Cytogenetic Abnormalities in Acute Leukemia Patients from Occupied Palestine

Ahmad Zaid\textsuperscript{1,2,3}, Khawla Abualia\textsuperscript{1}, Areej A.S. Khatib\textsuperscript{1}, and Mazin B. Qumsiyeh\textsuperscript{1,2}

Abstract

Cytogenetic data in acute myeloid leukemia and acute lymphoblastic leukemia are important for diagnosis, therapy design, and prognosis. This is the first report of a series of cytogenetic studies on patients with acute leukemia from central Palestine compared with data from other geographic areas. Cytogenetic analysis was done on 45 patients with acute myeloid leukemia and 111 patients with acute lymphoblastic leukemia. Bone marrow samples were collected from all patients and cultured for 24 hours. Metaphase chromosomes were banded by GTG conventional banding technique and karyotyped. Forty five acute myeloid leukemia cases referred for cytogenetic studies showed a male to female ratio of 1.6:1, 71.1% were above 18 years old, and 28.9% had an abnormal karyotype. Of the 111 cases referred with acute lymphoblastic leukemia, 37.8% were 2-6 years old, male to female ratio was 1.2:1, 54.1% were of B-cell and 12.6% T-cell lineage (others undetermined). ALL age distribution in our cases were tri-modal with three peaks of incidence; one from 2 to 6 years, a second from 14-17, and a third from 49-64. Of the ALL cases, 32.4% had abnormal karyotype with a mix of interesting abnormalities falling under three categories: pre-B, B, and T cell ALL. Some differences with the literature were noted in cytogenetic findings and age distribution between our data and that from other countries, which likely reflect either referral differences or ethnic and environmental differences.

Keywords: AML, ALL, Leukemia, Karyotype.

Introduction

Leukemia caused 265,400 deaths worldwide in 2012 and their incidence and effect are higher in less developed countries.\textsuperscript{(1)} Acute leukemias fall into the categories of myeloid and lymphoid, with the latter impacting children more. The leukemia and lymphomas are now classified by the World Health Organization based on morphological, molecular, and cytogenetic criteria.\textsuperscript{(2, 3)} In developed countries, targeted therapies based on accurate classification using molecular and cytogenetic methods have significantly reduced mortality in the past two decades.\textsuperscript{(4)}

A review of literature on chromosomal

1. Cytogenetics Laboratory and Master Program in Biotechnology, Bethlehem University, Bethlehem, Palestine.
2. School of Nursing and Allied Health, Birzeit University, Palestine.
3. Faculty of Allied and Medical Sciences, Arab American University, Jenin, Palestine.

*Correspondence should be addressed to:
Mazin B. Qumsiyeh
E-mail: mazinq@bethlehem.edu

© 2018 DAR Publishers / The University of Jordan. All Rights Reserved.
abnormalities in leukemia patients in Palestine and nearby countries showed scarce data. Abbasi et al.\(^5\) found that 16.1% of adult ALL cases in Jordan had BCR-ABL translocation and was associated with significantly poorer prognosis, which is similar to studies in developed Western countries. Mustafa Ali et al.\(^6\) showed that subdural hematoma is likely due to platelets dysfunction in a Dasatinib treated Jordanian patient with t(9;22) and ALL. Al-Bahar et al.\(^7\) studied the frequency of chromosomal abnormalities by karyotyping and FISH in 164 Kuwaiti pediatric ALL patients, showing findings not too different from European studies. A report on childhood AML in patients referred to an Israeli center also showed similar cytogenetic abnormalities, as reported in Western studies.\(^8\) In Syria, Mahayri and Monem\(^9\) studied all cytogenetics referral to their lab, including leukemias, and found referral patterns different than what was seen in Saudi Arabia by Al Husain and Zaki\(^10\) and Turkey\(^11\). The occupied Palestinian territories had limited access to modern health care facilities and patient outcomes were poor in many diseases as a result of prolonged Israeli occupation.\(^12\) No cytogenetic studies were reported from these territories. The clinical and research cytogenetic laboratories at Bethlehem University were established in 2009 and are now publishing papers on issues related to human health.\(^15\) Here, we review referral patterns to this cytogenetic laboratory of leukemic patients.

