The Role of Type I Insulin Like Growth Factor Receptor (IGF-IR) in Adult and Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Type 1 insulin like growth factor receptor (IGF-IR) is over expressed in many tumors including hematological cancers. It is a critical signaling molecule for tumor cell proliferation and survival. Data suggest that IGF-IR antibodies can effectively and specifically inhibit cancer cell growth in vitro and in vivo. Blockage of IGF-IR expression could be a promising therapeutic approach for the management of cancer patients.

Aim of Work: To characterize the expression pattern of IGF-IR gene in malignant lymphoblasts of children and adults suffering from ALL in relation to clinical features at diagnosis.

Patients and Methods: The expression of IGF-IR was analyzed in 60 patients with ALL, 30 childhood ALL (16 newly diagnosed and 14 in complete remission) and 30 adulthood ALL (15 newly diagnosed and 15 in complete remission) together with 20 normal age and sex matched healthy controls using a Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RTQ-PCR) to assess the possible relation, association or correlation between IGF-IR expression and ALL clinical and laboratory features at diagnosis.

Results: IGF-IR was expressed in all 60 patients with ALL; the expression levels of IGF-IR were significantly higher in newly diagnosed patients than in patients in complete remission (CR) and controls (p<0.001). There were no statistically significant differences in the expression of IGF-IR between patients with different clinical and laboratory features.

Conclusion: IGF-IR seems to play a crucial role in patients with ALL since it is expressed in all ALL cases (adulthood and childhood). Therefore, new therapeutic agents targeting IGF-1R may provide a better chance for those patients.

Key Words: IGF-IR – Adult ALL – Childhood ALL – RT-PCR – Prognosis.

INTRODUCTION

The first evidence indicating that local autocrine or paracrine production of growth factors might be important during the development of cancer comes from the observation that transformed or tumor derived cell lines require less serum for proliferation in vitro than their normal counterparts. From these early reports emerged the concept of de novo synthesis of polypeptide growth factors by the tumor cells themselves. Among the cytokines that are involved in growth regulation, differentiation and cell metabolism, members of the insulin-like growth factor (IGF) signaling system have been shown to be potentially important in most cell types. The IGF system is a complex network, consisting of the two IGF peptides (IGF-I and IGF-II), two IGF receptors, six well characterized IGF binding proteins (IGFBPs), at least six IGFBP related proteins (IGFBP-rPs), IGFBP and IGFBP-rP cell surface receptor proteins, in addition to IGFBP and IGFBP-rP proteases [1].

IGF-I and IGF-II are members of the insulin related peptides family, diverged from a common ancestor through evolution. Circulating IGF-I is growth hormone dependent and is produced by the liver and extra hepatic tissues. IGF-I stimulates DNA synthesis as a progression factor in the cell cycle. In addition, it has the ability to promote the differentiation of mesen-
chymal cells and has acute insulin-like metabolic effects. The biological activity of the IGF signaling system is mediated by specific membrane associated glycoprotein receptors: Type 1 and 2 IGF receptors, insulin receptor, and the chimeric insulin-IGF-I receptor. The type 1 IGF receptor is closely related to the insulin receptor [2].

The insulin receptor (isoforms IR-A and IR-B) and type 1 insulin-like growth factor receptor (IGF-1R) are homologous, multi-domain tyrosine kinases that bind insulin and IGF-1 with differing specificity. IR is involved in metabolic regulation and IGF-1R in normal growth and development. IR-A also binds IGF-2 with an affinity comparable to IGF-1R and like the latter, is implicated in a range of cancers [3].

Insulin-like growth factors and their receptor (IGF-1R) have been implicated in cancer pathophysiology. It was demonstrated that IGF-1R is universally expressed in various hematological malignancies (multiple myeloma, lymphoma, leukemia) and solid tumors such as breast, prostate, lung, colon, thyroid, renal, adrenal cancer, retinoblastoma and sarcomas [4].

IGF and IGF-1R play an important role in mitogenesis, apoptosis, growth and proliferation of several types of cancers. Recent studies have shown that over expression of IGF-1R is associated with increased cancer cell proliferation, migration and inhibition of apoptosis. These results suggest the involvement of insulin-like growth system in the regulation of apoptosis in many tumors [5,6].

Specific IGF-1R inhibition with neutralizing antibody, antagonistic peptide, or the selective kinase inhibitor has in vitro activity against diverse tumor cell types particularly hematological cancers [4].

In this study, we analyzed the expression level of type 1 insulin like growth factor receptor in 60 patients with acute lymphoblastic leukemia (ALL), 30 childhood and 30 adult type, together with 20 normal healthy age matched controls, using a Real-Time Quantitative Reverse–Transcriptase Polymerase Chain Reaction (RTQ-PCR) technique with a specific aim of determining there role in the development and progression of ALL.

