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del(4)(p14) + 4ace, t(4;15) (p14;pter), NEW CHARACTERISTIC CHROMOSOME ANOMALIES OF ACUTE LYMPHOBLASTIC LEUKEMIA?

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ABSTRACT

In this work we report a patient with acute Lymphoblastic Leukemia (ALL) whose bone marrow presented chromosome anomalies cells when submitted to cytogenetic examination, which have been previously reported for this condition (del(6) (q23); t(9;22) (q34;q11)) as well as others as yet unreported for ALL (del(4) (p14) + 4ace and t(4;15) (p14;pter)). We discuss the hypothesis that these anomalies influence the origin of the malignancy, patient response to treatment and prognosis, possibly through oncogene activation.

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is recognized as a proliferating and self-perpetuating accumulation of lymphoblastic cells in bone marrow and peripheral blood, with infiltration of other organs and tissues characterized by histomologous invasiveness.

With an incidence of approximately two to four cases per 100,000 inhabitants/year, ALL accounts for 85% of all childhood and adolescent leukemias. It may sporadically occur in adults and rarely in newborn infants.

Cytogenetic analysis of human leukemias, using standard staining techniques and chromosome banding, has revealed that 50% of ALL patients exhibit karyotype anomalies (Whang-Peng, 1977; Sandberg and Hossfeld, 1974). Several investigators have observed specific chromosome anomalies in ALL and have estimated their effects

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on hematological neoplasms as well as their importance in terms of medical conduct and prognosis (Williams *et al.*, 1982; Third Workshop on Chromosomes in Leukemia, 1981).

The most frequent alterations are:

- t(4;11) (q21;q23) translocation associated with types L_1 and L_2, although this alteration may be present in other types of leukemia as well (Van den Berger *et al.*, 1979).

- del(6) (q23) deletion and (q15) and (q21) variables, which are present in 5% of all ALL cases and are observed most frequently in children (Morphologic, Imunologic and Cytogenetic, 1985).

- $14q^+$ marker chromosome, which results most often from a t(8;14) translocation (Mitelman, 1981; Roth *et al.*, 1989).

- Philadelphia chromosome (Ph^1) (Proop and Lizzi, 1970) which results from a t(9;22) (q34;q11) translocation. Variables of this translocation (Prakash and Yunis, 1984) are present in patients with L_1 and L_2 (Kowalezyk and Sandberg, 1983; Morphologic, Imunologic and Cytogenetic, 1985). Less frequent alterations such as translocations or deletions involving 12p12 are mainly observed in L_1, and 9p translocations or deletions in bands p21 and p22 which occur in L_1 and L_2. The t(8;14) (q24;q32), t(8;22) (q24;q11) and t(2;8) (p12 or p13;q24) translocations are associated with L_3 (Burkitt type ALL) (Berger and Berheim, 1982).

Here we report a case of L_2 type ALL in which we correlated clinical and hematological data with cytogenetic examination. The examination revealed chromosome anomalies of the del(6) (q23); t(9;22) (q34;q11) types, which have already been reported in the literature. In addition we found del(4) (p14) + 4ace and t(4;15) (p14;pter) alterations, which are possibly new in this condition.

CASE REPORT

J.C.S.S., a white 12-year old boy, was referred to the Hemotherapy and Hematology Center of Para (HEMOPA-Belém/PA) from the Naval Hospital of Belém in March of 1989. He presented anemia of no apparent cause, hepatomegaly, low platelet counts, bone pain and fever. The medical history showed no exposure to toxic agents or previous radiotherapy or chemotherapy. Blood counts were as follows: leucocytes, 2100/mm^3; granulocytes, 6%; lymphocytes, 94%; hemoglobin, 10.2 g%; platelets, 3000/mm^3. Cytomorphologic and cytochemical examination of bone marrow cells (1st puncture) revealed hypercellularity. Approximately 96% of the cells were peroxidase-negative blastic cells, similar to lymphoblasts of L_2 morphology. Red, granulocyte and platelet cell lines accounted for the remaining 4%. Thus, the patient was diagnosed as having ALL/L_2 (French-American-British Classification - FAB, 1976).

