PROGNOSTIC RELEVANCE OF LEUKOCYTE ASSOCIATED IMMUNOGLOBULIN LIKE RECEPTOR-1 (CD305) IN CHRONIC LYMPHOCYTIC LEUKEMIA

Hayder Abdul Ridha, Ameer Kadhim Al-Humairi, Liqaa M. Al Sharifi, Sinan Y. Al Shemmery

ABSTRACT

Background: Chronic lymphocytic leukemia (CLL), is a malignant disorder of lymphocytes, that affects lymph cells or lymphocytes that make lymphatic tissue. Leukocyte associated immunoglobulin like receptor-1 (LAIR-1) or (CD 305) expressed by B-cells as an immune inhibitory marker of B cell receptor (BCR)-mediated signaling, that control cell proliferation pathways. Objectives: Assessment the role of CD 305 as a prognostic factor. Its relation with other clinic-pathological variables. Subjects and Methods: This cross sectional study was conducted on 98 newly diagnosed CLL patients based on peripheral blood film finding, bone marrow morphology and flow cytometry depend on the antigenic profile, with high scoring system (Matutes score > 3) (2). Clinical and laboratory assessment were done including staging by modified Rai staging system into low, intermediate and high risk groups, absolute lymphocytes count in addition to Hb and platelets count assessment, follow up for about 6-12 months including all patients whose stage need treatment or not and assessment of remission state is done by complete blood count, bone marrow morphology and evaluation of minimal residual disease, CD38 assessment and LAIR-1 expression on B cells were measured by flow cytometry at diagnosis, the latter was related to clinical and laboratory prognostic variables. Results: LAIR-1 was negatively expressed in those of high risk group according to Rai staging system (stage III-IV) and positive expression in those of low and intermediate risk groups (stage 0-II), the P value, 0.009. LAIR-1 was negatively correlate to age, hemoglobin, platelets count. LAIR-1 was significantly present in relation some prognostic factors like CD 38 and lymphocytes count, the P value was 0.037 and 0.021 respectively.

Conclusion: CD305 expression can be used as simple reliable, inexpensive independent prognostic factor in CLL patients in addition to predict at presentation patients who will initiate chemotherapy early.

Keywords: CD305, CLL patients, chemotherapy, LAIR-1

I. INTRODUCTION

Chronic lymphocytic leukemia (CLL), is a malignant disorder of lymphocytes, that affects lymph cells or lymphocytes that make lymphatic tissue. It is the most common chronic lymphoproliferative disorder (LPD). It is often diagnosed by a matching clinical, cytological and immunophenotypic picture (3). Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a exceedingly variable clinical course. Some patients have a life expectancy which resembles that of the age corresponding general population, while others advancement and need treatment within a few months of diagnosis. Several clinical and biological variables, some of which authenticated in prospective studies, have been reported to predict the outcome of CLL patients when assessed at presentation of the leukemia. Among them, the old-fashioned but still widely used clinical staging systems firstly proposed by Rai and/or Binet, or the more demanding mutational status of the variable region of the heavy-chain locus of the immunoglobulin genes (IGHV) and fluorescent in situ hybridization are the stamps for discriminating patients with an hostile or indolent clinical course. While the latter methods have been standardized, tests are still expensive and cannot be provided by all laboratories (4). Mainstream of patients affected by chronic lymphocytic leukemia (CLL) are diagnosed by flow cytometry. Numerous immunophenotypic markers have been recognized to be a significant and independent prognostic variables; especially from retrospective cohorts. Recognition of these markers was found to be feasible and inexpensive in
most laboratories but only few have been validated by independent series. Leukocyte associated immunoglobulin like receptor 1 (known as LAIR1, LAIR-1 or CD305), has been described to be of lower expression in high risk CLL patients. Studies addressing CD305 in CLL patients and its relation to biological variables and standard prognostic factors had been so infrequent till now(5,9).

The natural history of CLL is variable, with survival times ranging from 2 to over 20 years from diagnosis. In 1975, Rai et al. developed a staging system consisting of five stages (Rai 0 to IV) based on Dameshek's model of orderly disease progression in CLL. The Rai staging system is modified into a three-stage system: low risk (Rai 0), intermediate risk (Rai I, II), and high risk (Rai III, IV) (17). LAIR, is an inhibitory receptor present on most of hematopoietic cells, particularly on immune system cells. After the binding of its known ligands(10,11), LAIR1 prevents the activation of immune cells using two immune receptor tyrosine-based inhibitory motifs situated in the cytoplasmic tail of the receptor. LAIR1 is expressed during B-cell ontogenesis, but is misplaced on a subset of memory B cells, in all germinal center B cells, in plasmablasts, and in plasma cells (11). On the other hand, prolonged BCR- or CD40-stimulation induces down-regulation of LAIR1 on naïve B cells in vitro, suggesting an inhibitory role for LAIR1 on BCR signaling (12). Furthermore, in vitro studies set that collagen produced by lymph node-derived mesenchymal stromal cells could inhibit B-cell functions through LAIR1 engagement (9).