Material and Methods

Patients: The study included 60 patients with ALL and 20 normal age and sex matched controls. Patients were recruited from the National Cancer Institute (NCI), Cairo University and Beni Suef University hospital. The diagnosis of ALL was made based on the morphology from Geimsa stained smears of bone marrow (BM) aspirates, cytochemical stains criteria such as: Negativity for myeloperoxidase (MPO) and Sudan black B (SBB) and a positivity for acid phosphatase for (T-ALL) and immunophenotyping.

The study included 31 male and 29 female, 30 children (16 were newly diagnosed and 14 were in complete remission) and 30 adults (15 were newly diagnosed and 15 were in complete remission), 36 were precursor B-lineage and 24 were T-lineage. The RTQ-PCR was used to assess the expression rates and levels of IGF-IR and to investigate a possible relation, association, or correlation with the clinical features of ALL patients at diagnosis such as: Sex, age, lineage (B or T), hemoglobin (HB), TLC, platelets count and BM blast cell infiltration.

RNA isolation and real-time quantitative RT-PCR:

Mononuclear cells (MNCs) were isolated from 2ml BM aspirate at diagnosis by Ficoll density gradient centrifugation. Total RNA was extracted from MNCs using a QIAamp RNA blood kit (Qiagen, Germany) according to manufactures criteria. Complementary DNA (cDNA) was synthesized using (dt) 15-mer primer by superscript III Reverse transcriptase and stored at −20°C till use.

The mRNA expression levels of type I insulin like growth factor receptor (IGF-IR) and gliceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured by quantitative RT-PCR using an ABI PRISM 7000 Sequence Detector System (Applied Biosystems, Foster City, CA). The quantitative RT-PCR amplification was performed using the predeveloped Assays-on-demand Gene Expression Set for the IGF-IR and TaqMan GAPDH control reagents (Applied Biosystems) for the GAPDH gene in combination with the TaqMan Universal PCR Master Mix (Applied Biosystems).
All reactions were performed in triplicate using 20 µl samples containing 50 ng cDNA. The reaction protocol used involved heating for 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles of amplification (15 seconds at 95°C and 1 minute at 60°C). Analysis was performed using ABI PRISM 7000 Sequence Detection Software (Applied Biosystems).

The expression levels of IGF-IR gene in all tested samples and healthy controls were expressed in the form of CT (cycle threshold) level. A negative control without template was included in each experiment.

The expression rates of IGF-IR were recorded as a percentage (%) of patients with IGF-IR expression among total patients regardless of the expression level and the expression levels were represented as the mean values ± SD. Differences in expression levels of IGF-IR were correlated with other clinical factors using the Mann-Whitney U test. Results were considered significant at \( p < 0.05 \).

**RESULTS**

**Clinical characteristics of ALL patients:**

There were 30 children, 14 male and 16 female; 17 B lineage and 13 T lineage ALL. Fourteen cases were in complete remission (6 males and 8 females) and 16 cases were newly diagnosed (8 males and 8 females).

Concerning the children in complete remission (CR), the mean age was 6.2 ± 3.5 years, the mean TLC was 7.2 ± 3.5x/L, the mean Hb was 11.5 ± 2.5gm/dl, the mean platelet count was 128 ± 20x/L, the mean BM blast concentration was 3 ± 2%, 9 cases (64.2%) were B lineage and 5 (35.8%) were T lineage ALL.

Concerning the newly diagnosed children, the mean age was 10.5 ± 5 years, the mean TLC was 75.2 ± 50x/L, the mean Hb was 6.7 ± 2.5gm/dl, the mean platelet count was 46.4 ± 30x/L, the mean BM blast concentration was 89.9 ± 10%. 8 cases (50%) were B lineage and 8 (50%) were T lineage ALL. Seven cases (43.7%) had enlarged lymph nodes, 9 (56.2%) had hepatosplenomegaly and 7 (43.7%) had both (Table 1).

There were 30 adult patients, 17 male and 13 female; 19 B lineage and 11 T lineage ALL. Fifteen cases were in complete remission (8 males and 7 females) and 15 cases were newly diagnosed (9 males and 6 females).

Concerning the adults in complete remission (CR), the mean age was 25.5 ± 8 years, the mean TLC was 8 ± 4x/L, the mean Hb was 12 ± 3.5gm/dl, the mean platelet count was 120 ± 20x/L, the mean BM blast concentration was 2 ± 1%, 11 cases (73.3%) were B lineage and 4 (26.7%) were T lineage ALL.

