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ORIGINAL ARTICLE

Hyperdiploid chromosomes in patients with B cell Acute lymphoblastic leukemia

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Abstract

Introduction: Hyperdiploidy (more than 50 chromosomes) is an important cytogenetic abnormality found in B cell acute lymphoblastic leukemia (B cell ALL). The presence of hyperdiploidy > 50 is considered to be a good prognostic marker since it has increased sensitivity to standard chemotherapy. Patients with hyperdiploidy have the longest disease-free survival compared with any other cytogenetic group. **Aim of study:** To estimate prevalence of hyperdiploid chromosomes in patients with B cell ALL through conventional karyotyping and Fluorescent in situ hybridization (FISH). **Materials and methods:** Retrospective study was done on 200 bone marrow samples diagnosed with B cell Acute lymphoblastic leukemia referred to Division of Human Genetics, Department of Anatomy, St. John's Medical college, Bangalore. Samples were subjected to standard protocol for karyotyping and FISH. Percentage of patients who had hyperdiploidy was calculated. **Results:** Out of 200 samples, 30 samples had hyperdiploidy accounting to 15% of total patients diagnosed with B cell ALL. **Conclusion:** Conventional cytogenetics and FISH play a very important role in detecting hyperdiploidy since it is vital in risk stratification and treatment in patients with B cell ALL.

Keywords: Karyotyping, hyperdiploidy, FISH, B cell ALL, chromosomes

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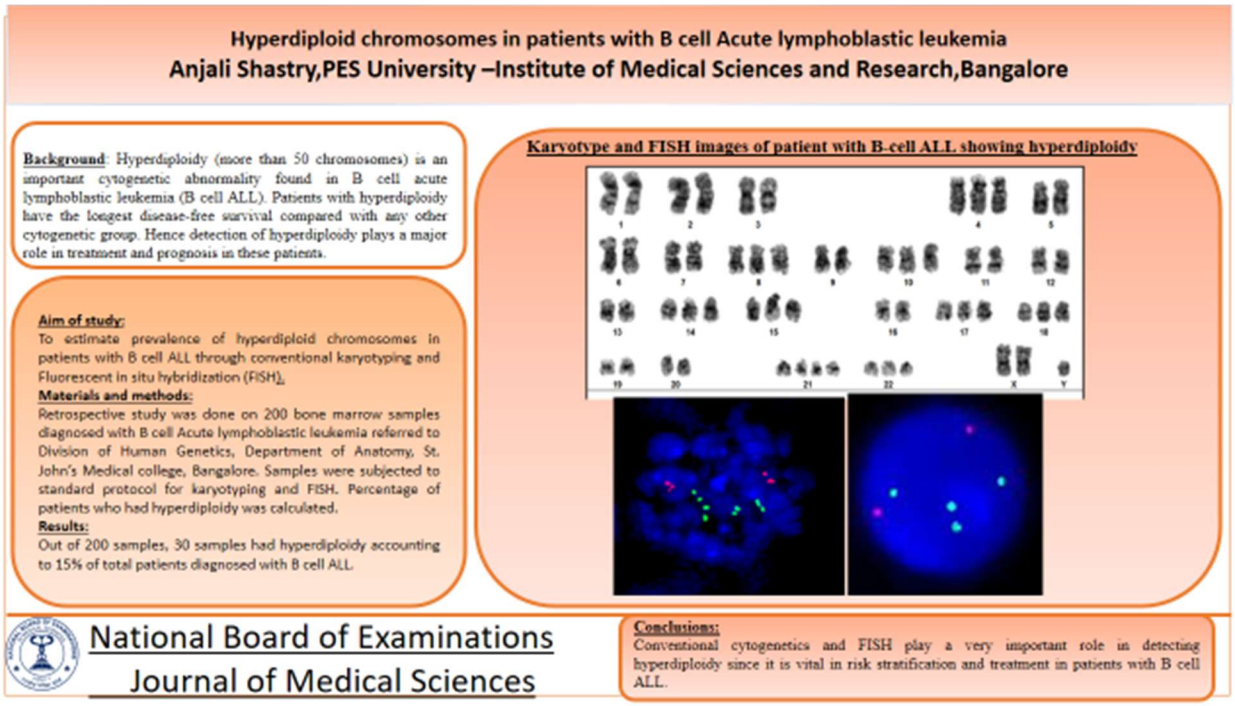
Graphical Abstract