The patient initiated treatment soon after diagnosis in accordance with the protocol of the Brazilian Group for Treatment of Childhood Leukemia, 1987. Part of the

treatment was administered at HEMOPA, but the greatest part was administered at the "Marcílio Dias Naval Hospital" in the city of Rio de Janeiro. Clinical evolution was complicated by several episodes of infection such as sinusitis and otitis. The patient suffered complications related to chemotherapy such as severe vincristine-induced neuropathy, Lasparaginase - induced prancreatitis, psychogenetic vomiting and medullary aplasia induced by ametopterin and 6-mercaptopurine. The patient was not treated during the month of February of 1990 due to the complications and to medullary aplasia. Another cytomorphologic examination of bone marrow was performed in March of 1990 after clinical hematologic and laboratory evaluation, revealing the presence of more than 5% blastic cells characterizing a relapse. A second induction was performed but the patient died in May of 1990, 14 months after diagnosis.

MATERIAL AND METHODS

The bone marrow aspirate were cultured in RPMI 1640 medium supplemented with 20% human serum and incubated at 37°C for 24 hrs. Colchicine was then added at a concentration of 0.1 mcg/ml medium. After 20 minutes, the preparation was centrifuged at 1800 rpm/min for five minutes and the supernatant was discarded. The sediment was submitted to hypotonic treatment for 30 minutes using 0.075 M KCl and cells were fixed in Carnoy.

Karyotype analysis was performed by G banding, according to the protocol of Yunis *et al.* (1973). The nomenclature recommended by the International System Cytogenetic Nomenclature - ISCN (1978) was used. Slides were prepared by dropwise addition of the material from a 1.2 meter height and flame dried.

RESULTS AND DISCUSSION

In the present case, cytomorphologic and cytochemical bone marrow tests suggested that this was type L_2 ALL (FAB, 1976).

In addition, bone marrow cell cultures were used for two cytogenetic examinations, one at the time of diagnosis (1st puncture) and one during the relapse (2nd puncture). The results of the cytogenetic examinations presented in Table I. A total of eight metaphases were analysed in the first sample. In seven of them we detected deletion of the short arm of chromosome four associated with the 4p acentric fragment resulting from the break in the p14 region of this chromosome (t94;15) (p14;pter)). Six metaphases were analysed in the second sample and all six exhibited the del(4) (p14) + ace deletion. In both samples, these alterations were usually located in cells with various aneuploidies which also presented deletion of chromosome six (del(6) (q23)) and Philadelphia translocation (t(9;22) (q34;q11)). The del(6) (q23) and t(9;22) markers, as well as the

del(4) (p14) + 4ace and t(4;15) (p14;pter) alterations are illustrated in Figure 1 and the translocation idiogram in Figure 2.

Table I - Karyotype abnormalities observed in patient with ALL/L_2.

Condition	No. cells analyzed	Karyotype
Diagnostic	1	47,XY,+10,del(4)+4ace,del(6),t(9;22)
	1	46,XY,t(4;15),del(6),t(9;22)
	2	46,XY,del(4)+4ace,del(6),t(9;22)
	1	45,XY,-14,del(4)+4ace,del(6),t(9;22)
	1	45,XY,-17,del(4)+4ace,del(6),t(9;22)
	2	44,XY,-14,-17,del(4)+4ace,del(6),t(9;22)
Subtotal	8	
Relapse	1	40,XY,-1,-C,-C,-D,-D,-D,del(4)+4ace,del(6),t(9;22)
	1	40,XY,-5,-C,-C,-C,del(4)+4ace
	1	44,XY,-D,-F,del(4)+4ace,del(6)
	1	44,XY,-D,-F,del(4),del(6),t(9;22)
	1	46,XY,del(4)
	1	50,XY,+C,+E,+E,+G,del(4)+4ace
Subtotal	6	
Total	14	

Most neoplasias are known to be associated with specific chromosome alterations (Yunis, 1983). The cytogenetic examination performed at diagnosis showed markers which frequently occur in ALL, including those associated with the L_2 subtype such as del(6)(q23) and t(9;22). These anomalies were detected along with the del(4)(9p14) + 4ace alteration in all cells, except in one cell where a t(4;15)(p14;pter) translocation was found. With the exception of this last rearrangement, all other alterations were detected again in the second examination which was performed at the time of relapse. Cells with a normal karyotype were not detected and, as reported previously, structural alterations were usually detected in predominantly aneuploid cells of the hypodiploid type.

