RESEARCH ARTICLE

Serum Human Leukocyte Antigen-G and Soluble Interleukin 2 Receptor Levels in Acute Lymphoblastic Leukemic Pediatric Patients

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Abstract

<u>Aims and Background</u>: Human leukocyte antigen-G and interleukin-2 receptor play pivotal roles in the proliferation of lymphocytes, and thus generation of immune responses. Their overexpression has been evidenced in different malignant hematopoietic diseases. This study aimed to validate serum soluble human leukocyte antigen-G (sHLA-G) and serum soluble interleukin-2 receptor (sIL-2R) as an additional tool for the diagnosis and follow up of acute lymphoblastic leukemia (ALL). <u>Subjects and Methods</u>: Both markers were determined by ELISA in the serum of 33 ALL pediatric patients before treatment and after intensification phase of chemotherapy as well as in the serum of 14 healthy donors that were selected as a control group. <u>Results</u>: ALL patients showed abnormal CBC and high serum lactate dehydrogenase, which were improved after chemotherapy. Also, there was a non-significant increase in serum sHLA-G in ALL patients compared with the control group. However, after chemotherapy, sHLA-G was increased significantly compared with before treatment. On the other hand, serum sIL-2R in ALL patients was increased significantly compared with the control group. After chemotherapy, sIL-2R decreased significantly compared with before treatment. <u>Conclusions</u>: From these results it could be suggested that measurement of serum sHLA-G might be helpful in diagnosis of ALL, while sIL-2R might be useful in diagnosis and follow-up of ALL in pediatric patients.

Keywords: Soluble human leukocyte antigen-G - soluble interleukin-2 receptor - acute lymphoblastic leukemia

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of lymphoid disorders that result from a monoclonal proliferation and expansion of immature lymphoid cells in the bone marrow, blood, and other organs (Faderl et al., 2010). It accounts for about 4.400 new cases each year in the United States (Pui, 2006). In Egypt, the National Cancer Institute (NCI) receives about 300 new cases of ALL every year (Elattar et al., 2007). ALL is subclassified according to the recognized steps of normal maturation within the B-cell lineage (pro-B, early pre-B, pre-B, transitional pre-B, and mature B cells) or T-cell lineage (pre-T, mid, and late thymocyte) pathways (Pui and Evans, 2006).

Human leukocyte antigen-G (HLA-G) is a non-classical major histocompatibility complex (MHC) class I molecule that functions as an immunomodulatory molecule capable of protecting fetal tissues from the maternal immune system (Yan, 2010). Its main function in physiological conditions is to abrogate maternal natural killer cell activity against fetal tissue and to establish immune tolerance at maternal-fetal interface. The HLA-G

is expressed not only as a membrane bound molecule on the surface of cells, but also as a soluble moiety in body fluids (Pistoia et al., 2007). HLA-G expression is highly tissue-restricted: besides being expressed in fetal tissues, such as trophoblast cells (Rizzo et al., 2011), HLA-G constitutive expression was found in adult thymic medulla (Mallet et al., 1999), cornea (Le Discorde et al., 2003), pancreatic islets (Cervera et al., 2010) and erythroid and endothelial-cell precursors (Rebmann et al., 2010). However, HLA-G expression can be induced in cancers (Rouas-Freiss et al., 2005), transplantation (Sheshgiri et al., 2010), multiple sclerosis (Wiendl et al., 2005), inflammatory diseases and viral infections (Fainardi et al., 2011). Expression of HLA-G has been evidenced in different malignant hematopoietic diseases, but most particularly in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and B-chronic lymphocytic leukemia (B-CLL) (Yan, 2010).

Interleukin-2 (IL-2)/IL-2 receptor (IL-2R) system plays important roles in the activation and proliferation of lymphocytes. IL-2R is expressed on the cell membrane of lymphocytes and is formed of at least three different subunits, namely alpha, beta and gamma chain (Bien and

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Balcerska, 2003). Among them, alpha chain is known to increase also in a form of soluble type receptor (sIL-2R) in association with various immunological response and inflammatory process (Bien and Balcerska, 2008). Even in neoplastic states, the elevation of serum levels of sIL-2R has been reported in patients with a variety of hematological malignancies such as adult T-cell leukemia (ATL) (Katsuya et al., 2012), chronic lymphocytic leukemia (CLL) (Pavlidis et al., 1992), hairy cell leukemia (HCL) (Kitaoka et al., 2003), Hodgkin's disease (Bien and Balcerska, 2008), non-Hodgkin's lymphoma (NHL) (Jo et al., 2010) and also in patients with solid malignancy such as lung cancer (Kasprzak et al., 2007).

