Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Leukemia Research 34 (2010) 1617–1621

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Genomic instability and rapid clinical course in adult T-cell lymphoma/leukemia patient

Adriana Zámečníková[a,*], Soad Al Bahar[a], Samah E. Elshinnawy[b]

[a] Department of Hematology, Laboratory of Cancer Genetics, Kuwait Cancer Control Center, Shuwalkh, Kuwait 70653, Kuwait
[b] Clinical Laboratory Department, Chest Diseases Hospital, Kuwait, Kuwait

ARTICLE INFO

Article history:
Received 6 January 2010
Received in revised form 10 February 2010
Accepted 10 February 2010
Available online 7 March 2010

Keywords:
Adult T-cell leukemia/lymphoma
Human T-cell leukemia virus type I
Genomic instability
Carcinogenesis

ABSTRACT

Adult T-cell leukemia/lymphoma is a distinct clinical entity characterized by a clonal proliferation of malignant T-lymphocytes. The etiologic agent of the disease is a Human T-cell lymphotropic virus type I. It occurs almost exclusively in areas where the virus is endemic; however the disease develops only in the minority of patients who are virus carriers. Karyotyping findings and their correlation with clinical features are still limited in T-cell malignancies, complicated by clinical heterogeneity and a plethora of secondary abnormalities. This study describes detailed chromosomal and fluorescence in situ hybridization results observed in a patient with adult T-cell leukemia/lymphoma and correlates them with clinical characteristics.

1. Introduction

Adult T-cell leukemia/lymphoma (ATLL) is an aggressive peripheral T-lymphocytic malignancy mediated by the Human T-cell lymphotropic virus type I (HTLV-I) [1,2]. The disease occurs almost exclusively in areas where the virus is endemic where approximately 6–37% of the population is infected [3]. Evidence from clinical studies suggests that fewer than 5% of infected individuals develop ATLL, usually after decades of relatively symptom-free infection. The clinical and prognostic diversity of patients has led to its subclassification such as acute, lymphoma, chronic, and smoldering types. Over two-thirds of patients present with leukemia (leukemia form), while approximately 25% have no blood involvement (lymphoma form). Noteworthy, unlike most leukemias, bone marrow infiltration is not always present in ATLL. The usefulness of clinical subclassification may be limited due to the heterogeneity of genomic abnormalities within the host genome, which may account for further diversities within clinical subgroups [4–7]. We present here detailed cytogenetic and fluorescence in situ hybridization (FISH) findings in an ATLL patient, the first reported case from Kuwait. Our case may be of interest due to: (1) rare occurrence of ATLL in this region, (2) detailed genomic findings in a patient and (3) correlation of genomic findings with clinical course, the first described from this region.

2. Materials and methods

2.1. Case summary

The patient was a 32-year-old Kuwaiti female with 1-month history of progressive dyspnea, dry cough and bilateral ankle pain. She denied any previous transfusion history or history of travel abroad. Her past history is significant for recurrent abortions and hypertension diagnosed 10 days back prior presentation. At presentation, physical examination was normal and abdominal ultrasound showed no organomegaly or lymphadenopathy. Chest X-ray and computerized tomography showed bilaterally ill defined opacities with fibrosis and bilateral bronchiectatic changes. Serum chemistry profile was normal, except raised lactate dehydrogenase, which was more than twice (399 U/L) the upper limit of the normal range (190). Serum calcium level was normal on repeated occasions. Collagen work up, thyroid function and tuberculosis tests were negative. Virology studies were negative for HIV, HBV, adenovirus, influenza, parainfluenza virus, Rhino virus, Corona virus, RSV virus and CMV virus.

