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Soluble HLA-G: A Novel Marker in Acute Myeloid Leukemia Patients

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ABSTRACT

Objective: to study soluble HLA-G (sHLA-G) in Egyptian acute myeloid leukemia (AML) patients. HLA-G is speculated to be a tumor-driven immune escape mechanism. In addition, it might be a promising target for future immune therapeutic approaches.
Methods: Thirty AML patients and 15 healthy controls of matched age and sex were the subject of the study. sHLA-G was done to all patients and controls by ELISA.
Results: Statistically significant increase in sHLA-G level was present in AML patients compared to controls, being higher in relapsed cases. HLA-G levels was correlated to bone marrow blast percentages but not affected by age, gender, WBCs or response to chemotherapy. HLA-G had a sensitivity of 100% and a specificity of 62% to detect AML cases.
Conclusion: HLA-G may be an additional marker for AML especially relapsed cases

Key words: AML, HLA-G, prognosis, bone marrow blasts, newly diagnosed, relapsed,

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INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous clonal stem cell malignancy in which immature hematopoietic cells proliferate and accumulate in the bone marrow, peripheral blood and other tissues resulting in bone marrow failure. AML accounts for 90% of all adults' acute leukemias. The annual incidence is approximately 3.5 per 100,000 and increases with age.[1] Most patients with AML are over 60 years old, and their prognosis is dismal, with median survival times of only 8-12 months among the most "fit" patients.[2]

HLA-G is a non-classical HLA class I molecule that plays a beneficial role in pregnancy, transplantation and autoimmune diseases by turning down immune reaction.[3] However, it plays a deleterious role in malignancy. HLA-G is speculated to be a tumor-driven immune escape mechanism[4] that protects tumor cells from NK and cytotoxic T lymphocyte -mediated cytolysis.[5]

In the past few years, HLA-G protein expression has been the focus of extensive research in the diagnosis and prognosis of several human malignancies. In addition, it might be a promising target for future immune therapeutic approaches.[3]

The immune clearance of tumor cells might be enhanced by blocking HLA-G at the level of expression (by RNA interference) or function (by neutralizing antibodies).[5] However, data are limited and conclusions remain controversial in leukemic patients.[3]

The aim of this work was to study soluble HLA-G as a novel marker in acute myeloid leukemia patients and its relation to response to therapy.

MATERIALS AND METHODS

Thirty adult AML patients were selected from Hematology Departments of Alexandria Armed Forces Hospital, Alexandria Main University Hospital and Maadi Armed Forces Medical Compound during the period from May 2013 to December 2013. The patients were subdivided into 2 groups: 15 newly diagnosed AML patients (group B), 15 relapsed AML patients (group C). 15 healthy controls of matched age and sex were considered group A. Patients with hepatic or renal impairment, concomitant

chronic illness and pregnant females were excluded from the study.

All patients were subjected to:

Thorough history taking and clinical examination

Complete blood count.[6]

Bone marrow examination.[7]

Immunophenotyping and conventional cytogenetics.[8,9]

Measurement of sHLA-G was done by ELISA **according to manufacture manual:** peripheral blood samples were allowed to clot and then were centrifuged for 10 min, 2400xg. Serum was frozen immediately at -20°C. Then, levels of sHLA-G (sHLA-G1 and sHLA-G5) were performed using a commercial ELISA kit (Glory Science, USA). All samples tests were performed at duplicate. The optical density (OD) of samples was measured at 450 nm by Dynatech plate reader. The quantity of sHLA-G was determined by constructing a calibration curve of HLA-G1 and sHLA-G5, catalogue#:11455, Glory Science Co., Ltd (USA).

Patients received conventional chemotherapy in form of 3+7 regimen.[10] Response to therapy was assessed after 1 course of chemotherapy. Complete remission (CR) was defined as bone marrow blasts less than 5%, an absolute neutrophil count $\geq 1.0\times10^9$/L, platelet count $\geq 100 \times 10^9$/L and no extra medullary leukemia. Relapse was defined as more than 5% bone marrow blasts unrelated to recovery from the preceding course of chemotherapy or new extra medullary leukemia in patients with previously documented CR.[10] Informed written consent was obtained as proposed by the medical ethics community in Alexandria Faculty of Medicine.

Statistical analysis was done using statistical package for the social sciences (SPSS, version 20.0; SPSS Inc., Chicago, Illinois, USA). Data were analyzed for normality using Kolmogorov-Smirnov test and Shapiro-Wilk test. Parametric data were presented as mean ± SD and compared using t-test (for 2 means) and F test (for more than 2 means). Non parametric data were presented as median value and compared using Mann-Whitney U and Kruskal Wallis. Qualitative data were presented as percentages and tested by Pearson's Chi Square. Spearman bivariate correlation analysis was used for analyzing correlation between sHLA-G and different parameters. $p<0.05$ indicated statistical significance. The ROC curve shows the sensitivity and specificity of sHLA-G in detecting AML cases. If the area under the curve was more than 0.50; the HLA-G was significantly sensitive to detect the incidence of AML.

