Investigation of chromosomal alterations in patients with Alzheimer's disease in the state of Amazonas, Brazil

Investigação de alterações cromossômicas em pacientes com doença de Alzheimer no estado do Amazonas, Brasil

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ABSTRACT

Alzheimer's disease (AD) has as its main characteristic the deterioration of cerebral functions. Its etiology is still complex and undefined despite the progress made in understanding its neurological, infectious, biochemical, genetic and cytogenetic mechanisms. Considering this, the aim of this study was to investigate the presence of chromosomal alterations in the peripheral blood lymphocytes, and to verify if there was a high frequency of these alterations in patients diagnosed with AD at the University Hospital Getúlio Vargas Outpatient Clinic Araújo Lima in Manaus, Amazonas, Brazil. Among the nine patients in the AD group, only one patient did not have metaphases with chromosomal alterations (2n = 46,XX), while eight patients with AD showed numerical chromosomal alterations, classified as X chromosome aneuploidy (2n = 45,X) and double aneuploidy (2n = 44,X,-X,-10; 2n = 44,X,-X,-13 and 2n = 44,X,-X,-21). In the control group, no chromosomal changes were found in the karyotypes of these individuals. Therefore, the karyotypes of patients with AD undergo chromosomal alterations at different levels. These findings are being described for the first time in the population of Amazonas, and they highlight the importance of the inclusion of cytogenetic investigations in the routine management of patients with AD.

Keywords: Alzheimer's disease; genetics; chromosome detetion.

RESUMO

Doença de Alzheimer (DA) tem como principal característica a deterioração das funções cerebrais. Quanto a sua etiologia ainda é complexa e indefinida, apesar do progresso alcançado na compreensão de seus mecanismos neurológicos, infecciosos, bioquímicos, genéticos e citogenéticos. Considerando isto, nós investigamos a presença de alterações cromossômicas nos linfócitos de sangue periférico e verificamos se há uma alta frequência dessas alterações em pacientes já diagnosticados com doença de Alzheimer no Hospital Universitário Getulio Vargas / Ambulatório Araújo Lima, Manaus / Amazonas / Brasil. Assim, dos 09 pacientes do grupo DA, somente 01 paciente não apresentou metáfases com alterações cromossômicas (2n = 46,XX) enquanto que 08 pacientes com DA apresentaram alterações cromossômicas numéricas, sendo classificadas como aneuploidia do cromossomo X (2n = 45,X) e aneuploidia dupla (2n = 44,X,-X,-10; 2n = 44,X,-X,-13 e 2n = 44,X,-X,-21). No grupo controle, não foram encontradas alterações cromossômicas nos cariótipos desses indivíduos. Estes achados para a população do Amazonas/ Brasil estão sendo descritos pela primeira vez. Os cariótipos de pacientes com DA sofrem alterações cromossômicas em diferentes níveis e demonstraram a importância das investigações citogenéticas no manejo rotineiro de pacientes com DA.

Palavras-chave: Doença de Alzheimer; genética; deleção cromossômica.

One of the most common types of dementia among older adults is Alzheimer's disease (AD). The main characteristic of this disease is the deterioration of cerebral functions, with the occurrence of memory loss, which can irreversibly progress, affecting the motor functions, even leading to the death of the individual[1]. Neuropathological alterations include mainly

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the formation of neurofibrillary tangles and alterations in the amyloid metabolism, causing synaptic loss and neuronal death[1,2]. The etiology of AD is still considered complex and undefined, despite the progress made in the understanding of its biochemical and genetic mechanisms[3,4]. Genetic studies have highlighted four genes responsible for AD: *amyloid precursor protein*, *presenilin-1*, *presenilin-2* and *apolipoprotein E*[5].

Cytogenetic analyses in patients diagnosed with AD have demonstrated a significant increase in numerical chromosomal alterations in different cell types of these patients[6]. Subsequent studies have also shown numerical chromosomal alterations related to the X chromosome, indicating a characteristic to be evaluated in these patients[7,8,9]. To date, there is no cytogenetic information available about patients with AD in the state of Amazonas, Brazil. The genes associated with AD have already been chromosomally mapped. The *apolipoprotein E* gene is located on chromosome 19q13.32, the *presenilin-1* gene on chromosome 14q24.2, the *presenilin-2* gene on chromosome 1q42.13 and the *amyloid precursor protein* gene is located on chromosome 21q21.3[10,11,12].

