Serum Immunoglobulin Paraprotein in Patients with Chronic Lymphocytic Leukemia

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Abstract

Background: Chronic Lymphocytic Leukemia (CLL) is the most common chronic lympho-proliferative disorder. This study was done to explore the frequency of serum Immunoglobulin (Ig) paraprotein in patients with CLL and its correlation with other prognostic factors as expression of CD38 and zeta-chain-associated protein kinase 70 (ZAP70) on malignant lymphocytes.

Aim of Study: To study the frequency of serum immunoglobulin paraprotein in patients with CLL and its correlation with other prognostic factors as expression of CD38 and ZAP70 on malignant lymphocytes.

Patients and Methods: Clinico-hematological profiles were done to 30 CLL patients. Serum Protein Electrophoresis (SPE) and Immunofixation Electrophoresis (IFE) were performed to measure serum Ig paraprotein. ZAP-70 and CD 38 were tested by flow cytometry.

Results: In this study CLL patients were classified according to modified Rai staging system into: Low risk 10%, intermediate risk 40% and high risk 50%. Four CLL patients (13.4%) had monoclonal gammopathy (M-band), the type of M-band in the 4 patients was IgG-Kappa. One patient (3.3%) had diffuse band (polyclonal gammopathy). ZAP-70 and CD 38 positivity were detected in 7 patients (23.3%) and 15 patients (50%), respectively. There was significant relation between presence of Ig paraprotein in CLL patients and ZAP 70 expression \((p=0.034)\). On the other hand there was no relation between presence of M-band in CLL patients and CD38 expression nor modified Rai staging system.

Conclusion: Monoclonal gammopathy was found in 13.4% of CLL patients. The detection of Ig paraprotein might be applied for the assessment of prognosis in patients with CLL.

Key Words: Chronic lymphocytic leukemia – Immunoglobulin paraprotein – Prognosis – CD38 – ZAP-70.

Introduction

CHRONIC Lymphocytic Leukemia (CLL) is a monoclonal expansion of small mature B lymphocytes accumulating in the peripheral blood, bone marrow, and lymphoid organs. CLL is one of the most common types of leukemia in the Western world, however, infrequent in the Eastern. It is the most common types of leukemia diagnosed in adult [1]. CLL cells are positive for monotypic surface immunoglobulin (dim intensity), CD5, CD19, CD20 (dim intensity) and CD23 and usually negative for CD22, CD79b and FMC-7 [2].

The limitation of clinical staging systems (Rai and Binet staging systems) in CLL, which fail to identify early-stage patients who most likely to progress, has led to the search for new prognostic markers with highly predictive capabilities like thymidine kinase, beta2-microglobulin (B2-M), CD38, zeta-chain-associated protein kinase 70 (ZAP70) and detection of monoclonal gamopathy [3].

Expression of CD38 on CLL cells has been shown to be increased in more proliferative clones. There is a close association between CD38 expression and increased Ki-67 proliferation index along with increased ZAP70 positivity [4].

Serum immunoglobulin paraprotein can be detected in a subset of patients with CLL by Serum Protein Electrophoresis (SPE) and immunofixation electrophoresis (IFE). The World Health Organization (WHO) classification recognizes that a "small M component" can be found in some patients with CLL, but no mention is made about the fre-
quency of this occurrence or the range of serum paraprotein levels in patients with CLL. An earlier study reported that patients with CLL with Immunoglobulin (Ig) paraproteinemia had an inferior survival compared with patients with CLL without serum paraprotein. However, other studies have not confirmed this observation. Therefore, how does the serum Ig paraprotein actually affect the CLL patients' survival is still unknown [8].

Patients and Methods

The study was done on 30 CLL patients and 20 sex and age matched control. Those patients were presented to South Egypt Cancer Institute Assiut University Hospital in the period between December 2014 and May 2016. The study was approved by the Institutional Review Board of Faculty of Medicine, Assiut University. An informed written consent was taken from of all cases and controls.