Introduction

Hyperdiploidy is defined by the presence of 51-65 chromosomes, has been classified as a distinct subtype of B-Acute lymphoblastic leukemia (ALL) in the World Health Organization classification of tumors of hematopoietic and lymphoid tissues [1]. Most of B cell ALL with current chemotherapy will go for remission but relapse rate is higher in adults when compared to pediatric age group. Relapse usually occur within two years of chemotherapy. Hyperdiploidy is a common numerical chromosomal abnormality in ALL whereas structural chromosomal abnormalities like translocations [t(9;22),t(12;21),t(1;19)] and deletions are common in these patients. Hyperdiploidy involves addition of chromosomes whereas polyploidy refers to addition of new set of chromosomes. Commonly involved chromosomes in hyperdiploidy are 4, 6, 10, 14, 17, 18, 20, 21, and X [2]. Reason for hyperdiploidy is still debatable. The extra chromosomes may result from specific mutation or it can be vice versa stating that increase in chromosomes can cause proliferation of blasts due to increase or change in dosage of genes. According to literature, clinical outcome in patients with hyperdiploid karyotype is favorable due to increased sensitivity of these lymphoid cells to standard chemotherapy [3]. Hence hyperdiploidy is considered as good prognostic marker in patients with B cell ALL. When compared with other cytogenetic abnormalities, patients with hyperdiploidy have disease free survival for long period of time probably due to increased accumulation of polyglutamates which makes it more sensitive to chemotherapy. In present study, importance was laid on detection of hyperdiploidy through conventional

karyotyping and Fluorescent in situ hybridization (FISH).

Materials and methods

After obtaining ethical clearance, informed consent was taken from patient or his/her relatives before the test. Age group of patients ranged from one year to sixty years. There were 124 pediatric patients ranged from 1 year to 15years in which 78 were males and 46 were females. Age of adult patients ranged from 18 to 60 years. Out of 76 adult patients, 48 were males and 28 were females. After confirmation of B cell ALL through flow cytometry study, 200 samples were randomly selected from samples referred to Division of Human Genetics, Department of Anatomy, St. John's Medical college, Bangalore. Study period was from October 2019 to March 2020. Statistical method used was calculation of percentage of patients showing positive result.

Culture was done on bone marrow samples without Phytohemagglutinin to prevent growth of normal cells and stimulate growth of cancer cells. Samples were incubated for one night and one day followed by harvesting. Cells were fixed on slide and Giemsa banding was done for karyotyping. FISH procedure was done using Metasystem probes [4]. Once slides were ready, images were captured using florescent microscope. Probes used were t(9;22), t(12;21),11q23 breakapart.

Results

Out of 200 samples 30 showed hyperdiploidy. Out of 30 patients who had hyperdiploidy, 25 were pediatric ALL and remaining 5 were adult B cell ALL concluding that hyperdiploidy is more common in pediatric patients when compared to adult ALL. Also, commonly seen abnormalities were trisomy 6, trisomy 10, tetrasomy and trisomy of 21 and 22, gain/loss of X and Y chromosomes. Structural abnormalities seen in these patients along with hyperdiploidy were t(9;22) and t(1;19). In cases where conventional karyotyping could not be cultured, FISH showed increase in number of signals revealing hyperdiploid status of chromosomes. Out these 30 patients with positive results 20 were males and 10 were females which implies hyperdiploidy was more prevalent in males than females (Table 1).

Table 1: Showing number of patients showing only hyperdiploidy and other Chromosomal variants along with hyperdiploidy

Chromosomal variants	**Number of patients**
Only hyperdiploidy	23
Hyperdiploidy along with Translocation	6
Hyperdiploidy along with deletion	1