Complete blood count results showed a leukocyte count of $37.4 \times 10^9\ L^{-1}$ (lymphocytes 3%, atypical lymphocytes 47%, monocytes 8%, neutrophils 41%, basophils 1%). The peripheral blood smear showed a polymorphous population of atypical lymphocytes with high N/C ratio of polylobulated and irregular nuclear borders described as clover leaf cells (ATLL cells). The cytoplasm was deeply basophilic with occasional vacuoles. Bone marrow aspirate was moderately hypercellular with diffuse infiltration of lymphoid elements (23%) and similar morphology as in the peripheral blood (Fig. 1). Flow cytometry of the bone marrow biopsy was tested positive for CD2 (83%), CD3 (78%), CD4 (79%), CD38 (89%), CD45 (96%), CD25 (45%) and CD38 (89%), whereas immunophenotype results from the blood were: CD2 (80%), CD3 (81%), CD4 (78%), CD38 (81%), CD45 (99%), CD25 (85%) and CD38 (81%). The expression of CD5 was reduced (29% in bone marrow and 41% in blood sample). CD7 and CD8 were negative both in bone marrow and blood samples. Based on clinical and laboratory findings the co-existence of Human T-cell lymphotrophic virus type-1 serology was sought, and was positive, giving a diagnosis of HTLV-1-induced adult T-cell leukemia/lyphoma (ATLL).

While no generalized lymphadenopathy, hepatosplenomegaly, cutaneous disease or elevation of calcium were observed in our patient, the presence of an elevated

* Corresponding author. Tel.: +965 248 21363; fax: +965 248 10007.
E-mail address: annaadria@yahoo.com (A. Zámečníková).

0145-2126/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2010.02.011

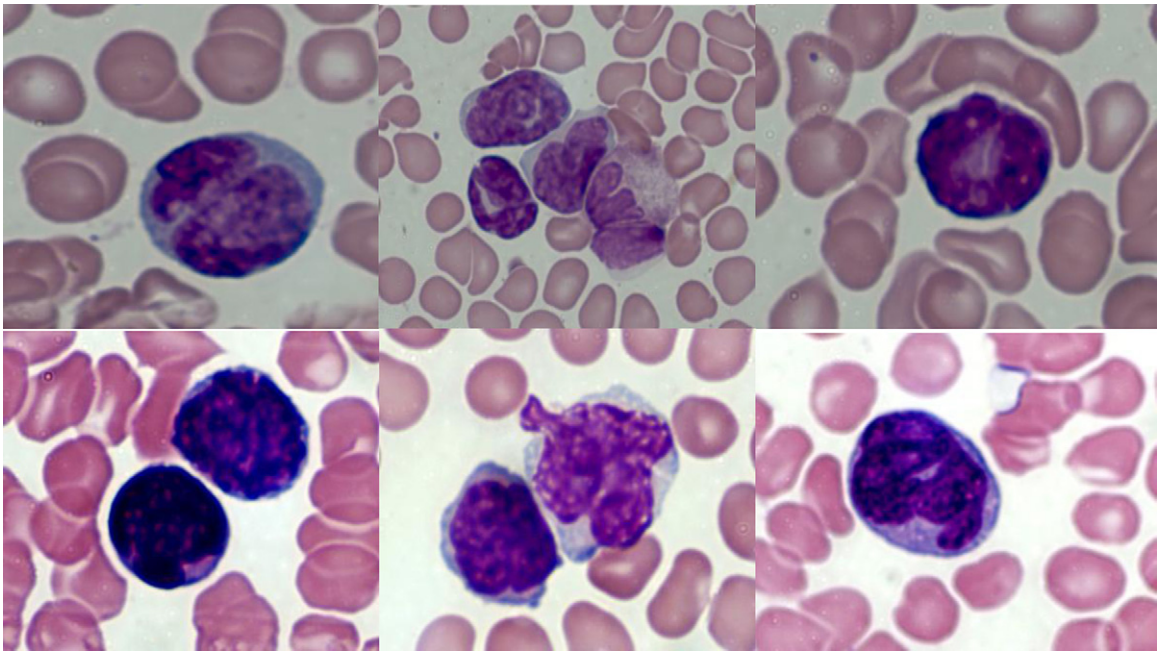

Fig. 1. Peripheral blood smear illustrating abnormal lymphocytes with indented or lobulated nuclei and irregular nuclear borders described as clover leaf cells as well larger cells with more dispersed chromatin and a cerebriform-shaped nucleus and clumped chromatin.

