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NERVE CELL DEPOPULATION IN CHRONIC CHAGAS' DISEASE. A QUANTITATIVE STUDY IN THE CEREBELLUM

Hugo J. Silviano BRANDÃO (1) and Ronaldo ZULIAN (2)

SUMMARY

A Purkinje cell counting was done in cerebellar samples taken from adult chagasic and non-chagasic individuals. This quantitative study revealed a neuronal depopulation in 52 per cent of the chagasic cases studied. The numerical Purkinje cell reduction presents a wide range of variation from slight to severe cell destruction.

INTRODUCTION

The "nervous form" of the American trypanosomiasis was described by Chagas himself[6, 7], who stressed the fact that the disease may be responsible for the greatest number of organic affections of the central nervous system in man. At that time and further investigations confirmed the central nervous system involvement in this disease [22, 23, 25]. A "chronic nervous form", howerer, is denied by most of the Authors today, and except for some few papers in the literature pointing out its existence[3, 17, 18], and some experimental studies, nothing was done since Chagas.

A depopulation of ganglion cells of the peripheral autonomic nervous system can be found in chagasic patients[5, 8, 11,12, 13, 14, 19]. This has also been observed in experimentally infected animals[1, 2, 4, 9], and in naturally infected dogs and cats[15]. A striking relation between tissular parasitism and nerve cell lesions was experimentally observed in the acute phase of the disease[16], and this neuronal damage was shown to exist even before any others changes could be demonstrated.

Spinal cord involvement with neuronal destruction was observed in chagasic rats in the chronic[21], and in the acute phase[24], of the disease. A severe depopulation of Purkinje cells was shown during the acute phase in the mouse[10].

It can be concluded that many evidences point out that Chagas' disease affects essentially the nervous system both central and peripheral. So, the chance for the nerve cell destruction in the human chephalic-medullar axis, in chronic Chagas' disease, is prone to be proved. The present investigation intends to check this hypothesis through a quantitative study on the Purkinje cells of human individuals with chronic Chagas' disease. The choice for the Purkinje cells comes from its regular distribution making such a counting easier.

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From the Department of Pathology (Prof. F. Köberle), Ribeirão Prêto, Faculdade de Medicina, São Paulo, Brasil.

(1) Assistent Professor

(2) Academic Monitor

MATERIAL AND METHOD

Forty human cerebellums of adults of both sexes were used in this study, fifteen non-chagasic (control group), and twenty-five chagasics. In this first group twenty-four were from patients in the chronic phase and one in the acute phase (case 5). The control group consisted of cerebellums of patients who had not shown Chagas' disease manifestation, both clinically or morphologically at post-mortem examination, and who had negative MACHADO & GUERREIRO reaction when alive and after death. Table I shows this group with the respective data. The chagasic group is presented in Table II which shows besides age and sex, the titers of the MACHADO & GUERREIRO reaction and the main Chagas' disease manifestations; not all the chagasic cases displayed organic changes of the disease (cases 4, 12 and 24). The brain was removed and preserved "in totum" in a 10 per cent formalin solution for at least 24 hours. Afterwards the cerebellum was cut, both hemispheres samples being taken, including the superior and the inferior semilunaris lobes. After paraffin embedding one 7-micra-thick section was taken from each sample and H. and S. stained.

The Purkinje cells were counted in consecutive microscopic fields through the straight segments of the intermediate layer, excluding the curvatures. The cellular quantitative study was done in two parts, In the first, the Purkinje cells of 21 chagasic and 11 non-chagasic cerebellums were counted using 80 X magnification which corresponds to a microscopic field diameter measuring 1,470 micra; one hundred fields were examined from each hemisphere in this series that corresponds to a linear extention of 147 millimeters in each side. In the second part of the counting, the eight remainding cases (four chagasic and four controls) were counted using 160 X magnification which corresponds to a microscopic field of 735 micra diameter, also counting the cells of one hundred consecutive fields which corresponds to a linear extention of 73.5 millimeters. The final results in each case were taken in cell number per linear millimeter. The counting was performed by the two Authors who ignored the case identification (if chagasic or not) at the moment of the counting.

