The Role of Chromosomal Aberration in Childhood Leukemia

M. Hassanzadeh-Nazarabadi,* A. Modarressi, and H. Miranpour

Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Islamic Republic of Iran

Abstract

Leukemia is referred to a group of heterogeneous neoplasia, which results from the hematopoietic cells transformation. Various chromosomal abnormalities have been recognized as primary patogenetic changes. Clonal chromosomal aberration is found in approximately 80% of childhood acute leukemia. Favourable and poor prognosis karyotypes are evident in such patients. Since cytogenetic play a dominant role in outcome of patients with childhood acute leukemia it is important to associate characteristic cytogenetic with prognostic value in relation to chromosomal abnormalities. Cytogenetic analysis from 51 childhood diagnosed patients under the age of 14 has been performed on the basis of G-banding technique. Each pair of chromosome show unique banding pattern. Various chromosomal abnormalities were evident in this study, these include; numerical disorders such as: monosomy, trisomy, tetrasomy, pentasomy and pseudo-diploidy. Among them monosomies were more prominent than the other aneuploidy. Structural aberrations such as philadelphia chromosome, gain and loss, inversion and breakage were seen. Each kind of childhood acute leukemia shows different responses to chemotherapy following cytogenetic studies. This finding strongly suggests that, a close association exists between chromosomal abnormalities and the prognostic value and prediction of outcome.

Keywords: Chromosomal abnormalities; Childhood acute leukemia; ALL; AML

Introduction

Leukemia is one of the most common cancers of childhood which is referred to a group of heterogeneous neoplasia which results from hematopoietic cells transformation [8,25]. Leukemic cells propagate initially in bone marrow and lymphatic tissue and then invade peripheral blood and other tissues [8,25]. Studies from various countries have revealed an increasing incidence of childhood leukemia in recent decades. A large set of population-based data from the Nordic society Of Paediatric Haematology and Oncology (NOPHO) revealed an acute leukemia database covering a population of approximately 5 million children aged 0-14 years [17]. Another study indicated that, the annual incidence

* E-mail: nazarabadi@hotmail.com
was 30-40 per million children under 15 years of age [23]. In the age group 1-4 years, a statistically significant annual 2.6% increase in incidence rate of childhood acute lymphocytic leukemia was seen in another study [30].

According to the type of affected cells (myeloid or lymphoid) and the disease process, leukemia divided into four different type, including:
4. Chronic myelocytic leukemia (C.M.L) [8,9,25].

Nearly 75% of childhood leukemia are classified as ALL, 15% as AML whereas CML is relatively rare and only accounts for 2-4% of childhood leukemia [32].

Although the cause of childhood leukemia remains unknown and there is no way to predict or prevent leukemia, environmental and genetic factors play an important role [8,24,25].

Environmental factors are including: Physical agents such as radiations, Chemical agents such as benzene and antineoplastic drugs, viral agents such as Epstine barr virus, HTLVI and influenza infection [7,11,28,34,42]. Birth weight is one of the few factors that has been associated with childhood leukemia, children weighing 4000gr. or more at birth were at higher risk of ALL than children weighing less [18,29].

Genetics Factors

The risk of leukemia has been increased in some of the heritable and congenital malformations diseases, such as: neurofibromatosis, agammaglobulinemia, bloom syndrome, fanconi and ataxia telangiectasia as well as some chromosomal syndromes like Down and Klinefelter [4,18,25,27,32,37,39]. Further more, monozygotic twin concordance rate is about 25% beyond 5 years of age and the risk to sib in childhood leukemia is 2-4 higher than the population incidence [8,12,25]. This observation again indicates that the genetic factors play an important role in leukemia. One of the strongest pieces of evidence for the genetic basis of cancer at the cellular level is the observation that nearly all cancer are monoclonal in origin, like philadelphia chromosome which is found in the cancer cells of more than 90% of patients with CML [8,40]. Experimental study has shown that mouse fibroblast cells transformed by extracted DNA from the human tumor cell lines, revealed strong tumorgenecity property [5,40]. Such observation strongly suggests that, DNA from human cancer cells have a gene that dominantly shows cancerous properties. Such a gene called oncogene. This gene before being oncogenic, named proto-oncogene, which play an important role in cell differentiation and growth control [40].