**Materials and Methods**

Cytogenetic studies were performed on 45 Palestinian patients diagnosed with AML and 111 diagnosed with ALL as diagnosed initially by flow cytometry and/or hematopathology. Patients’ consent was obtained for all cases to take the samples for clinical diagnosis at Bethlehem University cytogenetics laboratory for routine clinical cytogenetic studies. The institutional review board policies allowed the use of data without identifying the patients to be statistically analyzed, since the samples were taken for clinical use. Bone marrow samples were received in Sodium Heparin tubes and processed by standard cytogenetic methodologies, including culture for 24 and 48 hours in appropriate medium. The rare T-cell neoplasms (T-ALL) also received a three-day culture with phytaehamagglutinin as a mitogen. After culture, colcemid was added at a final concentration of 0.1\(\mu\)g/ml for 45 minutes. Then the cells were treated with hypotonic solution (Potassium chloride 0.075 M) for 18 minutes and fixed with Carnoy’s fixative (methanol/acetic acid in 3:1) for three times and slides made in a humidified chamber and then dried for 1 hour at 95\(^\circ\)C. Metaphase chromosomes were banded, using the conventional GTG banding technique and karyotyped with the karyotype described according to the International System for Human Cytogenetic Nomenclature (ISCN).\(^18\) For each sample, an attempt to fully analyze twenty metaphases was made to define the nature of the aberrations for each one. Karyotypic abnormalities were recorded as clonal per ISCN if at least two cells had the same structural abnormalities or added chromosome, and in case of missing chromosomes, then three cells must be missing the same chromosome.

Patients’ history and their diagnosis were available from laboratory records received with the submitted clinical samples. The chromosomal aberrations of patients were sorted and tabulated with respect to each hematologic malignancy. Age, sex, and other epidemiologic and demographic information
about the patients were used when available from the medical records, but patients’ names and other private information were kept confidential and were not included in the analysis pertaining to this study.

Results

The median age of 45 AML patients examined was 30 years, with 71.1% of cases over 18 years old. Additionally, 28 out of 45 were males, presenting a male to female ratio of 1.6 to 1. Among AML patients less than 18 years old, the male to female ratio was even more distorted at 2.7 to 1. A normal karyotype was found in 32 (71.1%) cases compared to 13 (28.9%) with various abnormal karyotypes (Table 1). Two cases had inversion 16, two deletion 20q, three had t(15;17), and two t(8;21) (Fig. 1 as an example).

A total of 111 ALL cases, with ages from 2 months to 64 years were examined cytogenetically. Of those, 37.8% were 2 to 6 years old, 9% were 14-17 years old, and 2.7% were 49-64 years old (median of all cases 7 years). The disease was more prevalent in males with a male to female (M:F) ratio of 1.2:1. ALL of B cell linage (B-cell and pre-B-cell ALL’s) predominated with 60 out of the 111 cases (54.1%) compared to 14 (12.6%) of T cell phenotype (37 cases were not sub-classified). A normal karyotype was noted in 75 cases (71.1%) and an informative abnormal karyotype in 36 cases (32.4%, Table 2). Typical abnormalities were noted, such as hyperdiploidy (15 cases) and t(1;19) (four cases) in B and pre-B ALL.