This study aimed to investigate the validity of measuring soluble human leukocyte antigen-G and soluble interleukin-2 receptor, as markers for ALL, in the serum of Egyptian children with ALL at time of diagnosis and after an intensification phase of chemotherapy (IPC).

Materials and Methods

The present study included 33 newly diagnosed childhood acute lymphoblastic leukemic patients ages ranging from 5 months to 17 years (19 males and 14 females) selected from the pediatric unit at the National Cancer Institute (NCI), Cairo University. The study lasted from October 2009 to January 2011. Among these patients 13 patients were early pre B-cell ALL (cALL), 10 patients were B-cell ALL and 10 patients were T-cell ALL. Two blood samples were collected, one directly after the diagnosis of ALL, and the second after the intensification phase of chemotherapy which lasts for about 6 months. Fourteen healthy donors, (6 males and 8 females) with ages ranging from 2 to 11 years, were selected as control group.

Informed consent was obtained from the participating patients or their parents in adherence with the guidelines of the ethical committee of the NCI, Cairo University.

Blood Samples were collected from each patient; one portion for complete blood count (CBC) and in the other portion, sera were separated and stored at -80°C till the time of analysis.

The following parameters were then measured in the sera: 1. Liver function tests which included ALT, AST, total bilirubin, total proteins and ALP and kidney function tests included urea, uric acid and creatinine. 2. Lactate dehydrogenase (LDH) was determined spectrophotometrically by a kinetic kit (BioSystems S.A. Costa Brava 30, Barcelona, Spain, M11580i-0506). 3. sHLA-G was measured by enzyme linked immunoassay (ELISA) technique (BioVendor Research and Diagnostic Products Laboratorni medicina a.s., Karasek 1767/1, 621 00 Brno, Czech Republic, RD194070100R). 4. sIL-2R was detected by enzyme linked immunoassay (ELISA) technique (Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria, Europe, BMS212/2).

Statistical analysis

Data were analyzed using SPSS statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation (SD).

Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) followed by post-Hoc "Schefe test" on rank of variables for pair-wise comparison. Comparison of repeated measures (before and after measures) was done using Wilcoxon signed-ranks test (non-parametric paired t-test). A p-value≤0.05 was considered significant.

Results

The observed changes in liver and kidney functions parameters in ALL patients before and after IPC were within the normal range.

CBC fractions in ALL patients before and after IPC as well as in the control group

The data in Table 1 clarified that CBC fractions in ALL patients showed great changes. This was indicated by a significant decrease in hemoglobin concentration and platelets count amounting to 57.34% and 32.1% respectively and a high increase in WBCs count and blasts by 790% and 386 folds respectively compared with control.

After IPC there was an observed improvement in CBC fractions of ALL patients as indicated by the significant increase in hemoglobin concentration and platelets count and decrease in WBCs count and blasts nearly to the normal values.

Serum LDH activity in ALL patients before and after treatment as well as in the control group

It was shown in Table 1 that serum LDH activity was significantly increased in ALL patients before treatment by 577.67% compared with the control. After treatment, its level decreased significantly to 33.81% compared with before treatment and returned to normal control value.

Serum levels of sHLA-G in ALL patients before and after treatment as well as in the control group

From the results of Table 2, it was clear that serum level of sHLA-G in ALL patients was increased non-

Table 1. Hemoglobin Concentration, Platelets, WBCs Blasts Count and LDH Activity in ALL Patients Before and After Treatment and in the Control Group

Group (N)	Hemoglobin (g/dl)	Platelets (x103/μl)	WBCs (x103/μl)	Blasts (%)	LDH activity (U/l)
Control (14)	14.3±1.3	309.8±79.5	6.4±1.7	0.2±0.2	108.7±64.0
Before treatment (33)	8.2±2.5[b]	99.7±116.3[b]	50.6±93.8[b]	77.3±20.3[b]	493.0±272.0[b]
After treatment (33)	10.5±1.4[bd]	359.8±182.5[d]	5.0±3.3[ae]	1.1±1.2[d]	291.0±217.0[c]

