Is Heterochromatin Polymorphism Associated with Chronic Myeloid Leukemia

Abolfazl Movafagh, Ph.D. ☆ ♦

☆ *Genetics Department, Shahid Beheshti Medical University*

♦ **P.O.Box: 19385-378, Genetics Department, Shahid Beheshti Medical University, Tehran, Iran**

Abstract

Introduction: Heterochromatin consists of DNA sequences that are not transcribed, and are repeated in short tandem at heterochromatic regions of chromosomes 1, 9, 16 as well as the distal part of long arm of chromosome Y. Slightly large tandem repeats at the centromeres of human chromosomes are also considered as heterochromatin regions. The main aim of the present study is to evaluate the heterochromatin polymorphism associated with chronic myeloid and acute non-lymphocytic leukemias.

Material and Methods: 16 Leukemic patients and 10 normal healthy persons were selected. By applying Barium Hydroxide Saline Giemsa (BSG) method. The variant heterochromatin of chromosome 1, 9 and 16 on bone marrow samples and lymphocyte cultures were evaluated.

Results: the result of present study indicated no significant differences for heteromorphisms between Acute Non-Lymphocytic Leukemia (ANLL) Patients and normal individuals. But differences were significant on comparing the complete inversions in ANLL patients with the controls. There were significant differences in heterochromatin variant of C-segment in chromosomes 1 and 9 between Chronic Myeloid Leukemia patients (CML) and normal group. Partial and complete inversion between CML patients and the control group is significant.

Conclusion: A number of reports have indicated pronounced heteromorphism in size and localization in constitutive band region of chromosomes 1, 9 and 16 in many malignancies. However, this study showed similarities and dissimilarities with other investigators regarding heterochromatin variations and inversions.

Key words: Polymorphism, Heterochromatin, Chromosome, Leukemias.

Introduction

Variant chromosomes are polymorphic in areas that are rich in repeated sequences such as pericentric regions, or in the acrocentric short arm regions (1). The term heterochromatin is used to denote those regions of chromosomes that remain condensed throughout interphase as well as during mitosis, and remain relatively constant in size. Heterochromatin contains highly repeated fractions of DNA (1). Perhaps 10% of the genome or even more, depending on the species, consists of sequences that are not transcribed and are repeated hundreds of thousands or even million times. These sequences probably are clustered in short tandem repeats at the heterochromatic regions of chromosome 1, 9 and 16 as well as the long arm of the Y-chromosome; or slightly larger tandem repeats at the centromeres of human chromosomes (2).

Heterochromatin plays a major role in the evolution of the chromosomes, which leads to increase genomic size, while its loss produces extreme polymophisms. It also influences crossing over and probably sex differentiation (3).

Heterochromatic variants can also be one of the factors that influence fertility and the appearance of aneuploid component during meiosis (4). Atkin and Baker suggested that heterochromatic variants in chromosome 1 might increase the risk of developing a malignancy (5).

In view of the fact that differences in C-band patterns have been found among different ethnic groups, C-band have also been studied in tumor tissue, blood disorders, polycythemia vera, myeloid leukemia, acute Lymphoid leukemia and preleukemic states. More surveys have shown an increased frequency of heterochromatic polymorphisms in patients with different disorders, suggesting its possible role in the development of neoplasia (6, 7). A number of reports have indicated pronounced heteromorphism in size and localization in C-band region of chromosomes 1, 9 and 16 in individuals with different malignancies such as various types leukemia (8).

Materials and Methods

A study of the variant heterochromatin of chromosomes 1, 9 and 16 was performed on bone marrow and lymphocyte culture followed by C-banding from a total of 16 leukemic patients and 10 normal healthy persons from Postgraduate Institute Medical Education and Research (PGI) India. The samples were taken from the patient with their consent. Personal information of them kept confidential. Samples were taken in order to determine the proportion of individuals with heterochromatin variants. The group studied consisted of 8 patients with CML (the age ranged from 21 to 53 years, the mean was 36.4±13.45) and 8 patients with ANLL, the age ranged from 13 to 45, the mean was 29.4±12.4 years. The controls consisted of 10 healthy adult persons randomly selected (the age ranged from 27 to 45).

