Implications of intrachromosomal amplification of chromosome 21 on outcome in pediatric acute lymphoblastic leukemia: Does it affect our patients too?

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Abstract

Intrachromosomal amplification (iAMP) of chromosome 21 entity is associated with a dismal outcome in B cell Acute Lymphoblastic Leukemia (B-ALL). This cytogenetic abnormality is caused by a novel mechanism; breakage-fusion-bridge cycles followed by chromothripsis along with major gross rearrangements in chromosome 21. Charts of B-ALL diagnosed at King Faisal Specialist Hospital and Research Center between 2005 and 2015 were reviewed. iAMP is a rare entity occurring at around 2.4% of all pediatrics B-ALL. No statistically significant difference was found among patients with iAMP21, patients with extra copies of 21 and other patients with B-ALL. The reported adverse prognostic effect of iAMP21 could be due to other coexistent adverse factors, including older age at the time of diagnosis. The most common associated abnormality in our population in addition to the hyperdiploidy was ETV6/RUNX1.

Introduction

Comprehensive genomic analyses of B-ALL have identified different entities that are important in clinical decision-making, including recurrent chromosomal translocations.[1,2] Currently, around 75% of childhood ALL are associated with genetic abnormalities that have favorable or adverse clinical outcome.[3-5] Some abnormality like t(9;22)(q34;q11), and hypodiploidy are associated with adverse risk groups.[6-10]

Intrachromosomal amplification of chromosome 21 (iAMP21) is a novel genetic entity of B-ALL that was identified as a distinct subgroup back in 2003.[11-13] It was identified by chance during the initial screening of patients with B-ALL using the same fluorescence *in situ* hybridization (FISH) probe used for detecting ETV6/RUNX1; t(12;21)(p13;q22),[3,6,11,14-17] and is caused by breakage-fusion-bridge cycles followed by chromothripsis resulting in the tandem amplifications on chromosome 21.[16,19,20]

This entity occurs in approximately 2-5% of pediatric patients diagnosed with B-ALL, with more prevalent among older children who present with low WBC count.[4]

The diagnostic FISH criteria are defined as the presence of 5 or more copies of RUNX1 gene when using interphase FISH.[6,21] So far, FISH analysis remains the only reliable diagnostic method.[3,13,15,22,23] Secondary genetic abnormalities associated with iAMP21 include gain of chromosomes X, 10, or 14; or monosomy 7/deletion of 7q; deletions of 11q, including the ATM and MLL genes; and deletions of ETV6.[3,7]

Recent analyses concluded that patients with iAMP21 had a significantly inferior event-free and overall survival with higher relapse rate compared to other patients with B-ALL especially if treated with standard risk protocols.[9,13,23,24] They are now assigned into the high-risk group and, in case of a slow early response, are considered for allogeneic stem cell transplantation (SCT).[13,23] Moorman *et al.* observed in a multivariate analysis, that the presence of the iAMP21 mutation was an independent indicator of an adverse event-free and overall survival.[13]

Materials and Methods

We performed a retrospective study to evaluate the outcome of different B-ALL subtypes, in a cohort of pediatric patients (age at diagnosis ≤14 years) by reviewing the medical records of 411 patients, who were diagnosed and treated at our institute from 2005 to 2015. Only those who were treatment naïve were included in the study. Infants and patients with congenital disorders such as Down syndrome were excluded. We divided the patients into three distinct groups; the first group included those with iAMP21. The second group included patients with extra copies of chromosome 21 (not reaching the diagnostic limit for iAMP21), the last group included patients with B-ALL without extra copies of chromosome 21. The detection of this chromosomal abnormality was done at our cytogenetics laboratory using FISH and the criteria for the cytogenetic diagnosis was the presence of 5 or more copies of the *RUNX1*

gene on chromosome 21 by analysis of interphases. The probe used to detect the *RUNX1* gene is the same probe designed for the detection of the *ETV6-RUNX1* fusion. At our center, we use four probes in cases of B-ALL that can detect the most common mutations in BALL. Including: t(9;22)(q34;q11), t(12;21)(p13;q22), KMT2A 11q23 break apart probe and probes for centromeres 4,10 and 17. DNA index was calculated by flow cytometry. Approval from the Institutional Review Board of King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia, was sought prior to the initiation of the study (Approval Number 2141133), consent forms were waived. Patients' medical charts were reviewed and data was collected on specially designed Case Report Forms (CRFs). After Quality Assurance of the CRFs, data was entered into a computerized database, which was finally transported into IBM SPSS for Windows Version 20.0 for final analysis.