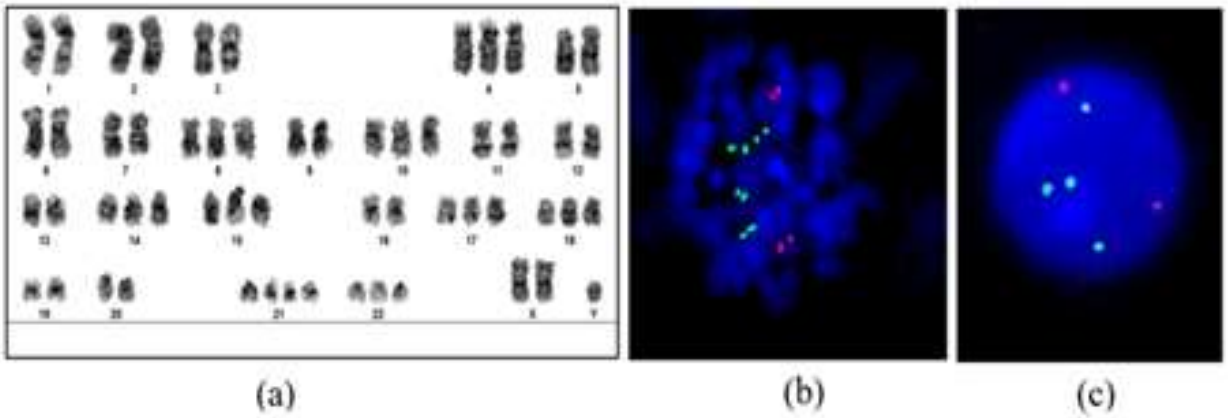


Figure 1. Hyperdiploid karyotype = 57,XXY,+4,+8,+10,+14,+15,+17,+18,+21,+21,+22 (a) Metaphase FISH (b) and interphase FISH (c) showing four green signals for chromosome 21.

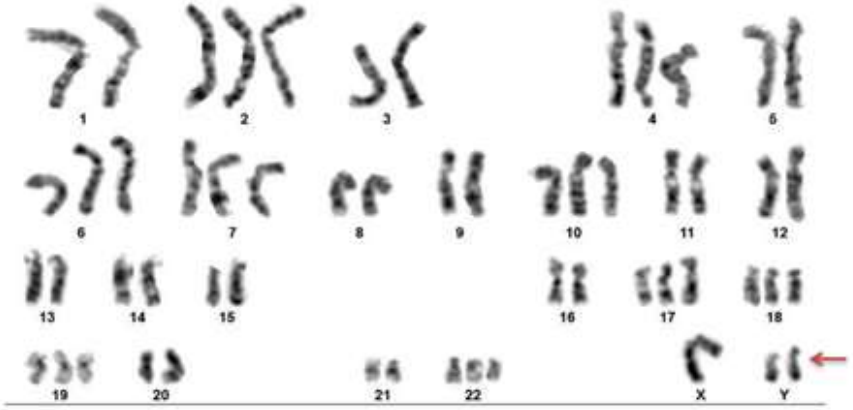


Figure 2. Hyperdiploid karyotype of a male child with additional Y chromosome

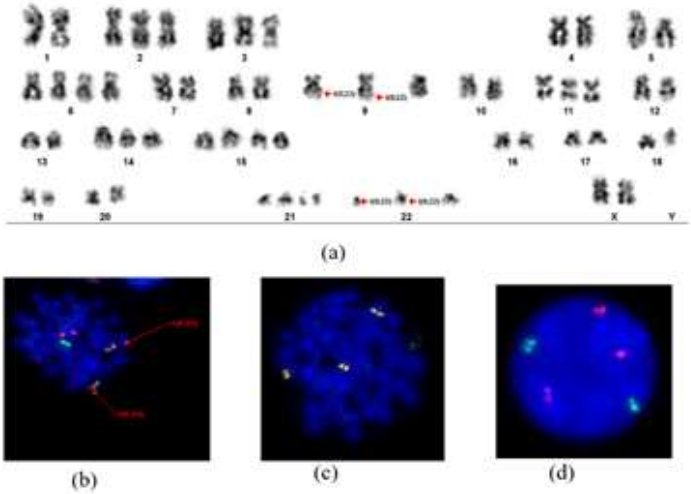


Figure 3. Karyotype of adult female showing Hyperdiploidy with positive double Philadelphia chromosome (a), Metaphase FISH showing positive BCR/ABL1 gene fusion (b), Metaphase FISH with three yellow signals for chromosome 11 at MLL breakpart (c), Interphase FISH with three signals for chromosome 21 (d)

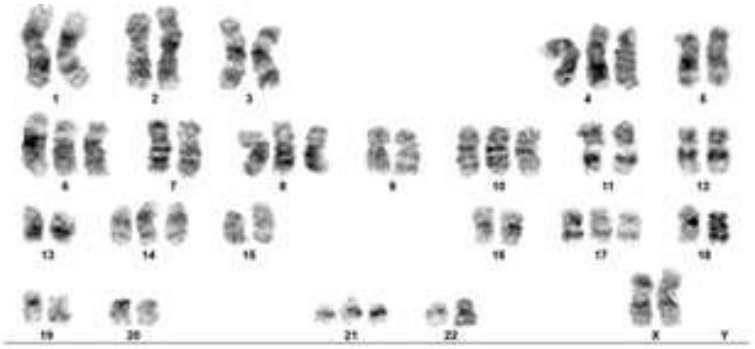


Figure 4. Hyperdiploid karyotype of a male child with additional Y chromosome and gain of X Chromosome

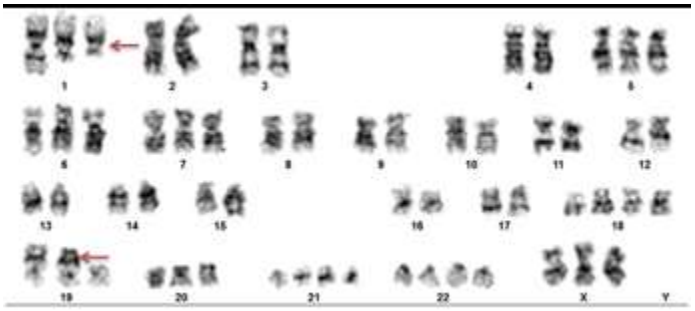


Figure 5. Hyperdiploid karyotype of a female child with double t(1;19).