WBC count, elevation of serum LDH and the rapidly progressive clinical course is therefore indicative of chronic subtype with terminal leukemic manifestations. Once hospitalized, the course of her disease was very acute and severe, as is seen in acute disease. She was started on interferon alpha, broad spectrum antibiotics and anti-retroviral therapy with Tazocin, Erythromycin and Septrin and as well as with Diflucan and Claxene. The patient developed rapidly progressive dyspnea with respiratory distress syndrome and type I respiratory failure, for what she was kept on mechanical ventilation. While on ventilation, bilateral surgical emphesema were noticed and chest X-ray showed bilateral pneumothorax, pneumomediastinum and pneumopericardium. The patient developed fever and persistant tachycardia, out of proportion to fever. The patient continued on sensitive antibiotics; however cultures and sensitivity tests revealed gram-negative and gram-positive bacteraemia (coagulase-negative staphylococci, pseudomonas and klebsilla pneumoniae) and Candida Dubleninesis. Her general condition did not improve and hemoglobin dropped despite being a febrile with no evidence of blood loss. The patient developed hypotension and progressed to slow bradycardia. Her condition continued to deteriorate and she died shortly afterwards due to cardiac arrest.

2.2. Chromosomal and fluorescence in situ hybridization studies

Chromosome studies were performed on peripheral blood and bone marrow cultures obtained at diagnosis. Direct and 24 h cultures were established and the analysis was performed using G-banding. Karyotypes were described according to the International system for Cytogenetic Nomenclature (1995) [8]. The presence of genomic anomalies was confirmed by fluorescence in situ hybridization on slides prepared for cytogenetic analysis. Interphase and metaphase FISH assay was performed using commercially available LSI *IGH*/*BCL2*, LSI D7S486/CEP 7, LSI 13S319 and LSI MLL probes (Vysis, Downers Grove, IL) according to Vysis, Inc. protocol. Hybridization signals were visualized using a Leitz Diaplan fluorescence microscope and images were captured and digitally recorded by Isis software (MetaSystems, Germany).

3. Results

Karyotyping and FISH from leukemic cells displayed extensive and varied forms of genomic anomalies involving chromosomes 3, 7, 11, 13, 14, 18, and 19 (Fig. 2). Of these abnormalities, involvement of 7q, 13q, 14q, and i(18) has been indicated to be specific for T-cell leukemias/lymphomas including ATLL. In the sideline karyotypes further anomalies such as abnormalities of 3q, 7p, 12p, 14q, 17q, abnormality of chromosome 19 were observed indicating these anomalies may be additional changes reflecting disease evolution. In the peripheral blood cultures only 3 normal metaphases were detected and the remaining metaphases displayed multiple numerical anomalies such as monosomy 13 and additional 18, hypotetraploidy as well as multiple structural anomalies. Among them, chromosome 3, 7q and 11q abnormalities, del(14)(q11q13), an additional isochromosome 18 and monosomy 13 were observed in the majority of examined metaphases. Bone marrow studies revealed similar cytogenetic pattern, however the proportion of normal metaphases was higher than in the peripheral blood sample (10 normal metaphases out of 20 examined were found) (Table 1).

Applying the LSI *IGH*/*BCL2* probe on PB interphase cells we detected in 80% of cells 2 signals for the *IGH* gene without *IGH*/*BCL2* rearrangement and 4 signals for the *BCL2* gene indicating the presence of 4 copies of 18q in 80% of cell confirming the formation of an extra isochromosome 18(q) in 80% of cells (Fig. 3A). Applying the LSI *Rb* probe on PB interphase cells we detected in 40% of cells only 1 signal for *Rb* gene indicating the loss of *Rb* gene from 40% of cells indicative of monosomy 13 and confirming the karyotyping result. 7q and 11q rearrangements were confirmed with the LSI 7p/7q and MLL probe (Fig. 3B and C). Hybridization with LSI *MLL* gene revealed normal signal pattern indicating that *MLL* is not rearranged but 11q is involved in chromosome rearrangement resulting in a der(11) chromosome. The green signal of the rearranged chromosome 7 hybridizing to 7p10 appeared double sized compared with the signal on normal chromosome 7 (Fig. 3D).

