Progranulin in the diagnosis of Acute Lymphoblastic Leukemia: A case-control study

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Abstract

Background: Acute Lymphoblastic Leukemia (ALL) is a highly aggressive hematological malignancy of the lymphoid line. Continuous search for new reliable diagnostic and prognostic markers in ALL could help better guide treatment and improve prognosis. Progranulin (PGRN) is novel marker with various physiological functions, that was found to be elevated in many solid and hematological tumors.

Objective: To examine PGRN's relation and possible role as a novel diagnostic and prognostic marker in ALL by measuring PGRN concentrations in serum samples from newly diagnosed ALL patients pre-chemotherapy and investigate the relationship of PGRN levels with established diagnostic and prognostic criteria.

Methods: A case-control study was conducted on 40 newly diagnosed treatment-naive ALL patients and 15 age matched healthy subjects who were attendants to Clinical Hematology and Oncology Unit, Internal medicine department, Ain Shams University. All the patients were subjected to the following: Full history, clinical examination, routine laboratory investigations, Bone marrow aspirate and flowcytometry, cytogenetics, radiographic studies, cerebrospinal fluid analysis and risk stratification. Serum PGRN was measured at time of diagnosis.

Results: The present study proved that PGRN levels in ALL patients were significantly elevated when compared to the control group. High PGRN levels were also associated with lower disease-free survival (DFS) in ALL patients

Conclusion: PGRN is useful in the diagnosis and predicting DFS in ALL. It is likely to be implicated in the pathology of ALL.

Keywords: Acute Lymphoblastic Leukemia, progranulin
**Introduction**
Acute lymphoblastic leukemia (ALL) is a malignant disorder originating from hematopoietic B- or T-cell precursors, characterized by bone marrow infiltration by lymphoblasts and accompanying depression of normal hematopoiesis. *(Baljevic et al, 2017)*

Bone marrow analysis as well as cytogenetics and molecular studies are cornerstones in diagnosis and risk stratification of ALL patients. *(Hoelzer et al, 2016)*

Progranulin (PGRN) is a 88 kDa secreted protein with pleiotropic functions including regulation of cell cycle progression, cell motility, wound repair and tumorigenesis. *(Cenik et al, 2012)*

PGRN was found to be elevated in many solid and hematological tumours, including chronic lymphocytic leukemia, diffuse large B cell lymphoma and multiple myeloma. *(Göbel M et al, 2013)*

**Materials and Methods**
This study was conducted on 65 subjects (40 patients and 15 age matched healthy subjects) who were attendants to Clinical Hematology and Oncology Unit, Internal medicine department, Ain Shams University Hospital during the period from March 2019 to September 2019.

**Subjects:**
- Sixty-five subjects included 40 patients diagnosed as ALL by:
  - Full history taking.
  - Through clinical examination.
  - Complete blood count plus stained blood film.
Routine metabolic profile; Kidney & Liver functions tests, LDH.
Coagulation profile: Prothrombin Time & Partial Thromboplastin Time.
BM aspiration (stained smears by lishman/Giemesa) with or without trephine biopsy (stained by H&E) for morphologic examination.
Immunophenotyping by immunohistochemistry or flowcytometry
Bone marrow cytogenetic studies (Karyotyping and FISH).
Radiographic studies for assessment of extramedullary disease.
CSF examination (Cytology)
Risk stratification

Measurement of PGRN level in serum using ELISA in 15 healthy persons and in the 40 patients at the time of diagnosis.

Statistical analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. The level of significance was taken at P value < 0.05 is significant, otherwise is non-significant.

Results

Table [1] shows the patients' group (26 males and 14 females) with ages ranging from 16 to 62 years (mean, 29.2±13.1 years) , the control group had (9 males and 6 females) with ages ranging from 17 to 60 years (mean, 33.2±13.7) Table (1): Comparison between patients and controls regarding sex and age:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control</th>
<th>Patient</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>60.0%</td>
<td>26</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>40.0%</td>
<td>14</td>
</tr>
<tr>
<td>Age</td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>33.2</td>
<td>13.7</td>
<td>17-60</td>
</tr>
</tbody>
</table>
Table [2] shows highly significant increase in PGRN level in patient group compared to control group (P<0.001), with median PGRN level in patient group (115 ng/ml) and median in control group (20 ng/ml).

Table (2): Comparison between control and patients regarding PGRN:

<table>
<thead>
<tr>
<th>PGRN level ng/mL</th>
<th>Control</th>
<th>Patients</th>
<th>Mann Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR)</td>
<td>20 (20-25)</td>
<td>115 (65-170)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>22 ± 6.5</td>
<td>161.3 ± 171.7</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>10-35</td>
<td>25-800</td>
<td></td>
</tr>
</tbody>
</table>

Table [3] shows significant decrease in disease-free survival (DFS) in patients with high PGRN levels (>115 ng/ml) (P=0.019).