Statistical considerations

All continuous data which did not conform to the normality assumptions tested using Shapiro-Wilk test, are presented as median of the data with minimum and maximum points. For categorical variables, chi square test for independence was used to test for the independence of relationship. Kaplan-Meier curves were drawn for survival analysis and tested for any difference between the survival time using Tarone-Ware test in univariate setting.

Results

Patients' characteristics of our cohort of 411 patients are given in Table 1. For comparison purposes, patients were divided into three groups: first group included patients with iAMP21 (n=10). The second group included patients with extra copies of chromosome 21 (not reaching the diagnostic limit for iAMP21) (n=16) and the third group included patients with B-ALL without extra copies of chromosome 21 (n=385). 80% of patient with iAMP21 were boys. While 56.2% and 57.1% were boys in the second and third groups, respectively. The majority of patients were less than 10 years at the time of diagnosis in all three groups with median ages of 5.9, 4.8 and 4.2 years in the first, second and third groups respectively. WBC at diagnosis was ≤50K in all patients in the first group, and in the majority of patients in the second and third groups. Most of the patients had no CNS involvement at the time of diagnosis in all three groups. While Patients with CNS-2 were 10% (n=1), 6.2% (n=1) and 15.6% (n=60) in the first, second and third groups respectively. Patients with CNS-3 were only seen in the third group (n=8) 2.1% DNA index was >1.16 in 44.4%, 50% and 26.5% in the first, second and third groups respectively.

In addition to the common occurrence of trisomies in chromosomes 4, 10 and 17, which is part of hyperdiploidy. The most common associated cytogenetic abnormality in patients with iAMP21was t(12;21) ETV6/RUNX1, occurring in 4 out of 9 patients. MLL gene rearrangements occurred in 17 of 312 (5.4%) of patients in the third group while it was not seen in patients in the first and second groups. Treatment regimens comprised of standard international protocols (Table 2). First Follow-up Bone Marrow was done on day14 of chemotherapy showed most of the patients 93.1% were M-1; <5% blasts in the bone marrow, 5.1% patients were M-2; 5-<25% blasts in the bone marrow and

remaining 1.8% patients were M-3; blasts ≥25% in the bone marrow. All patients achieved Complete First Remission. Relapse rate was 13.4% (n=55) with the first relapse occurring at a median of 27.9 (2.4-101.5) months from the time of diagnosis. 10.9% (n=6) of these were CNS Relapses. 54.5% (n=30) who had relapse were categorized as Standard Risk while the remaining 45.5% (n=25) were High Risk. 1.8% (n=1) who relapsed had iAMP21 while 1.8% (n=1) had additional copies of chromosome21, rest of them were from the third group. (P-Value: 0.891). With a medi-

Table 1. Patients characteristics at the time of diagnosis with CNS status on D14 of induction chemotherapy; n=411 (%)

	iAMP21	Extra Copies of 21	B-ALL with no iAMP21
Total	10 (2.4%)	16 (3.9%)	385 (93.7%)
Gender			
Female	2 (20)	7 (43.8)	165 (42.9)
Male	8 (80)	9 (56.2)	220 (57.1)
Age at diagnosis			
<10 years	8 (80)	15 (93.8)	333 (86.5)
10 and above	2 (20)	1 (6.2)	52 (13.5)
Median (Min – Max)	5.9 (2.7-13.9)	4.8 (1.7-13.7)	4.2 (1-14.8)
CNS Status*			
CNS-1	9 (90)	15 (93.8)	316 (82.3)
CNS-2	1 (10)	1 (6.2)	60 (15.6)
CNS-3	0 (0)	0 (0)	8 (2.1)
BCR-ABL (+)	2 of 10	2 of 15	19 of 274
MLL-Gene Rearrangement (+)	None	None	17 of 312
ETV6/RUNX1 (+)	4 of 9	5 of 15	94 of 269
Tri-4 (+)	5 of 7	10 of 13	79 of 184
Tri-10 (+)	4 of 7	10 of 13	79 of 185
Tri-17 (+)	5 of 8	9 of 13	70 of 182
Risk Group**			
Low Risk	0 (0)	0 (0)	11 (2.9)
Standard Risk	8 (80)	13 (81.2)	261 (67.8)
High Risk	2 (20)	3 (18.8)	111 (28.8)
Very High Risk	0 (0)	0	2 (0.5)
WBC 10⁹/L at presentation			
≤50 K	10 (100)	14 (87.5)	320 (83.1)
>50K	0	2 (12.5)	65 (16.9)
DNA index			
>1.16	4 (44.4)	8 (50)	98 (26.9)
≤1.16	5 (55.6)	8 (50)	266 (71.3)

*CNS Status for one patient was not available in the records. Values are in n(%) for discrete data. ** Risk group according to NCI and COG. CNS-1: No CNS involvement. CNS-2: <5 WBC/mul CSF with blast cells were 10% CNS-3: ≥5 WBC/mul CSF with blast cells, or signs of CNS involvement.