4. Discussion

Adult T-cell leukemia is one of the most aggressive human neoplasias and one of the few examples in which the primary etiologic agent, the human retrovirus HTLV-1 has been established [9,10]. HTLV-1 integrates into random sites in host chromosomal DNA, inducing genomic instability by interfering with mitotic checkpoints and reduced DNA repair [11,12]. The transformation is initiated by Tax, a regulatory protein encoded by the HTLV-I pX region [13]. This viral oncoprotein interacts with numerous cellular processes including transcription, cell cycle regulation, DNA repair, and apoptosis resulting in a state of genomic instability, which is thought to be a major contributor to the development of ATLL

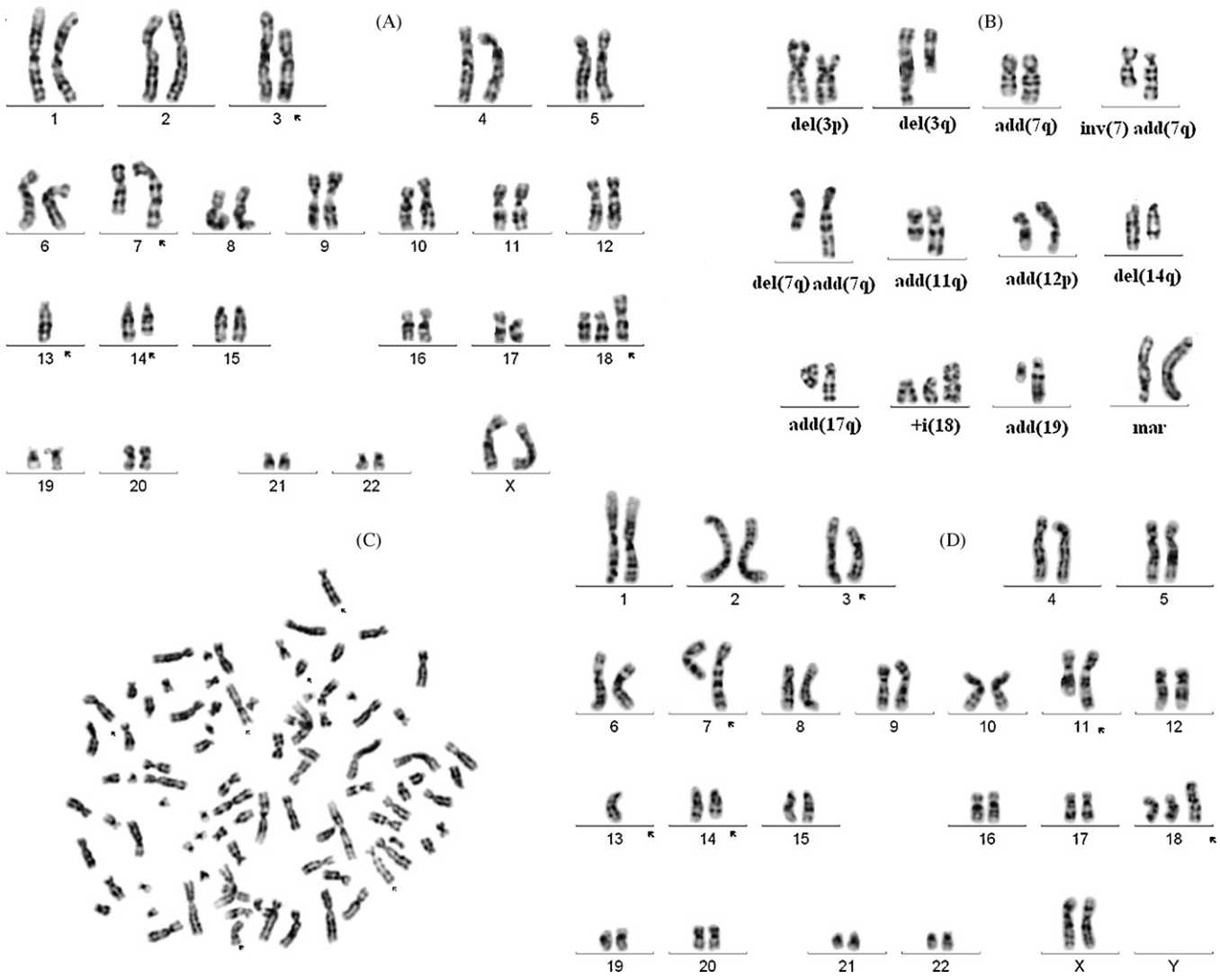

Fig. 2. Karyotype from the leukemic blood cell of a patient showing deletion of chromosome 3 and 14 as well as rearrangement of chromosome 7, monosomy 13 and an extra isochromosome 18 (A). Partial karyotypes displaying extensive and varied forms of genomic anomalies involving chromosomes 3, 7, 13, 14, 18 and 19 (B). Hypo-tetraploid metaphase with multiple anomalies (C). Clonal evolution in a patient detected in the sideline karyotype showing an abnormal chromosome 11 indicative of an additional chromosomal change in the ATLL karyotype (D).

