Original Article

# The Possible Association between Constitutive Heterochromatin Polymorphism and Human Leukemias

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**Abstract**

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**Objective:** Polymorphism of the size of heterochromatin region of chromosomes has been well documented in human genome and it consists of DNA sequences that are not transcribed. The prime aim of the present study was to evaluate the heterochromatin polymorphism associated with chromosomes in leukemic patients.

**Materials and Methods:** The study was conducted on 35 consecutive leukemic patients and 34 healthy individuals in Modaress and Taleghani hospitals, Tehran, Iran between 2004-2006. By applying Barium Hydroxide saline Giemsa (BSC) method with certain alterations, the variant heterochromatin polymorphism of chromosomes 1, 9 and 16 on bone marrow and peripheral blood lymphocyte cultures were evaluated. Chi-square and Fisher's exact tests were used for statistical analysis with SPSS software.

**Results:** Constitutive heterochromatin polymorphism of chromosomes 1 and 9 in leukemic patients revealed statistical significant differences when compared with chromosomes of healthy controls (p=0.0005) and (p=0.006) respectively. The differences were not significant for chromosome 16, it was 11.4% in leukemic patients and 0% in the control group (p=0.05). The frequency of partial and complete inversions did not show any significant differences between the leukemic patients and the control group.

**Conclusion:** The constitutive heterochromatin polymorphism blocks may provide an opportunity to serve as a marker for the detection and characterization of the chromosomes in leukemic patients.

***Keywords:*** Heterochromatin, Chromosome, Leukemia, Variant

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## Introduction

The term heterochromatin is used to denote those regions of chromosomes that remain condensed through interphase as well as during mitosis. Heterochromatin contains highly repeated fractions of DNA sequences that are not transcribed. These sequences probably are clustered in short tandem repeats at the heterochromtic regions of chromosome 1, 9 and 16 as well as the long arm of the Y-Chromosome (1-3). Deposition of heterochromatin on chromosome may be important for centromere function, segregation and gene silencing (4-6). A number of reports have indicated pronounced heteromorphism in size and localization in C-band regions of chromosome 1, 9 and 16 in individuals with different malignancies such as various types of cancer and leukemias (7-9), also the role of Y-chromosome C-band and male infertility have been described in the literature (10).

This study represents as the first report of heterochromatin polymorphism regions of chromosomes, ever investigated in Iranian populations. Hence, the purpose of the present study was to evaluate the heterochromatin polymorphism associated with chromosomes in leukemic patients and healthy normal individuals.

## Materials and Methods

A study of variant heterochromatin of chromosomes 1, 9 and 16 was performed on bone marrow and peripheral blood lymphocyte cultures followed by C- banding from a total of 35 leukemic patients, 16 males and 19 females, with the age range from 17 to 75 years, (the mean was 38.11±15.75) referred to Modaress and Taleghani referral hospitals in Tehran-Iran. Thus, the study group consisted of 16 CML; 14 AML and 5 patients with ALL. Informed consent was obtained from all the patients whose samples were taken. The patients' personal information has been kept confidential. The samples were used to determine the proportion of individuals with heterochromatin variants. The controls consisted of 34 randomly selected healthy individuals, with the age range from 18 to 45 (28.59 ±6.87).

Approximately 0.5 ml of peripheral blood was obtained from each participant. The blood sampling and cell culture procedures were essentially the same for all the participants. Briefly, heparanized blood was immediately mixed with 4 ml RPMI – 1640 (Gibco BRL) cell cultured medium supplement with 15-20% heat inactivated fetal bovine serum (Gibco BRL), 100mg/ml Phytohemagglutinin (PHA, Sigma) in a Vacutainer tube (Becton Dickinson Co, Ltd). This tube cultured for 70 hours at 37ċ under the aeration of 5% Co2 in the incubator. After an incubation period, the cultured cell harvested by 75 ml colcemide 10µg/ml (Gibco BRL) and incubated at 37ċ for 30 minutes. The contents of the tube were then centrifuged for 10 min at 1000 rpm and resuspended in 10 ml of 75 mM Kcl 0.56% (Sigma, Co) prewarmed to 37ċ for 20 min. At this stage 1ml of 20ċ Carnoys Fixative 3:1 methanol: acetic acid (Fisher Scientific) was added in to the tube to stop further cell swelling. This fixation repeated four times. Then cells dropped on to clean slides, and cultured for 3 days at 60ċ on the slide warmer. Slides then banded for 10 second with 0.2 X trypsin (Difco, Co) and stained for 3 min Giemsa (Harleco, Co). Slides were examined with an Olympus model BH-2 light microscope. Eighty well spread G-banded metaphases were analyzed.

