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Chromosome Aberrations Study in Human Lymphocytes from Marijuana Smokers

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One of the main problems affecting our society nowadays is drug consumption. Hemp derivatives (*Cannabis sativa)* are the drugs most commonly abused by the young Chilean population. This product is obtained from the leaves and dried up flowers, they contains the active product, tetrahydrocannabinol, which is five to six times lower in concentration than that found in hashish (Florenzano et al. 1982).

In Chile, there is easy access to marijuana. In the central zone of the country there are extensive plantations due to the industrial use of hemp fiber. The consumption of marijuana is prohibited by law, and the epidemiology of the use of this drug is only obtained by indirect information (Medina 1989).

An investigation carried out between 1968-1970 showed that from 734 students of secondary education, 6.96% smoked marijuana.(Gomberoff et al. 1972) A study done in 1981, demonstrated that out of 1,240 secondary education students surveyed;17% had smoked marijuana and 7.3% continued using it (Florenzano et al. 1982). In 1989 a survey applied to young people of between 12-19 years of age showed that about 40-50% of the adolescents had at some time, consumed marijuana (Medina 1989). These data show a striking increase in the consumption of this drug among the Chilean young population. Considering these facts we planned to establish if marijuana has some toxic effects on the genetic material. For this purpose the frequency of Chromosome Aberrations (CA) was evaluated in a group of university students.

MATERIALS AND METHODS

The study group contained a total of 45 subjects. The control group (CG) had 15 individuals (10 females and 5 males), with an average age of 24.5 years. The marijuana consumers was a group (MCG) of 30 individuals (21 male and 9 females), with an average age of 22.7 years. The average consumption time was 5.6 years (1-12 years) with a consumption of 4.2 marijuana cigarettes a week.

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The last date of consumption was in average 4.6 days. Before the cytogenetic analysis all the individuals answered a questionnaire about personal data, smoking and drinking habits, (classified as 1: non consumer; 2: they consumed less than 600mL (3 units) of beer a week; 3: they consumed around 600 and 1000 mL. of beer a week) drugs and medications used, health status, medical history and exposure to known genotoxic agents. Individuals with similar alcohol and smoking habits ware selected.

Venous blood was taken from each subject using heparinized vacutainer tubes. Cultures were set up by adding 0. 5 ml whole blood in 10 ml RPMI 1640 (Gibco) supplemented with 20% heat inactivated fetal calf serum (FCS) (Gibco) antibiotics, (penicillin and streptomycin) and glutamine. Lymphocytes were stimulated by adding l% phytohaemogglutinin (Gibco). Duplicates were setup for each subject. The cultures were incubated for 48 hr at 37° C. One our before harvesting Colcemide (Gibco) was added to arrest cells in metaphase. The cells were collected by centrifugation, resuspended in hypotonic solution at 37°C (0.075M KCI) for 20 min and fixed in methanol: acetic acid (3: 1 v/ v). Air-dried preparations were made and the slides were stained with 4% Giemsa.

A total of 100 well spread metaphases containing 46 chromosomes were examined blindly for each donor on coded slides. The structural aberrations analyzed included chromatid and chromosome-type (a: acentric fragments, f: fragments, b: breaks, del: deletions, dic: dicentrics, and e: exchange between non sister chromatids breaks). Gaps were scored, but not quantified in the statistical analysis because under the light microscope it is difficult to distinguish chromosome breaks from the achromatic regions (Garcia et al.1993). Cells containing structural altered chromosomes were classified as aberrant cells. The data was analyzed using CSS: Statistical WTM (1993). The differences between groups were evaluated for t-test ($p<0.05$).

RESULTS AND DISCUSSION

The analysis of CA was carried out in a total of 45 individuals selected from a bigger group of university student. MGC and CG group had similar smoking and drinking habits (Tables 1 and 2). In the CG of 15 individuals, 40% drank alcohol and 53.3 % smoke tobacco, with an average of 3.8 cigarettes per day. In the MCG of 30 individuals, 42.2% drank alcohol and 83.3 % smoked tobacco with an average of 5.2 cigarettes per day. There are not significant differences in both group with respect to drinking and smoking ($p>0.05$).