All patients and controls were subjected to:

- Thorough history taking and clinical examination, with careful assessment of clinical signs relevant to leukemia as hepatomegaly, splenomegaly, lymphadenopathy.
- Complete blood picture were performed by the fully automated blood counters Ruby Cell Dyn (American, Serial number: 36026BG) and Cell Dyn 1700 (American, Serial number: 513554). Blood Films were stained with Leishman stain, and were used for morphological identification of the differential count.
- Liver function tests, kidney function tests and estimation of LDH were performed by Cobas integra 400 plus (Swiss, Serial number: 500558).
- Bone marrow examination.
- Immunophenotyping analysis was done by multicolor flow cytometry (FACSCaliber, BD Biosciences-San Jose, CA, USA, serial number E5140). Foreward scatter and side scatter histogram were made to detect the lymphocyte population. Lymphocytes were then gated for further analysis of different monoclonal antibodies as CD5, CD10, CD19, Kappa, lamda, FMC7, CD23, CD3, CD38 and Zap70.

Immunophenotyping diagnosis of our CLL patients was done according to scoring system [6].

- Serum protein electrophoresis and acid blue immunofixation: Serum sample were collected and stored in –20°C until analysis. SPE was used as a screening test, while IFE was for confirmation and isotype identification. Pretty Interlab protein electrophoresis analyser (Italy, serial number 38405301) automatically performed all the steps of the analytical procedure in SPE: After application of the samples on the agarose gel plate, electrophoretic migration, gel denaturation, gel staining and de-staining and final gel drying were done.

Immunofixation was done after the electrophoretic migration by application of the fixative solution and the antisera, gel incubation at 20°C and gel blotting at 40°C.

Statistical analysis:

Statistical analysis was carried out using SPSS statistical software Version 18. Qualitative data are expressed frequency and percentage; quantitative data are expressed by mean ± Standard Deviation of mean (SD). Comparisons between variables were performed using the chi-square test and independent samples t-test. p-value <0.05 was considered significant.

Results

Fig. (1) shows the distribution of CLL patients according to modified Rai staging system. Table (1) show the base line characteristic of CLL patients and the controls.

The expression of CD38 on malignant lymphocytes was detected in 15 patients (50%) with mean expression of 64.84±23.60. However, the expression of ZAP70 on malignant lymphocytes was detected only in 7 patients (23.3%) with mean expression of 57.98±23.43 as in Fig. (2).

In the present study, 4 CLL patients (13.4%) had monoclonal gammopathy (M-band), the type of M-band in the 4 patients was IgG-Kappa Figs. (3,4). One patient (3.3%) had diffuse band (polyclonal gammopathy).

There was significant relation between presence of immunoglobulin paraprotein in CLL patients and ZAP 70 expression with p-value=0.034. On the other hand there was no significant relation between presence of M-band in CLL patients and CD38 expression. Also there was no significant relation between modified Rai staging system and the presence of M-band as shown in Tables (2,3).
Low risk
10.0%

Intermediate risk
40.0%

High risk
50.0%

Fig. (1): The distribution of studied patients according to Modified Rai staging system.

Fig. (2): Expression of CD38 and ZAP 70 on malignant lymphocytes in CLL patients.

Fig. (3): Serum protein electrophoresis strip showing M-band in our studied CLL patients.

Fig. (4): Immunofixation strip of one of our studied CLL patients showing M-band in SPE, the type of the M-band is IgG kappa.

Table (1): Baseline characteristic of CLL patients and the controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=30)</th>
<th>Control (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.53±12.03</td>
<td>58.85±10.17</td>
<td>0.416</td>
</tr>
<tr>
<td>Sex</td>
<td>17/13</td>
<td>12/8</td>
<td>0.815</td>
</tr>
<tr>
<td>Hemoglobin (gm/dL)</td>
<td>10.64±1.90</td>
<td>12.01±1.17</td>
<td>0.007</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>151.51±78.98</td>
<td>256.00±93.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBCs (10^9/L)</td>
<td>101.10±140.34</td>
<td>6.91±2.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PB lymphocytes (%)</td>
<td>74.13±25.88</td>
<td>33.30±6.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BM lymphocytes (%)</td>
<td>73.90±25.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>63.63±11.33</td>
<td>68.10±4.25</td>
<td>0.165</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>114.50±60.38</td>
<td>73.55±10.63</td>
<td>0.002</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>495.23±309.44</td>
<td>314.25±78.59</td>
<td>0.004</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>31.17±5.13</td>
<td>33.60±6.82</td>
<td>0.167</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.76±0.13</td>
<td>0.77±0.17</td>
<td>0.881</td>
</tr>
</tbody>
</table>

Independent samples t-test.
Significant p-value <0.05.
Data expressed as mean ± SD.
BM : Bone Marrow.
WBC : White Blood Cell.
ALP : Alkaline Phosphatase.
LDH : Lactate Dehydrogenase.

