



A Retrospective Observational Study of Dicentric (9;12): A Unique, Nonrandom Translocation Defining a Cytogenetic Subgroup with Favorable Outcome in Acute Lymphoblastic Leukemia

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Abstract

Introduction Cytogenetic abnormalities are integral to the risk stratification of acute lymphoblastic leukemia (ALL).

Objectives The present study aimed to highlight a rare, yet nonrandom cytogenetic abnormality notably dicentric (9;12), which was observed in ALL patients who presented to our institute. The study analyzed the frequency, clinicohematological features, and treatment response of these patients.

Materials and Methods A single-group observational study was conducted from April 2014 to April 2020. Cytogenetic analysis was done on bone marrow aspirate samples of the patients referred to the cytogenetics laboratory with clinical diagnosis of acute leukemia. Cytogenetic, clinical, and hematological data were collected from respective departmental records, case files, and patients.

Results Dic(9;12) was identified in 1.2% of ALL (19 out of 1,544 patients). They showed striking preponderance in teen and young adult males with characteristic precursor B cell immunophenotype. Majority of these patients displayed favorable risk profiles such as low total count, mild lymphadenopathy and splenomegaly, mild-to-moderate elevation of lactate dehydrogenase, and good response to first induction chemotherapy. Rare coexistence of dic (9;12) with well-established cytogenetic markers such as t(9;22) and t(1;19) was observed.

Conclusion Dic(9;12) is one of the most specific cytogenetic markers of precursor B cell (pre-B) ALL. It defines a subgroup with favorable clinical and biological profile. We

Keywords

- ▶ dicentric (9;12)
- ▶ precursor B cell acute lymphoblastic leukemia

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suggest inclusion of dic(9;12) in cytogenetic risk stratification of precursor B cell ALL. Long-term follow-up studies are recommended to establish the prognostic significance of this cytogenetic subgroup, which may benefit from less intensive chemotherapy.

Introduction

The World Health Organization (WHO) classification of B cell acute lymphoblastic leukemia (ALL)/lymphoma (2017) is refined by risk stratification model based on the recurrent chromosomal abnormalities. High hyperdiploidy, t(12;21), t(1;19), and t(9;22) are most commonly considered cytogenetic abnormalities in prognostication.¹

Only 110 cases of dicentric (9;12) have been reported to date since its first discovery in 1985 by Faderl et al,² although it was Carroll et al in 1987, who recognized this abnormality as a recurrent finding in childhood ALL.³ There has been no update until the last couple of years on this unique translocation, which is possibly associated with good prognosis. With this background, the present study aims to assess the overall frequency of dic(9;12) in ALL and comprehensively analyze the associated clinicohematological features.

Materials and Methods

This was a single-group retrospective observational study conducted from April 2014 to April 2020. Nineteen patients having dic(9;12) were retrieved from the cytogenetic records. Patients of ALL were identified based on the information obtained from cytogenetic registers (from 2014 to 2020) and departmental records of medical and pediatric oncology. Clinical and hematological parameters were obtained from the case files.

Cytogenetic Technique

Heparinized bone marrow aspirates of the patients were sent to the cytogenetics laboratory. Dual cultures of 24-hour and 48-hour incubation were set up in RPMI-1640 medium supplemented with 15% qualified, heat-inactivated fetal bovine serum (GIBCO, Invitrogen, United States). This was followed by mitotic arrest using Karyomax-Colcemid solution (0.10 µg/mL concentration) for 30 minutes. Subsequently, cells were subjected to hypotonic treatment in potassium chloride (0.075 M) at 37°C for 20 minutes and overnight fixation in Carnoy's fixative solution (3:1 concentration of methanol and glacial acetic acid). Giemsa-Trypsin-Giemsa banding was done on prepared slides, and chromosomal analysis was done in accordance with the International System for Chromosomal Nomenclature. Good quality metaphases were captured and analyzed using Olympus applied spectral imaging software version 8.1.0.47741.

Statistical Methods

Python 3.7 software was used for statistical analysis. Descriptive statistical analysis (frequencies, mean, median, and range) was done to analyze baseline parameters. Fisher's

exact test was applied to find the association between complex karyotype and risk stratification. Survival probabilities were estimated by Kaplan–Meier method.

