ORIGINAL ARTICLES

Cellular expression of CD54 and estimation of sIL-2R, sVCAM-1 and sE-selectin in Children with Acute Lymphoblastic Leukemia

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ABSTRACT

Measurement of some circulating endothelial adhesion molecules levels and interleukin receptors have been suggested as additional tools for assessment of patients with acute lymphoblastic leukemia (ALL). The aim of this study is to estimate circulating sVCAM-1, sE-selectin and sIL2-R in the serum of patients with acute lymphoblastic leukemia before and after chemotherapy and comparing them to the control group. Also to detect the cellular expression of CD 54 on bone marrow cells of children with acute lymphoblastic leukemia and comparing its level in responders and nonresponders to treatment. This study included seventy subjects attending National Cancer Institute; forty newly diagnosed patients with acute lymphoblastic leukemia (ALL), 10 patients in relapse and 20 apparently normal subjects within the same age and sex range, as a control group. Acute lymphoblastic leukemia diagnosis was made by bone marrow aspiration, cytochemistry and microscopic examination. Flowcytomer was used for immunophenotyping to confirm the diagnosis using monoclonal antibodies. Expression of CD54 on mononuclear cells is detected by monoclonal antibodies using flow cytometry. Soluble sIL2-R, sVCAM-1 and sE-selectin were estimated using enzyme-linked immunosorbent assay. There were significant increases in the estimated parameters in patients before chemotherapy as compared to after treatment as well as to the control group. Initial CD54 cellular expression was significantly higher in responders as compared to nonresponders to treatment. In conclusion, the levels of some soluble circulating adhesion molecule levels can be utilized for monitoring the disease activity of ALL and its response to treatment. Also the initial low cellular expression of CD54 is a predictor for adverse prognosis.

Key words: ALL, sVCAM-1, sE-selectin, CD54 and sIL2-R

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common pediatric leukemia accounting for 25-30% of all cases of childhood malignancies (Krajinovic et al., 1999). It is characterized by the neoplasm of immature haematopoietic precursor cells (Chowdhury et al., 2007).

CD 54, the intercellular adhesion molecule-1 (ICAM-1) is a glycoprotein. Through its binding to its physiologic ligand, the leukocyte function-associated antigen-1, CD54 plays a crucial role in cell-cell and cell-stroma interactions, which are important for the initiation of the immune inflammatory processes, cellular interactions with vessel endothelium and with malignant transformation of cells (Arkin et al 1991). Interaction of intercellular adhesion molecules (ICAMs) with their receptors has a key role in leucocyte adhesion and migration (Mustjoki et al., 2001). Altered expression or function of adhesion molecules on leukemic blasts may contribute to the evolution and biological behaviour of acute leukemia (Hafez et al., 2003).

Adhesion molecules on the cell surface play an important role in cell-cell interaction. Elevated levels of soluble forms of these molecules are found in a variety of inflammatory conditions (Nash et al., 1996).

The main function of interleukin-2 receptors (IL-2R) is the regulation of the immune response by binding interleukin-2, resulting in blocking the biological functions of this cytokine. By the competition with the cell surface interleukin-2 receptors, sIL-2R act as an immunosuppressive factor inhibiting IL-2R related lymphoblast growth (Siemińska, 2004).

The selectins mediate the adhesion of hematopoietic cells to vascular surfaces and to each other (Vestweber and Blanks, 1999). These interactions are important for host defense, hematopoiesis, immune cell surveillance, hemostasis, and inflammation (Zarbock et al., 2011). E-selectin is a cell adhesion molecule expressed on
endothelial cells activated by cytokines. Like other selectins, it plays an important part in inflammation. In humans, E-selectin is encoded by the SELE gene. E-selectin is also an emerging biomarker for the metastatic potential of some cancers and recurrences (Yago et al., 2010).

The vascular cell adhesion molecule 1 (VCAM-1) is a member of the immunoglobulin gene superfamily that plays an important role in the adhesion of leukocytes to the vascular endothelium. Cytokine activation dramatically up-regulates its expression on the cell surface where it supports the interaction of leukocytes and endothelial cells (Barreiro et al., 2002).

In addition to being expressed on the cell surface, soluble forms of adhesion molecules have been detected in circulating blood and have been shown to retain their functional ability (Ballantyne and Entman, 2002).