Table 1
Karyotyping findings in a patient displaying chromosomally unstable clones.

	No
Peripheral blood	
46,XX	4
46,XX,del(3)(p21),add(7)(q34),−13,del(14)(q11q13),+i(18)(q10)	15
46,XX, 46,XX,del(3)(p21),add(7)(q34),add(11)(q24),−13,del(14)(q11q13),+i(18)(q10)	15
46,XX,del(3)(p21),add(7)(q34),−13,del(14)(q11q13),t(18;?)(q23,?),+i(18)(q10)	4
46,XX,der(3)del(3)(p21)del(3)(q21),add(7)(q34),del(12)(p11),−13,del(14)(q11q13), add(17)(q25),+i(18)(q10),−18×2,−20,−22,+mar1,+mar2	1
45,XX,del(3)(p14), der(7)inv(7)(p15q34)add(7)(q34),−13,del(14)(q11q13),i(18)(q10)	2
46,XX,del(3)(p14),add(7)(q34),−13,add(14)(q23),del(14)(q11q13),del(18)(q21), +i(18)(q10)	1
45,XX,del(3)(p14),add(7)(q34),−13,del(14)(q11q13), i(18)(q10)	1
43,XX,del(3)(q13),add(7)(q34),del(7)(q31),+8,+10,−12,−13×2,add(17)(q22),−18, i(18)(q10),add(19)(p13?),−20	1
87,XXXX,del(3)(p14)×2,−5,−6,der(7)inv(7)(p15q34)add(7)(q34)×2,add(11)(q24),−13×2,del(14)(q11q13),−16,i(18)(q10)	11
Bone marrow	
46,XX	10
46,XX,del(3)(p14),add(7)(q34),−13,del(14)(q11q13),+i(18)(q10)	5
46,XX,del(3)(p14),add(7)(q34), add(11)(q24),−13,del(14)(q11q13),+i(18)(q10)	2
46,XX, der(3)del(3)(p14)del(3)(q),t(3;12)(q?;q24), add(7)(q34),−13,del(14)(q11q13), add(18)(q23),i(18)(q10),add(19)(p13?)	1
87,XXXX,del(3)(p14)×2,−5,−6,der(7)inv(7)(p15q35)add(7)(q34)×2, add(11)(q24),−13×2,del(14)(q11q13),−16, i(18)(q10)	2