Ethics

The procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki Declaration of 1964, as revised in 2013. Ethics Committee Waiver was obtained from Kidwai Memorial Institute of Oncology (dated 12–04–2020, KMIO/DPT/106/2021). The informed consent was waived due to the retrospective nature of the study.

Results

Nineteen patients of dic(9;12) were identified among 1544 patients of ALL and 1214 patients of B cell ALL, accounting for frequencies of 1.2 and 1.6%, respectively.

The median age of patients was 14 years (range: 1–57 years). Majority of patients (12 out of 19 patients; 63%) were adolescents and young adults (10–23 years). Males were more frequently affected than females (M:F = 4:1). Clinical features and hematologic and biochemical parameters are summarized in **Tables 1** and **2**, respectively.

Bone marrow examination revealed blasts having L1/L2 morphology of French-American-British classification. Normal bone marrow elements were suppressed. Immunophenotypically, the blasts were positive for Tdt, CD34, CD10, CD19, cCD79a, and human leukocyte antigen-DR and negative for T cell and myeloid markers. CD20-positive expression was noted in only six cases. Features were consistent with the diagnosis of precursor B cell ALL.

Cytogenetic Studies

Hypodiploidy (modal number of chromosomes ranging from 44 to 45) was observed in 16 out of 19 patients (84%) (**Table 3**). Patients with 45 chromosomes were considered

Table 1 Clinical features of patients with dic(9;12) at diagnosis

Signs and symptoms	Percentage of patients
Fever and fatigue	73–80
Others: cough, neck swelling, epistaxis	6
Moderate pallor	21
Severe pallor	79
Lymphadenopathy	87
Hepatomegaly	64
Splenomegaly	76

Abbreviation: dic(9;12), dicentric (9;12).

Table 2 Clinical, biochemical, and hematological parameters of patients with dic(9;12) at diagnosis

Parameters	Median	Range
Liver size on ultrasound	17 cm	13–20 cm
Spleen size on ultrasound	15.5 cm	10–20 cm
LDH	347	205–552
Hb (g/dl)	8	4–11
TC ($n \times 10^9/L$)	3	1–113
ANC ($n \times 10^9/L$)	0.45	0.02–15.36
Platelets ($n \times 10^9/L$)	25.5	2–71
PBL blast (%)	40	22–70
BM blast (%)	80	50–90

Abbreviations: ANC, absolute neutrophil count; BM, bone marrow; dic (9;12), dicentric (9;12); Hb, hemoglobin; LDH, lactate dehydrogenase; PBL, peripheral blood; TC, total count.

near-diploid since it occurred as a result of unbalanced translocation dic(9;12). **Fig. 1** represents the novel translocation dic(9;12). Complex karyotype is defined as metaphases having at least three chromosomal abnormalities, irrespective of their type. As per these criteria, 7 out of 19 patients (37%) in our study group had complex karyotype. The additional structural abnormalities observed in our study group included t(9;22), t(1;19), del(9p), del(7q), marker chromosomes, and numerical abnormalities. **Fig. 2**

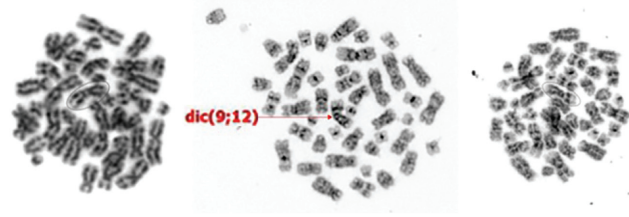


Fig. 1 Metaphases of dic(9;12) abnormality identified in different patients.