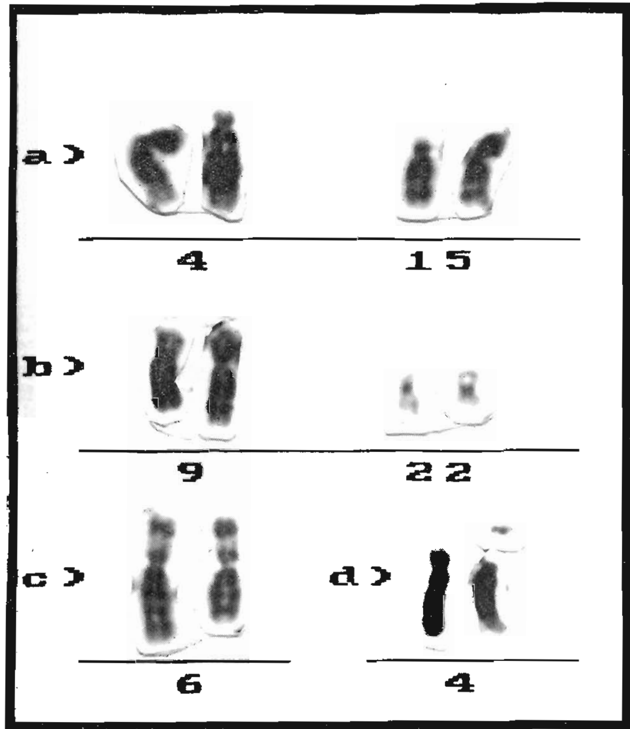

Figure 1 - G-banded bone marrow chromosomes from the patient with a diagnosis of ALL showing: a) t(4;15)(p14;pter); b) t(9;22)(q11;q34); c) del(6)(q23); d) del(4)(p14) + 4ace.

The significance of chromosome alterations in ALL and their possible relationship with the evolution of the disease are points of major interest and have been exhaustively analyzed by several investigators.

Data from the literature (Williams *et al.*, 1980) reveal that ALL patients with almost haploid (26-28 chromosomes) or numerically normal karyotypes with some structural alterations respond poorly to treatment. Considering the karyotype of our patient, the poor prognosis and the complicated clinical evolution, we suggest that the hyplodiploid cells detected may possibly represent random chromosome losses in numerically normal cells.

The relationship between specific chromosome anomalies and the activation of celular oncogenes has been well established. Clear examples of this situation are the t(8;14)(q24;32) translocation in Burkitt's lymphoma associated with activation of the c-myc oncogene, and the t(9;22)(9q34;q11) translocation in chronic myeloid leukemia associated with activation of the c-abl oncogene. In both cases, these oncogenes are located at the break points of these translocations (Trent *et al.*, 1989).

