

Assessment Of Soluble Fas Ligand In Patients With Chronic Myeloid Leukemia

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ABSTRACT

Background: Soluble FasL (sFasL) generating from Membrane-bound FasL (mFasL) cleaved by matrix metalloproteinases (MMPs). sFasL inhibits the apoptotic and inflammatory activity of mFasL because of sFasL competes with the mFasL and binds to Fas. To evaluate serum soluble Fas ligand (sFasL) level in patients with chronic myeloid leukemia.

Materials and Method: Serum levels of sFasL were measured by ELISA method after venous blood was collected from 56 CML patients (newly diagnosed and optimally treated) and 28 healthy subjects as control group.

Results: There were no significant increases in serum sFasL patient compared to healthy control with $P=0.07$. When the mean sFasL concentration was obviously highest in newly diagnosed (216.9pg/ml) followed by healthy control (152.5pg/ml) and lowest in optimally treated (147.7 pg/ml).

Conclusion: Production of sFasL in tumor patients may be a key mechanism to inhibit Fas-mediated apoptosis.

Keywords: Chronic Myeloid Leukemia, CML, Soluble Fas Ligand, Sfasl, Philadelphia Chromosome, Ph Chromosome.

Article Information

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INTRODUCTION

Chronic myeloid leukemia is a type of myeloproliferative neoplasm (MPN) [1]. It is a malignant clonal disorder of hematopoietic stem cells that results in increase in myeloid, erythroid cells, and platelets in the peripheral blood and marked myeloid hyperplasia in the bone marrow [2]. Patients most commonly present in chronic phase (CP), but without treatment, CP-CML will progress to accelerated phase (AP-CML) and blast crisis (BP-CML) within 3 to 5 years [3].

Philadelphia chromosome, results from the reciprocal translocation t (9; 22) (q34; q11), is the hallmark of chronic myeloid leukemia (CML) [4]. It accounts for approximately 15% of adult leukemias and its incidence increases with age [5, 6]. Fas ligand (FasL) is a type II transmembrane protein belonging to the tumor necrosis factor superfamily (TNFSF) [7]. It was principally expressed on activated T-lymphocytes, natural killer cells, tumor cells and in immune privileged sites such as the eye [8]. Fas-ligand plays a critical role in immune homeostasis by binding to its receptor Fas (CD95) and inducing apoptosis (programmed

cell death) [9]. Membrane-bound FasL (mFasL) can be proteolytically cleaved by matrix metalloproteinases (MMPs), generating soluble FasL (sFasL) [10]. Membrane-bound Fas ligand (mFasL) is proapoptotic and proinflammatory but soluble isoform, soluble Fas ligand (sFasL) inhibits the apoptotic and inflammatory activity of mFasL [11]. When soluble FasL (sFasL) binds to Fas, cell proliferation, but not apoptosis, is induced [12]. Soluble FasL competes with the membrane – bound counterpart; however, it can act even as an antagonist preventing apoptosis induction by the membrane integrated form of the ligand

[13]. Therefore the membrane-bound form of Fas ligand (FasL) signals apoptosis in target cells through engagement of the death receptor Fas, whereas the proteolytically processed, soluble form of FasL does not induce cell death [14]. The aim of this study was to present the first case of an elevated serum sFasL level associated with chronic myeloid leukemia and discuss the possible clinical value of sFasL in newly diagnosed and optimally treated CML.

MATERIAL AND METHODS

The study was conducted between November 2012 up to June 2013; during this period 56 Iraqi cases of chronic myeloid leukemia evaluated at Baghdad teaching hospital/hematology department. Of these CML patients, 28 cases were newly diagnosed CML while another 28 cases were optimal response to treatment (Glivec therapy). Age ranged for patients were 15 -78 years with mean age (42.6 ± 14.0). So the inclusion criteria including all patients were free of fever and other chronic illness such as diabetes mellitus, hypertension and infection; also they had no history of smoking and drinking of alcohol. An evaluation of these cases FISH BCR-ABL was used in diagnosis and assessment the responses states to glivec therapy.