The Barium Hydroxide Saline Giemsa (BSG) method was introduced by sumner (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 5°C in water bath (WB) for about 2-5 minutes, followed by rinsing with deionized water. Slides were incubated for 1 hour at 60°C (WB) in 2x SSC (0.3 M 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 was situated near the centromere, but on the short (P) and partly on the long (q) arm of chromosome. The results of the investigation were statistically analyzed, by applying Chi square 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) was less than 5. In routine practice, 15 G-banded metaphases from each preparation were

needed for scoring. In some cases it was not possible, while in others more metaphases were analyzed for better definition of a particular aberration. Well spread metaphases were photographed and karyotyped.

Results

Variant chromosomes are polymorphic in areas that are rich in repeated sequences such as pericentromeric regions or in acrocentric short arm regions (9).

Heteromorphism in CML: The proportion of individuals with quantitative C-band size of homologous chromosome in each 1, 9 or 16 chromosomes, and also the relative heteromorphism in CML patients was compared to the control group (table 1), the frequency of heterochromatin variants was significantly increased in the CML patients in homologous chromosomes 1 and 9 as compared with the controls ($p<0.01$) (table 2). On the other hand, the results of heterochromatin variants did not show significant differences between homologous chromosome 16qh in the series of CML patients and the control group. The results indicated partial inversion in 37% of CML patients and 10% in the controls, the differences being highly significant $p<0.01$,whereas 12% complete inversion were detected in CML patients compared with the controls, the differences again were significant ($p<0.05$). It is interesting to note that these inversions were more frequent in chromosome 1, compared to chromosomes 9 and 16.While analyzing the frequency of total inversions in both the CML and control groups, it was found to be 50% in CML patients (table 3) while the controls, showed 10% total inversions, the differences was highly significant ($p<0.01$).

Heteromorphism in ANLL: This work deals with the analysis of heteromorphism in chromosomes 1, 9 and 16; and also the frequency of inversions in ANLL patients (table 4) as well as the controls. The results indicated that no significant differences for heteromorphism between ANLL patients and the control group. C-band heterochromatin variants of chromosome 1, were detected in 25% of ANLL patients and 20% of the controls. The p-value was not significant for chromosome 9 as well, the frequencies were 25% in ANLL patients compared to 10 % in the controls. The differences were also not significant for chromosomes 16, it was 12% in ANLL patients and 10% in the control group. But the total distribution of heteromorphism incidence (62%) in ANLL patients and 40% in the control for chromosomes 1, 9 and 16 which was significant in ANLL compared to the control ($p<0.05$). No significant difference was noticed in the frequency of partial inversion of the C-band. However, the difference was significant in comparison with the complete inversions in ANLL patients with those of the controls ($p<0.05$). The frequency of total inversions, including partial and complete ones did not show any significant differences between the ANLL patients and the control group.

Heteromorphism in CML: The main aim of the present study was to analyse heterochromatic variant of the C-segment in chromosomes 1, 9 and 16. The results showed that there were significant differences in chromosomes 1 and 9 between the CML patients and the normal individuals used as controls ($p<0.01$).

Table 1: Distribution of Individuals by Heterochromatin Varints in Chromosomes 1, 9 and 16 in control Population

Sr. No.	Sex/Age Years	Chromosomes with C-band Variants			Chromosome Inversions					
					Partial			Complete		
	1	9	16	1	9 16	1	9	16		
1.	M/30	-	-	qh+	-	-	-	-	-	-
2.	F/27	-	-	-	-	-	-	-	-	-
3.	M/31	-	-	-	-	+	-	-	-	-
4.	M/34	qh+	-	-	-	-	-	-	-	-
5.	M/34	-	-	-	-	-	-	-	-	-
6.	F/33	-	qh-	-	-	-	-	-	-	-
7.	M/36	-	-	-	-	-	-	-	-	-
8.	M/41	-	-	-	-	-	-	-	-	-
9.	M/45	qh-	-	-	-	-	-	-	-	-
10.	M/31	-	-	-	-	-	-	-	-	-

Table 2: Distribution of C-variants in Chromosomes 1, 9 and 16

Group study	Number of individuals	Chromosome Pair			
		1(%)	9(%)	16(%)	(%)
Control	10	2(20)	1(10)	1(10)	4(40)
CML	8	4(50)*	3(37)*	2(25)	6(75)*
ANLL	8	2(25)	2(25)	1(12)	5(62)*