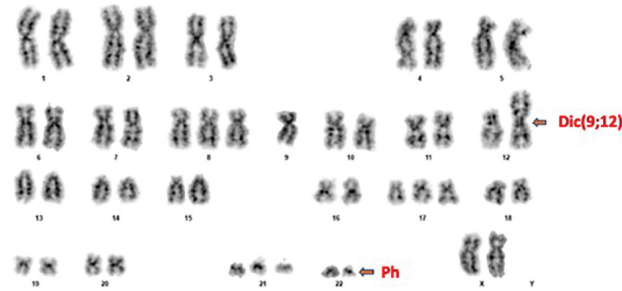


Fig. 2 Karyotype: 48,XX,+8,dic(9;12)(p13;p13),+17,+21,der(22)t(9;22)(q34;q11.2). dic(9;12); dicentric dic(9;12).

denotes coincidence of dic(9;12) and Philadelphia chromosome along with numerical abnormalities in a single metaphase. Although derivative chromosome 9 appeared cryptic in the metaphase, fluorescent in situ hybridization confirmed *bcr-abl* translocation (**Fig. 3**). Reverse transcriptase

Table 3 Cytogenetic findings of patients with dic(9;12) at diagnosis

Case number	Age (y)/gender	Karyotype	Complex karyotype
1	6/male	45,XY,dic(9;12)(p11;p11-12)[8]/46,XY[4]	No
2	10/female	48,XX,+8,dic(9;12)(p23;q13),del(9)(p21),+2mar	Yes
3	12/male	45,XY,dic(9;12)(p11;p12)	No
4	13/male	45,XY,del(7)(q31),dic(9;12)(p13;p13)	Yes
5	14/male	45,XY,dic(9;12)(p11-13;p11)	No
6	14/male	45,XY,dic(9;12)(p11;p11),t(1;19)(q23;p13)	Yes
7	15/male	45,XY,dic(9;12)(p13;p13)	No
8	15/female	44,XX,dic(9;12)(p13;p13),-17	No
9	17/male	45,XY,dic(9;12)(p13;p13)	No
10	21/male	45,XY,dic(9;12)(p13;p13)	No
11	23/male	45,XY,der(9)t(9;12)(p13;p13)t(9;22)(q34;q11.2)	Yes
12	20/male	44,X,-Y,dic(9;12)(p13;p13)	No
13	43/female	45,XX,dic(9;12)(p13;p13)	No
14	01/male	45,XY,dic(9;12)(p11-12;p13)	No
15	57/male	45,XY,-2,i(8)(q10),dic(9;12)(p11;p11),t(9;22)(q34;q11.2)	Yes
16	19/female	46,XX,dic(9;12)(p13;p13),-10,+2mar	Yes
17	11/female	46,XX,dic(9;12)(p13;q13),t(9;22)(q34;q11.2)	Yes
18	8/male	45,XY,dic(9;12)(p13;p13)	No
19	9/male	45,XY,dic(9;12)(p13;p13)	No

Abbreviation: dic(9;12), dicentric (9;12).

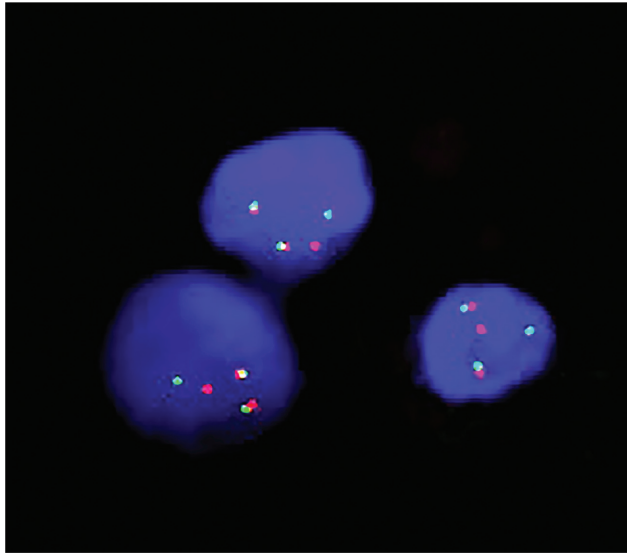


Fig. 3 Fluorescent in situ hybridization (dual color, dual fusion): Two red-green, one green, and one red signals confirm *bcr-abl* translocation.

polymerase chain reaction showed major *bcr-abl* transcripts in three patients harboring t(9;22).