Accordingly, the aim of this study was to perform cytogenetic analyses in nine patients diagnosed with AD, as well as in nine healthy individuals, attending the University Hospital Getúlio Vargas Outpatient Clinic Araújo Lima in Manaus, Amazonas, Brazil. Conventional cytogenetic markers (conventional staining and G-banding) were used to investigate the presence of chromosomal alterations in peripheral blood lymphocytes and to verify whether there was a high frequency of these alterations in AD patients of Manaus.

METHODS

The study was approved by the Research Ethics Committee of the Amazonas State University, under the authorization number 2.456.885 (CAAE: 66630616.6.0000.5016). The individuals studied were referred by doctor Talísia Vianez from the Department of Neurology at the University Hospital Getúlio Vargas Outpatient Clinic Araújo Lima. The cohort included nine individuals (one man and eight women), aged between 62 and 85 years, who had previously been diagnosed with AD, for at least three years; and nine healthy individuals (control group, one man and eight women) of the same age group. The purpose of the research was explained to all patients, and all signed the Informed Consent Term, as required by the National Health Council. All the patients were submitted to the Mini-Mental State Examination (MMSE) to evaluate their cognitive functions[13]. The MMSE scores were used to classify the cognitive impairment of the patients into three levels: 24–30 = no cognitive impairment; 18–23 = mild cognitive impairment; and 0–17 = severe cognitive impairment[14].

Cytogenetic analyses were performed at the Laboratory of Cytogenetics of the Amazonas State University. Metaphase mitotic chromosomes were obtained from lymphocyte culture[15]. G-banding was obtained using trypsin solution[16]. Metaphases were karyotyped using the GeneALL program, and the chromosomal alterations were classified according to the International System for Human Cytogenetic Nomenclature.

RESULTS

The characteristics of the AD patients (age, MMSE test scores and diagnosis year) and the chromosomal alterations found in these patients are shown in the Table. Based on MMSE scores, eight patients were in the category of severe cognitive impairment (0–17 points), of whom two did not score any points; and one patient was in the category of mild cognitive impairment (18–21 points).

A total of 100 metaphases were analyzed from each AD patient and individual in the control group. Among the nine patients of the AD group, only one (B-09) did not have metaphases with chromosomal alterations (2n = 46,XX; Figure 1A). The other eight showed metaphases with numerical chromosomal alterations related to the loss of one of the X chromosomes (2n = 45,X), as well as with the concomitant loss of a X chromosome and an autosomal chromosome (2n = 4,X,-X,-10; 2n = 44,X,-X,-13 and 2n = 44,X,-X,-21) (Figure B, C). Chromosomal aneuploidies identified in the AD group were classified as X chromosome aneuploidy and double aneuploidy (loss of one X chromosome and autosomes) (Table). In the control group, all individuals underwent the same process of chromosomal analysis and no chromosomal alterations were found in their karyotypes.

DISCUSSION

Alzheimer's disease is a chronic neurodegenerative disorder, which compromises cognitive functions and the individual's behavior, social and professional life[17]. Chromosome studies in AD patients have shown the presence of chromosomal alterations, as well as a high frequency of these changes in the karyotypes of patients with AD, suggesting that these alterations may be associated with the disease[18,19,20].