The Barium Hydroxide Saline Giemsa (BSG) method, with some alterations, was applied (1). Chromosome preparations were treated with 0.2 N HCL for 1 hour at room temperature, followed by a rinse with deionized water. The Slides were placed in a freshly prepared 5% aqueous solution of Barium hydroxide Octahydrate [(Ba(OH) 28H20] at 56Ċ in water bath (WB) for about 2-5 minutes, followed by rinsing with de-ionized water . Slides were incubated for 1 hour at 60 (WB) in 2X SSC (0.3M Sodium chloride containing 0.03M tri-sodium citrate) Followed by a rinse with water. The treated chromosomes were stained with Giemsa for about 45 minutes. A Minimum of five well spread metaphases were photographed from each individual. To eliminate the variations in C- segment lengths in chromosomes 1, 9 and 16 the presence of heterochromatin variants were estimated visually when at least 25% variation in C–band size was observed between homologues chromosomes. Heterochromatin region differences of the abnormal C–block were recorded as $qh^+$ or $qh^-$. Two groups of localization of C-segment inversion were distinguished as total inversion when the whole C-segment inversion were distinguished as total inversion when the whole C-segment was situated near the centromere, but on the short (P) and partly on the long (q) arm of chromosome. In routine practice, 15 G banded metaphases from each preparation were needed for Scoring. In some cases this was not possible, while in others more metaphases were analyzed for better definition of a particular aberration. Well spread metaphases were saved by Yvisis software and karyotypes were described according to ISCN (11).

### *Statistical Analysis*

The results of the investigation were statistically analyzed , by applying chi square and Fisher's Exact test , where the frequency ( percentage) was greater than 5 and chi square test of goodness of fit with Yate's correlation , where the frequency percentage 0 was less than 5. Data analysis was performed by SPSS (version 11.5, Inc. USA).

## Results

The study reveals the proportion and analysis of constitutive heterochromatin polymorphism in chromosomes 1, 9 and 16 and the frequency of inversions in leukemic patients (Table 1) as well as the healthy controls (Fig 1) (Table2).