How are proto-oncogenes activated to oncogenes? By mutation, amplification and chromosomal aberration [5].

A large number of publications deal with various oncogenes associated with chromosomal abnormalities [10]. So the primary chromosomal event appeared to determine the biological bases of the disease. Clonal chromosomal abnormalities are found in approximately 80% of children with AML. Favourable prognosis karyotype in patients with AML include: trisomy 21 (Down syndrome) and translocation of 8;21 (q11.2;q13) which show good response to therapy and relatively long survival, in contrast, the AML patients with reciprocal translocation of chromosomes 9:22 (q34;q11) (philadelphia chromosome) show rather poor prognosis. However, among the patients with all kinds of translocations appeared with poor prognosis compare to those with normal karyotype [26,38,41].

Most patients with CML, who have philadelphia chromosome and bcr/abl product, show a good clinical manifestation whereas true philadelphia -negative patients who have no bcr/abl product, appear with worse prognosis [8,25].

Objective

Since cytogenetic characteristics play a dominant role in outcome of patients with childhood acute leukemia, it is important to correlate cytogenetic characteristic and prognostic values to chromosomal abnormalities. In this study chromosomal analysis have been performed on 51 childhood patients under the age of 14.

Materials and Method

Chromosomal cultures from 51 childhood patients were performed, according to the Armes Verma & Arvind Babu [3]. Five drops of whole peripheral blood and bone marrow aspiration sample were placed separately in 5 ml of full addition media (RPMI1640), supplemented with 20% fetal calf serum, penicillin and streptomycin but without phytohemaggutinin. Cultures were incubated in slant position at 37°C for 12 and 24 h. After this period of time 0.2 ml of colcemid was added to each culture tube and then incubated at 37°C for another 2 h. The culture tubes were centrifuged at 800 rpm for 8 min, the supernatant was discarded and 5 ml of 0.075 M potassium chloride was added to each culture tube. Tubes were incubated for additional 15 min at 37°C in water bath. The samples were
centrifuged at 800 rpm for 5 min, the supernatant was discarded and the pellet was resuspended in 5 ml of fresh fixative (3 part ethanol and one part acetic acid).

The tubes were centrifuged at 800 rpm for 5 min, the supernatant was discarded. This step was repeated for 3 times, after the final centrifugation, the pellet was resuspended in 0.5 ml of fresh fixative, 2 to 4 drops was put on wet cold slide by pasteur pipet, then the cells were treated with trypsin solution (10 gr/100cc BSS) at 37°C for 15 sec and stained with Gimsa. The slides were studied under the high magnification phase objective (100×) of photomicroscope for karyotype preparation. Each chromosome was analyzed for its presence and pairing chromosome in well defined order, based on characterised band and number in order of size. The 23 paris differed in the length of the arms and each showed a unique banding pattern (Plate 1).

Results and Discussion

Cytogenetic characteristics play a dominant role in childhood acute leukemia and has emerged as one of the most important prognostic factor [35]. In the present study chromosomal study was performed on 51 childhood diagnosed patients under the age of 14 before receiving any therapy.

In Table 1 the patients are classified as sex and age of incidence. The proportion of male and female include, more female (67.7%) than male (35.3%), whereas Cartwright and his colleagues showed equal sex ratio in their study [6]. In another investigation Jackson et al. have claimed that, acute leukemia is more common in males at almost every age and this fact remains unexplained [19]. However such a discrepancy results in terms of sex ratio might be indicated that, sex can not be an incidence factor.

The age of onset was more significant at 3-5 years group (Table 1).These results are similar to those of Magnani et al., in north west Italy [30].