*Data are represented as mean±SD. Significantly different from the control at [a]P≤0.01 or [b]P<0.001. Significantly different from before treatment at [c]P≤0.01 or [d]P<0.001. Results are compared with control using Mann-Whitney test for hemoglobin level, platelets and blasts count and Fisher's Extract test for WBCs count. Results are compared with before treatment using Wilcoxon signed Rank's test. N=number of cases

significantly to 232.16% compared with the control group. After treatment, serum sHLA-G showed significant increase by 152.81% compared with before treatment and also increased significantly by 354.77% compared with the control group.

Serum levels of sIL-2R in ALL patients before and after treatment as well as in the control group

From the results of Table 2, it was clear that serum level of sIL-2R in ALL patients was increased significantly to 216% compared with the control group. After treatment, sIL-2R decreased significantly by 49.55% compared with before treatment.

sHLA-G and sIL-2R in the different subtypes of ALL patients, in males and females and at different ages before and after treatment group

It was clear from the results of Tables 3 and 4 that there was no significant difference in sHLA-G and sIL-2R in the different subtypes of ALL or between males and females or between different age groups before and after treatment.

Table 2. Serum Level of sHLA-G Concentration in ALL Patients Before and After Treatment and in the Control Group

Group (N)	sHLA-G concentration (Units/ml)	sIL-2R concentration (ng/ml)
Control (14)	31.0±21.1	10.5±3.4
Before treatment (33)	71.9±82.6	22.7±13.9[a]
After treatment (33)	109.9±91.3[ab]	11.2±7.7[c]

*Data are represented as mean±SD. Significantly different from the control group at [a]P≤0.01. Significantly different from before treatment group at [b]P≤0.05. Results are compared with control group using Mann-Whitney test. Results are compared with before treatment group using Wilcoxon signed Ranks test. N=number of cases

Table 3. Comparison between sHLA-G and sIL-2R in the Different Subtype and at Different Age Stages of ALL Before and After Treatment

Group (N)	sHLA-G		sIL-2R	
	Before	After	Before	After
ALL Subtypes				
cALL (13)	49.4±80.0	98.6±96.5	28.9±13.2	12.4±8.2
Pre B-cell ALL (10)	89.7±71.4	123.6±98.2	20.2±14.9	9.2±3.8
T-cell ALL (10)	83.6±96.8	111.0±84.6	17.0±11.7	11.8±10.2
P value	0.278	0.67	0.136	0.643
Different Age Stages				
<5 (14)	59.5±90.9	88.3±97.9	26.8±15.1	11.7±8.9
5-10 (14)	81.1±86.8	123.4±99.1	19.3±11.1	12.3±7.6
>10 (5)	81.5±47.4	132.9±27.7	20.6±17.7	7.1±3.8
P value	0.460	0.176	0.505	0.304

*Data are represented as mean±SD. Results are compared using non-parametric ANOVA by Kruskal-Wallis. N=number of cases

Table 4. Comparison of sHLA-G and sIL-2R in Males and Females ALL Patients Before Treatment

Group (N)	sHLA-G		sIL-2R	
	Before	After	Before	After
Males (19)	66.6±78.3	104.8±103.3	24.5±13.6	12.0±19.5
Females (14)	79.3±90.5	116.9±75.1	20.1±14.6	10.2±4.7
P value	0.788	0.339	0.377	0.872

*Data are represented as mean±SD. Results are compared using non-parametric ANOVA by Kruskal-Wallis. N=number of cases

Discussion

In the present study ALL patients, at the time of diagnosis, showed abnormal CBC fractions characterized by decreased hemoglobin concentration and platelets count with increased WBCs count and blasts. These results were in accordance with previous work of Sarmento-Ribeiro, (2012), Brito-Babapulle, (2003), and Ling et al. (2007).

Furthermore, serum LDH activity was highly increased in ALL patients compared to control. This was in agreement with Hafiz et al. (2008) who stated that increased cellular LDH activity reflects a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of malignant cells accompanied by high cellular turnover rate. After IPC these CBC fractions and serum LDH activity were greatly improved.