Treatment and Follow-Up

Pediatric patients were treated according to the MCP-841 chemotherapeutic regimen until 2018 and Indian Childhood

Collaborative Leukemia Group ALL-14 protocol since 2019. Adult patients were given chemotherapy according to BFM-95 ALL regimen. A 23-year-old male of Ph-positive ALL with dic(9;12) received R-Hyper-CVAD protocol along with imatinib. Majority of patients belonged to standard and intermediate-risk group (81%; 13 out of 16 patients) (►Table 4). All patients achieved complete hematologic and cytogenetic remission within 4 to 6 weeks of first induction chemotherapy. Minimal residual disease status was evaluated for six pediatric patients, who showed leukemic cell count <0.01% in the bone marrow.

Out of 19 patients, 10 were selected for survival analysis because other nine patients had still not completed treatment. The median duration of follow-up was 36 months (range: 7–51 months) (►Table 4). The overall survival was estimated to be 67% for 3 years. The survival curve is depicted in ►Fig. 4. A 15-year-old girl died of disease following discontinuation of treatment for 19 months. Deaths of a 43-year-old female and a 57-year-old male were attributed to their comorbid conditions.

Discussion

A dicentric chromosome is a single chromosome that has two centromeres, causing genomic instability and evolution of the disease in cancer.⁴ There is considerable heterogeneity in breakpoints and the fusion gene partners in dicentric chromosomal rearrangements involving chromosome 9p.⁵ Despite the

Table 4 Risk stratification, duration of follow-up, and survival status of patients with dic(9;12)

Case number	Risk stratification	Duration of follow-up in months	Survival status
1	Standard	36	Remission
2	Standard	51	Remission
3	Standard	7	On treatment
4	Standard	6	On treatment
5	Standard	37	Lost follow-up
6	Not available	36	Remission
7	Standard	40	Remission
8	High	19	Dead
9	Intermediate	12	On treatment
10	Intermediate	16	Lost follow-up
11	High	36	Remission
12	Intermediate	9	On treatment
13	Intermediate	7	Dead
14	Not available	9	On treatment
15	High	28	Dead
16	Intermediate	9	On treatment
17	Intermediate	8	On treatment
18	Not available	2	On treatment
19	Standard	2	On treatment

Abbreviation: dic(9;12), dicentric (9;12).

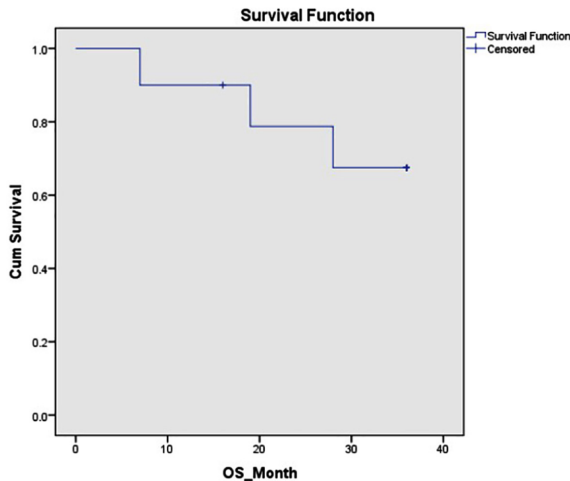


Fig. 4 Survival outcome analysis of patients with dic(9;12) by Kaplan–Meier method. dic(9;12); dicentric dic(9;12).

heterogeneity, molecular investigations have shown 9p rearrangements including deletion or gene fusions resulted in partial or complete deletion and under expression of *PAX5* gene.⁵ The usual partner chromosomes are 7, 12, and 20.⁶

Dic(9;12)(p11–13;p11–13) is an unbalanced translocation with partial loss of short arms of chromosomes 9 and 12. This results in fusion of *PAX5* gene on short arm of chromosome 9 with *ETV6* gene on short arm of chromosome 12, resulting in *PAX5-ETV6* chimeric fusion protein.

Similar to earlier studies on dic(9;12),^{7–9} the peak incidence was noted in teenage and young adult males in the present study. This translocation is rarely encountered in infants and the elderly. Only a single case of a 66-year-old woman with dic(9;12) in pre-B-ALL has been reported so far.⁹ Two patients in our study group were older adults (aged 43 and 57 years).

