The Role of Soluble HLA-G Serum Level in Therapeutic Response of Chronic Myeloid Leukemia Patients

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Abstract

Human leukocyte antigen-G molecules (HLA-G) have been suggested to play a role in immune evasion and progression of different malignancies by their tolerogenic activity, through interaction with inhibitory receptors on surface of immune cells.

The aim is to evaluate the role of the s HLA-G serum level in the prognosis and therapeutic responses to TKIs in CML patients.

Serum level of soluble HLA-G was measured for a total of 61 adult patients with CML, who were on regular TKI for at least 6 months whom attended the out-patient's clinic of the Hematology Center in Basra, compared with 20 apparently healthy controls matched in ages and sexes to the patients using Enzyme Linked Immuno-Sorbent Assay (ELISA) technique.

Serum levels of HLA-G in CML patients was significantly higher than that in healthy controls (p=0.006). Elevated serum level of s HLA-G was significantly correlated with sex, BMI, duration of disease, Sokal scoring system.On the other hand, low s HLA-G serum level was significantly correlated with event free status (EF) of CML patients.

Lower level of serum soluble HLA-G in CML patients compared to healthy controls and it might be proved as a prognostic biomarker for CML patients.

Keywords: *CML, soluble HLA-G, TKI, ELISA.*

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm with an incidence of 1-2 cases per 100 000 adults. It accounts for approximately 15% of newly diagnosed cases of leukemia in adults. CML is characterized by a balanced genetic translocation, t (9;22) (q34;q11.2), this translocation result in the generation of a BCR-ABL1 oncoprotein which has a tyrosine kinase activity. [(1)]

CML has a variable clinical course and prognosis. Therefore, the identification of prognostic factors to recognize risk groups of CML patients would be of major interest and one of the studied factors was HLA-G antigen. The expression of which was evaluated previously in different solid and hematologic tumors[(2,3)].

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Human leukocyte antigen G (HLA-G) is a nonclassical MHC class I antigen with very low polymorphism and limited expression in physiological conditions [4,5]. HLA-G exerts multiple immunoregulatory functions such as inhibition of natural killer (NK) cell or T-cell–mediated cytolysis, induction of T-cell apoptosis, or inhibition of trans endothelial NK cell migration.[6,7] Since the net result of these effects is immunosuppression, the ectopic HLA-G expression in tumor cells may favor their escape from antitumor immune responses, thus allowing tumor progression.[8]

All above high lighting the possible role of soluble HLA-G as a prognostic parameter in CML patients. So, we tried to investigate the impact of s HLA-G level on CML patients as it may serve as a possible marker for tumor sensitivity to chemotherapy and as a prognostic indicator for advanced disease and clinical outcome.

Material and Methods

Study population:

This case-control study was conducted at outpatient clinics in Hematology Consultation in Basra city during the period from October 2019 to the end of April 2020. Peripheral blood samples were collected from 61 patients aged ranges between (18-70) years, who were 32 males and 29 females diagnosed as CML, on regular use of TKI treatment for at least six months were included in this study.

Patients who were newly diagnosed, irregularly use the treatment, had history of transplantation, or viral infection were excluded from the study. All patients were assessed for hematologic response by doing complete blood count test (CBC), BMI was calculated for each patient, the most recent RT- PCR tests (within 3months) were recorded. Grouping of patients according torisk stratification scores using Sokal, Hasford or EUTOS scoring systems was done. Also, 20 apparently healthy subjects were included in the study.

Blood sample collection and preparation

five mls of blood were collected from each subject under a septic technique. The blood samples were evacuated into gel tubes, centrifuged to get serum then the supernatant was carefully transferred to Eppendorf tubes and immediately frozen in aliquots at -20°C till use for the s HLA-G ELISA test.

s HLA-G Enzyme-linked Immunosorbent Assay (ELISA)

s HLA-G serum level was measured according to the manufacturer's directions using s HLA-G ELISA kit (CUSABIO/ China).

Statistical Analysis

Statistical Package for Social Science program (SPSS version 18, Chicago, IL, USA) was used for statistical analysis of the results. Chi. Square test, ANOVA test and t-test were used to determine the significance of the differences. Logistic regression analysis was done to detect association of s HLA-G level in CML patients with their variables. P value $\leq$ 0.05 was considered statistically significant.

Results

In the present study the most frequent age group of CML patients was (40-49) years old which comprised (31.1%) of the patients, followed by the age group (50-59) years old and ($\geq$ 60) years old which constituted (29.5% and 21.3 %) of the patients respectively. The number of males were 32 (52.5%) while females were 29 (47.5%) (table 1& fig. 1).

This study shows significant statistical difference in the serum level of s HLA-G between patients and controls with a mean ± SD of (4.85± 5.01) among cases compared to (1.60± 0.37) of healthy controls with (P= 0.006) (Fig. 2). However, serum level of s HLA-G increased with age although difference among age groups was statistically not significant (P=0.591) (Table 2).

As well as this work revealed a female sex predominance over male sex but the difference was statistically not significant (p= 0.373) (table 3).