Table (2): Relation between the expression of CD38 and ZAP 70 and the pattern of protein electrophoresis in CLL patients.

<table>
<thead>
<tr>
<th>Protein electrophoresis pattern and immunofixation</th>
<th>CD38:</th>
<th>ZAP70:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pattern (n=25)</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>M-Band (IgG-Kappa) (n=4)</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Diffuse band (polyclonal) (n=1)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>No.</td>
<td>50.0</td>
<td>84.0</td>
</tr>
<tr>
<td>%</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>p-value</td>
<td>0.624</td>
<td>0.034*</td>
</tr>
</tbody>
</table>

Chi-square test.
Significant p-value <0.05.
M-Band : Monoclonal Band.
CD : Clusters of Differentiation.
ZAP70 : Zeta-chain-Associated Protein kinase 70.

Table (3): Relation between the protein electrophoresis and immunofixation pattern and modified Rai staging system in CLL patients.

<table>
<thead>
<tr>
<th>Modified Rai staging system</th>
<th>Low risk</th>
<th>Intermediate risk</th>
<th>High risk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Protein electrophoresis and immunofixation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Pattern</td>
<td>3</td>
<td>10.0</td>
<td>10</td>
<td>83.4</td>
</tr>
<tr>
<td>M-Band (IgG-Kappa)</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>Diffuse band (polyclonal)</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>8.3</td>
</tr>
</tbody>
</table>

M-Band: Monoclonal Band.
Chi-square test.
Significant p-value <0.05.
Discussion

Although M-protein secretion is typically a feature of plasma cell dyscrasias, monoclonal gammopathy has been recognized in a wide variety of lymphoproliferative disorders. It has been described in patients with B-CLL, well-differentiated lymphoma, Burkitt’s lymphoma and other histological subtypes of lymphomas [2].

The expression of ZAP 70 on malignant lymphocytes was detected in 23.3% of our CLL patients. This result is near the result found by Morabito et al., who reported that ZAP-70 was positive expressed in 34.9% of all studied CLL cases [7].

Also, Del Principe et al., found that ZAP-70 was positive expressed in 36% of all CLL cases [8]. Otherwise, other study done by Cruse et al., and found that ZAP-70 expression was positive in (64.7%) of CLL cases [9]. In contrast, Deaglio et al., reported that ZAP-70 expression was positive in (9.52%) of their studied CLL patients [10]. This variation in the expression of ZAP-70 in CLL patients between our results and previous studies may be due to the low number of our studied patients and also indicate that more large study is required.

Regarding CD38 expression, 50% of our CLL patients were CD38 positive. This result was nearly in agreement with Cruse et al., [9] and Del Principe et al., [8] studies, who found that the expression of CD38 was detected in (40%) and (36%) respectively, of their CLL cases. In addition to, Deaglio et al., found that CD38 expression was detected in (58.92%) of his studied cases [10]. In contrast, only (19.5%) of cases were positive for CD38 expression in Morabito et al., study [7].

In this study, we found a significant relation between presence of M-band by protein electrophoresis and ZAP 70 positivity. This may indicate that the detection of immunoglobulin paraprotein may be added as a prognostic factor in CLL. Our results is in agreement with Xu et al., who suggested the possibility of interaction between serum immunoglobulin paraprotein in CLL patients and their prognostic impact might be due to different therapeutic regimens, shorter clinical follow-up and smaller number of patients.

In this study, we found a significant relation between presence of M-band by protein electrophoresis and ZAP 70 positivity. This may indicate that the detection of immunoglobulin paraprotein may be added as a prognostic factor in CLL. Our results is in agreement with Xu et al., who suggested the possibility of interaction between serum immunoglobulin paraprotein and other known prognostic factors, such as serum level of B2-M, LDH, ZAP-70 [8].