Concerning the newly diagnosed adults, the mean age was 34.5 ± 15 years, the mean TLC was 75.6 ± 40x/L, the mean Hb was 7.5 ± 3gm/dl, the mean platelet count was 54 ± 30x/L, the mean BM blast concentration was 73 ± 25%, 8 cases (53.3%) were B lineage and 7 (46.7%) were T lineage ALL. Seven cases (43.3%) had enlarged lymph nodes, 8 (53.3%) had hepatosplenomegaly and 7 (43.7%) had both (Table 2).

The analysis of IGF-IR expression showed that all newly diagnosed ALL patients exhibited IGF-IR above controls, while none of the ALL cases in complete remission exhibited IGF-IR above controls (Fig. 1).

The mean expression level of IGF-IR was at 22.9 ± 2.5 CT for newly diagnosed childhood cases compared to 34.1 ± 4 CT for childhood cases in complete remission.

The mean expression level of IGF-IR was at 22.5 ± 2.5 CT for newly diagnosed adulthood cases compared to 37.7 ± 2 CT for adulthood cases in complete remission.

The mean expression level of IGF-IR was at 34.7 ± 4 CT for controls.

The expression levels of IGF-IR were higher in newly diagnosed ALL patients (both childhood and adulthood) than in ALL patients in complete remission (CR) & controls (\( p < 0.001 \)). On comparing ALL childhood and adulthood cases in complete remission (CR) with controls, there was no statistically significant difference (\( p > 0.05 \)).

There were no statistically significant differences in the expression levels of IGF-IR between patients with different clinical and laboratory features including sex, age, lineage, hemoglobin, TLC, platelets and BM blast concentration (\( p > 0.05 \)).
DISCUSSION

Type 1 insulin-like growth factor receptor (IGF-IR), which mediates cancer cell proliferation and metastasis, is over expressed or activated in many human cancers including haematological malignancies [7]. Several studies indicate that blocking IGF-IR expression can inhibit tumour cell proliferation and metastasis [8].

In this study, the expression level of type 1 insulin like growth factor receptor (IGF-IR) was analyzed in 60 patients with ALL (30 childhood and 30 adulthood) together with 20 normal age matched healthy controls.

IGF-IR was expressed in all 60 patients with ALL (adulthood and childhood). The expression levels of IGF-IR were higher in newly diagnosed ALL patients than in ALL patients in complete remission (CR) and controls. However, on comparing ALL childhood and adulthood cases in complete remission (CR) with controls, there was no statistically significant difference. Also, there were no statistically significant differences between ALL patients (childhood and adulthood) regarding gender, age, lineage, hemoglobin, TLC, platelets and BM blast infiltration.

Vorwerk et al., [2] studied the expression pattern of genes of the IGF system in malignant

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**Table (1): Childhood patients’ characteristics and IGF-IR expression rates and level.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
<th>Level</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total:</strong></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly diagnosed</td>
<td>16</td>
<td>22.9CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td>CR</td>
<td>14</td>
<td>34.14CT</td>
<td>p1&gt;0.05</td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>21.5CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>22.1CT</td>
<td>p2&gt;0.05</td>
</tr>
<tr>
<td><strong>Immunophenotyping:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>23.54CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td>T</td>
<td>13</td>
<td>25.62CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p2&gt;0.05</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>14</td>
<td>26.26CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td>Hepatosplenomegally</td>
<td>16</td>
<td>25.54CT</td>
<td>p1&lt;0.001</td>
</tr>
</tbody>
</table>

p1: Relation of each group to control calibrators.  
p2: Relation of each category to the other in the same group.  
p<0.001 HS.  
p>0.05 NS.

**Table (2): Adulthood patients’ characteristics and IGF-IR expression rates and levels.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
<th>Level</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total:</strong></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly diagnosed</td>
<td>15</td>
<td>22.54CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td>CR</td>
<td>15</td>
<td>37.69CT</td>
<td>p1&gt;0.05</td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>22.44CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>23.12CT</td>
<td>p1&lt;0.001</td>
</tr>
<tr>
<td><strong>Immunophenotyping:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>19</td>
<td>23.54CT</td>
<td>p1&lt;0.001</td>
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<tr>
<td>T</td>
<td>11</td>
<td>26.3CT</td>
<td>p1&lt;0.001</td>
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<tr>
<td>Lymph nodes</td>
<td>14</td>
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</tr>
</tbody>
</table>

p1: Relation of each group to control calibrators.  
p2: Relation of each category to the other in the same group.  
p<0.001 HS.  
p>0.05 NS.