It is generally accepted that bone marrow relapse in ALL results from residual leukemic cells that have survived therapy. The level of minimal residual disease at the end of induction chemotherapy is therefore an indicator of disease chemoresistance and has proven to be a powerful tool in predicting relapse in childhood ALL (Borowitz et al., 2003). Relapse following remission induction chemotherapy remains a barrier to survival in approximately 20% of children suffering from acute lymphoblastic leukemia (Choi, 2007).

The aim of this study is to investigate the changes in serum levels of soluble forms of IL-2R, vascular cell adhesion molecule-1 and E-selectin in children with ALL in newly diagnosed cases in comparison to patients after treatment as well as cases in relapse and normal control children. Also, the bone marrow cells expression of CD54 as regards responders and non responders to treatment.

Materials and Methods

This study included seventy subjects attending National Cancer Institute within the year 2009. Their age ranged from 2 to 14 years old. They were 48 males and 22 females. Informed consents were obtained from children’s parents and the protocol was approved by the Medical Ethics committee of the National Cancer institute. Forty newly diagnosed patients with acute lymphoblastic leukemia (ALL) before starting chemotherapy (Group 1), follow up was done and by the end of the 120 weeks of therapy, complete re-evaluation was again confirmed by bone marrow analysis, then patients were put under follow-up once monthly by clinical examination and CBC. Complete remission is defined as the disappearance of organomegaly, normal hematological indices and bone marrow normocellular with <5% lymphoblasts (Group 2). Ten patients in relapse were included in this study (Group 3) and 20 apparently normal subjects within the same age and sex range, as a control group (Group 4). Patients were subjected to full clinical examination. Acute lymphoblastic leukemia diagnosis was made by bone marrow aspiration, cytochemistry and microscopic examination. Flowpartec PASIII flow cytometer was used for immunophenotyping to confirm the diagnosis (Coustan-Smith et al., 2002). Fluorescent labeled monoclonal antibodies for CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD19, CD22, CD33, CD54 as well as anti kappa, anti lambda and cytoplasmic mu chain were used. The antibodies were purchased from Serotec DAKO, they were either fluorescein-labelled (FITC) or phycoerythrin-labelled (PE) (Moon et al., 2004). Soluble IL2-R, sVCAM-1 and sEselectin were estimated using enzyme-linked immunosorbent assay (Moon et al., 2004; Glowinska et al., 2005).

SPSS package (version 13) was used for data analysis. Mean and standard deviation were used to describe quantitative data by student’s t test. P-value is considered significant at 0.05 or less.

Results:

Initial cellular expression of CD54 showed a significant higher level (P<0.05) in responders as compared to non responders to treatment (Table 1).

The mean ± SD levels of sIL-2R, sVCAM-1 and sE-selectin at the onset of the disease (Group 1) were 159.55±80.31 pg/ml, 204.2 ± 71.9 ng/ml and 55.7±15.9 ng/ml, respectively. These values were significantly higher (P < 0.05) than those of the control group (Group 4); 29.4±13.21 pg/ml, 40.4 ± 32.7 ng/ml and 14.4 ± 11.3 ng/ml respectively. In patients after treatment (Group 2), the mean level of sE-selectin did not differ significantly from those of the control group, while sIL-2R and sVCAM-1 were significantly elevated as compared to the control group (p<0.05). In relapse (Group 3) sIL-2R, sVCAM-1 and sE-selectin mean ± SD levels; 60.1±20.3 pg/ml, 203.6±108.1 ng/ml and 46.4±16.4 ng/ml respectively were significantly higher (P < 0.05) than those of the control group (Table 2). Pearson's correlation coefficient r was calculated and positive correlations were detected between bone marrow blasts count in group 1 and both the sVCAM-1 and sE-selectin (Figure 1&2), P<0.05.
Table 1: Initial CD 54 cellular expression in patients with ALL as regards their response to treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial CD54 (% Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients in remission (n=40)</td>
<td>12.5 ± 5.1</td>
</tr>
<tr>
<td>Patients in relapse (n=10)</td>
<td>7.8 ± 4.2</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.05</td>
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</tbody>
</table>