TABLE I

Control Group

Cases	Autopsy	Age (y)	Sex	Diagnosis
1	A-163/64	66	♂	Diabetes mellitus. Pulmonary embolism
2	A-315/64	30	♂	Neurocysticercosis
3	A-196/64	63	♂	Leukemia. Encephalic arteriosclerosis
4	A-338/64	24	♀	Bronchopneumonia
5	A-168/64	27	♂	Rheumatic heart disease
6	A-108/64	38	♂	Adrenal hemorrhage. Essential hypertension
7	A-159/64	63	♂	Carcinoma of the gall bladder. Arteriosclerosis
8	A-116/64	57	♂	Amebiasis. Peritonitis
9	A-105/64	32	♂	Acute pulmonary edema. Arterial hypertension
10	A-189/64	67	♂	Bronchopneumonia. Arteriosclerosis
11	A-117/63	30	♀	Neurocysticercosis
12	A-223/64	34	♀	Tracheoesophageal fistula. Bronchopneumonia
13	A-186/64	38	♂	Acute pulmonary edema. Arterial hypertension
14	A-216/64	24	♂	Typhoid fever. Peritonitis
15	A-160/64	23	♂	Ancylostomiasis. Anemia

TABLE II

Chagasic Group

Cases	Autopsy	Age (y)	Sex	MG	Diagnosis
1	4-156/64	66	♂	2.8	Chagasic cardiopathy. Megacolon. Megaesophagus
2	A-328/63	34	♂	2.3	Chagasic cardiopathy
3	A-212/64	50	♂	2.6	Chagasic cardiopathy
4	A-204/64	60	♂	2.0	Bronchopneumonia *
5	A-330/64	40	♀	2.3	Chagas' disease (acute fase)
6	A-308/64	58	♂	3.0	Chagas' disease. Megacolon
7	A-483/64	42	♂	3.0	Chagasic cardiopathy
8	A-233/63	31	♂	2.8	Chagasic cardiopathy
9	A-274/63	53	♀	3.0	Chagasic cardiopathy
10	A-301/64	52	♂	2.1	Chagasic cardiopathy. Megacolon
11	A-261/63	35	♂	2.3	Chagasic cardiopathy
12	A-495/63	33	♂	1.9	Hodgkin disease *
13	A-188/63	48	♂	2.6	Chagasic cardiopathy
14	A-359/63	37	♀	3.0	Chagasic cardiopathy
15	A-236/63	47	♀	2.6	Chagasic cardiopathy
16	A-346/63	51	♂	2.5	Chagasic cardiopathy
17	A-217/64	58	♀	2.3	Chagasic cardiopathy. Megaesophagus
18	A-104/64	60	♀	2.6	Chagas' disease. Megacolon
19	A-227/64	42	♀	2.6	Chagas' disease. Megacolon
20	A-157/64	34	♂	3.0	Chagasic cardiopathy
21	A-319/64	24	♀	3.0	Chagasic cardiopathy. Cirrhosis
22	A- 41/64	40	♀	2.6	Chagasic cardiopathy
23	A-218/64	52	♂	3.0	Chagasic cardiopathy. Megaesophagus
24	A-551/63	19	♀	1.9	Lymphosarcoma *
25	A-472/64	33	♀	2.8	Chagasic cardiopathy