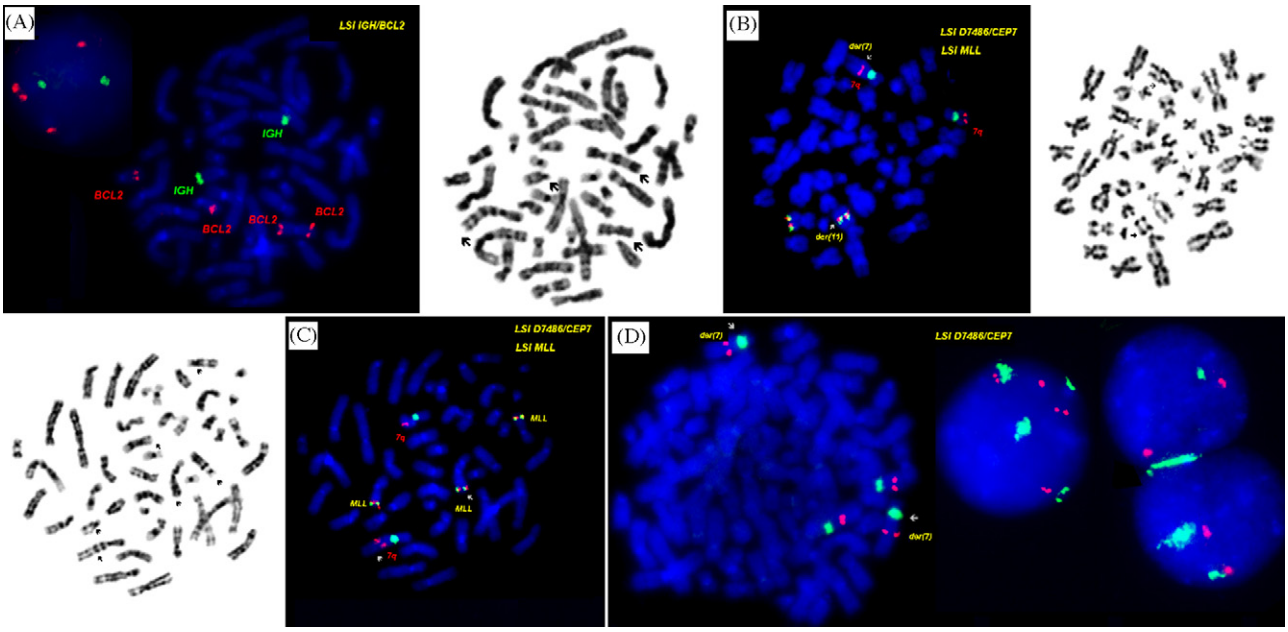

Fig. 3. Fluorescence in situ hybridization studies applying the LSI IGH (green)/BCL (red) probe showing two normal green signals of IGH on chromosome 14 and 4 red signal of the BCL gene indicative of the presence of two normal chromosomes 18 and an additional isochromosome 18 (A). Combination of LSI 7p (green)/7q (red) and MLL probe showing normal signal pattern on the add(7)(q) and add(11) chromosomes confirming the rearrangements of chromosomes 7 and (B and C). The green signal of the rearranged chromosome 7 hybridizing to 7p10 appeared double sized compared with the signal on normal chromosome 7 indicating duplication of the centromeric region (Fig. 2D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

[14–16]. The presence of a variety and extensive forms of genomic aberrations observed in leukemic cells of our patient is compatible with this concept. A number of breaks and multiple numerical rearrangements were observed including aneuploidy, hypotetraploidy, derivative chromosomes, balanced and unbalanced translocations, deletions, complicated by a plethora of additional abnormalities.

Some of these alterations observed in our patient, including a chromosome 14 anomaly with a break at band q11 and break in band 7q35 where the *TCRB* gene has been assigned, appear to be rather ATLL specific [17]. Rearrangement of 14q11 in ATLL was first reported by Sadamori et al., and its specificity has been confirmed in further studies [18–20]. The *TCRA*/*D* gene is located at 14q11; however its involvement has not been confirmed in ATLL [21]. Moreover, the deletion of chromosomal 14q11-13 region observed in our patient is a known karyotypic anomaly in ATLL, suggesting location of tumor suppressor gene(s) in this region. Several deletion abnormalities and alterations of several known tumor suppressor genes have been found in ATLL [22,23]. Chromosome 13 abnormalities involving the retinoblastoma gene and their association with structural abnormalities of 7q chromosomes as it was found in our patient have been reported to occur in ATLL. Chromosome 3 anomalies and isochromosome of 18q found in the majority of clones in our patient are known anomalies in T-cell malignancy including ATLL. In most of reported cases, chromosome 18 rearrangements were observed in the sideline karyotypes, indicating they may occur as additional changes in the ATLL karyotype [22–24].

Another important characteristic of HTLV-I is its restricted geographic prevalence. Until the beginning of the 1990s, the Middle East has been considered as a region virtually free from HTLV-I infection. Later it has been shown; that a relatively high prevalence of HTLV-1 infection can be found in Iran and the region of Mashhad has been recognized as an endemic area of the virus. Subsequent studies confirmed, that a relatively high prevalence of HTLV-I infection can be detected in Israel, confined almost exclusively to the immigrants from Mashhad, in so-called Mashhadi Jews. Then it has been shown that the high prevalence of HTLV-I infection is also characteristic for the Muslim population of Mashhad and for additional regions in Iran [25,26]. A low prevalence and sporadic cases of HTLV-I has been described in Iraq and, more recently, in Kuwait [26–28]. The family roots in three described Kuwaiti patients were located to Iraq in Najaf, which population had historically strong ties with the Mashdad region of Iran. Molecular characterization of HTLV-1 found revealed close similarity with HTLV-1 isolates originating from Mashhad in these Kuwaiti patients, suggesting the common origin of Kuwaiti (Najafi) and Mashhadi HTLV-1 infection [26,27]. In addition, a relatively high prevalence of HTLV-1 infection (1:4648) was found among volunteer blood donors in Kuwait [28]. The infection was more prevalent among non-Kuwaiti Arab donors (Indians and Iranians) than among Kuwaiti nationals (1:3782 vs. 1:6379). These findings indicate that HTLV-I infection is quite prevalent among blood donors in Kuwait, raising the possibility of another pocket of HTLV-1 infection in the Middle East.