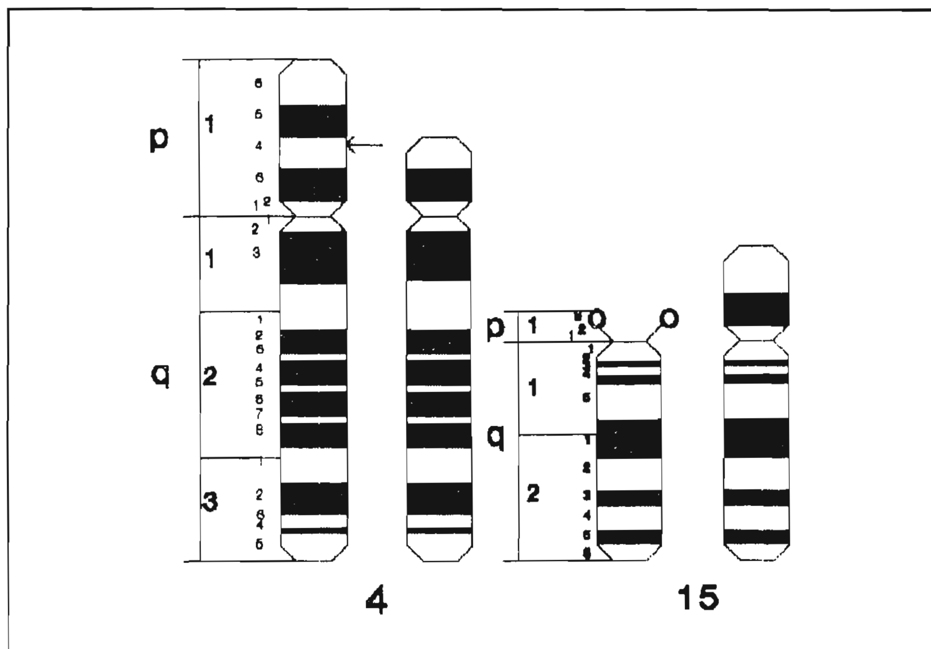

Figure 2 - Translocation t(4;15)(p14;pter). Arrow: Breakpoint 4p14.

Furthermore, Braekleer *et al.* (1985) demonstrated that in most cases the analysis of chromosome anomalies observed in leukemias, lymphomas and certain carcinomas revealed an association between the breakpoints involved in the chromosome rearrangements and fragile sites and the oncogenes.

Chromosome 6 alterations are frequently associated with a large number of malignancies, including ALL. The del(6)(q14-q27) variables are also detected in four other types of cancer (Reeves, 1973; Oshimura *et al.*, 1977; Mark *et al.*, 1979; Prigogina *et al.*, 1979; Third International Workshop on Chromosomes in Leukemia, 1981; Bloomfield *et al.*, 1983; Nowell *et al.*, 1982; Johnson *et al.*, 1985; Shapiro *et al.*, 1987).

According to some investigators, the del(6)(q23) alteration detected in our patient is more common in patients with ovarian carcinoma in which the del(6)(q15-q23) variables are observed (Trent and Salmon, 1981; Kusyk *et al.*, 1981; Atkin and Baker, 1981). However, it is considered by others to be an ALL marker.

It is important to point out that the MYB proto-oncogene is located in the 6q22-23 region (Trent *et al.*, 1989).

Considering the observation that the break points in the del(6)(q23) and t(9;22) alterations coincide with the cellular oncogenes MYB and c-abl, activation of these genes

by the chromosome rearrangement could explain the occurrence of the neoplastic process in the case under study.

Other considerations, however, are probably of importance. One of these is that del(4)(p14) could represent a malignant lymphoma (ML) marker (Bloomfield *et al.*, 1983; Levine *et al.*, 1985; Kristofferson *et al.*, 1985; Maseki *et al.*, 1987). Since the malignant process occurs in the stem line and the lymphoid cell line common to ALL and ML originates from the stem line, the possibility of this being a leukemic lymphoma cannot be excluded. Another consideration is the hypothesis that these alterations may represent new chromosome anomalies characteristic in ALL. Oncogenes RAF 1p1 and KIT are located on the short arm of chromosome four in the 4p16.1 and 4p11 regions, respectively, (Trent *et al.*, 1989) and the break point is located in 4p14. Although this break point does not exactly coincide with the location of the oncogenes mentioned above, it might activate them because of their proximity to it.

Thus, the occurrence of the del(4)(p14) + 4ace deletion, which may result in a t(4;15)(p14;pter) translocation, supports the hypothesis that oncogenes and chromosome anomalies play an important role in the etiology of human cancer and perhaps represent mechanisms that act on oncogene activity at the molecular level. In addition, when present in ALL, this deletion may, together with the other alterations detected, be responsible for the occurrence of the neoplastic process and the possible activation of oncogenes. This may explain the poor response to treatment and the poor prognosis observed in our patient.

RESUMO

Os autores relatam um caso de leucemia linfoblástica aguda (ALL) que no exame citogenético de células de medula óssea apresentou anomalias cromossômicas já descritas nesta condição del(6)(q23); t(9;22)(q34;q11), ao lado das alterações cromossômicas del(4)(p14) + 4ace e t(4;15)(p14;pter) ainda não relatadas em ALL. Discutem a hipótese destas alterações influenciarem na origem da malignidade, na pobre resposta ao tratamento e mau prognóstico observado no paciente pela possível ativação de oncogenes em consequência das anomalias observadas.

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