Rev. Biol. Trop. 50(1): 347-353, 2002
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Chromosomal defects in 34 male homosexuals, half of them with HIV antibodies

Isabel Castro-Volio and Luisa Valle-Bourrette
Sección de Genética Humana, Instituto de Investigaciones en Salud (INISA), Universidad de Costa Rica, 2060 San José, Costa Rica. Fax (506) 207-5130; icastro@cariari.ucr.ac.cr

Recibido 13-III-2001. Corregido 21-VIII-2001. Aceptado 21-IX-2001.

Abstract: Most of the research about viral interactions with human chromosomes was done during the sixties and early seventies and very few was performed after the human immunodeficiency virus (HIV) appearance as an epidemic in the eighties. The objective of this work was to estimate if particular chromosomal changes follow the infection of homosexual males by HIV and to determine if the lifestyle, habits, sexual practices, of our sample of male homosexuals predisposes them to chromosomal abnormalities at a higher rate than the background level of cytogenetic damage the general population has. This was a double blinded case-control study, 17 individuals positive for HIV antibodies (HIV+) detected by enzyme-linked immunosorbent assay (ELISA) and confirmed by western blot (WB) were the cases, and 17 individuals negative for HIV antibodies (HIV-) the controls. These men were a very homogeneous population in terms of age, social status, lifestyles, drug abuse, sexual practices and education. Blood was collected between September 1988 and October 1989. Fresh whole blood was cultured in duplicate for 72 hr. Cell harvest followed conventional methods. Once all cell cultures were gathered, the tubes were picked up at random and air dried chromosome preparations were trypsin-giemsa banded (GTG) after overnight incubation at 60Cº. The percentage of gaps and breaks these men had was not different from the reported for the general population, nor were there significant difference among both groups (O.R. = 1.8) in terms of amount of chromosomal fragility. The distinction among them was at the level of the specific chromosomal sites where the gaps and breaks located, being sites at 2p21 and at 3p21 four times more frequent among HIV+. These probably represent viral modification sites on chromosomes which are known to look like non-staining gaps which are caused by the virus or viral products. This presumption is supported by an earlier report of repeated breaks at 3p21.1 , in fact this was the most common lesion site in this study of chromosomal aberrations of male homosexuals and the authors even considered the probability of “a new type of chromosome marker” . Furthermore, years later the *CKR5* structural gene was mapped to human chromosome 3p21. This gene codes for the chemokine receptor 5 (CKR5) protein which serves as a secondary receptor on CD4+ T lymphocytes for certain strains of HIV-1. It is possible that this gene was being transcribed in HIV+ men and the consequent “staggering” of DNA contributed to the production of gaps and breaks at 3p21.

Key words: AIDS, HIV, homosexuals, cytogenetics, chromosomes 2 and 3, Costa Rica.

Viral interactions with human chromosomes produce cytogenetic effects by both RNA and DNA viruses, by both transforming and tumorogenic viruses and by non transforming and non tumorogenic viruses (Nichols 1983). Much research in this field was done during the sixties and early seventies, reviewed by Makino (Makino 1975). Human chromosome damage caused by viral infection varies from single chromosome and chromatid breaks to rearrangements and total pulverization of the chromosome complement. Cells containing viruses also suffer alterations of the spindle and mitotic mechanism. A most remarkable finding of the viral cytogeneticists is the nonrandom distribution of chromosome breaks.

From the seventies to our date, the attention has focused mainly on oncogenic viruses, particularly at the molecular level (Heim and Mitelman 1991).

The human immunodeficiency virus (HIV), a cytopathic retrovirus, is the etiologic agent of the acquired immunodeficiency syndrome (AIDS). This fatal disease has spread since 1981 specially among homosexual males, intravenous drug abusers, recipients of HIV infected blood products and more recently among the general population. HIV infection produces depletion of T_4 lymphocytes resulting in profound immunosuppression that renders the body highly susceptible to opportunistic infections and neoplasms. AIDS virus isolation coincided with the "boom" of molecular genetics and the cytogenetic approach to the new pathogen has been restricted to a few studies of AIDS related tumours (Gyger *et al.* 1985, Delli-Bovi *et al.* 1986, Alonso *et al.* 1987, Offit *et al.* 1989, Sawyer *et al.* 1995). The specific chromosomal aberrations observed are not different from those expected according to the histological type of the neoplasm, since the AIDS virus role in malignancy is basically an indirect one through diminished cancer cell surveillance by the depressed immune system. To our knowledge, there is only one report of cytogenetic studies in AIDS performed in peripheral lymphocytes of 19 gay men probably infected with HIV or with AIDS related complex (ARC) or AIDS (Manolov *et al.* 1985). The authors observed various kinds of chromosomal structural aberrations among 12 healthy homosexual males and very little cytogenetic damage among the five ARC and two AIDS individuals. The virus carrier status of the healthy gays is not stated, although they point out they isolated a retrovirus in almost all gay men they studied from the population where this 19 homosexuals came from. The cytogenetic findings among this individuals are attributed to recreational drug exposure, viral infections or immunologic reactions to sperm which crossreacts with lymphocytes.