Significant difference with respect to control: *p<0.01, **p<0.05

Table 3: Distribution of Polymorphism in Chromosome 1, 9 and 16 in CML Patients

Sr. No.	Sex Age Years	Chromosomes with C-band Variants			Chromosome Inversions					
					Partial			Complete		
		1	9	16	1	9	16	1	9	16
1.	M/52/	-	qh+	qh+	-	-	-	+	-	-
2.	M/21	qh-	-	-	+	-	-	-	-	-
3.	M/50	qh+	qh-	qh+	-	-	-	-	-	-
4.	M/53	qh+	-	-	-	-	-	-	-	-
5.	M/25	qh+	-	-	-	+	+	-	-	-
6.	M/36	-	qh+	-	-	-	-	-	-	-
7.	M/30	-	-	-	-	-	-	-	-	-
8.	F/24	-	-	-	-	-	-	-	-	-

Table 4: Distribution of Polymorphism in Chromosome 1, 9 and 16 in ANLL Patients

Sr. No.	Sex Age Years	Chromosomes with C-band Variants			Chromosome Inversions					
					Partial			Complete		
		1	9	16						
1.	M/40	qh-	-	-	-	-	-	-	-	-
2.	F/13	-	-	-	-	-	-	-	-	-
3.	F/22	-	-	-	-	-	-	-	-	-
4.	F/36	qh+	-	-	-	-	-	-	-	-
5.	F/45	-	-	qh+	-	-	-	-	-	-
6.	F/41	-	-	-	-	-	-	-	-	-
7.	M/20	-	qh+	-	-	-	-	-	-	+
8.	M/38	-	qh+	-	-	-	-	-	-	-

Discussion

One of the most important results is the higher frequency of intrapair differences in the 1qh which have been supported by many investigators (10, 11).

Higher frequency of intrapair heteromorphism of 1qh in various blood disorders has been reported (12, 7). Other investigators did not find a higher frequency of intrapair heteromorphism of chromosome 1qh in their studies (13). In the course of this study, an excess of chromosome 9 C-band heteromorphism was found in CML patients compared to the controls. An increase in the C-band regions of chromosome 1 and 9 has been described in CML patients and in various blood disorders (14).Furthermore, increase of the C-band regions of the chromosome 1, 9 and 16 was reported in the breast cancer patients (15). In contrast to the investigations of Berger et al (16) who did not demonstrate greater frequency of inversion of C-bandin CML patients, analysis of the inversions in chromosomes 1, 9 and 16 of CML patients and the control group in the present study found significant increase in partial and total inversions in CML patients. Chromosomal polymorphism depends mainly on heterochromatin and is transmitted in a Mendelian way. The significance of heterochromatin variants is not well understood, and the interpretation of the incidence variation among different groups of patients or families is thus difficult and it remains to be clarified. In fact, the possibility of the uses of heterochromatin size variation measurements of repetitive DNA represents a better approach for the problem. However, these studies may be helpful in understanding the possible predisposing role of heterochromatin variation regarding cancer or leukemia risk. A number of reports have indicated pronounced heteromorphism of size and localization in

the C-band region of chromosomes 1, 9 and 16 in individuals with different malignancies such as ovarian and colorectal carcinoma (16, 17, 18). According to many investigators, there is an increase in the C-band region of chromosome 1, 9 and, in 1, 9 and 16 in various malignancies (16, 8, 19, 20). The motive of the present study, however, was the analysis of C-band heteromorphism in chromosomes 1, 9 and 16. The results did not show increases in C-band heterochromatin variants in chromosomes 1, 9 and 16 in ANLL patients, compared to the controls. However, extensive works of previous investigators suggested the role of heterochromatin variants and possible risk of developing various blood disorders in human population (8, 17). Hence, the present results on heterochromatin variant in chromosomes 1, 9 and 16 in ANLL patients disagreed with above studies. Although, one reason may be the number of ANLL patients (cases) not being large enough to reach the level of significance. On the other hand, the results of the present work is in agreement with that of the other group of investigators who could not find any correlation between C-band heteromorphism and cancer susceptibility (21, 22). Frequency of complete inversion was noticed to be significantly higher in ANLL patients as compared with the controls ($p<0.05$). This disagreed with the results of Le Coniat and colleagues (23) who failed to demonstrate increased frequency of inversions between a group of 100 ANLL patients and the controls for the three chromosomes 1, 9 and 16.

The main aim of the present research work was to detect the role of heterochromatic variants as well as the frequency of partial and complete C-band on chromosome 1, 9 and 16. Many reports indicated pronounced constitutive heterochromatin polymorphism and variant localization inversion of chromosomes 1, 9 and 16. This study concluded similarities and dissimilarities with other investigators regarding heterochromatic variants and inversions in chromosomes 1, 9 and 16. It may be due to different geographical localization. Although, it needs more research work to be done to draw firm conclusions.