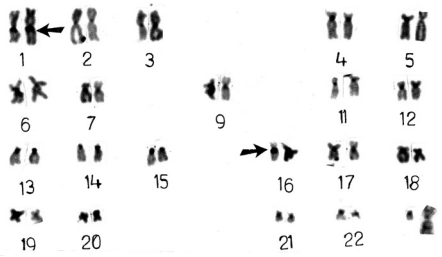


***Fig 1: C-banding karyotype with normal heterochromatin below the centromere***

***Table 1: Distribution of polymorphism in chromosome 1, 9 and 16 in leukemic patients***

| Sr | Sex/age | Chromosomes with C-band variants | | | Chromosome inversion | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No | Years | | | | Partial | | | Complete | | |
| | | 1 | 9 | 16 | 1 | 9 | 16 | 1 | 9 | 16 |
| 1 | F/38 | qh+ | - | - | - | - | - | - | - | - |
| 2 | F/75 | qh+ | - | - | - | - | - | - | - | - |
| 3 | M/73 | - | - | - | - | - | - | - | - | - |
| 4 | F/57 | - | qh+ | - | - | - | - | - | - | - |
| 5 | F/68 | - | - | - | - | - | - | - | - | - |
| 6 | M/28 | qh+ | qh+ | - | - | - | - | - | - | - |
| 7 | M/27 | - | - | qh+ | - | - | - | - | - | - |
| 8 | F/38 | - | qh+ | - | - | - | - | - | - | - |
| 9 | M/24 | - | qh+ | - | - | - | - | - | - | - |
| 10 | M/41 | - | - | - | - | - | - | - | - | - |
| 11 | F/35 | qh+ | - | - | - | - | - | - | - | - |
| 12 | M/37 | qh+ | - | qh+ | - | - | - | - | - | - |
| 13 | M/40 | qh+ | - | - | - | - | - | - | - | - |
| 14 | M/42 | qh+ | - | - | - | - | - | - | - | - |
| 15 | M/40 | - | qh+ | - | - | - | - | - | - | - |
| 16 | F/24 | qh+ | qh+ | - | - | - | - | - | - | - |
| 17 | F/40 | - | - | qh+ | - | - | - | - | - | - |
| 18 | M/20 | - | - | - | - | - | - | - | - | - |
| 19 | M/40 | - | qh+ | qh+ | - | - | - | - | - | - |
| 20 | F/26 | qh+ | qh+ | - | + | - | - | - | - | - |
| 21 | F/19 | qh+ | qh+ | - | - | - | - | - | - | - |
| 22 | F/40 | qh+ | - | - | - | - | - | - | - | - |
| 23 | F/34 | - | qh+ | - | - | - | - | - | - | - |
| 24 | F/18 | qh+ | qh+ | - | - | - | - | - | - | - |
| 25 | F/23 | qh+ | - | - | - | - | - | - | - | - |
| 26 | M/18 | - | - | - | - | - | - | - | - | - |
| 27 | M/20 | qh+ | - | - | - | - | - | - | - | - |
| 28 | M/17 | qh+ | - | - | - | - | - | - | - | - |
| 29 | F/26 | - | qh+ | -qh+ | - | - | - | - | - | - |
| 30 | F/40 | qh+ | - | - | - | - | - | - | - | - |
| 31 | F/52 | qh+ | - | - | - | - | - | - | - | - |
| 32 | M/40 | - | qh+ | - | - | - | - | - | - | - |
| 33 | M/41 | qh+ | qh+ | - | - | - | - | - | - | - |
| 34 | F/60 | - | qh+ | - | - | - | - | - | - | - |
| 35 | F/37 | qh+ | - | - | - | - | - | - | - | - |

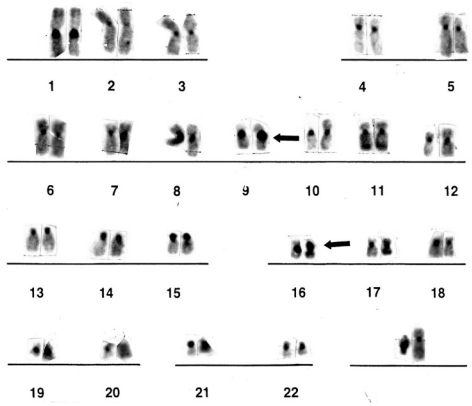

*Fig 2: C-banded bone marrow metaphases showing: 1q+, 16q+ in leukemic patient. Table 1 (Case 12)*

Constitutive heterochromatin variants of chromosome 1 (Fig 2 case 12), were detected in 54.3% of leukemic patients and 11% of the control healthy group, the results revealed significant differences of variant heterochromatins between leukemic patients and the control group (p=0.0005).The differences were also significant for chromosome 9, it was 43% in leukemic patients and 9% in the control group (p=0.006). The differences were not significant for chromosome 16, it was 11.4% in leukemic patients (Fig 3 case 9) and 0% in the control group (p=0.05).