* Cases without morphologic evidences of Chagas' disease

RESULTS

About 50,400 Purkinje cells were counted in this work. Table III shows the results of the non-chagasic group. It can be seen that in all but one case of this group the righ recebellar hemisphere showed numerically more Purkinje cells than the left one. The difference between the right and the left means was submited to the "t" significance test of Student and noted to be statiscally significant ($P < 0.01$); in this group the mean value (the mean of the whole group) was found to be 5.75 Purkinje cells per millimeter with a standard deviation (s) of 0.41 which gives the range limits of 6.61 and 4.89 cells ($\bar{x} \pm 2.1$ s, considering 14 degrees of freedom and 5 per cent the critical level). An average of 8.48 cells with range of 0 to 20 cells were found per microscopic 80 X magnification field. The chagasic group (Table IV) presents a general mean of 4.63 Purkinje cells per millimeter and Fig. 2 stresses the difference between the cell population in the two groups, showing a cellular depopulation in the chagasic one (the "t" test of Student for diference between means revealed $P < 0.02$, i.e., statiscally significant). Thirteen chagasic cases (52 per cent) presented the mean below the inferior limit of the control range. In the chagasic group no difference was noted between the right and the left means. Some chagasic cases showed a severe depopulation which was found to be variable; not all cases showed depopulation. In one case (case 1) an extreme depopulation was verified and a diffuse cortical atrophy of the molecular and granular layers without glial reaction.

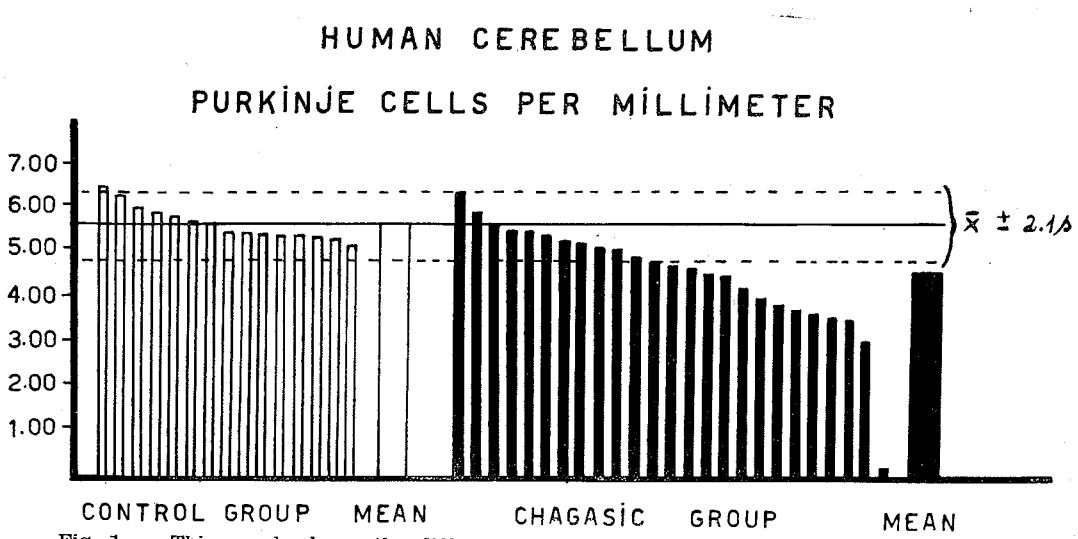

Fig. 1 — This graph shows the difference between the Purkinje cell population in the non-chagasic and chagasic groups. It can be noted cellular depopulation in thirteen chagasic cases (52%), the means of which are below the inferior control range limit.

DISCUSSION

The control group used in this work does not constitute a uniform sample considering sex, age, and specially the pathological changes found in these brains, which can be considered implicated with neuronal damage, such as arteriosclerosis, cerebellar leukemic infiltration, severe and longstanding anemia, hypertensive disease, and cerebral hypertention by neurocysticercosis. However, through the quantitative analysis used, the Purkinje