On the other hand, elevated level of s HLA-G with a mean value (8.89±9.70) was detected among CML patients received first line treatment with nilotinib 150 mg without statistically significant difference compared to other groups (P=0.117).

Furthermore, no statistically significant differences in the level of s HLA-G were detected among optimum, warning and failure molecular responder group in the current study (p=0.532) (table 3). However, this study revealed that the serum level of s HLA-G in patients survive event free status was significantly lower than that in those who developed bad events (became accelerated) (p=0.012) (table 3)

On the other hand, a logistic regression analysis was done, where the dependent variable s HLA-G was significantly correlated with the five variables Sex, Duration of disease, BMI, Sokal score and Event development that were independent predictors which are significantly affected serum level of s HLA-G with p-values (0.017, 0.023, 0.024, 0.033 and 0.040) respectively. While age, type of treatment, CCI, Hasford, EUTOS scoring systems and MR were non significantly affected s HLA-G level as shown in Table 4.

Table1: Comparison of cases and controls according to age groups

Age group	Patients		Controls		Total	
	No.	%	No.	%	No.	%
< 30	6	9.8	1	5	7	8.6
30-39	5	8.2	4	20	9	11.1
40-49	19	31.1	9	45	28	34.6
50-59	18	29.5	5	25	23	28.4
≥ 60	13	21.3	1	5	14	17.3
Total	61	100	20	100	81	100

Table 2: comparison of s HLA-G levels in different age groups of patients

Age groups	Mean ±SD of s HLA-G	No. of cases
<30	1.82± 0.449	6
30-39	4.97± 3.02	5
40-49	4.62 ±5.20	19
50-59	5.31± 5.89	18
≥ 60	5.91 ±5.49	13
Total	4.85 ±5.1	61

Table 3: Serum level of s HLA-G among patients according to their different clinical and sociodemographic characteristics

Patient characteristics	No.	s HLA-G mean ±SD
Sex		
Male	29	4.234 ±4.493
Female	32	5.4084± 5.585
Type of treatment		
Imatinib 400 mg	29	4.25± 4.11
Nilotinib 200 mg	26	4.59±4.47
Nilotinib 150 mg	6	8.89±9.70
MR (molecular response)		
Optimum	36	5.408± 5.83
Warning	2	1.78 ±0.134
Failure	21	4.39 ±4.001
Event		
EF patients	50	4.09 ± 4.596
Patients had event	11	8.3 ±5.987

Table 4: Logistic regression analysis to predict value of s HLA-G

Variable	Regression coefficient	P value	CI	
	B		Lower	Upper
Significant				
Male Sex	-3.260	0.017	0.003	0.558
Duration of disease	0.047	0.023	0.917	0.994
BMI	0.270	0.024	1.036	1.655
Sokal score	3.234	0.033	1.301	494.734
ED	4.796	0.040	1.246	11749.966
Insignificant				
Age	-0.168	0.146	0.674	1.060
Type of treatment	-0.762	0.320	0.104	2.098
CCI	2.409	0.307	0.109	1136.891
Hasford	0.847	0.504	0.194	28.023
EUTOS	-2.023	0.053	0.017	1.029
MR	-1.220	0.084	0.074	1.179