The AIDS epidemic has not spared Costa Rica and adequate clinical and laboratory diagnostic capacity, legislation, education of the general public and of high-risk groups have developed (Mata and Herrera 1988).

The objective of this work was to estimate if particular chromosomal changes follow the infection of homosexual males by HIV and to determine if the lifestyle, habits, sexual practices, of our sample of male homosexuals predisposes them to chromosomal abnormalities at a higher rate than the background level of cytogenetic damage the general population has.

MATERIALS AND METHODS

This is a doubled-blinded case-control study, 17 individuals positive for HIV antibodies (HIV+) detected by enzyme-linked immunosorbent assay (ELISA) and confirmed by western blot (WB) are the cases, and 17 individuals negative for HIV antibodies (HIV-) conform the controls. All 34 are apparently healthy homosexual men of about the same age, the average age of cases is 26 years old and that of controls is 28 years. Socioeconomic status of both groups is similar as well, middle to high level, and so are lifestyles. Most of this gays have an university degree or are studying to achieve it, attend discotheques and gay bars regularly, travel abroad and have contact with foreign homosexuals, work, study or both. Around 35% of them consume one or more drugs, particularly marihuana, cocaine, benzodiazepines, hallucinogen mushrooms and nitrites or "poppers" in that order of frequency. Their sexual practices are not different from those common among north american and european gays (Mata *et al.* 1988)

These men are a subsample of a bigger cohort of cases and controls recruited to detect infection and colonization by opportunistic and pathogenic microbes in the presence of HIV infection, through periodical (every three to four months) tests. During each visit they provided blood samples, faeces, sputum, urine and pharyngeal secretions to perform: hemoculture, Monotest for Epstein-Barr virus

detection, hepatitis B surface antigen (HbsAg) detection, V.D.R.L. test for syphilis diagnosis, coproculture and parasitological examination, *Pneumocystis carinii* was looked for in sputum smear and *Candida albicans* in sputum culture. Urine and pharyngeal secretions were used for viral culture. During their visit, these men were also interviewed concerning their education, sexual practices, habits, drug abuse, lifestyles, etc.

The subsample of 34 homosexual men were randomly assigned for the cytogenetic studies and provided an extra blood sample of 3ml of heparinized blood, collected between September 1988 and October 1989. All blood samples were collected and handled in the same way. Fresh whole blood was cultured in duplicate for 72 hr in 5ml McCoy's5A medium supplemented with 10% virus-free fetal bovine serum and 1% M-phytohemagglutinin (all reagents from GIBCO, same batch all). Cell harvest followed conventional methods, using 0.56% KCl as the hypotonic solution and methanol:acetic acid 3:1 as the fixative. After the third fixation, the cells were centrifuged and stored in the freezer for one to two years (after one year all samples were collected, and it took another year to complete the evaluation). Once all cell cultures were gathered, the tubes were picked up at random and air dried chromosome preparations were trypsin-giemsa banded (GTG) after overnight incubation at 60ºC. The samples and slides were coded and scored in such a way that the HIV antibody status of each man was unknown at the time of scoring. Assessment of all preparations was systematically done by one of us (L.V.) according to the same criteria: the slides were scanned at low magnification to select suitable cells for analysis. Once the cell was observed at high magnification, it was rejected only if overlapping of chromosomes or non-chromosomal material over it, prevented an accurate analysis and if the centromere number was different from 46 ± 2. Any cell considered aberrant by the permanent scorer, was confirmed by a second more experienced scorer (I.C.) and photographed. The goal was to analyze 50 cells per sample, but since this was not always possible, due to bad quality of some of the preparations, the average number of cells analyzed was 40 cells per sample with a range of 10 to 50. Although all preparations were banded, some under-digested cells prevented the precise localization of every lesion, in such cases, the assessment of the distribution of lesions in chromosomes was performed according to the method of Aula modified by Makino (Makino 1975). The banding resolution was around 400 bands and the international nomenclature was used (Anonymous 1978). Working definitions used for chromatid / chromosome lesions: break is the discontinuity of one chromatid / both chromatids of a chromosome with clear displacement of the resulting fragment; gap is a nonstaining region of a single chromatid / both chromatids without any apparent displacement of a chromatid / both chromatids.