Table 2: Estimated parameters in the four groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>sVCAM-1 (ng/ml)</th>
<th>sE-selectin (ng/ml)</th>
<th>sIL2-R (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=40)</td>
<td>204.2 ± 71.9(^a)</td>
<td>55.7± 15.9(^a)</td>
<td>159.55± 80.31(^a)</td>
</tr>
<tr>
<td>Group 2 (n=40)</td>
<td>94.4± 29.8(^b)</td>
<td>22.4± 18.1(^b)</td>
<td>57.27± 24.03(^b)</td>
</tr>
<tr>
<td>Group 3 (n=10)</td>
<td>203.6± 108.1(^a)</td>
<td>46.4± 16.4(^a)</td>
<td>60.1± 20.3(^a)</td>
</tr>
<tr>
<td>Group 4 (n=20)</td>
<td>40.4± 32.7</td>
<td>14.4± 11.3</td>
<td>29.4± 13.21</td>
</tr>
</tbody>
</table>

\(^a\) _p_ < 0.05 as compared to the control group

\(^b\) _p_ < 0.05 as compared to the newly diagnosed patients group

Fig. 1: The correlation between sVCAM-1 and bone marrow blasts in Group 1

Fig. 2: The correlation between sE-selectin and bone marrow blasts in Group 1
Discussion:

The adhesion molecule expression has a relevant role for the mechanisms of homing, trafficking and spreading of malignant cells (Mengarelli 2001). High expression of the adhesion molecule CD54 (ICAM-1) seems to play an important role in the anti-leukemic immunity as some authors report a positive correlation between survival and the presence of this molecule on blast cells (Luczyński et al., 2006). In this study the mean cellular expression were significantly high in patients in remission as compared to patients in relapse. This was in agreement with Hafez et al., 2003 results who stated that low CD54 is a significant predictor of mortality in children with ALL. Also, Mustjoki et al., (2001) showed that ICAM-1 mediated leukaemia cell extravasation, and that cellular ICAM-1 had an important role in the disease progression. Mielcarek et al., (1997) demonstrated that CD54 expression has an impact on dissemination patterns and outcome of childhood ALL, and emphasized the potential importance of adhesion mechanisms in influencing clinical characteristics and prognosis of ALL. While Mengarelli et al., (2001) found CD54 was expressed in variable proportion by ALL blasts. They stated that expression of adhesion molecules on lymphoblasts is not relevant for the clinical aspects of the disease and for prognosis.

In this study, sVCAM-1 and sE-selectin were significantly elevated in newly diagnosed ALL cases as compared to after treatment and to the control group, this was in agreement with Hatzistilianou et al., (1997) results. They stated that the concentration of soluble adhesion molecules may be dependent on different factors such as the rate of synthesis of the receptor by cells, the rate of shedding from cells and their capacity to bind ligands expressed on cells and vascular endothelium. Also, Sulicka et al., (2012) showed increased levels of sVCAM-1 in ALL survivors than controls.

In this study, sIL-2R was significantly elevated in newly diagnosed ALL cases as compared to patients after treatment and to the control group, this was in agreement with Nakase et al., (2005) who showed that hematological neoplasms displayed a wide range of sIL-2R levels and extremely elevated values of sIL-2R. Also, Moon et al., (2004) detected significantly higher serum levels of sIL-2R than in normal people, in addition its level was higher in patients with a poorer outcome, and decreases after induction chemotherapy. They suggested that the serum sIL-2R level is a valuable marker for monitoring ALL after chemotherapy. Siemińska, (2004) reported elevated levels of sIL-2R both in immunodeficiency and immunostimulation state and stated that the level of sIL-2R may be used as a valuable prognostic marker in patients treated with chemotherapy.

In this study, statistically positive correlations were detected between bone marrow blasts count in newly diagnosed cases and both the sVCAM-1 and sE-selectin, which indicate that these soluble molecules are correlated with the tumor cell burden.

Conclusion:

The levels of some soluble circulating adhesion molecules can be utilized for monitoring the disease activity of acute lymphoblastic leukemia and its response to treatment. Also the initial low cellular expression of CD54 is a predictor for adverse prognosis.

References


Glowinska, B., M. Urban, J. Peczynska, B. Florys, 2005. Soluble adhesion molecules (sICAM-1, sVCAM-1) and selectins (sE selectin, sP selectin, sL selectin) levels in children and adolescents with obesity, hypertension, and diabetes. Metabolism., 54(8): 1020-1026.