Table 1. Abnormal karyotypes found in AML cases in our series

<table>
<thead>
<tr>
<th>Age</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>46,XY,t(4;9)(q34;q13),t(8;21)(q21;q21),del(11)(q13)[10]/46,XY[10]</td>
</tr>
<tr>
<td>17</td>
<td>46,XY,t(15;17)(q22;q22)[10]/46,XY[10]</td>
</tr>
<tr>
<td>20</td>
<td>46,XX,inv(16)[20]</td>
</tr>
<tr>
<td>3</td>
<td>47,XY,+mar?,der(22)[7],46,XY[13]</td>
</tr>
<tr>
<td>21</td>
<td>46,XX,del(20)(q12)[10]/46,XX[10]</td>
</tr>
<tr>
<td>60</td>
<td>46,XY,del(20)(q12)[5]/46,XY[15]</td>
</tr>
<tr>
<td>29</td>
<td>46,XY,t(8:21)(q22;q22)[15]/46,XY[5]</td>
</tr>
<tr>
<td>30</td>
<td>46,XY,del(2)(p13),t(15:17)(q22;q21)[15]/46,XY[5]</td>
</tr>
<tr>
<td>46</td>
<td>46-49,XY,del(1)(p22),-5,-14,-19,?der(19)t(1;19),+3-4 mar[cp19]/46,XY[1]</td>
</tr>
<tr>
<td>NA</td>
<td>46,XY,inv(16)(p13q22)[8]/46,XY[7]</td>
</tr>
<tr>
<td>39</td>
<td>46,XY,t(15;17)(q22;q12)[5]/46,XY[12]</td>
</tr>
<tr>
<td>33</td>
<td>48,XY,+4,+21[2],46,XY[18]</td>
</tr>
<tr>
<td>29</td>
<td>46,XY,t(15;17)(q22;q21)[2]/46,XY[3]</td>
</tr>
</tbody>
</table>
Table 2. Abnormal karyotypes found in different ALL subtypes in our series

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Cell ALL</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>59-62,XY,+X,+3,+4,+6,+8,+10,+11,+13,add(14)(q32),+20,+21,+2-3mar[cp10]/46,XY[8]</td>
</tr>
<tr>
<td>4</td>
<td>55,XX,del(1)(q32)+6,+8,add(9)(p24),+10,+11,+18,+21,+21,+2,+3mar[cp20]</td>
</tr>
<tr>
<td>2</td>
<td>58-59,XY,+X,+Y,+4,+5,+6,+8,+13,+14,+15,+21,+21[10]/46,XY[10]</td>
</tr>
<tr>
<td>1</td>
<td>54,XY,+6,+8,+10,+18,+21,+22,+mar(3)/46,XY[17]</td>
</tr>
<tr>
<td>4</td>
<td>46,XY,del(5)(p12)[20]</td>
</tr>
<tr>
<td>19</td>
<td>45,XX,-5,t(7;14)(q11;q32),add(11)(p12),der(12)?inv(12),-13,-16,-17,-20,+mar[19]/46,XX[1]</td>
</tr>
<tr>
<td>16</td>
<td>46,XX,add(14)(q32)[4]/46,XX[16]</td>
</tr>
<tr>
<td>16</td>
<td>47-48,XX,add(7)(p13),+8,der(19)(19;21)(q23;p13),+21[10]/46,XX[10]</td>
</tr>
<tr>
<td>3</td>
<td>50,XY,t(1;19)(q23;p13),+5,+8,+11,der(19)t(1;19),+22[12]/46,XY[2]</td>
</tr>
<tr>
<td>14</td>
<td>56-58,XY,+X+4,+6,+8,+8,t(9;22)(q34;q11.2),+10,+13,+15,+2mar,</td>
</tr>
<tr>
<td></td>
<td>+other[cp18]/46,XY[2]</td>
</tr>
<tr>
<td>5</td>
<td>54,XY,+4,+6,+8,+10,+18,+21,+1-6mar[cp16]/46,XY[2]</td>
</tr>
<tr>
<td>1</td>
<td>70-72,XY,+2,+3,del(3),+4,+6,+8,+10,+11,+12,+13,+16,+18,+18,+21,+21,+22,+4-6mar[cp17]/46,XY[3]</td>
</tr>
</tbody>
</table>

Figure 1: The karyotype of the AML sample showing t(8;21)
1 46,XY,t(1:19)(q23;p13)[4]/46,XY[16]
2 50,XY,+6,+11,+20,+21[7],46,XY[8]
3 47-48,XY,?13q,add(8)(p11),del(13)(q14),+19,+mar[6]/46,XY[5]

**pre-B cell ALL**

2 55-57,XY,+4,+8,+10,+14,1(17)(q10),+18,+21,+45mar,(cp4)/46, XY[20]
4 46,XX,t(4;12)(q12;p13)[2]/46,XX[18]
4 46,XY,t(1:19)(q23;p13)[15]/46,XY[5]
21 49-60,XY,+3+4+6+8+10+18+1-6mar[cp12]
0.2 58,XY,+X+Y+4+6+7+10+13+15+16+18+21/46XY[15]
2 46,XY,der(19)t(1;19)(q21;p13)[5]/46,XY[10]
3 50-67,XY,+8,+18,+20,+21,+other[5]/46,XY[10]