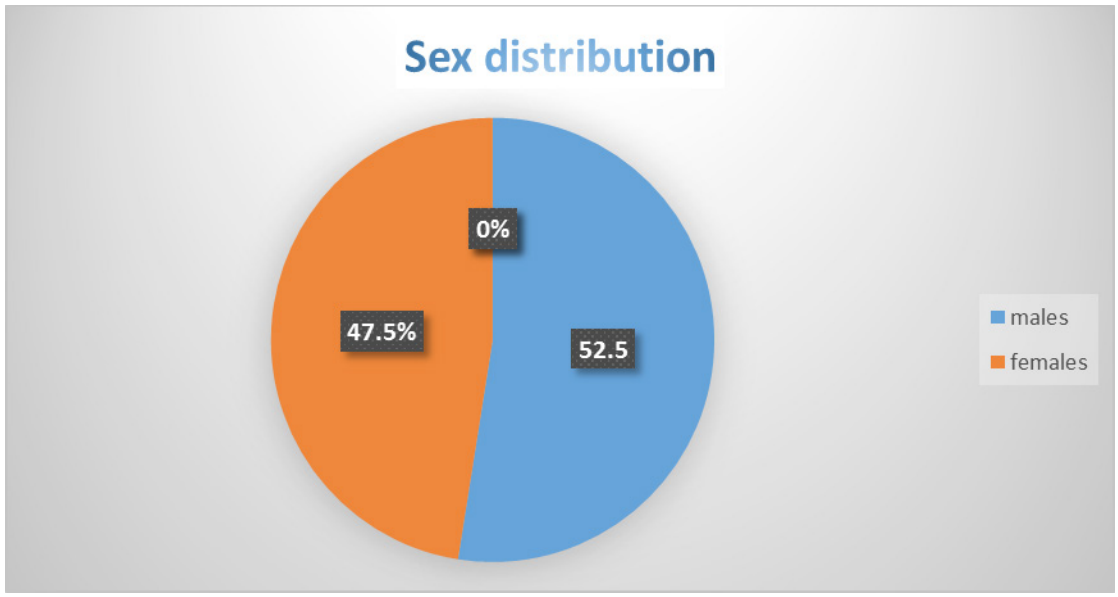

Fig 1: Sex distribution of CML patients

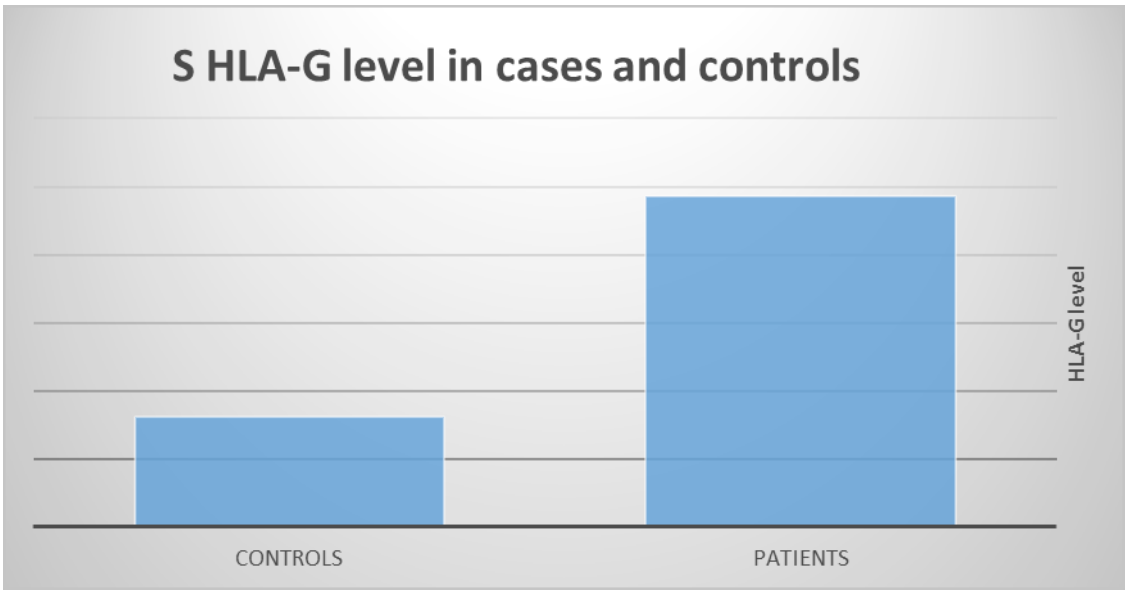

Figure 2: S HLA-G level in cases and controls

Discussion

In current study the mean age of patients with CML was 48.7± 0.597 yearsthat is agreed with previous studiesreported that CML is detected in younger age population (median age 47 years) in developing countries than that in developed countries (median age 72 years) [9, 10].

On the other hand, the incidence of CML was higher in males than females which is comparable with previous literatures [2, 3, 10].

Data regarding s HLA-G secretion in CML are limited and controversial despite the relatively small number of patients, the current study is the second one in the world to assess the association between s HLA-G level and clinical outcome of CML patients.

We detected a statistically significant higher level of s HLA-G in CML patients compared to controls. This result is compatible with previous studies on hematological and solid malignancies [13,14,15,16]. So, the elevated s HLA-G level in CML group is

consistent with speculations on type of secreting cells.

The univariant analysis of the association between s HLA-G level and different clinical parameters revealed absent significant relation of s HLA-G level with age and sex, whereas in multiple regression test a significant correlation was detected between sex and s HLA-G level of CML patients. Similarly, Zidi et al (2014) reported absence of association between s HLA-G level and age or sex of patients, but Calini et al (2013) reported a significant correlation [17, 18].

Furthermore, the highest mean value of s HLA-G level was detected in obese patient group and in multiple regression test a significant association between s HLA-G level and BMI was reported. The effect of obesity on s HLA-G level may be hormonally directed [19]. This result is agreed with previous studies done by Solini et al (2010) and beneventi et al (2016) [20, 21].

Regarding the type of treatment, we noticed that the highest level of s HLA-G was among patients using nilotinib (150 mg) which is higher than that among patients using imatinib (400 mg) and nilotinib (200mg) but we cannot depend on such result that's may be related to the small number of patients using nilotinib (150 mg).

In the current study, different levels of s HLA-G were detected among different scoring system groups and the most accepted one was that seen among low-risk group of Sokal scoring system where the lowest s HLA-G level was seen in single test and in multiple regression test it was significant association.

Furthermore, patients with higher s HLA-G level were seem to have significant lower event free survival in both single and multiple regression test. Also, a negative correlation was found between s HLA-G level and molecular response (MR) in multiple regression test, but not in univariant analysis that is agreed with Cocci et al (2017) [22].

Conclusion

The higher level of s HLA-G may play a role in suppression of immune response to CML cancer cells, while its lower level may account for the event free survival of CML patients and their benefit of TKI program discontinuation.

Declarations: Conflicts of interest related to the study.

Source of Funding: Nil

Ethical Clearance: This research has exemption as it aroutine treatment (none materials were used).

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