The present study results showed that serum level of sHLA-G in ALL patients was increased non-significantly compared with the control group. After IPC, sHLA-G still increased significantly compared with before treatment and failed to return to control value. This result agreed with Yang et al., (2012) who stated that there was an increase in plasma sHLA-G of ALL patients compared to the control.

Rouas-Freiss et al. (2005) stated that cancer cells may likely use HLA-G expression to escape host immunosurveillance similar to what extravillous (intermediate) trophoblastic cells do in the maternal–fetal interface. HLA-G expression would favor tumor development by impairing antitumor immunity (Singer et al., 2003). This is achieved by inhibiting the cytolytic function of uterine and peripheral blood NK cells (González et al., 2012), the antigen-specific cytolytic function of cytotoxic T lymphocytes (Ksouri et al., 2009), the alloproliferative response of CD4+ T cells (Mitsdoerffer et al., 2000), the proliferation of T cells and peripheral blood NK cells (HoWangYin et al., 2010) and the maturation and function of dendritic cells (Wehner et al., 2011).

The mechanism explaining how HLA-G help tumor cells escape immunosurveillance was explained by HoWangYin et al. (2011) who showed that following immunological synapse formation, HLA-G1 containing membrane patches can be transferred from antigen presenting cells to T cells or from tumor cells to NK cells. This mechanism is also known as trogocytosis. The acquisition of HLA-G1 at the surface of T or NK cells not only inhibits their immune functions but also renders them immunosuppressive as they could inhibit T cells alloproliferation as well as NK cytolytic functions. Thus, trogocytosis of HLA-G may explain how a low proportion of tumoral cells expressing HLA-G can protect the whole tumor against immune system.

The present study also showed that serum level of sIL-2R in ALL patients was increased significantly compared with the control group but returned to normal level after IPC.

This study agreed with Nakase et al., (2005) who found that sIL-2R was elevated in serum of patients with hematological malignancies including ALL. Also, similar results were obtained by Moon et al. (2004) who found that

plasma IL-2R levels were significantly higher in the ALL patients than in the normal controls and it decreases after induction chemotherapy. Furthermore, Eiji et al. (2000) concluded that measurement of serum sIL-2R level is useful in bone marrow monitoring of patients with ALL.

IL-2 is a lymphocytotrophic cytokine that is involved in the growth and differentiation of T and B cells and enhances the cytolytic functions of natural killer (NK) cells; it is also known to have some function in the proliferation of several non-lymphoid cells (García-Tuñón et al., 2004). The release of sIL-2R appears to be a characteristic marker of T-lymphocyte activation and might serve an immunoregulatory function during both normal and abnormal cell growth and differentiation (Arioz et al., 2009).

Although the physiological and pathological function of sIL-2R is not well defined, it can bind IL-2 and might downregulate the host immune response by competing with the normal lymphocyte cellular IL-2R. Therefore, the increased levels of sIL-2R have been suggested to enhance neoplastic growth by suppressing the host antitumor immunity in patients with a malignancy and this may be one possible explanation for the leukocytosis (leukemic cell proliferation) (Nakase et al., 2005). There were many cases, particularly ALL patients, who had elevated plasma sIL-2R levels without IL-2R expression on their leukemic cells. This suggests that the plasma sIL-2R originates from other cells (possibly activated T lymphocytes) but not from leukemic cells (Moon et al., 2004).

In the present study, there was no significant difference in HLA-G and sIL-2R among the different subtypes of ALL indicating that these parameters might support diagnosis and follow up in any of the different subtypes of ALL, but could not differentiate between them.

In conclusion, 1. This study showed that sHLA-G was increased in ALL patients before treatment and further increase was observed after IPC. 2. On the other hand, serum LDH and sIL-2R were increased in ALL patients at diagnosis, and decreased significantly after IPC compared to before treatment. 3. These results suggest that measurement of serum sHLA-G can be helpful in diagnosis of ALL in children. 4. sIL-2R can provide an additional tool in diagnosis as well as prognosis of ALL. 5. Finally, the estimation of sHLA-G and sIL-2R could be a promising tool for the diagnosis and prognosis of ALL; however, further investigations with greater number of patients and for longer times of chemotherapy are required to evaluate the beneficial use of sHLA-G and sIL-2R as additional markers for the diagnosis and follow up of ALL patients.

Acknowledgements

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