In this study patients were grouped into acute lymphocytic leukemia (ALL) and acute myelocytic leukemia (AML). The incidence of ALL (74%) was more prominent than AML (26%). This result is in agreement with the results of the work which carried out by knox-Macaulay and Brown in the Sultanate of Oman who showed that 66% of their patients diagnosed as ALL, of the remaining (34%) as AML [21].

Cytogenetic Analysis

Four samples appeared with an apparently normal karyotypes (6%), the remaining 47 cases appeared with an abnormal karyotypes (92%). Such frequency of chromosomal abnormalities are comparable with those of Jarsova et al. [20] who indicated that, 96% of 79 childhood acute leukemia had abnormal karyotypes. Also the results are in agreement with those reported by Amare and his colleagues [40] in which chromosomal aberration were detected in 95% of 109 cases of acute leukemia.

Various types and frequency of chromosomal aberrations were classified as numerical (aneuploidy) and structural abnormalities in both kind of leukemia (Figs. 1 & 2).

Within each cytogenetic category, aneuploidies are listed in descending order. These include: monosomies of 4,5,6,7,9,10,11,18,21,22 (60%) trisomies of 4,5,6,7,17,18,21 (19.8%), tetrasonies of 12, 19,21 (7.4%) pentasonies of 21,13 (2.8%) pseudodiploidy (10%) (Fig. 1). These results are different with the cytogenetic finding of Susana et al., who showed that,
the frequency of monosomies and trisomies are 1.9% and 3.6% respectively [38]. The reason for such a discrepancy remains unknown.

Monosomies and trisomies were more evident in ALL, whereas tetrasomy and pseudodiploidy were more frequent in AML (Figs. 3 & 4). Alteration in chromosome number have a strong impact on outcome in childhood leukemia [16].

Structural abnormalities include: Translocation 9.22 (q34;q11) (philadelphia chromosome) (12.8%), addition (8%), deletion (8%), paracentric inversion (4%), breakage (2%) (Fig. 2).

Chromosomal breakage was not evident in AML compare with those of ALL, but on the other hand philadelphia chromosome in AML was prominent in contrast to ALL (Figs. 5 & 6). This result is comparable with the results of others studies, in which the philadelphia chromosome was only present in 3% of pediatric ALL patients [1,35].

Responses to chemotherapy following cytogenetic studies were different in each type of childhood acute leukemia (AML & ALL). Most of the ALL patients with the philadelphia chromosome-negative (Ph−) showed a good remission (Fig. 7), while AML patients with Ph+ appeared with poor response and unfavourable prognosis (Fig. 8). These results were comparable with those reported by Arico et al. and Riberior et al. [1,35] who observed that Ph− in pediatric ALL patients confers a favourable prognosis. In another study Susana et al. [38] claimed that, favourable prognosis karyotypes in patients with AML include: constitutional trisomy 21 (Down syndrome) and t (8;21), whereas poor prognosis karyotypes involved Ph+. Similar conclusion claimed by Hann et al. [15] who explained that, presence of philadelphia chromosome and t (4;11) were unfavourable karyotype and poor prognosis outcome.
Figure 5. Distribution of cytogenetic finding (structural abnormalities) in childhood acute myelocytic leukemia (AML).

Figure 6. Distribution of cytogenetic finding (structural abnormalities) in childhood lymphocytic leukemia (ALL).

Figure 7. Representative of treatment outcome in childhood acute lymphocytic leukemia (ALL).

Figure 8. Representative of treatment outcome in childhood acute myelocytic leukemia (AML).

With recurrent treatment protocol only 30-50% of children with AML will have successful outcome [13]. This finding may suggest that hematopoietic stem cell transplantation from a matched sibling donor is the treatment of choice for patient with Philadelphia chromosome positive [31]. Therefore it is important to correlate cytogenetic characteristics with disease outcome to identify patients that may benefit from prospective individualized therapy.

Acknowledgement

We wish to express our appreciation to vice president for research of Mashhad University of Medical Science for providing the expense of this project.

Our thanks to Mrs S. Mirzaie, Mrs Z. Rabani and Mrs F. Hassanzadeh for their technical assistance at Medical Genetic Department.

References