The method is a solid phase sandwich ELISA (Abcam Company, Uk). It utilizes a monoclonal antibody (capture antibody) specific for human sFas ligand coated on a 96-well plate. Standards and samples are added to the wells, and any human sFas ligand present binds to the immobilized antibody. The wells are washed and biotinylated polyclonal anti-human sFas ligand antibody (detection antibody) is added. After a second wash, avidin-horseradish peroxidase (avidin-HRP) is added, producing an antibody-antigen - antibody sandwich. The wells are again washed and a substrate solution is added, which produces a blue color in direct proportion to the amount of human sFas ligand present in the initial sample. The stop solution is then added to terminate the reaction. This results in a color change from blue to yellow. The wells are then read at 450 nm.

STATISTICAL ANALYSIS

The Statistical Analysis System- SAS (2012) program was used to affect different factors in study parameters (15)

RESULT

We present here the first case of an elevated serum soluble Fas ligand (sFasL) level associated with chronic myeloid leukemia and discuss the possible clinical value of sFasL. So 56 CML patients were included in the study. These CML patients, 28 cases were newly diagnosed CML while another 28 cases were CML on glivec therapy (400 mg/day imatinib mesylate) for at least 12 months with optimal response (complete cytogenetic response (CCgR)) = FISH Ph cells result $< 1\%$ or by major molecular response (MMoR) = BCR-ABL: ABL $\leq 0.10\%$ by International Scale, on RT-Q-PCR.

We work to estimate the level sFasL and their effect on the disease progress and response to treatment. These finding, besides FISH analysis of the two patients group, was used in diagnosis and assessment the responses states to glivec therapy and that led to indicate a very good response to treatment. So this study including CML patients (newly diagnose and optimal response) as well as, apparently healthy subjects were examined to estimate the differences in sFasL among them. So this study included 56 CML patients, 25 (44.6%) were males and 31 (55.4%) were females. It was found that male to female ratio is 0.8: 1. At the time of diagnosis, the age of the patients ranged from 15 -78 years and the mean age of the patients was 42.6 ± 14.0 years. Almost half of the patients (46.4%) were in the age group (40 – 59 years), which represents middle age. So CML is a disease of middle age and early elderly. Twenty eight samples of apparently healthy volunteers were included and evaluated as control samples, 14 (50.0%) were males and 14 (50.0%) were females (M: F ratio 1:1). Age ranged from 19 years to 73 years (mean age 42.9 ± 14.8). The mean $\pm$ SD of serum sFas ligand level for the newly diagnosed, optimally treated CML cases and healthy control were 216.9 ± 165.7 , 147.7 ± 91 and 152.5 ± 98 respectively. That mean sFas ligand

concentration were increased in newly diagnosed in comparison to other groups with no statistically significance for sFas ligand ($P=0.07$) as in (Table 1).

Table 1: The mean level of sFas ligand concentration for CML cases and healthy group with statistical analysis results with each other.

sFasL concentration (pg/ml)	Study group			
	Healthy controls	Newly diagnosed CML cases	Optimally treated CML cases	P value 0.07[NS]
Range	(55.8 - 437)	(22.6 - 626.7)	(11.9 - 426.7)	NS
Mean	152.5	216.9	147.7	NS
SD	98	165.7	91	NS
SE	18.9	31.3	17.2	NS
N	27	28	28	NS
Newly diagnosed CML cases x Healthy controls:				
Difference in mean =		64.4		
Effect size (Cohen's d)=		0.47		
Optimally treated CML cases x Healthy controls				
Difference in mean =		-4.8		
Effect size (Cohen's d)=		-0.05		
Optimally treated CML cases x Newly diagnosed CML cases				
Difference in mean =		-69.2		
Effect size (Cohen's d)=		-0.52		

In this study sFasL were increase, such an increase was noted in other studies for patients with hematopoietic malignancies and that due to the fact that, there is a direct correlation between the expression and function of Fas in hematopoietic cells with the rate of proliferation, suggesting a potential role for Fas and its ligand in the regulation of hematopoietic homeostasis [16, 17]. Also it is similar with other studies which found increased sFas ligand in patients with pancreatic carcinoma and small cell lung

cancer (SCLC) when compared with healthy controls [18, 19]. High levels of soluble FasL have been observed in some colon cancer and esophageal cancer [20, 21].