RESULTS

The number of men, samples and cells studied in each group is shown in Table 1.

TABLE 1
Number of men, samples and cells studied

	HIV antibodies		
	HIV +	HIV -	Total
Homosexual men	17	17	34
Blood samples	34	23	58
Cells scored	1315	956	2271
One blood sample	5	12	
Two blood samples	8	4	
Three blood samples	3	1	
Four blood samples	1	0	

Chromatid and chromosome gaps and breaks were the usual lesions found in the population (Table 2). We detected only one case of pulverization in a HIV+ man.

To test for statistical difference in the proportions of abnormal metaphases and of the different types of aberrations, among the totality of

TABLE 2
Percentage of chromosome gaps and breaks in HIV+ and HIV- homosexual men

	Gaps*		Breaks*		HIV+**		HIV-**	
N observations	HIV+	HIV-	HIV+	HIV-	breaks	gaps	breaks	gaps
1	13.79	10.00	3.08	7.84	3.08	11.54	7.84	3.92
2	11.54	5.00	1.78	4.28	1.78	1.78	4.28	0.00
3	7.25	3.92	1.72	4.00	1.72	13.79	4.00	10.00
4	6.35	3.91	1.65	2.86	1.65	0.00	2.86	0.00
5	5.77	2.56	1.61	2.56	1.61	1.61	1.56	2.56
6	3.85	1.79	1.47	1.66	1.47	1.47	1.66	1.66
7	2.03	1.66	1.45	1.59	1.45	7.25	1.59	1.59
8	2.00	1.59	0.96	0.78	0.96	5.77	0.78	3.91
9	2.00	0.00	0.00	0.59	0.00	6.35	0.59	1.79
10	1.78	0.00	0.00	0.00	0.00	3.85	0.00	5.00
11	1.61	0.00	0.00	0.00	0.00	2.03	0.00	0.00
12	1.47	0.00	0.00	0.00	0.00	2.00	0.00	0.00
13	1.32	0.00	0.00	0.00	0.00	2.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	1.32	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	3.57	1.79	0.81	1.54				

* Figures in decreasing order of magnitud.
** Both gaps and breaks belong to the same individual.

cells scored in both groups of men, the Fisher's exact test was used and results shown in Table 3. Using McNemar's x^2 to test for chromosomal fragility in general, both subjects and controls again showed no statistically significant difference among them, as well as using the median test in a subset of ten HIV+ men and ten HIV- men, matched according to the number of analyzed cells of each one (the median of the ratio or the number of cells with gaps or breaks divided by the total of analyzed cells).

When examining the chromosomal sites of the gaps and breaks we found non-random distribution which correlated with the localization of common fragile sites on chromosomes, with fra(7)(q22) as the most common. However, we identified two frequent sites (four times more frequent than the rest of sites with fra 7q22 as the sole exception) almost exclusively of HIV+ men: one in (2)(p21) and the other in (3)(p21) (Table 4).

TABLE 3

Estimation of the strength of association among cytogenetic defects and the presence or abscence of HIV infection

				95% confidence intervals	
Class	HIV+*	HIV-**	O.R.	lower	upper
Gaps	54	23	1.73	1.06	2.89
Breaks	14	19	0.53	0.25	1.06
Total	68	42	1.18	0.80	1.77
Chromatid	48	33	1.06	0.67	1.67
Chromosome	20	9	1.62	0.74	3.76
Total	68	42	1.18	0.8	1.77

* Corresponds to number of lesions in the pool of 1315 cells.
** Number of lesions in 956 cells.

TABLE 4
*Chromosomal lesions in lymphocytes from male homosexuals according to HIV infection**

Chromosome number	Location of the lesion sites on the chromosomes	
	HIV +	HIV-
1	p21 q21 q22 q31 q32 q43	p21 p22 q21 q32
2	p26 q21(4x) q24 q3(2x)	p4 q23 q31(2x) q37
3	p21(4x) p23	p21 p26 q24 q26
5	q15 q31	q35
6	p23 q15 q26(2x)	p5 q2 q21 q22
7	q22	p22 q22(4x)
8	q23	q24
9	q34	p13 q32 q34
10		q24
11		q21
12		q21
15	q24	q15
16	q24	
17		p11
19	p13	

* Only those lesions identified by band and sub-band have been assigned.

DISCUSSION

The chromosomal fragility found in cases as well as in controls is not significantly different among them. Several questions arise now:

1. Is it different from the population background level of cytogenetic damage?
2. Does viral infection at high doses produce excessive T lymphocyte lethality which masks the damage?
3. Is it a time-response matter rather than a dose-response matter? In other words, is there an optimal time to take the blood sample which allows quantification of chromosomal consequences in the lymphocytes of the infected individual?

