Parallel Increases in Heterochromatin Variants and Chromosome Damages in Drugs Addicts

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ABSTRACT

Polymorphisms of the size of heterochromatin regions and abnormalities of chromosomes have been well documented in human genome; they consist of DNA sequences that are not transcribed. The prime aim of the present study was to evaluate the heterochromatin polymorphism and numerical and structural abnormalities associated with chromosomes in drug addicts. No data exists in Iran regarding the cytogenetic characteristics of drug addiction lymphocytes. Therefore, cytogenetic investigations were performed in 93 drug addiction lymphocytes and 93 normal controls. This randomized collected study was conducted on 93 consecutive drug addiction individuals and 93 healthy individuals in Loghman and private hospitals, Tehran, Iran between the years 2007-2009. By applying Barium Hydroxide saline Giemsa (BSC) method, the variant heterochromatin polymorphism of chromosomes 1, 9 and 16 on lymphocyte cultures were evaluated. Cytogenetic analysis was performed in drug addicts' lymphocytes cultures. Constitutive heterochromatin polymorphism of chromosomes 1 in drug addicts revealed statistical significant when compared with chromosome of healthy controls (P=0.004). The differences were significant for chromosome 9 (P=0.029), it was 94.1%   in drug addiction and 5.9% in the control group (P=0.196). The differences were also significant for chromosome 16: it was 91.9% in addicts and 8.1% in the control group (P=0.052). Also the frequency of partial and complete inversion did not show any significant differences between the drugs addicts and the control group. The occurrence of chromosomal defects including, chromatid break (12 addicts), chromatid gap (8 addicts), dicentric (2 addicts), was higher in our observation. Our constitutive heterochromatin polymorphism blocks and chromosomal abnormalities in drugs addicts' chromosome may provide an opportunity to serve as a marker for the detection and characterization of the damages chromosomes in drug addicts.

Keywords: Addiction; Drugs; Chromosome; Abnormalities; Heterochromatin; Polymorphism

INTRODUCTION

Heterochromatin as the library of different Satellite DNA is one of fragment in which it becomes spread and fix in different species genome [1]. Heterochromatin contains highly repeated fractions of DNA sequences that are not transcribed .The term heterochromatin is used to denote those regions of chromosomes that remain condensed through inter-phase as well as during mitosis. These sequences are probably clustered in short tandem repeats at the heterochromtin regions of chromosomes 1, 9 and 16 [2-4]. Deposition of heterochromatin on chromosome may be important for centromere function, segregation and gene silencing [5-7]. A number of reports have indicated pronounced hetero-morphism in size and localization in C–band region of chromosomes 1, 9, and 16 in individuals with different malignancies such as various types of cancers and leukemia [8], also the role of Y chromosome constitutive heterochromatin band and male infertility have been describe in the literature [9].

This work represent the first report of cytogenetic study and heterochromatin polymorphism regions of chromosomes 1, 9 and 16 together, ever investigated in Iranian and Middle East populations. Hence, the motive of the present study was to evaluate the heterochromatin polymorphism associated with chromosomes in addicts and healthy normal controls.

MATERIALS AND METHODS

We applied Constitutive heterochromatin and G-banding method with minor modified development in our laboratory to enhanced detection of the cytogenetic damage in heroin, opium, etc, addicts. In order to draw conclusions concerning a possible effect of narcotic drugs on Addicts chromosomes, the

relevant exposed chromosomal analysis and heterochromatin of chromosomes 1, 9, and 16 was carried out on 93 drugs addicts and 93 controls. A Study of the variant heterochromatin of chromosomes 1, 9, and 16 was performed on lymphocyte culture followed by C- banding from a total of 93 drugs addicts and 93 normal healthy persons. The samples were taken from the addicts with their consent and personal information of them kept confidential. Samples were taken in order to determine the proportion of individuals with heterochromatin variants and chromosomal analysis. There were no significant differences of sex distribution between drug addicts and normal healthy persons**.** The age of addicts ranged from 17 to 75 years with a mean of 32.4±11.4. The controls consisted of 93 healthy randomly selected adults (the age ranged from 18 to 48) the mean was 29.2±6.8. No medicines and drugs were taken by the blood donors for control group for at least one month prior to sampling, also those who were suffering from genetic disorders due to cancers, leukemia, syndromes, and infections were excluded from the present study.

Approximately 0.5 ml of peripheral blood was obtained from each addict at Shahid Beheshti Medical University affiliated Loghman Hospital, and private Hospitals in Tehran between the years 2007-2009.
The blood sampling and cell culture procedures were essentially the same for all the participants (addicts and controls).
Briefly, heparanised 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 was cultured for 70 hours at 37° C under the aeration of 5% Co_2 in the incubator. After an incubation period, the cultured cell was harvested by 75 ml colcemide 10µg/ml (Gibco BRL) and incubated at 37° C for 30 minutes. The contents of the tube were then centrifuged for 10 min at 1000 rpm and re-suspended in 10 ml of 75 mM Kcl 0.56% (Sigma, Co) pre-warmed to 37° C 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 was repeated four times. Then, cells sere dropped on to clean slides, and cultured for 3 days at 60° C on the slide warmer. Slides were 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 [2]. Chromosome preparations were treated with 0.2 N HCL for 1 hour at room temperature followed by a rinse with de-ionized water. The Slides were placed in a freshly prepared 5% aqueous solution of Barium hydroxide Octahydrate $[Ba(OH)_2 .8H_20]$ at 56°Ċ in water bath (WB) for about 2-5 minutes followed by rinsing with de-ionized water . Slides were incubated for 1hour 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 was photographed from each individual. To eliminate the variations in C-segment lengths in chromosomes 1, 9, and 16, the presence of heterochromatin variants was 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 1) total inversion, when the whole C-segment inversion is located in one arm; it was distinguished as total inversion. 2) 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 inversion was named as partial inversion. 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 [10].