***Table 2: Distribution of individuals heterochromatin variants in chromosome 1, 9 and 16 in control population***

| Sr | Sex/age | Chromosomes with C-band variants | | | Chromosome inversion | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No | Years | | | | Partial | | | Complete | | |
| | | 1 | 9 | 16 | 1 | 9 | 16 | 1 | 9 | 16 |
| 1 | M/45 | - | qh+ | - | - | - | - | - | - | - |
| 2 | F/39 | - | - | - | - | - | - | - | - | - |
| 3 | F/25 | qh+ | - | - | - | - | - | - | - | - |
| 4 | F/45 | - | - | - | - | - | - | - | - | - |
| 5 | M/42 | qh+ | qh+ | - | - | - | - | - | - | - |
| 6 | M/31 | qh+ | - | - | - | - | - | - | - | - |
| 7 | F/34 | - | - | - | - | - | - | - | - | - |
| 8 | F/27 | - | - | - | - | - | - | - | - | - |
| 9 | F/33 | - | - | - | - | - | - | - | - | - |
| 10 | M/31 | - | - | - | - | - | - | - | - | - |
| 11 | M/28 | - | - | - | - | - | - | - | - | - |
| 12 | M/30 | - | - | - | - | - | - | - | - | - |
| 13 | F/22 | - | - | - | - | - | - | - | - | - |
| 14 | M/30 | - | - | - | - | - | - | - | - | - |
| 15 | F/22 | - | - | - | - | - | - | - | - | - |
| 16 | F/26 | - | - | - | - | - | - | - | - | - |
| 17 | M/27 | - | - | - | - | - | - | - | - | - |
| 18 | M/29 | - | - | - | - | - | - | - | - | - |
| 19 | M/30 | - | - | - | - | - | - | - | - | - |
| 20 | F/25 | - | - | - | - | - | - | - | - | - |
| 21 | M/25 | - | - | - | - | - | - | - | - | - |
| 22 | F/25 | - | - | - | - | - | - | - | - | - |
| 23 | M/29 | - | - | - | - | - | - | - | - | - |
| 24 | F/38 | - | - | - | - | - | - | - | - | - |
| 25 | F/21 | - | - | - | - | - | - | - | - | - |
| 26 | F/23 | - | qh+ | - | - | - | - | - | - | - |
| 27 | F/20 | - | - | - | - | - | - | - | - | - |
| 28 | F/18 | - | - | - | - | - | - | - | - | - |
| 29 | M/28 | - | - | - | - | - | - | - | - | - |
| 30 | M/30 | qh+ | - | - | - | - | - | - | - | - |
| 31 | M/25 | - | - | - | - | - | - | - | - | - |
| 32 | F/19 | - | - | - | - | - | - | - | - | - |
| 33 | F/22 | - | - | - | - | - | - | - | - | - |
| 34 | M/28 | - | - | - | - | - | - | - | - | - |

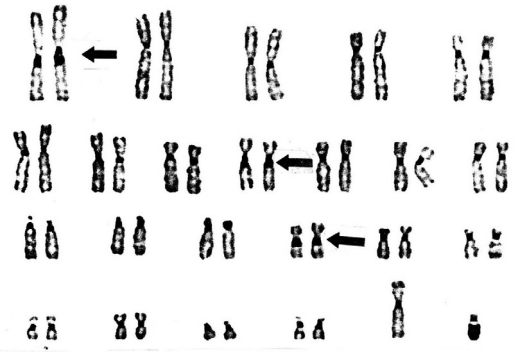

*Fig 3: C-banding Karyotype with normal heterochromatin below the centromere*

The frequency of partial and complete inversion (Fig 3) did not show any significant differences between the leukemic patients and the control group.

## Discussion

Polymorphism of the size of heterochromatin regions of chromosomes have been documented in the human genome (12). In the course of this study, an excess of constitutive heterochromatin polymorphism of chromosome 1 and 9 C-band was found in leukemic patients when compared to the control group. This is in an agreement with previous studies suggesting the role of heterochromatin variants of chromosome 1 and 9 and possible risk of developing cancer and leukemia (7, 9, 13). Other investigators did not find a higher frequency of 1qh and 9qh in their studies (14, 15). Furthermore, the results of this study did not exhibit an increase in C- band heterochromatin variants in chromosome 16 in leukemic patients when compared to the control. This is in close agreement with that of the other investigators who could not find any correlation between C- band heterochromatin polymorphism of chromosome 16 and cancer susceptibility (16, 17). Our investigation of C- banding provides an opportunity to better characterize some autosomal types, and to detect differences in heterochromatin polymorphism between the leukemic patients and the healthy individuals. In this study, frequency of partial and complete inversion were not significantly higher in leukemic patients. This finding is in accordance with the study conducted by Leconiant and colleagues (18) who failed to demonstrate increased frequency of inversions between a group of leukemic patients and the control group. In contrast to the present investigation, previous reports demonstrated greater frequency of inversion of C-band heterochromatin in chromosomes 1, 6 and 16 in cancer and leukemias patients (15, 19).The ability to distinguish these two types of inversion may help to resolve the question of the clinical significance of leukemias and malignancies.

## Conclusion

The constitutive heterochromatin polymorphism blocks may provide an opportunity to serve as a marker for the detection and characterization of tchromosomes in leukemias patients. The present study may be helpful in understanding the possible predisposing role of heterochromatin variation regarding leukemias. We recommend further investigations in this regard.

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