Having a newly diagnosed CML was increase sFasL concentration by a mean of 64.4pg/ml compared to healthy controls. This disease effect was evaluated as moderately strong effect (Cohen's d 0.47). And that similar with level of sFasL in patients with esophageal cancer was significantly higher than that in healthy donors. And that indicate that elevated serum sFasL levels might be associated with a disease progression and circulating soluble FasL (sFasL) has been suggested to provide protection from Fas-mediated apoptosis. [22]. Optimally treated CML was reducing sFasL concentration by a mean of 4.8pg/ml compared to healthy controls. This disease effect was evaluated as weak effect (Cohen's d 0.05). And optimally treated CML was reducing sFasL concentration by a mean of 69.2pg/ml compared to newly diagnose. This disease effect was evaluated as moderately strong effect (Cohen's d 0.52). And that similar with sFasL level in patients after gastrectomy which was significantly lower as that of controls [23]. So the reduction in the level of sFasL concentration in optimal patients' serum could indicate a very good response to imatinib treatment. Imatinib inhibit BCR-ABL, by this, it will decrease the number of malignant cells. So that the sFasL will be low in optimally when we compare it with the newly diagnosed although non-significant, but it showed a moderately strong effect (Cohen's d 0.52). This is mostly because of persistent disease and decrease the sensitivity of sFas ligand to bind with Fas so there is no negative feedback to inhibit its excretion by malignant cells. sFasL was markedly decreased with the tumor regression induced by the glivec therapy and that agreement with Kanda Y. et al., 1999 studies which including sFasL was markedly decreased with the tumor regression induced by the chemotherapy [24]. Urbaniak-Kujda D. et al., 2002 show in there studied that the concentration of sFasL in acute leukemia patients at diagnosis was significantly higher than in healthy control group, decreased to normal values in remission and rose again in

relapse and that similar with my result about sFasL in chronic myeloid leukemia [25]. Soluble forms (sFas and sFasL) are thought to be one of the mechanisms preventing the immune system from rejecting the tumor cells [26]. The effect of optimally treated compared to newly diagnosed CML cases on sFas Ligand concentration was evaluated after adjusting for the possible confounding effect of age and gender in a multiple liner regression model as in (Table 2). The model was statistically significant ($P=0.02$) and able to explain 0.17% of observed variation in the response variable. Optimally treated CML was associated with statistically significant ($P=0.024$) decrease in sFas ligand concentration by a mean of 80.706pg/ml compared tonewly diagnosed CML cases, after adjusting (controlling) for the confounding effect of age and gender.

Table 2: sFasL concentration /Multiple liner regression model with sFasLconcentration as the dependent (outcome) variable and study group (optimally treated and newly diagnosed CML patients) in addition to age and gender as explanatory (independent) variable.

(Constant)	Unstandardized regression coefficients	P value
	368.879	<0.001
Optimally diagnosed CML cases compared to Newly diagnosed CML cases	-80.706	0.024
Age (years)	-3.175	0.015
Gender	-24.703	0.48[NS]

$P(\text{Model})=0.02$; $R^2=0.17$

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In linear correlation coefficients among newly diagnosed CML cases, sFasL conc. shows statistically significant direct linear correlation with age as in (Table 3) and that similar with Jiang et al, 2008 who show increased sFasL with aging [27].

Table 3: Linear correlation coefficient among newly diagnosed CML cases with Age.

sFasL conc.	
Age (years)	$r=-0.54$ $P=0.003$

CONCLUSIONS

This study demonstrates that newly diagnosed CML patients' shows a statistical no significance increase in sFas ligand when compared with optimally and healthy control groups. The identification of sFas ligand levels as a predictor of outcome in malignant disease further establishes a connection between Fas loss-of-function and tumor progression that is poised for detailed exploration.

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