5. Conclusions

Our study revealed extensive karyotypic complexity and several breakpoints clustered at regions characteristic of ATLL. The occurrence of chromosomally unstable clones or single cell abnormalities in our patient may constitute steps in the genesis and progression of the disease. To characterize these breakpoints may be helpful in identifying the responsible genes in the stepwise process of ATLL carcinogenesis. Our findings confirmed that genomic instability, which is thought to be a major contributor to the HTLV-1 associated ATLL, clearly correlated with karyotypic complexity and poor prognosis [29]. To our knowledge the present case is the first reported of ATLL from Kuwait in which these features are associated with HTLV-1 infection.

Conflict of interest

No conflicts of interest.

Acknowledgements

Contributions. AZ designed and wrote the manuscript, designed and performed genetic testing methods, result interpretation, final approval; SAB interpretations, collecting and final approval of patients clinical data, treatment and disease related information, final approval of manuscript and SEE collected and interpreted clinical data, final approval of laboratory and clinical data and patient diagnosis, contribution in writing.

References

[1] Rizvi MA, Evens AM, Tallman MS, Nelson BP, Rosen ST. T-cell non-Hodgkin lymphoma. Blood 2006;107:1255–64.
[2] Vose JM. Update on T-cell lymphoma. Ann Oncol 2008;19:74–6.
[3] Rodriguez-Abreu D, Bordoni A, Zucca E. Epidemiology of hematological malignancies. Ann Oncol 2007;18:3–8.
[4] Choi YL, Tsukasaki K, O'Neill MC, Yamada Y, Onimaru Y, Matsumoto K, et al. A genomic analysis of adult T-cell leukemia. Oncogene 2007;26:1245–55.
[5] Nakagawa M, Nakagawa-Oshiro A, Karnan S, Tagawa H, Utsunomiya A, Nakamura S, et al. Array comparative genomic hybridization analysis of PTCL-U reveals a distinct subgroup with genetic alterations similar to lymphoma-type adult T-cell leukemia/lymphoma. Clin Cancer Res 2009;15:30–8.
[6] Tsukasaki K, Tanosaki S, DeVos S, Hofmann WK, Wachsman W, Gombart AF, et al. Identifying progression-associated genes in adult T-cell leukemia/lymphoma by using oligonucleotide microarrays. Int J Cancer 2004; 109:875–81.
[7] Tagawa OH, Ohshima K, Karube K, Uike N, Tashiro Y, Utsunomiya A, et al. Identification of subtype-specific genomic alterations in aggressive adult T-cell leukemia/lymphoma. Blood 2006;107:4500–7.
[8] Mitelman F. Basel SK. ISCN 1995: an international system for human cytogenetic nomenclature; 1995.
[9] Suzumiya J, Ohshima K, Tamura K, Karube K, Uike N, Tobinai K, et al. The International Prognostic Index predicts outcome in aggressive adult T-cell leukemia/lymphoma: analysis of 126 patients from the International Peripheral T-cell Lymphoma Project. Ann Oncol 2009; http://annonc.oxfordjournals.org/misc/terms.shtml0, doi:10.1093/annonc/mdn696.
[10] Tsukasaki K, Hermine O, Bazarbachi A, Ratner L, Ramos J, Harrington W, et al. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an International Consensus Meeting. J Clin Oncol 2009;27:453–9.
[11] Liu M, Yang L, Zhang L, Liu B, Merling R, Xia Z, et al. Human T-cell leukemia virus type 1 infection leads to arrest in the G1 phase of the cell cycle. J Virol 2008;82:8442–55.
[12] Tsukasaki K, Tsushima H, Yamamura M, Hata T, Murata K, Maeda T, et al. Integration patterns of HTLV-I provirus in relation to the clinical course of ATL: frequent clonal change at crisis from indolent disease. Blood 1997;89: 948–56.
