken from all participants with the appropriate Ethical Committee permissions. Serum TNF-alpha concentrations were determined using the commercially available enzyme-linked immuno-sorbent assay (ELISA Ultra sensitive), kit supplied by BOSTER Immunoleader (USA). The assays employ the quantitative sandwich enzyme immunoassay technique using recombinant

human TNF-alpha with antibodies raised against the recombinant proteins .

Results

Our study has confirm that there were no significant differences in TNF- α serum levels according on age and gender in patients and control groups ($P > 0.05$). as shown in tables (1,2).

Also it has observe that the TNF- α serum levels were significantly raised in CML cases in comparison to controls (35.73 ± 19.97 pg/mL and 10.01 ± 3.24 pg/ml).table (3). A marked significantly lower of TNF- α serum levels in CML patients who had given Imatinib treatment has been confirmed in most cases of CML when compared with a other cases who had not received Imatinib therapy .

Discussion

TNF- α , is a pro-inflammatory cytokine plays several important roles in the establishment of inflammation⁽¹²⁾, cell growth control, and involved in the pathogenesis of many human diseases,. When dysregulated in the circulation, it mediates a wide variety of diseases include cancers,⁽¹³⁾. It was suggested that TNF- α neutralization seems to be a useful to control a variety of disorders and hematological malignancies⁽¹⁴⁾. Our study was confirmed that the TNF- α serum level was significantly higher in CML patients than normal controls , it may be due to high expression of enzymes that block the TNF- α surface receptors , or may be due to over expression by malignant clones to enhance angiogenesis⁽¹⁵⁾.

In the present study the mean serum TNF- α level for CML patient who had taken Imatinib therapy was found to be $25.86 \pm$

9.65 pm/ml while the mean serum TNF- α level for CML patient who had not received Imatinib therapy was found to be 54.37 ± 21.29 pm/ml. Therefore there is a significant lower in treated than untreated CML patients group regarding to serum TNF- α level ,so the effect of treatment with Imatinib on TNF- α serum level in CML patients was very clear. So our study provides another evidence that imatinib inhibits the TNF- α production of human myeloid cells . These data might define an important role of imatinib in malignant diseases , this findings was totally agree with many studies who confirmed that there were a significant increase in TNF- α serum level in patient with CML than control suggesting that the change in TNF- α before and after chemotherapy in leukemic patients was previously explained due to changes in the immunological response. They have implied that the release of some pro-inflammatory cytokines may promote tumor growth and hence influence survival^(16,17). TNF- α induce diverse effects as apoptosis, necrosis, angiogenesis, immune cell activation, differentiation, and cell migration. These processes are of great relevance in tumor immune surveillance, and also play crucial roles in tumor development and tumor progression⁽¹⁸⁾.

Imatinib is known to suppress the phosphorylation of *LCK* and *ERK1/2*, both kinases associated with TCR-mediated signaling and may account for the suppression of cytokine synthesis by CD4 T cell of CML patient treated with Imatinib . also suppressed the tyrosine phosphorylation of *ZAP70*.⁽¹⁹⁾ Imatinib inhibits SCF-induced c-Kit phosphorylation and

downstream activation of MAPK pathways in the cells. Further, imatinib inhibited SCF induced macrophage cell production of the inflammatory cytokines include TNF- α . Other study demonstrated that imatinib inhibits SFMC production of TNF- α in response to LPS. ^(22,21) Imatinib-mediated inhibition of monocyte and macrophage proliferation, differentiation, and TNF- α production could reduce disease activity. It may be involved in more complex interactions between a leukemic clone and normal bone marrow cells which provide a favorable environment for leukemic cells to survive ⁽⁶⁾.

therapeutic doses of imatinib dramatically reduced LPS-induced production of the proinflammatory cytokine TNF- α in human myeloid cells, LPS-induced transcriptional activation of TNF- is critically governed by the transcription factor NF-B. Activation of NF-B is regulated by phosphorylation of the inhibitor of B, which is induced by IB kinases and subsequently degraded via the ubiquitin-proteasome pathway. Also the production of IL-10 was not significantly regulated, thereby excluding an IL-10-mediated suppression of TNF- α ⁽²¹⁾.

In conclusion, we have demonstrated that imatinib strongly inhibits TNF- α production as form of its antitumor activity also confirm that TNF- α could be used as a good indicator for assessment of therapeutic response of Imatinib in CML patients.

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Table (1) the mean and St.D of TNF- α serum levels in CML patients and healthy control groups depend upon age groups

Age group	TNF- α serum level	
	control mean \pm SD pm/ml N=	Patients mean \pm SD pm/ml N=
30 - < 40	9.0 \pm 1.86 N=4	31.48 \pm 14.1 N=6
40 - < 50	11.2 \pm 5.14 N=5	34.58 \pm 16.7 N=12
50 - < 60	9.96 \pm 2.25 N=9	35.3 \pm 22.3 N=8
60 - < 70	10.45 \pm 3.36 N=7	37.2 \pm 25.1 N=16
70 - < 80	9.0 \pm 4.0 N=5	37.65 \pm 20.2 N=10
P value	P > 5%	P > 5%

Table (2) the serum levels of TNF- α in both female and male of patients with CML and control groups.

Gender	TNF- α serum level	
	Control group Mean \pm SD pm/ml N=	Patients group Mean \pm SD pm/ml N=
Male	10.69 \pm 3.19 N=17	33.23 \pm 17.7 N=30
female	9.12 \pm 3.2 N=13	39.14 \pm 22.6 N=22
P value	0.962	0.230

Table 3: comparison of TNF- α serum levels in group of CML patients and healthy control group.

	Control Mean \pm SD N=30	CML patients Mean \pm SD N=52	P value
TNF-α (pm/ml)	10.01 \pm 3.24	35.73 \pm 19.97	0.001

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Table 4: comparison of TNF- α serum levels in both treated and untreated groups of CML patients and healthy control group.

	Control N=30 Mean \pm SD	CML cases N = 52		P value
		Untreated N=18 Mean \pm SD	Treated N=34 Mean \pm SD	
TNF- α (pm/ml)	10.01 \pm 3.24	54.37 \pm 21.29	25.86 \pm 9.65	0.001