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Amplification Plot

Fig. (1): Curve of IGF-IR gene in ALL cases and controls by RT-PC.
lymphoblasts of 142 children with ALL, together with 127 cord blood samples and 55 age matched controls using a reverse transcription polymerase chain reaction (RT-PCR) of Ficoll separated mononuclear cells. The expression of IGF-I, IGF-II, IGF binding protein 5 (IGFBP-5) and CTGF (IGFBP-rP2) was found in a higher proportion of cells in patients with ALL than in controls. Patients with ALL who were in continuous remission had a lower percentage of IGFBP-2 and IGFBP-3 expressing mononuclear cells at diagnosis than those who developed a relapse. Only malignant lymphoblasts of B cell origin showed expression of (IGFBP-rP2). Malignant lymphoblasts of T cell origin expressed mainly IGFBP-2 and IGFBP-5, whereas IGF-II and IGFBP-3 expression was frequently seen in lymphoblasts of B cell origin.

A significant higher expression of IGF-I was reported in the study of Vorwerk et al., [9] in ALL (29%) compared to controls (7%) and cord blood samples (4%). They found a statistical significant difference between ALL patients and both controls and cord blood samples as \( p \) value was (0.001).

As for IGF-IR it was expressed in 68% of ALL patients compared to 87% for controls and 39% in cord blood samples with a significant difference between ALL patients and cord blood samples (\( p=0.01 \)). Also, there was a significant difference between IGF-IR expression in ALL patients and cord blood samples (\( p=0.01 \)).

Vorwerk et al. [9] compared the expression level of genes of IGF family in 142 ALL patients, 118 ALL patients with no relapse and 24 patients who have developed relapse. IGF I and IGF-IR expression, there was no significant difference in the expression level of patients with relapse versus those without relapses. However they found that, in general the percentage of patients expressing IGFBPs in their mononuclear cell fraction at diagnosis was higher in the relapsed group. Consequently, they concluded that malignant lymphoblasts of patients with ALL express components of the IGF family and therefore promote their own growth in an autocrine, paracrine, or endocrine manner. Whether these components will be useful as prognostic factors in the stratification of ALL treatment in children needs to be evaluated.

On the other hand Vorwerk et al. [9] found elevated IGFBP-2, low IGFBP-3, low IGF-I and low normal IGF-II, but normal IGFBP-1 levels in a series of 162 children with ALL (88 boys and 74 girls). Highly elevated IGFBP-2 and low IGFBP-3 levels at the time of diagnosis correlated with a higher risk of relapse or lack of remission.

They suggested that the cure for ALL may not require elimination of all leukemic cells and that other mechanisms control the proliferation of persisting leukemic cells. If different local concentrations of IGF/IGFBP complexes are responsible either for sensitivity or resistance to chemotherapy, new therapeutic options in leukemia may include the interaction with the IGF regulatory pathway.

In 2008 Whelan et al. [10] mentioned the homeobox (Hox) gene family, which encodes a group of transcription factors that are preferentially expressed during embryonic development and hematopoiesis and are commonly deregulated in acute lymphoblastic leukemia (ALL). They studied whether HoxA9 gene can induce IGF-IR expression in B-lineage ALL or not. They found that HoxA9 over expression induces IGF-1R expression and subsequently promotes leukemic cell growth. As for IGF-I, their results suggest that HoxA9 may not regulate the expression of IGF-1, but this growth factor is expressed by both the leukemic cells and the supporting stromal cells [10].

In 2007, Ciampolillo et al. [11] demonstrated that IGF-IR is involved in the pathogenesis of a variety of human neoplasia via the mitogenic and anti-apoptotic properties of its cognate receptor.

Several studies have shown that high concentrations of serum IGFs and/or lower levels of IGFBPs are associated with increased risk for several cancers [12-20].

Several lines of evidence suggest a role for IGF-1 and IGF-1R in leukemia. High levels of serum IGF-1 correlate with childhood leukemia and high birth weight [21]. Signaling via IGF-1/IGF-1R interactions has been shown to participate in the growth and survival of multiple myeloma cells [22,23].

IGF-1R expression has been reported in AML blasts and IGF-1 is capable of stimulating
AML cell proliferation. Several studies have indicated that inhibition of IGF-1R signaling results in a reduction of cell proliferation and induction of apoptosis, particularly in AML [24,25].

We conclude IGF-1R seems to play a crucial role in patients with ALL. Since, all cases of ALL patients expressed IGF-1R and that the IGF-1R level is higher in newly diagnosed cases than in patients in CR or controls. Therefore, new therapeutic agents targeting IGF-1R may provide a better chance for those patients. However, extended clinical trials are needed to confirm this.

REFERENCES