[13] Twizere JC, Springael JY, Boxus M, Burny A, Dequiedt F, Dewulf JF, et al. Human T-cell leukemia virus type-1 Tax oncoprotein regulates G-protein signaling. Blood 2007;109:1051–60.
[14] Hidaka T, Nakahata S, Hatakeyama K, Hamasaki M, Yamashita K, Kohno T, et al. Down-regulation of TCF8 is involved in the leukemogenesis of adult T-cell leukemia/lymphoma. Blood 2008;112:383–93.
[15] Bellon M, Nicot C. Central role of PI3K in transcriptional activation of hTERT in HTLV-I–infected cells. Blood 2008;112:2946–55.
[16] Ly H. Telomerase regulation in HTLV-I infection. Blood 2008;112:2603–4.
[17] Mitelman F, Johansson B, Mertens F. Mitelman database of chromosome aberrations in cancer. 2004; http://cgap.nci.nih.gov/Chromosomes/Mitelman.
[18] Sadamori N, Kusano M, Nishino K, Tagawa M, Yao E, Yamada Y, et al. Abnormalities of chromosome 14 at band 14q11 in Japanese patients with adult T-cell leukemia. Cancer Genet Cytogenet 1985;17:279–82.
[19] Sadamori N, Nishino K, Kusano M, Tomonaga Y, Tagawa M, Yao E, et al. Significance of chromosome 14 anomaly at band 14q11 in Japanese patients with adult T-cell leukemia. Cancer 1986;58:2244–50.
[20] Miyamoto K, Tomita N, Ishii A, Miyamoto N, Nonaka H, Kondo T, et al. Specific abnormalities of chromosome 14 in patients with acute type of adult T-cell leukemia/lymphoma. Int J Cancer 1987;40:461–8.
[21] Sadamori N, Isobe M, Shimizu S, Yamamori T, Itoyama T, Ikeda S, et al. Relationship between chromosomal breakpoint and molecular rearrangement of T-cell antigen receptors in adult T-cell leukaemia. Acta Haematol 1991;86: 14–9.
[22] Tsukasaki K, Krebs J, Nagai K, Tomonaga M, Koeffler P, Bartram CR, et al. Comparative genomic hybridization analysis in adult T-cell leukemia/lymphoma: correlation with clinical course. Blood 2001;97:3875–81.
[23] Itoyama T, Chaganti RSK, Yamada Y, Tsukasaki K, Atogami S, Nakamura H, et al. Cytogenetic analysis and clinical significance in adult T-cell leukemia/lymphoma: a study of 50 cases from the human T-cell leukemia virus type-1 endemic area, Nagasaki. Blood 2001;97:3612–20.
[24] Kamada N, Sakurai M, Miyamoto K, Sanada I, Sadamori N, Fukuhara S, et al. Chromosome abnormalities in adult T-cell leukemia/lymphoma: a karyotype review committee report. Cancer Res 1992;52:1481–93.
[25] Karimi A, Nafici MR, Imani R. Comparison of human T-cell leukemia virus type-1 (HTLV-1) seroprevalence in high risk patients (Thalassemia and Hemodialysis) and healthy individuals from Charmahal - Bakhtiari Province, Iran. Kuwait Med J 2007;39:259–61.
[26] Voevodin A, Gessain A. Common origin of human T-lymphotropic virus type-I from Iran, Kuwait, Israel, and La Réunion Island. J Med Virol 1997;52:77–82.
[27] Voevodin A, al-Mufti S, Farah S, Khan R, Miura T. Molecular characterization of human T-lymphotropic virus, type 1 (HTLV-1) found in Kuwait: close similarity with HTLV-1 isolates originating from Mashhad, Iran. AIDS Res Hum Retroviruses 1995;11:1255–9.
[28] Al-Muftia S, Voevodinc A, Ahmeda S, Al Hamdanb S, Al-Basheer AA. Seroprevalence of human T-cell leukemia/lymphoma virus Type I and Type II (HTLV-I/HTLV-II) infection among volunteer blood donors in Kuwait. Med Principles Pract 1999;8:45–50.
[29] Tsukasaki K. Genetic instability of adult T-cell leukemia/lymphoma by comparative genomic hybridization analysis. J Clin Immun 2002;22:57–63.