Table 2. Treatment protocols given to patients.

	iAMP21	Extra Copies of 21	B-ALL with no extra copies of 21
Total n(%)	10 (2.4)	16 (3.9)	385 (93.7)
CCG-1882 Reg A	0	0	22
CCG-1882 Reg B	0	0	107
CCG-1882 Reg C	0	0	1
CCG-1891	0	0	24
Modified CCG 1961 Regimen C	4	6	146
Modified CCG 1991 Regimen IS	6	9	70
Modified CCG 1961 Regimen D	0	1	6
St Jude XIII	0	0	1
St Jude XV (Infants,BALL,Ph+ALL)	0	0	5
DFS 105-001	0	0	1
COG Protocol (AALL0331)	0	0	2

an follow-up time of 59.1 (95% CI: 53.0-65.2) months, our 10 years overall survival was (0.911±0.016) with 28 deaths (Figure 1). All patients with iAMP21 or having Extra Copies were alive at the last follow-up. Ten-year Event Free Survival of our cohort of patients with relapse (n=55) and Death in Remission (n=5) as events, was (0.783±0.029) (Figure 2). No statistically significant difference was found among the iAMP21 (0.800±0.179, with 1 relapse), with Extra Copies (0.900±0.095, with 1 relapse) or the third group (0.785±0.029, with 53 relapses and 5 deaths in remission) in terms of their Event Free Survival (P-Value: 0.896, Figure 3).

Figure 1. Overall survival of the whole cohort.

Discussion

In the past few decades, efforts have been made to identify prognostic factors and to stratify patients diagnosed with B-ALL for risk-adapted therapy.[6] They include; clinical features such as white cell count WCC, age at the time of diagnosis and gender of the patient, and biological factors like early response to treatment regimen, DNA index, and cytogenetic findings.[8-10] iAMP21 is a novel genetic entity that was identified as a distinct cytogenetic subgroup of B-ALL in 2003.[9] It has been associated with an adverse prognosis and event free survival (EFS), especially in patients treated with standard chemotherapy protocols.[6,8,9,20,25,26]

The majority of patients with iAMP21 were boys 80%. This is slightly more common than what was observed by Moorman *et al.*, where the prevalence was 54% in boys in a prospective analyses' of 1630 patients, this is may be related to ethnicity and older age were recruited, the cut off for pediatrics at our institution is 14 year.[13]

Most of patients with iAMP21 were diagnosed at an age of less than 10 years with a median of 5.9 years. Although the observed median age in our study was slightly bigger than median age in the other two groups with median ages of 4.8 and 4.2 years in the second and third groups, respectively. This is much less than the reported median age in many studies including Moorman *et al.* observation in their large prospective analyses of a median age of 9 years which was significantly older than other patients with B-ALL included in their study.[13,21]

All patients with iAMP21 in our study presented with low WCC <50,000, whereas, around 16.9% of patients in the third group presented with high WCC >50,000. The
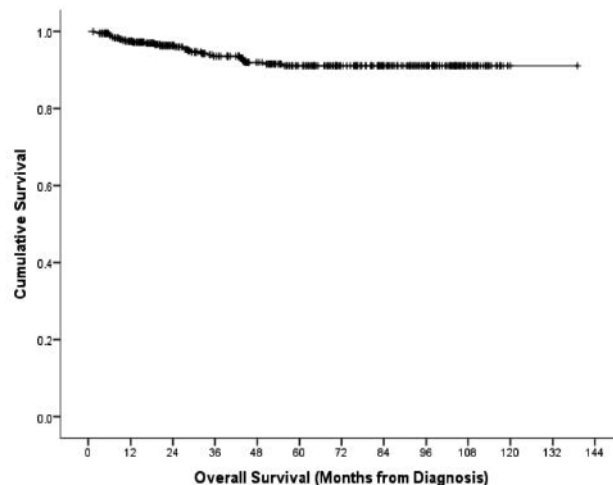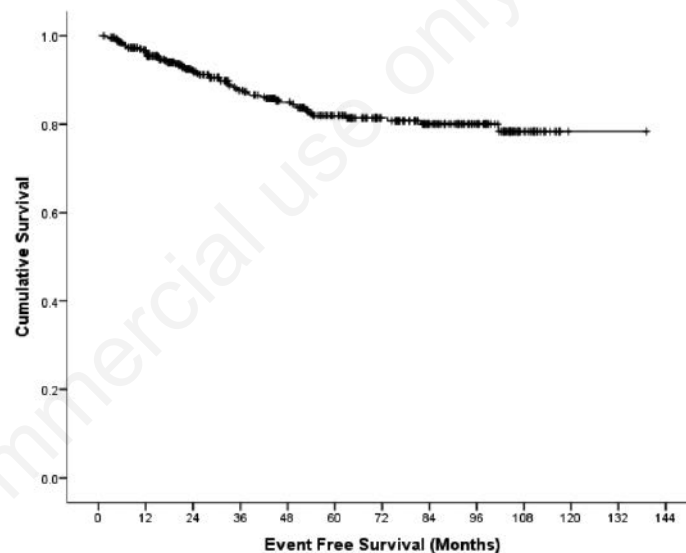