RESULTS

Table 1 shows comparison between the two AML groups and controls regarding age and sex. M4 was the commonest subtype in both groups (6 cases, 40%) (X^2= 0.98, p=0.365). Table 2 shows the results of complete blood count, bone marrow blasts and sHLA-G level in the studied groups. There was no statistically significant difference between patients as regards different FAB subtypes (F=2.96 p=0.685) and response to therapy (H=14.1; p=0.158). The highest mean value was observed in M6 subtype (605.3±116.5 ng/L) while the lowest mean value was observed in M2 subtype (365.2±98.9 ng/L) as shown in Table 3. Relapsed patients (551.63 ±109.18ng/L) had higher mean value than patients in complete remission (359.67±58.29 ng/L) (data not shown). The area under the curve (Figure 1) was 0.839. The cutoff value of sHLA-G was 368.84; this value shows sensitivity of 100.0% and specificity 62.0%. 24/30 (80%) of all studied AML patients had higher levels than the cutoff point, 10/24 were newly diagnosed AML and 14/24 were relapsed AML.

Table 4 shows the relation between sHLA-G and gender. There was no statistically significant difference between sHLA-G and gender in the same group ($p > 0.05$). Comparing males and females of the three groups together revealed statistically significant increase in sHLA-G in both males and females of newly diagnosed and relapsed group compared to their corresponding controls.

There was statistically significant correlation between sHLA-G and bone marrow blasts percentage (r=0.521*, p= 0.021) while no statistically significant correlation was present between sHLA-G and age (r= 0.125, p = 0.365) or WBCs count (r=0.232, p=0.126).

The mean value of sHLA-G was 365.5, 276.45 and 561.6 ng/L in patients with normal cytogenetics, 20q del (2 patients) and trisomy 8 (2 patients) respectively.

Table 1: Comparison between the clinical data of the studied groups

Parameter	Group A Controls	Group B New AML	Group C Relapsed AML	Test
Age (years) Mean ± SD	48.5 ±16.98	50.1 ±17.5	49.8 ±19.5	F=0.03 p=0.968
Sex Male	7 (46.67%)	6 (40%)	8 (53.33%)	X^2 =0.53
Female	8 (53.33%)	9 (60%)	7 (46.67%)	p=0.765

Table 2: laboratory findings in the studied groups

Parameter	Group A Controls	Group B New AML	Group C Relapsed AML	Test of significance P value
WBCs (x10^9/l) Median	11.0	5.7	8.0	H=2.9 p=0.233
Hb (g/dl) Mean ± SD	12.3 ± 1.57^{bc}	6.91 ± 2.06^{ac}	10.07 ± 2.10^{ab}	F=32.066* p=0.000
Platelets (x10^9/l) Median	148.0 ^{bc}	44.0	48.0	H=25.6 * p=0.001
BM blasts (%) Median	-	34.0	36.0	Z=1.54 p=0.571
sHLA-G (ng/L) Mean± SD	329.8^{bc}±57.54	451.15^{ac}±163.99	551.63^{ab}±109.18	F= 12.21* p=0.001

BM: bone marrow, H: Kruskal-Wallis test, Z: Mann-Whitney test, F: One Way ANOVA; p is significant if < 0.05

Table 3: Comparison between sHLA-G and FAB subtypes

sHLA-G (ng/L)	Mean ±SD
M2 (n=4)	365.2±98.9
M3 (n=5)	402.6±125.0
M4 (n=12)	526.1±105.1
M5 (n=4)	553.6±124.3
M6 (n=5)	605.3±116.5

P is considered significant if <0.05

Table 4: Relation between sHLA-G and gender

s-HLA-G (ng/L)	Controls		Newly diagnosed AML		Relapsed AML	
	Male n=7	Female n=8	Male n=6	Female n=9	Male n=8	Female n=7
Mean ± SD	330.96^{bc} ± 48.26	328.43 ^{bc} ± 61.33	445.63 ± 140.92	455.91 ± 204.59	568.65 ± 135.37	540.39 ± 105.28
t (p)	0.09 (0.930)		0.11 (0.913)		0.46 (0.655)	
F (p); males			7.7 (0.004)*			
F(p);females			4.6 (0.022)*			

t: independent samples t-test, F: One Way ANOVA, * p < 0.05 (significant)

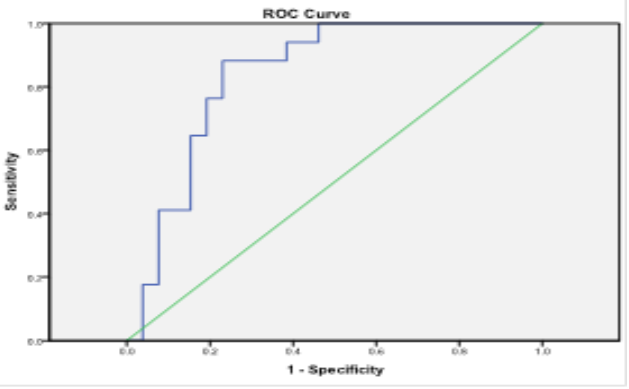

Figure 1: ROC curve for detecting the sensitivity and specificity of sHLA-G in AML cases.