Discussion

Conventional karyotyping is a gold standard technique in detection of chromosomal abnormalities in patients diagnosed with leukemia. Hyperdiploidy is a numerical chromosomal abnormality commonly seen in patients with acute lymphoblastic leukemia. In present study, hyperdiploidy was present in 15% of cases diagnosed with B cell ALL. In this FISH was an additional tool used to detect hyperdiploidy where karyotyping showed few numbers of spreads. Hyperdiploidy as a sole abnormality is considered as good prognostic marker. However when it is combined with structural abnormalities like t(9;22) the treatment outcome will differ. In present study, we observed that most of cases had only hyperdiploidy as seen in Figure 1. In two cases there was addition (Figure 2) and loss of Y chromosome (Figure 4) which is rare finding in literature. This suggests increase and decrease in sex chromosomes are also seen in B cell ALL which should be taken into consideration during treatment. One case of adult ALL had double Philadelphia chromosome which might have a variable outcome (Figure 3). Even though t(1;19) is a common structural abnormality in B cell ALL, one of our cases had double t(1;19) (Figure 5) which is a rare finding. This shows that along with increase in normal chromosomes there is tendency for cancer cells to multiply translocated chromosomes as well during mitotic event. This also increases gene dosage which might call for alteration in chemotherapy. But in general, most of studies showed good response to

chemotherapy in patients who had hyperdiploid chromosomes.

According to previous studies, hyperdiploid karyotype was present in 23-42% of newly diagnosed cases of ALL [5,6,7].Onordera et al discussed mechanism of formation of hyperdiploid karyotype. They used restriction fragment length polymorphism in 15 patients with hyperdiploidy to understand pathophysiology. They concluded that it happens due to sudden gain in number of multiple chromosomes [2].

In a study done by Kaspers et al., on 74 patients, 22% had hyperdiploid ALL. They observed that number of cells in S phase of cell division are more in hyperdiploid patients when compared to non hyperdiploid cases. They also studied drug sensitivity of hyperdiploid cells towards standard chemotherapeutic agents. They concluded that patients with hyperdiploidy had increased sensitivity towards antimetabolites, glucocorticoids and l-asparaginase when compared to non hyperdiploid patients probably hinting towards more number of cells in S phase [3].

Chikako Ito et al., stated that hyperdiploid cells have marked intensity to undergo apoptosis since they rapidly died in stromal cultures. They concluded that pathogenesis of hyperdiploid ALL could involve molecular defects leading to both DNA content abnormalities and a propensity to undergo apoptosis [8].

In a review done by Barbara Gibbons, author stated that presence of hyperdiploidy is dependent on age of patient. As age of patient advances chances of hyperdiploidy decreases. Also they mentioned hyperdiploidy is a secondary change and structural abnormalities like translocations are primary event which will lead to increase in number of chromsomes [9].

Anthony V. Moorman et al., did a study on ALL patients in which 32% had hyperdiploidy karyotype. In 8 cases along with hyperdiploidy additional structural abnormalities were present. Number of associated structural abnormalities with hyperdiploid karyotype was lower as observed in our study[10].

Ritterbach et al., used FISH as a quick screening method for identification of hyperdiploid karyotypes. They specifically used DNA probes for chromosomes 6, 10, 17, and 18. 28.8% patients had high hyperdiploid karyotype. FISH is a quick screening technique but using DNA probes for all chromosomes is not cost effective also it fails to detect any structural abnormalities if critical region /fusion probes are not used. [11]

Limitation of our study includes there is no correlation of our results with treatment, prognosis relapse of patients whom we have included in our study. Also, further studies are required to understand these abnormalities at molecular level by next generation sequencing.

Conclusion

We conclude that karyotyping and FISH are useful diagnostic tools in detecting hyperdiploidy in patients with B cell ALL. Further molecular studies need to be done to understand pathogenesis as well as reason for increased sensitivity of leukemic cells to chemotherapy.

Conflicts of interest

The authors declares that they do not have conflict of interest.

Acknowledgements

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