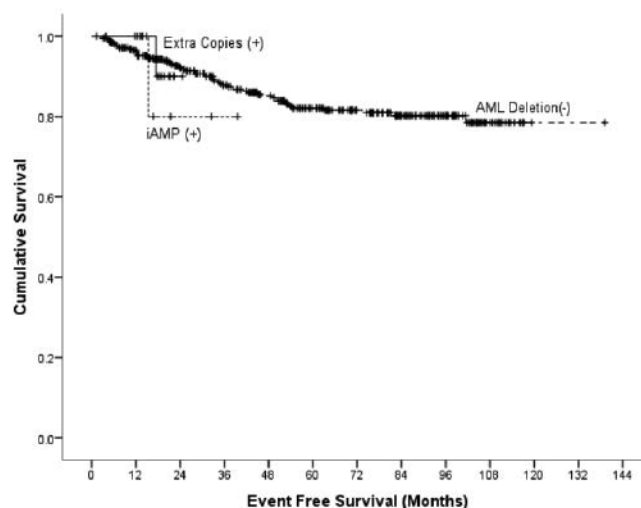**Figure 2. Event free survival of the whole cohort.**

Figure 3. Event free survival of the three groups.

same observation was noted by Moorman *et al.* when they compared the median WCC at the time of diagnosis in patients with iAMP21: 3.9×10^9/L, to the median in patients without iAMP21: 12.4×10^9/L.[13]

Most of patient with iAMP had no CNS involvement (n=9), The same observation was noted by Moorman *et al.* where all of the patients with iAMP21 included in their study showed no CNS involvement.[13]

The most cytogenetic changes associated with iAMP21, other than Trisomies of chromosomes 4, 10 and 17 which are part of the hyperdiploidy, was t(12;21) ETV6/RUNX1 which occurred in 4 out of 9 patients with iAMP21(44.44%). MLL gene rearrangement which is associated with an adverse prognosis and EFS,[27] and is reported to occur in 10% of all childhood B-ALL,[28-30] was not seen in any of the patients with high copy number of chromosome 21(patients in the first and second groups) . However, it was seen in around 5.5% of patients with normal copy number of chromosome 21. After treatment and achieving complete remission, 55 patients had relapse. Only one patient of them had iAMP21, one had extra copies of chromosome 21. The rest of relapses occurred in patients of the third group, n=53 patients. (P-Value: 0.891). Ten-year overall survival of our patients was (0.911±0.016) with 28 deaths. No statistically significant difference was found among all three groups in terms of their Event Free Survival (P-Value: 0.896). This contradicts what Moorman *et al.* observed in their study, where there was a 3-fold increase in relapse risk in patients with iAMP21compared to other patients with B-ALL. They compared the 5-year overall survival between patients with iAMP21 and other patients with B-ALL, and it was 71% *versus* 87%.[13] The discrepancy in the overall survival between our study and the study of Moorman *et al.* could be due to the median age difference. In our study the median age (5.9 years) *versus* (9 years) in their study.

Conclusions

In conclusion, our study confirms the rare occurrence of the iAMP21 among B-ALL patients and suggest that iAMP21 by itself is not an independent prognostic factor, and the reported adverse outcome in this group could be partly due to the presence of other adverse risk factors including older age at the time of diagnosis, which was not seen in our patients. A larger and longer-term follow-up study is needed to verify or refute our finding.

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