**T-cell ALL**

17 46,XY,del(6),(q21q27)[18]
8 47,XX,+19[3]/46,XX[17]
4 46XX,-7,add(9)(p12),add(21)(p11.2)?t(7;9;21)[15]/46,XX[5]

Figure 2: The karyotype of the sample SB-12-037 shows hyperdiploid and structurally abnormal B-cell ALL

**Discussion**

Leukemic patients referred to our center included roughly a quarter (28.8%) AML and the rest were ALL cases. Median age of AML at diagnosis in Palestinian patients was 30 years old compared to 67 years old in the USA(19). This wide difference may be due to exposure to mutagens earlier in life, which results in the disease; such as ionizing radiations, occupational exposures to chemicals, smoking,
diets, and infection. Our study showed male predominance in AML patients, which is similar to the results of the US studies(20). One fourth of our AML cases showed abnormal karyotype, while the rest were normal. The WHO classification includes fourteen types of AML(2). Two of our samples showed t(8;21)(q22;q22), which is the common abnormality in AML-M2 and is associated with a relatively good prognosis with therapy(21,22,23). Three of our cases had M3 with t(15;17)(q24;q21), which respond well to targeted therapies with all-trans retinoic acid (ATRA)(24,25). Two cases had inv(16) seen with AML-M4eo subtype, which has favorable prognosis(26).

Of our ALL cases, the median age was 7 years old and 72.9% were 18 years old or younger as is common in ALL in other countries(1,4,5,7). ALL age distribution in our cases were tri-modal with three peaks of incidence; one from 2 to 6 years, a second from 14-17, and a third from 49-64. A bimodal incidence distribution was suggested in the literature as in the USA population with a first peak of incidence among infants <1 year, followed by a decrease in childhood and then an exponential rise beginning in young adulthood and advancing with age with a slight peak around 60 years(27). This heterogeneity in age of incidence may reflect the etiological heterogeneity among our patients, who present various types of abnormalities with different outcomes since ALL likely has both environmental and genetic influences(28). Chromosomal abnormalities are usually found in 60-70% of ALL cases(29). However, in our study, the majority (67.6%) of ALL cases demonstrated a normal karyotype, and only 32.4% of the cases demonstrated an abnormal karyotype, either numeric abnormalities or structural changes, such as translocations, inversions, or deletions or both numerical and structural abnormalities. This could reflect sample-related issues and/or geographic differences as discussed above pertaining to environment and background genetics, see(28).

Among the notable abnormalities in our series were 7 patients with hypodiploidy or pseudodiploidy and 24 patients with hyperdiploidy (nine with 2n=47-50 and the rest 2n=51 or more) (see Fig. 2). Hyperdiploidy of more than fifty one chromosomes represents 41.7% of all abnormal ALL cases and is considered to be of a favorable prognosis(30).

We had some abnormalities in ALL reported earlier and commonly in other countries such as t(7;14) and t(1;19). However, we had one case of pre-B ALL with an unusual isolated t(4;12). The breakpoint on 12 is a common leukemic associated breakpoint affecting ETV6 gene, but we neither note that in the most recent review reports of pre-B cell ALL with t(4;12)(31) nor in internet searches. Further studies are needed to determine if this translocation is found in other patients (recurrent), which warrants molecular analysis for potential new oncogenes. The diversity of abnormalities resulting in very few cases (two to three) with each kind of abnormality meant that the numbers we have are too small to draw other conclusions, such as the correlation between cytogenetic abnormalities in Palestine, on the one hand, and outcome or age, on the other. This could be an interesting follow-up study. Studies of cytogenetics of acute leukemia in Palestine are still in their infancy. Cytogenetic data is now being used in developing countries like ours by clinicians in targeted therapy applications. This can make a significant difference in outcomes and is now considered as standard medical therapy for these cancers(21,32,33).
Conclusion
Our study shows decreased median age of AML patients compared to the USA. Common translocations were found in our patients including t(8;21), t(15;17), and inv(16). The majority of ALL cases show normal karyotype and heterogenic age incidence. Many translocations were found in ALL, such as t(7;14), t(1;19), and one unusual pre-B cell t(4;12). Further studies with larger series could elucidate some remaining questions and help physicians in Palestine improve referral patterns.