Table (3): Correlation of PGRN with DFS of patient's group:

<table>
<thead>
<tr>
<th>PGRN level ng/mL</th>
<th>Mean</th>
<th>95% CI</th>
<th>Log rank test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% survival</td>
</tr>
<tr>
<td>&lt;115</td>
<td>142.500</td>
<td>128.17 - 156.83</td>
<td>95%</td>
</tr>
<tr>
<td>&gt;115</td>
<td>112.500</td>
<td>72.98 - 152.02</td>
<td>55%</td>
</tr>
<tr>
<td>Overall</td>
<td>142.152</td>
<td>118.78 - 165.52</td>
<td>93%</td>
</tr>
</tbody>
</table>

**Discussion**

In our study, we found that patients with ALL had higher serum PGRN level compared to control subjects with statistically significant difference (P < 0.001). To our knowledge, this is the first report to demonstrate that PGRN levels are increased in newly diagnosed ALL patients before start of chemotherapeutic treatment.
These results are in agreement with a number of studies that identified similar increase in serum PGRN in lymphoid malignancies. In the study done by Göbel et al, it was found that serum PGRN levels are elevated in CLL patients ($P < 0.0001$). \textbf{(Göbel et al, 2013)} In another study by Yamamoto et al, the median serum PGRN concentration of malignant lymphoma patients was 91.3 ng/ml, and was significantly higher than that of the control group (median, 57.7 ng/ml) ($p<0.0001$). \textbf{(Yamamoto et al, 2018)}

Wengang et al examined PGRN expression profile in hematological cell lines, and detected an increase in expression in hematological malignancies of the B-cell lineage (B cell lines and MM cell lines) but not in T-cell lineage (Jurkat and KOPT-K1) and promyelocytic leukemia (HL-60). \textbf{(Wengang et al, 2003)}

In the study done by Kimura et al, it was demonstrated that CSF PGRN levels are increased in lymphoma and carcinomas with CNS involvements ($P<0.05$), when compared to lymphoma and carcinomas without CNS involvement. \textbf{(Kimura et al, 2018)}

In our study, we found that PGRN level before treatment higher than a mean of 115 ng/ml correlated with a statistically significant decrease in DFS of ALL patients ($P = 0.019$). Göbel et al also showed in their study, that increase in PGRN (using a mean of 165 ng/ml) correlated significantly with poor outcome in CLL patients ($P < 0.0001$). The median TTFT in CLL patients with high PGRN plasma levels (19 months) was significantly shorter than in patients with low PGRN concentrations (125 months, $p<0.0001$). \textbf{(Göbel et al, 2013)}

They however observed statistically highly significant differences in terms of OS between the two groups, where the median survival of PGRN high patients was 124 months, whereas the median survival for the PGRN low subgroup was not reached during follow-up ($P = 0.0003$) \textbf{(Göbel et al, 2013)} This is in contrast
to our study where no statistically significant difference was found in OS between the two groups (P = 0.34). This could be due to small sample size and different disease progression.

Association between serum PGRN concentrations and overall survival (OS) was also examined by Yamamoto et al in patients with diffuse large B cell lymphoma (DLBCL). Results showed that patients with serum GP88 concentrations of ≤116 and >116ng/ml, had 5-y OS rates of 70% and 50%, respectively (p=0.02).

In our study, we found that the optimal cut-off value for serum PGRN in diagnosis of ALL was 34 ng/mL with an area under the curve value of 0.989 (P = 0.0001). This result has to be validated with a larger sample size and different study groups.

In the present study we found that in ALL patients, there was no statistically significant correlation between serum PGRN level and initial WBC count, absolute blast count, co-morbidities, subtype of ALL, risk status, cytogenetics or extramedullary affection, (P = 0.519), (P = 0.718), (P = 0.281) (P = 0.197), (P = 0.871), (P = 0.709) and (P = 0.115) respectively. A multivariate analysis comparing PGRN and other risk factors regarding DFS showed no statistical significance.

**Conclusion**

PGRN plays an important role in pathogenesis of ALL and lymphoid malignancies. We can conclude from our study that PGRN is increasingly expressed in ALL before treatment and serum PGRN at diagnosis can be used as an indicator of DFS in patients with ALL.
Ethical considerations

The study followed the ethical considerations according to the ethical committee of Ain Shams, faculty of medicine. An informed written consent was taken from all patients participating in the study.

Disclosure Statement

There is no conflict of interest.

References:


6. Wengang Wang, Jun Hayashi, Wes E. Kim, and Ginette Serrero, PC Cell-derived Growth Factor (Granulin Precursor) Expression and Action in Human Multiple Myeloma, University of Maryland, Baltimore, Maryland 21201, 2003