The statistical analysis of the percentage of aberrant cells in the CG (mean:2.03) did not show significant differences when comparing it with the MCG (mean:2.8). There are no differences in the type of CA in both groups mainly breaks and fragments were found (Table 3).

Concerning the genotoxic action of the marijuana, the literature does not give information about genotoxic effects, except for Chiesara et al. (1983) who described an increase CA frequency in addicts to marijuana and heroine. Vassiliades et al. (1986) working with 14 addicts to the marijuana, with average age of 28.9, found a significantly higher frequency of SCE in the study group when compared to the control group, so much in consumers of cannabis as in the consumers of heroine. This research did not show an increase of CA in marijuana smokers' peripheral lymphocytes. It is interesting to point out that the exposed group only consumes marijuana, tobacco and small amounts of alcohol, and not other illicit drugs.

Table 1. Frequency of chromosome aberrations in control group

Case N°	Sex	Age (yr)	Tobacco Smoking (d)	alcohol intake	CA (%)
1	f	20	5	1	4
2	f	22	0	1	2
3	m	23	0	1	4
4	m	25	4	2	4
5	m	23	2	2	4
6	f	22	0	2	3
7	f	27	0	1	0
8	f	22	20	1	2
9	f	30	0	2	3
10	f	22	5	1	1
11	m	22	0	1	0
12	m	29	10	1	2
13	f	30	1	2	2
14	f	28	0	1	2
15	f	22	10	2	2
Mean		24.50	3.8		2.03
(SEM)		3.3	5.5		1.3

Alcoholic habits 1:non consumers;2: <3 units/week; 3>3 units/ week.
SEM: Standard Error Mean

Table 2. Chromosome aberrations from marijuana consumer group

Case	Sex	Age (yr)	Duration of Exposure (yr)	Last Consumption (d)	Frequency Consumption (wk)	Smoking habits (d)	Alcohol intake	CA 100 cells
1	m	28	5	1	7	10	2	4
2	m	22	4	5	3	2	1	4
3	m	23	8	4	3	6	1	4
4	f	22	2	30	1	2	2	4
5	f	23	2	15	1	0	1	8
6	m	22	4	1	7	2	3	0
7	m	22	3	1	7	10	2	6
8	m	26	5	3	2	4	2	0
9	f	24	7	4	2	3	2	4
10	f	26	10	4	1	1	2	4
11	f	23	6	3	6	0	1	2
12	m	21	8	1	4	10	2	2
13	m	19	3	14	4	20	2	4
14	m	18	4	1	2	3	2	0
15	m	21	6	4	7	10	1	4
16	m	19	1	4	7	0	1	4
17	m	25	8	1	8	2	2	0
18	m	19	6	1	5	8	2	2
19	m	19	6	4	5	3	3	4
20	m	18	4	1	6	6	1	0
21	m	24	12	1	4	6	2	0
22	m	18	4	1	2	1	2	0
23	f	25	2	1	1	1	1	0
24	m	26	10	1	5	20	2	4
25	m	28	10	1	6	5	2	0
26	f	26	9	1	2	10	2	6
27	m	19	7	4	7	10	2	8
28	f	23	6	20	2	0	1	2
29	f	30	4	2	5	1	1	2
30	m	23	4	6	4	0	1	2
Mean		22.7	5.6	4.6	4.2	5.2		2.8
SEM		3.2	2.7	6.4	2.2	5.2		2.3

Alcoholic habits 1:non consumers;2: <3 units/week; 3>3 units/ week. SEM :Standard Error Mean

Table 3. Types and frequencies of chromosome aberrations observed in the lymphocytes of 30 marijuana consumers and 15 controls

Group	N° of subjects	N° of cells scored	No of cells with CA	Chromosome type b	del	ace	dic	Chromatic type b	del	e
Exposed	30	3000	85	26	6	4	4	37	4	4
Control	15	1500	35	8	4	0	0	22	1	0

Ace: acentric fragments; b: breaks; del: deletions; dic: dicentrics; e: exchange between non sister chromatids.

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