Marked elevation of lactate dehydrogenase (LDH) serves as a surrogate for high leukemic blasts.¹⁰ The present study showed marginal-to-moderate elevation of LDH and median blast of 40% (►Table 2). Dicentric translocations are seemingly most prevalent in leukemias of B cell lineage.¹¹ Dic(9;12) is predominantly a marker of precursor B cell ALL, though there are anecdotal reports of this abnormality in chronic myeloge-

nous leukemia (CML)-blast crisis and T cell lymphoid malignancies.^{3,8,9} All 19 patients in our study were diagnosed as precursor B cell ALL.

UKCCG 1992 reported better prognosis in patients with dic(9;12) than in patients with other translocations involving 9p and 12p. Patients with dic(9;12) possibly achieve complete remission with a 5-year survival rate of more than 95%, abrogating the need for bone marrow transplantation.^{12,13}

Mahmoud et al identified 15 cases of dic(9;12) supposed to be dic(9;12)(p11;p12) in their study of 2,303 pediatric ALL patients. On median follow-up of 57 months, all were in remission.⁸ Behrendt et al reported 31 cases of leukemia with dic(9;12)(p11–13;p11–12). Fourteen patients had pre-B-ALL with clinical and hematologic profile similar to our study group. Despite the presence of one or more adverse prognostic factors, 94% of these patients remained in remission on 5-year follow-up.⁹ Illade et al reported a 14-year-old male with t(9;12)(p13;p13), who was in remission on 4-year follow-up.¹⁴ In concordance with these studies, we observed overall survival of 67% for 3 years (►Fig. 4). Considering the death of two patients not directly related to the disease, the 3-year overall survival could be deduced as 90% in our study.

In general, low hypodiploidy having chromosomes <40 and complex karyotype is considered to be independent poor prognostic factors in ALL.^{15,16} Majority of cases in our study were near-diploid or diploid, which is not considered as a poor prognostic factor. The relationship between presence or absence of complex karyotype and risk stratification was not statistically significant (Fisher’s exact test, *p* = 0.504; ►Table 5). However, patients with complex karyotype also showed good initial treatment response.

Dic(9;12) and dic(7;9) frequently coexist with t(12;21) (*ETV6-RUNX1* fusion) and t(9;22) (*BCR-ABL1* fusion), respectively, which are considered to be important cooperating genetic events.⁵ Bargetzi et al have reported dic(9;12) involving derivative chromosome 9 of t(9;22) in patients with CML-blast crisis.¹⁷

Conversely, three patients in our study showed dic(9;12) and t(9;22) as coexisting abnormalities (►Fig. 3) and all were diagnosed as de novo precursor B-ALL. One of these patients who aged 57 years and had comorbid conditions died at 28 months of treatment.

Table 5 Fisher’s exact test to find the association between dic(9;12) with or without complex karyotype and risk stratification

Risk stratification of patients against presence and absence of complex karyotype	Standard	Intermediate	High	Total
Dic(9;12) with complex karyotype	2	2	2	6
Percentage within risk stratification	29	33	67	38
Dic(9;12) without complex karyotype	5	4	1	10
Percentage within risk stratification	71	67	33	63
Total	7	6	3	16
<i>p</i> = 0.504				

Abbreviation: dic(9;12), dicentric (9;12).

Conclusion

The current study showed that dic(9;12)(p13;p13) is a rare, yet recurrent chromosomal abnormality, with an overall frequency of 1.2% in ALL and 1.6% in B cell ALL. Based on the findings of this study and review of literature, we believe that patients with this abnormality show standard risk profile with initial good response to induction chemotherapy. Hence, we suggest incorporation of dic(9;12) in risk stratification of precursor B cell ALL as it represents a group with favorable risk profile and good initial response to treatment. We observed rare concurrence of two nonrandom translocations, dic(9;12) and t(9;22). Until the prognostic impact of dic(9;12) is well established by larger studies, it is better to consider t(9;22) over dic(9;12) in risk stratification of such cases. The current study is limited by small sample size and short duration of follow-up. Survival analysis could be confounded by different treatment protocols used among pediatric and adult patients. Additional studies are being sought to evaluate the prognostic value of this unique cytogenetic aberration, which may benefit from less intense chemotherapy.

Funding
None.

Conflict of Interest
None declared.

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