Acknowledgements: We are grateful to Reem Hanona for technical assistance and to two anonymous reviewers for their comments. The funding was covered by internal resources from the Cytogenetics Laboratory at Bethlehem University.

References
10. Al Husain M, Zaki OK. A survey of 1,000 cases referred for cytogenetic study to King Khalid University Hospital, Saudi Arabia. Hum Here. 1999; 49: 208-14.
17. Qumsiyeh MB, Borqan H, Obeid T.


الاختلالات العليلية لدى مرضى سرطان الدم الحاد في فلسطين المحتلة

أحمد زيد1,2، حلاوة أبو عابا1، أريج خطي1، مازن قصبة1,2

1- مختبر الوراثة الحيوية وبرنامج الماجستير في التكنولوجيا الحيوية، جامعة بيت لحم، بيت لحم، فلسطين.
2- كلية التمريض والعلوم الصحية المساندة، جامعة بيرزيت، فلسطين.
3- دائرة التكنولوجيا الطبية، كلية العلوم الطبية المساندة، الجامعة العربية الأمريكية، فلسطين.

الملخص

تعد البيانات الحيوية الوراثية مهمة في التشخيص وتصميم العلاج والتوقع المرضي في سرطان الدم النقوي وسرطان الدم الليمفاوي الحادين، ويعتبر هذا التقرير الأول لمسلسلة من الدراسات الوراثية الحيوية على مرضى الوراثة الحيوية الحادة من وسط فلسطين وممارستها بينات من مناطق جغرافية أخرى. تم إجراء التحليل الوراثي الحيوية على 45 مريضاً بسرطان الدم النقوي الحاد و111 مريضاً بسرطان الدم الليمفاوي الحاد، حيث تم جمع عينات من خلايا العظام من جميع المرضى واستراعة لها لمدة 24 ساعة ثم اتباع الألفاظ الطبية للكروموسومات في مرحلة الظهور الانقسامي الوسيط من خلال تقنية الألفاظ الطبية التقليدية ثم تم تناول النتائج النتائج. 

اظهرت الخمسة وأربعون حالة من حالات سرطان الدم النقوي الحاد التي تم تحليلها للتخلص من الوراثة الحيوية أن نسبة الألائم للذكور فيها 1:6، مع ما نسبته 71.1% فوق الثامنة عشرة من العمر، وظهر لنا ما نسبته 28.9% من الحالات ذات خطر حيوي محتمل. من بين المعدل واحد عشر حالة التي تم تحليلها بسبب سرطان الدم الليمفاوي الحاد فإن 37.8% كانت اعمارهم من 2-6 أعوام، وأيضاً فان ما نسبته 54.1% كان من نوع خلايا B مع نسبة البالانسات للذكور فيها 1:2.1. في المقابل فإن 12.6% كان من خلال نوع T (في حين بقيت النسبة المتبقية غير محددة).

لقد كانت النتائج العريفي للإصابة لدى مرضى سرطان الدم الليمفاوي الحاد ثلاثة القسم: واحد من عمر 2-6 أعوام، وثاني من 14-17 عاماً، وثالث من 49-64 عاماً. من بين حالات سرطان الدم الليمفاوي الحاد هذه فإن 32.4% كانت ذات خطر وراثي حيوي محتمل، تمثلت الاختلافات في تشكيلة ميزة للاهتمام، وكانت تقع تحت ثلاثة نفات: نوع ما قبل النوع، نوع خلايا A، نوع خلايا T. لقد نرى في بنود نتائج الألفاظ الوراثية الحيوية بعض الاختلافات عما هو معروف في الدراسات العلمية وكذلك أشارنا أن التوزيع العريفي في بياناتنا مقارنة بذلك التي من دول أخرى ربما يعكس تناوبات في تحول المرضى أو اختلافات نية وبيئة.

الكلمات المفتاحية: سرطان الدم النقوي الحاد، سرطان الدم الليمفاوي الحاد، سرطان الدم، النظام الوراثي الحيوي.