Probably the best report on normal spontaneous background frequency of chromosome aberrations in populations (Bender *et al.* 1988), reports a frequency of gaps and breaks of 5.48%, not significantly different from our figures of 4.38% for HIV+ men and 3.33% for HIV- ones (Table 2). However, they scored non-banded preparations and might have seen more gaps than we did.

Supposing phytohemagglutinin stimulates primarily uninfected lymphocytes since infected ones are scarce, the analysis is done in healthy lymphocytes from both HIV+ and HIV- men and thus their chromosomal fragility is about the same. Probably this is not the case, since we observed no alteration in the quantity of mitosis in any group.

Considering the optimal timing for sampling, two facts must be taken into account: first the incubation period of the virus is quite variable and usually large, from two to 12 years or more, and second, the long half-life of T lymphocytes is responsible for the persistence of chromosomal damage for years or even decades after cessation of exposure (Forni 1984). The mean time that elapsed from the date HIV+ men of this study were detected positive to the date of first sampling was eight months (range 0 to 2.7 years). It might be that our cytogenetic studies in this population were precipitated.

On the other hand, qualitative differences were exhibited regarding the sites of lesions in

chromosomes, one at 2p21 and another at 3p21. These are two sites which can be considered virus-associated markers or virus insertion sites. There are four categories of virus interaction with the human genome: (1) integration, (2) sensitivity, (3) regulation and (4) chromosome modification (Shows *et al.* 1987). Modification identifies site-specific morphological changes in human chromosomes resulting from virus infection. Viral modification sites on chromosomes are non-staining gaps (which can resemble fragile sites) which are caused by the virus or viral products (Sutherland and Mattei 1987).

This presumption is supported by the report of repeated breaks at 3p21.1, in fact this was the most common lesion site in the study of chromosomal aberrations of male homosexuals and the authors even considered the probability of "a new type of chromosome marker" (Manolov *et al.* 1985). Furthermore, years later the *CKR5* structural gene was mapped to human chromosome 3p21. This gene codes for the chemokine receptor 5 (CKR5) protein which serves as a secondary receptor on CD4+ T lymphocytes for certain strains of HIV-1 (Dean *et al.* 1996, Samson *et al.* 1996, Cheng-Mayer *et al.* 1997, Maho *et al.* 1999). It is possible that this gene was being transcribed in HIV+ men and the consequent "staggering" of DNA contributed to the production of gaps and breaks at 3p21. Concerning the fragile site at 2p21 we report, a recent study of HIV proviral integration in chromosomes of persistently infected cells, shows 2p21 as the most frequent proviral integration site (Glukhova *et al.* 1999). These results were achieved through precise localization of fluorescent signals on the chromosomes using fluorescent *in situ* hybridization (FISH). Moreover, this area is frequently involved in cytogenetic alterations in human cancers (Kirschner *et al.* 1999).

A prospective design in which the subject is studied before and after the infection takes place, thus becoming the case and his own control, is probably the best choice to reach the study objectives. However, the population needed for this kind of studies has to be bigger since not all subjects are going to get infected, the risk of loosing cases is high considering the long time this infection might take to occur, and another drawback is that cytogenetic studies are costly, since they are time consuming and require the work of skilled personnel.

ACKNOWLEDGMENTS

This work received financial support from the Vicerrectoría de Investigación - University of Costa Rica- through project Nº 742-90-047.

RESUMEN

En este estudio se determinaron las consecuencias citogenéticas de la infección con el virus de inmunodeficiencia humana (VIH), mediante un estudio tipo doble-ciego de casos infectados o VIH+ (N = 17) y controles VIH- (N = 17), en 34 hombres de una población muy homogénea de homosexuales, estudiada de 1988 a 1991. Se encontró fragilidad cromosómica no diferente del porcentaje informado para la población general ni diferente entre casos y controles (O.R. = 1.18). Las diferencias entre ambos grupos fueron más bien cualitativas, en los sitios de lesión cromosómica, ya que los hombres infectados mostraron cuatro veces más lesiones en dos sitios en particular: 2p21 y 3p21. Este último coincidió con el sitio de fragilidad más común informado con anterioridad. Este sitio en 3p21 fue posteriormente indentificado como el locus cromosómico donde se ubica el gen CKR5 el cual codifica para un receptor secundario en el linfocito T del VIH -1. Se propone que este gen se estaba transcribiendo activamente en los hombres infectados en los que se evidenciaron lagunas o fracturas cromosómicas en 3p21.

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