II. MATERIAL AND METHOD

This study was conducted on 98 adult patients of newly diagnosed chronic lymphocytic leukaemia from January 2020 to December 2020. These patients were admitted to hematology department in Baghdad teaching hospital, the immunophenotyping was done in the flowcytometry department, teaching laboratories in Baghdad, Iraq. Peripheral blood and bone marrow samples were prepared for staining using leishman stain. The diagnosis depend on clinical suspicion, peripheral and bone marrow morphology which showed absolute mature lymphocytosis, immunophenotypic analysis showed high MS score > 3, CD38 expression was also measured in addition to CD305 expression. Clinical data include age, gender, physical sign, clinical presentation and examination such as organomegaly, generalize or local lymphadenopathy were collected from patient laboratory request and case sheet of patient. Hematological data include hemoglobin, WBC, absolute lymphocytes count, and platelets count was also collected.

Flowcytometry

Four color flow cytometric analysis was performed using a BD FACS Calibur™ flow cytometer (Becton Dickinson, Bio) and FACSCanto II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA). CellQuest software (Becton Dickinson, San Jose, CA) and FACSDiva software were used to analyze the data (7). CD45+ lymphocytes were gated on CD45 and SSC dot plot, then B cells were isolated by differentiated on CD19+ cells. A cut-off of CD30% on gated B-lymphocytes, then within the CD19+ B cell population, the subset of cells expressing LAIR-1 was determined as LAIR-1+ CD19+ B cells and their percentage evaluated on quadrant histogram (7). All patients were evaluated by CBC, lymphocytes count and bone marrow for assessment of remission for a minimal period of 6 months after beginning of treatment (if patients had a treatment) and relate that to CD 305 expression.

III. RESULTS

There are 98 patient with CLL of them 62.2 were male and 37.8 were female, Overall, LAIR1 was positive in 22(22.4%) and negative in 76 (77.6%) of the 98 CLL patients. There were significantly different distributions of some clinical and biological features between the LAIR1 positive (LAIR1+) and negative (LAIR1-) patients, as reported in Table 1.

Table 1. Table show the correlation between the prognostic factors (laboratory features) and the expression of LAIR-1

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>ALL PATIENTS (98)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAIR-1+ (22)</td>
<td>LAIR-1- (76)</td>
</tr>
<tr>
<td>Age &lt; 60</td>
<td>8 (36.4)</td>
<td>28 (36.8)</td>
</tr>
<tr>
<td>Age ≥ 60</td>
<td>14 (63.6)</td>
<td>48 (63.2)</td>
</tr>
</tbody>
</table>

www.turkjphysiotherrehabil.org
There is a clinical significant between LAIR-1 expression and lymphocytes absolute count (*10⁹/L) in which LAIR-1 positive expression patients have 31 *10⁹/L while those of LAIR-1 negative expression patients have 71*10⁹/L. So show a negative expression with high ALC the P value are 0.021. LAIR-1 expression was inversely correlate to CD38 it was positive in 13 patients (13.2%) all of them show negative LAIR-1 expression, P value is 0.037. There were no significant co-relation between LAIR-1 and age, hemoglobin and platelets counts. LAIR-1 expression was clinically significant in those of high risk group according to Rai staging system (stage III-IV), in which more than 90% of patients show negative expression, while those of low and intermediate risk (0-I-II) LAIR-1 was negatively expressed in 50%, 61.3% and 87.5% respectively, the P value 0.009.

Table 2 Association between study variables and Rai staging system among CLL patients (N=98)

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Clinical Stage</th>
<th>Total</th>
<th>X²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 305</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4 (50.0)</td>
<td>12 (38.7)</td>
<td>3 (12.5)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>4 (50.0)</td>
<td>19 (61.3)</td>
<td>21 (87.5)</td>
<td>21 (91.3)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (100.0)</td>
<td>31 (100.0)</td>
<td>24 (100.0)</td>
<td>23 (100.0)</td>
</tr>
</tbody>
</table>

Figure 1: show LAIR-1 expression and clinical stage of disease.
Figure 2: show number of patient who need treatment 64 (65.3%), and those who not need 34 (34.7%).