References

1. Sumner AT : The nature and mechanisms of chromosomes banding. Cancer Gent Cytogent 1982; 6: 59-87

2. Thompson and Thompson : Genetics in medicine. Thompson Js. and Thompson MW (ed). Philadelphia, Saunders Company Press, 2001, P.31. 3. Kuznetsova SM, : Polymorphism of heterochromatin areas on chromosomes 1, 9, 16 and Y in long-lived subjects and persons of different ages in two regions of the Soviet Union. Arch Gervant Geriat 1987; 6: 172-186

4. Jacobs PA, Frackleslez A, Law P, Hilditch CJ, Morton NE: The effect of structural aberrations of the chromosomes on reproductive fitness in man. Clin Genet 1975; 8: 162-178.

5. Atkin NB, Baker Mc: Pericentric inversion of chromosome 1: Frequency and possible association with Cancer. Cytogenet Cell Genet 1977; 18: 180-184

6. Atkin NB, Brito-Babapulle V: Heterochromation polymorphism and human cancer. Cancer Genet Cytogenet 1981; 3: 261-272

7. Ranni NSM, Vinuesa ML, Pargament M, Slavutsky I, Larripa I: Heterochromatic variants and their association with neoplasia III. Multiple myeloma. Cancer Genet Cytogenet 1987; 28:101-105

8. Sampaio DA, Mattevi SM, Cavalli IJ, Erdtmann B: Densitometric measurement of C-bands of chromosome 1, 9, 16 and Y in leukemic and preleukemic disorders. Cancer Genet Cytogenet 1989; 41: 71-48

9. Ready KS, Sulcova V: The mobile nature of acrocentric elements illustrated by unusual chromosome variants . Hum Genet 1998; 102: 653-662

10. Verma RS, Costa M, Gogineni SK, Palermo G: New translocation involving homologous chromosome 3 in acute myelogenous leukemia (M2). Am J Hematol 1993;42: 398-399

11. Adhvaru SG, Jain KH, Trivedi AH, Dave BJ, Rawal UM: Y chromosome in leukemia patients. A study on the heteromorphic nature of its heterochromatic segment. Neoplasma 1989; 36: 343-437

12. Atkin NB, Britio-Babapulle V: Chromosome 1 heterochromatin variants and cancer: A reassessment . Cancer Genet Cytogenet 1981;18:325-331

13. Rey JA, Bello MJ, Campos JM, Kausak ME, Valcarcel E, Benitez J: C-band pattern in patients with nervous system tumors. Cancer Genet Cytogenet 1987; 27: 185-190

14. Rajasekariah P, Garson OM: C-banding studies in ptients with Ph1 chronic granulocytic leukemia. Pathol 1981; 13: 197-203

15. Berger R, Beruheim A, Kristoffersson U, Mitelman F,

Olsson H: C-band heteromorphosm in breast cancer patients. Cancer Genet Cytogenet 1985; 18: 95-102

16. Berger R, Berhein A, Le Conniat M, Vecchione D: C-banding studies in variants blood disorders. Cancer Genet Cytogent 1979; 195-102

17. Nanjoks II, Weil II: Heterochromatic C- bands of chromosome 1 in ovarian cancer patients. Cancer Defect Prev 1985; 8: 151- 153

18. Shabtai F, Antebi E, Klar D, Kimichi D, Hart J, Halberecht I: Cytogenetic study in patients with carcinoma of colon and rectum: Particular C-band variants as possible markers for cancer proneness. Cancer Genet Cytogenet 1985; 15: 73-77

19. Chen PY, Wu HM, Kuo PL, Tzeng CC: Unusal chromosome 9 variant with an extra G-dark and C-negative Segment in the short arm, proximal to the centromere. Zhonghua Yi Xue Za Zhi 1999; 62: 121 124

20. Verma RS , Kleyman SM, Conte RA: An unusul G negativebandwithin 1qh region a rare variant or an abnormality ? Ann Genet 1997; 40: 229-231

21. Aguilar L, Lisker R, Ruz L and Mutchinick O: Constitutive heterochromation polymorphisms in patients with malignant diseases. Cancer 1981; 47:2432-2439

22. Rivera H, Gutierrez AM, Gonzales GJR: Chromosome 9qh inversions may not be true inversions. Hum Genet 1999; 105: 181-184

23. Le Coniant M, Veccione D, Bernheim A, Berger L: C-banding studies in acute nonlymphocytic leukemia. Cancer Genet Cytogenet 1982; 5: 327-331