DISCUSSION

HLA-G may be produced by tumor cells or by tumor-infiltrating cells. HLA-G expression has never been found in healthy tumor surrounded areas, in tissues or effusions from benign disease or in the corresponding tissues from healthy individuals.[11] It had local effects at its site of expression and systemic inhibitory activity through its distribution in blood circulation.[11] The induced expression of HLA-G on the surface of tumor cells has been associated with greater tumor morbidity, tumor progression, and spreading.[12] HLA-G has been shown to inhibit all the actors of the anti-tumor response.[11]

We detected statistically significant difference between sHLA-G level in new and relapsed AML cases compared to controls (p=0.001), being higher in relapsed than in new AML cases

($p < 0.05$). 80% (24/30) of our AML patients had HLA-G levels higher than the cutoff point (10/24 were newly diagnosed AML and 14/24 were relapsed AML).

There are contradictory results about HLA-G in AML in various reports. While it was undetectable in some studies;[13,14] it could be detected by western blot in another study.[15]In a large study, 18.5% (10/54) of the leukemic blast cells in AML patients was positive for HLA-G expression. The proportion of HLA-G expression on leukemic cells of AML samples varied from 3.47% to 99.69% in one study [16] and from 0% to 93.96% in another study done by Guo *et al*.[15]

The discrepancy in results can be related to different ethnic population analyzed. A discrepancy can be also explained by higher sHLA-G (shed HLAG1 and HLA-G5) in plasma treated with EDTA compared to plasma treated with heparin or serum.[17]

The analysis of the association between HLA-G level and different clinical parameters, revealed absent of significant relation between HLA-G levels and age, WBCs count, FAB subtypes and response to therapy. There was also no significant difference in the present study between gender in the same group but sHLA.G expression in both males ($p=0.004$) and females ($p=0.022$) of new and relapsed groups was higher than that in the control group that is explained by higher tumor burden. Similarly, Guo *et al*[15] reported absence of association between HLA-G expression status on leukemic blasts and patient age or FAB subtype at diagnosis but Locafaro *et al*[17] reported HLA-G+ blasts in all their studied leukemic males.

The highest mean HLA-G level value in our patients was present in M6 subtype while Gros *et al*[18] showed increased soluble HLA-G antigen in M4 more than M5. They attributed their finding to the influence of GM-CSF and IL-10 on HLA-G secretion and the presence of a more mature monocytic component in FAB M4, in contrast to FAB M5.[18] However in Yan study,[16] 3 out of 11 cases of AML-M5 patients were HLA-G-positive, while HLA-G was not detected in AML-M4.

Two biologic features could be correlated with a high sHLA-G level: absence of myelodysplasia and high-level leukocytosis. Myelodysplasia is one of the factors that are relevant to prognosis in acute leukemias and, is now included in the World Health Organization classification.[18]

No correlation was detected between white blood cell count and sHLA-G level in our study ($p=0.126$). The correlation between leukocytosis and sHLA-G merely reflects higher levels of sHLA-G expressed by the hypercellular pattern.[17] Gros *et al*[18] study failed to reveal any correlation between sHLA-G levels at diagnosis with prognosis. There is absent dysplastic features among our cases. There is an inverse correlation between sHLA-G level and myelodyplasia that can be explained by a potential link between sHLA-G secretion and a de novo acute leukemic process unrelated to chronic pathology, such as dysplasia.[18]

We found a positive correlation between HLA-G and percent blasts in the bone marrow, being higher with higher percent blasts in the bone marrow. This was also reported by Yan *et al*[16] ($p < 0.01$) while not reported by Locafaro *et al*[17] and Guo *et al*.[15]

Locafaro *et al*[17] demonstrated increased HLA-G-expressing CD4+ regulatory T cells in the peripheral blood of leukemic patients with HLA-G-expressing blasts. This lends support to the hypothesis that the expression of HLA-G on blasts may be a strategy by which leukemia limiting anti-tumor responses. This mechanism of immune escape has been previously proposed for solid tumor.[19]

In this study sHLA-G was statistically unrelated to response to therapy. The lowest mean value of sHLA-G was observed in patients in complete remission when compared to relapsed patients. This is similar to that reported by Guo *et al*[15] whose study results indicated that HLA-G expression is of no significance for the prognosis of patients with AML.

CONCLUSION

HLA-G may be an additional marker for AML especially relapsed cases. Further studies on a wider scale are still needed to confirm these findings.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

ABBREVIATION USED

S: soluble, AML: acute myeloid leukemia, BM: bone marrow, H: Kruskal-Wallis test, Z: Mann-Whitney test, F: One Way ANOVA

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