Figure 3: show percentage of LAIR-1 expression (positive and negative) in patients not need treatment.

Table 3 Association between LAIR-1 and state of remission during studied period (N=98)

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Patients not need treatment (34.6%)</th>
<th>Patients die early (9.1%)</th>
<th>Treated Patients without remission (14.2%)</th>
<th>Treated Patients with remission (41.8%)</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 305</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02*</td>
</tr>
<tr>
<td>Positive</td>
<td>14 (41.2%)</td>
<td>2 (14.3%)</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>34 (58.8%)</td>
<td>14 (100.0%)</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41 (100.0%)</td>
<td>16 (88.9%)</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 (100.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thirty four (34.6%) of patients were in low stage of disease and not need treatment, LAIR-1 was negatively expressed in (58.8%), while those who need treatment account for 64 (65.3%) patients, 9 patients (9.1%) died early after diagnosis, 8 (88.9 %) of them showed negative LAIR-1 expression. Patients on treatment but not achieving remission constituting 14 patients (14.2%), 12 (85.7%) of them negatively expressed LAIR-1 while 41 (41.8%) of patients who achieving remission after treatment showed 36 patients (87.8%) negative LAIR-1 expression, so there is correlation between LAIR-1 expression and early death of patients and early need of treatment but have no relation with remission state, the P value was 0.02.

IV. DISCUSSION:

Chronic lymphocytic leukemia (CLL) has a clinical course which is highly undulant. Some patients had a usual expectancy of life, while others need chemotherapy and follow up. Several clinical and biological variables when assessed at presentation can expect outcome(3). The aim of this study was to evaluate LAIR-1 expression in peripheral blood of newly diagnosed CLL patients and to relate it with the laboratory, clinical and biological prognostic criteria of the disease.

Interestingly LAIR-1 decrease significantly with advanced modified Rai staging which means that the positive expression are found in earlier clinical stages (mostly of low and intermediate stages). Similarly, Ahmedy et al. and Rawstron et al. found that CD305 positive patients were presented in early stages than in advanced stages (stage 2 and 3)(4,12). Moreover Ahmedy et al. and Poggi et al. confirmed that its expression is lower in high risk patient(4,13). AsLAIR-1 constrain B-cell receptor (BCR)-mediated signaling and control kinase pathways of cell proliferation. It was reported that CD 305 inhibits the activation of immunological cells using two immune receptor tyrosine-based inhibitory motifs located in the cytoplasmic tail of the receptor(12). This study showed a significant decrease in LAIR-1 expression in those patients who need early medical intervention similarly Ahmedy et al. and Perbellini et al. (3,4) found a significantly lower proportion of LAIR1+ patients who initiated cytotoxic treatment during follow-up compared to LAIR1− patients. On the other hand, CD38 expression has a positive significant in inversely correlated to LAIR-1 expression similarly withHammad R, Ahmedy et al. and Perbellini et al. (1,3,4). Our study show no significant correlation between LAIR-1 and both hemoglobin level and platelets count in contrast to Hammad R andAhmedy et al. that both show significant correlation to haemoglobin level and platelets count(1,3), also no significant correlation with age of patients. Similar results were obtained by to Hammad R, Ahmedy et al., Thornton et al. and Ibrahim et al (1,3,14,15); similarly there was a positive significant relationship between LAIR-1 expression and lymphocytes count in accordance with those Ahmedy et al. and Perbellini et al. (3,4). After we correlated LAIR-1 with many of the standard prognostic factors in CLL and from our multivariate analysis, we can conclude that CD305 expression can be used as simple reliable, inexpensive independent prognostic factors in CLL patients and they can even predict at presentation patients who will initiate chemotherapy early.

V. CONCLUSIONS

Our results support the request of LAIR-1 to having an active role in the pathophysiology of CLL. LAIR-1 expression in CLL cells carries the potential factor of being a prognostic marker. negative LAIR-1 expression in advanced CLL patients might be a potential factor in the process of tumor genesis of CLL which might qualify it as a prospective therapeutic measure.

Acknowledgement: We thanks all subjects whom participated in this study and the doctors staff of the flowcytometry department, bone marrow transplant center in Baghdad, Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of collage of medicine/ Babylon university/ Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Hammad R. Role of Leukocyte Associated Immunoglobulin Like Receptor-1 (CD305 ) in Predicting Clinical Variables of Chronic Lymphocytic Leukemia. 2020;1(March).

www.turkjphysiotherrehabil.org