Statistical Analysis
The difference between two the groups was analyzed with student's *t-test*, *chi*-square and *Fisher's* Exact test. *P-values* less than 0.05 were considered statistically significant. Data analysis was performed by SPSS (Version 11.5, Inc.USA).

RESULTS
This work has dealt with the proportion and analysis of constitutive heterochromatin polymorphism in chromosomes 1, 9, and 16 and the frequency of inversions in drug addicts (Table 1) as well as the healthy controls (Table 2). Constitutive heterochromatin variants of chromosome 1, were detected in 86.6% of addicts and 13.4% of the controls in the healthy group, the results revealed

significant statistical differences for variant heterochromatin between addicts and the control group (P=0.004). The differences were significant for chromosome 9: it was 94.1% in addicts and 5.9% in the control group (P=0.029). The differences were also near significant for chromosome 16 which was 91.9% in addicts, and 8.1% in the control group (P=0.052).

The frequency of partial and complete

inversion did not show any significant differences between the addicts and the control groups. The occurrence of chromosomal defects including, chromatid break (12 addicts), chromatid gap (8 addicts), and dysenteric (2 addicts) was higher in our observation. The incidence of marker chromosome, translocation and numerical abnormalities did not observer in either addicts or normal controls.

Table 1. Distribution of polymorphism in chromosome 1, 9 and 16 in Drugs Addicts

No: of Drug Addicts Individuals	Chromosomes with C-band of Drugs Addicts qh$^+$ / qh$^-$ variants			Partial Inversion			Complete Inversion		
				+	+	+	+	+	+
	1	9	16	1	9	16	1	9	16
93	16	9	11	3	4	3	-	-	-

Table 2. Distribution of polymorphism in chromosome 1, 9 and 16 in control population

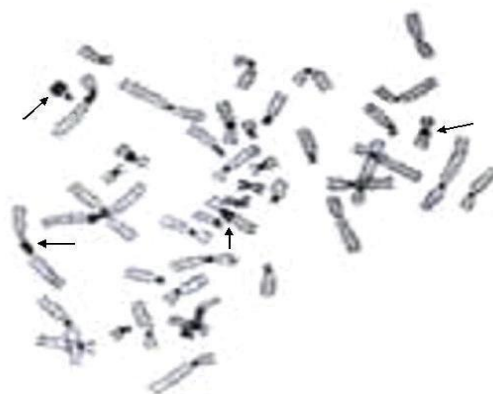| No: of control population	Chromosomes with C-band of Drugs Addicts qh$^+$ / qh$^-$ variants			Partial Inversion			Complete Inversion		
				+	+	+	+	+	+
	1	9	16	1	9	16	1	9	16
93	4	2	4	1	-	1	-	-	-

Figure 1. Arrow indicates Heterochromatin polymorphism in chromosome 1, 9, 16 and distal part of chromosomeɣ

DISCUSSION

Polymorphism of the size of heterochromatin regions of the human chromosomes have been documented in the literature [11]. Our results have shown that opiate, heroin and ….. drug addicts have higher heterochromatin variants in chromosomes 1,9 and 16 and other chromosome damages . These damages revealed such as break, gap and dicentrics are more pronounced in the addicts when we compared them with healthy controls. In the course of this study, an excess of heterochromatin polymorphism

of chromosome 1 was found in drugs addictscompared to the controls. This result is in agreement with previous investigators that suggested the role of heterochromatin variants of chromosome 1 and possible risk of developing malignancies [12, 13]. Furthermore, the results of this study exhibited an increase in C-band heterochromatin variants in chromosome 9 and 16 in addicts when compared to the controls.

These results were not in agreement with those of the other investigators who could

not find any correlation between C- band heterochromatin polymorphism of chromosome 16 and cancer and leukemia [14, 15]. Our C- banding studies provide an opportunity to better characterize some autosomal types, and to detect differences in heterochromatin polymorphism variants between the addicts chromosomes and those of healthy individuals'.

In this study frequency of partial and complete inversion was not found to be significantly higher in addicts. This result is in accordance with the research works of Leconiant and colleagues [16] who failed to demonstrate increased frequency of inversions between a group of malignancy and the controls group. In contrast to the present investigation, previous reports demonstrated greater frequency of inversion of C-band heterochromatin in chromosomes 1,9,16 in malignant patients [15, 17]. Therefore, exposure to these drugs and perhaps other environmental agents may cause heterochromatin variants and / or chromosomal damages. More data are needed in this field to draw firm conclusion.

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