Conflict of interest:
The authors declare no conflict of interest.

References
3- ZENZ T., S. FROHLING, D. MERTENS, H. DOHNER and S. STILGENBAUER: Moving from prognostic to


Serum Immunoglobulin Paraprotein in Patients with CLL

البَارا بروتَين المناعي

في مرضى سرطان الدم الليمفاوي المزمن

المقدمة: ينتج مرض سرطان الدم الليمفاوي المزمن عن إضطراب في الخلايا الليمفاوية الناضجة B، ويختلف تطور المرض في بعض المرضى. قد تدمه حالتهم الصحية بسرعة بينما البعض الآخر يعمر عدد من الأسرة دون الحاجة إلى العلاج، ويعتبر وجود CD38 من علامات النتائج السلبية لمرض سرطان الدم الليمفاوي المزمن. حيث أن الخلايا الليمفاوية التي يوجد عليها أكثر قابلية للتكاثر والتزايد من الخلايا السلبية CD38. وبالمثل الخلايا الليمفاوية التي يوجد عليها ZAP70 تكن أكثر شرارة في المرض.

وقد رجح منظمة الصحة العالمية إمكانية وجود البَارا بروتَين المناعي "عنصر M" في مرضى سرطان الدم الليمفاوي المزمن ولكن لم تبين معدل ظهوره في حالات المرضى. ويمكن الكشف عن مصل البَارا بروتَين المناعي في حالة المرضى عن طريق الفصل الكهربائي لمصل البروتينات. وتشير بعض التقارير إلى أن وجود هذا البَارا بروتَين له تأثير سلبي على سير المرض، وتتأثر الدراسات نسبياً بوجود إعلان غامضات ثانية السبيل في مرضى سرطان الدم الليمفاوي المزمن.

الهدف من هذه الدراسة: تهدف هذه الدراسة إلى الكشف عن البَارا بروتَين المناعي في مرضى سرطان الدم الليمفاوي المزمن وعلاقته بعوامل النتيجة الأخرى كوجود CD38 وZAP70 على الخلايا الليمفاوية.

طريق الدراسة: وقد أجريت الدراسة على 20 مريض من مرضى سرطان الدم الليمفاوي المزمن، بعد الحصول على موافقة لجنة الأخلاقيات في كلية الطب جامعة أسوان، للكشف عن مصل البَارا بروتَين المناعي عن طريق الفصل الكهربائي لمصل البروتينات Immunfixation وZAP70 وCD38 على الخلايا الليمفاوية عن طريق التنقيح الخلوى.

نتائج الدراسة: وقد وجد أن نسبة حيوية المريض في الثورة (52.6%) أكثر من الإناث (26%) وأن معدل عمر المرضى ينقر بين 25 سنة إلى 87 سنة. تم تصنيف المرضى في الدراسة وفقاً لنظام المعدل لتطور مرض سرطان الدم الليمفاوي المزمن (Rai) إلى 10% من المرضى لون خطرة متقدمة و4% لون خطرة متوسطة و5% لون خطرة متوسطة.

وقد كشفت الدراسة عن وجود CD38 في 50% من مرضى سرطان الدم الليمفاوي المزمن ووجود ZAP70 في 20.3% من المرضى، ولم تكن هناك علاقة ذات إصبعائية بين وجود CD38 ونظام المعدل لتطور المرض (Rai).

وقد كشفت نتائج الدراسة أيضاً أن نسبة البَارا بروتَين المناعي في مرضى سرطان الدم الليمفاوي المزمن في 13.4%.

ويمكن أن يكون هذه العلاقة ذات إصبعائية بين وجود مصل البَارا بروتين المناعي في مرضى سرطان الدم الليمفاوي المزمن ووجود البَارا بروتين المناعي في مرضى سرطان الدم الليمفاوي المزمن ZAP70 من ناحية أخرى لم تثبت الدراسة وجود علاقة ذات إصبعائية بين وجود مصل البَارا بروتين المناعي ووجود البَارا بروتين المناعي ZAP70.