TABLE III

Purkinje cells per millimeter
Control Group

Cases	Autopsy	Right	Left	Mean
1	A-163/64	5.84	4.65	5.24
2	A-315/64	5.40	5.40	5.40
3	A-196/64	5.48	5.46	5.47
4	A-338/64	5.78	5.22	5.50
5	A-168/64	5.80	5.21	5.50
6	A-108/64	5.96	5.16	5.56
7	A-159/64	5.67	5.46	5.56
8	A-116/64	6.11	5.06	5.58
9	A-105/64	5.85	5.72	5.78
10	A-189/64	5.97	5.63	5.80
11	A-117/64	6.01	5.84	5.92
12	A-223/64	6.28	5.68	5.98
13	A-186/64	6.41	5.82	6.11
14	A-216/64	6.54	6.23	6.38
15	A-160/64	7.20	5.95	6.57
	MEAN	6.02	5.49	5.75

TABLE IV

Purkinje cells per millimeter
Chagasic Groups

Cases	Autopsy	Right	Left	Mean
1	A-156/64	0.17	0.14	0.15
2	A-328/63	3.02	3.22	3.12
3	A-212/64	3.65	3.59	3.62
4	A-204/64	3.59	3.70	3.64
5	A-330/64	4.38	3.13	3.75
6	A-308/64	3.93	3.71	3.82
7	A-483/64	3.98	3.95	3.96
8	A-233/63	4.08	4.15	4.11
9	A-274/63	4.20	4.53	4.36
10	A-301/64	4.65	4.62	4.63
11	A-261/63	5.00	4.28	4.64
12	A-495/63	4.89	4.69	4.79
13	A-188/63	5.07	4.65	4.86
14	A-359/63	4.80	5.00	4.90
15	A-236/63	4.96	5.09	5.02
16	A-346/63	5.23	5.14	5.18
17	A-217/64	5.52	4.95	5.23
18	A-104/64	5.18	5.55	5.36
19	A-227/64	5.43	5.37	5.40
20	A-157/64	5.55	5.37	5.46
21	A-319/64	5.51	5.68	5.59
22	A- 41/64	5.50	5.82	5.66
23	A-218/64	6.04	5.31	5.67
24	A-551/63	5.91	6.19	6.05
25	A-472/64	6.47	6.36	6.41
	MEAN	4.62	4.56	4.61

cell population showed in this group mean values quite constant regardlees of the pathological changes pointed out, making this sample uniform to the point of being an actual control group for the planned investigation. The method used, through deficient in demonstrating small populational differences, which certainly occur in the non-chagasic group, proves to be adequate for demonstrating the statiscally significant difference between the population means of the two groups, pointing out the depopulation of Purkinje cells in the chagasic group. Figure 1 shows thirteen chagasic cases (52 per cent) the means of which are below the inferior control range limit, but almost all theses cases exhibit only a slight cellular depopulation.

Nerve cells lesions and destruction were shown in the acute phase of Chagas' disease[6, 7, 22]. The tissue parasitism may be heavy in this phase, becoming mild in the subsequent ones. Parasites in tissues are extremely difficult to find in the chronic phase. According to KÖBERLE[12], the fate of the chagasic patient, as concerned to further manifestations of the disease, is decided during the acute phase because it is in this phase that occurs the major destruction of nerve cells. This postulate was confirmed by ALCÂNTARA[1] who found the greatest cardiac ganglion cells depopulation in chagasic rats in the acute phase as compared to chronic one. JARDIM[10] showed a severe Purkinje cells depopulation in the acute phase in the mice experimentally, infected with *Trypanosoma cruzi*. Therefore the believe that the Purkinje cells depopulation found in the present investigation could be explained by the nerve cell destruction that occured in the acute phase of the disease. Thus nerve cell depopulation is a "reliquat" of the acute phase of the Chagas' disease. Other parts of the central nervous system might be affected in the same way.

RESUMO

Redução do número de células nervosas, na doença de Chagas crônica. Estudo quantitativo realizado no cerebelo

Foram praticadas contagens de células de Purkinje em amostras padronizadas de cerebelos de indivíduos não chagásicos e chagásicos. Observou-se redução numérica celular em 52% dos casos chagásicos. Esta deprivação neuronal mostrou grande variação quantitativa de caso para caso, sendo geralmente pouco acentuada, havendo casos com grande redução.

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