Chromosomal alterations of aneuploid type in AD patients have been reported in the different cell types, suggesting that there is a systemic chromosome instability in AD. In the neuronal cells in AD patients, aneuploidy potentially contributes to functional decline and predisposition to the disease, and a two-fold increase in X chromosome aneuploidy rates has been shown in neural cells of the hippocampus and cerebrum, which are dramatically affected by neurodegeneration[9]. Non-neural cells (peripheral blood lymphocyte cells, buccal cells and fibroblast tissue) were isolated from AD patients and showed increased aneuploidy X chromosome and autosomal chromosomes[21]. Chromosome instability
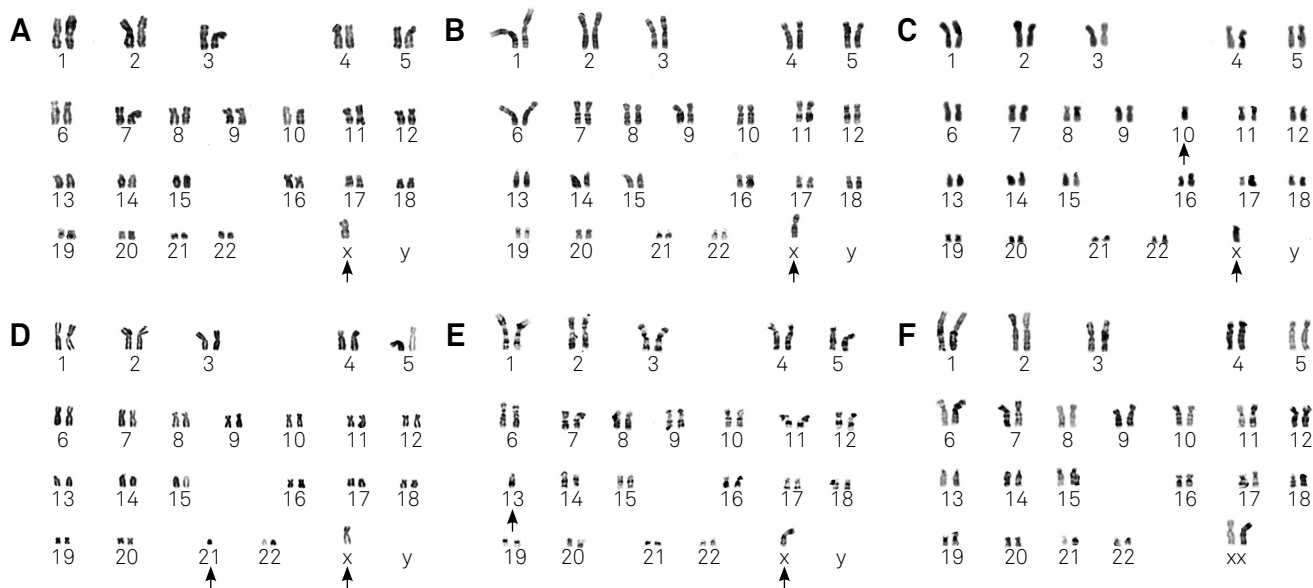
Figure. Karyotypes of patients diagnosed with Alzheimer's disease: (A and B) loss of one homologue of the sex chromosome X; (C) loss of one homologue of the chromosomal pair 10 and the sex chromosome X; (D) loss of one homologue of the chromosomal pair 21 and the sex chromosome X; (E) loss of one homologue of the chromosomal pair 13 and the sex chromosome X; (F) Patient without chromosomal alteration.

Table. Characteristics of the patients in the Alzheimer's disease group. Age, the scores of the MMSE test, year of diagnosis of the disease, chromosomal alterations of patients with Alzheimer's disease.

Patient	MMSE score	Age	Year of diagnosis	Aneuploidy		
				Monosomy of X chromosome	Double monosomy	n
B-01	6	71	2012	45,X	-	5
B-02	0	79	2010	45,Y	-	4
B-03	0	79	-	45,X	-	3
B-04	8	86	2011	45,X	-	3
				-	44,X,-X,-10	1
B-05	16	67	2013	45,X	-	5
B-06	5	79	2010	-	44,X,-X,-21	1
B-07	21	62	-	45,X	-	1
				-	44,X,-X,-13	1
B-08	12	74	2011	45,X	-	1
B-09	14	69	-	-	-	-

MMSE: Mini-Mental State Examination; N = number of cells found in the total of 100 cells analyzed. For data not observed, the symbol "-" was used.

results in loss or gain of chromosome in different cells and tissue as well as harmful effects on organism physiology, and is considered a risk factor for AD neuropathogenesis[21,22].

Cytogenetic analyses performed in the present study with patients diagnosed with AD revealed that only one patient did not show any numerical and/or structural chromosomal alterations (2n = 46,XX). On the other hand, the presence of aneuploidies was observed in the karyotypes of eight patients diagnosed with AD (2n = 45,X), which corroborates the literature data available for other localities[7,8,9]. When comparing these results with the healthy elderly, no type of chromosomal alteration was observed, presenting with only normal metaphase cells (46,XX and 46,XY). This demonstrates that these alterations are associated with AD, which seems to favor the loss of one of the X chromosomes and one of the

autosomal chromosomes (chromosomes 10, 13 and 21). This is the first record of chromosomal alterations in AD patients of the state of Amazonas.

The X chromosome contains some genes that are associated with the nervous system. Chromosomal syndromes resulting from the loss of X can influence the cognitive capacity of the individual, and intellectual deficiency is the main characteristic of these syndromes[23,24]. Karyotype analysis performed in patients with non-Down syndrome intellectual disability, attending the APAE-Manaus, identified one patient with mosaic Turner syndrome (45,X/46,XX) detected by classical cytogenetics, demonstrating a correlation between the loss of X chromosome and intellectual deficiency[25]. Although a relationship between X chromosome loss and cognitive/intellectual impairment in certain

diseases is evident, in the present study, no direct evidence of a correlation between the X chromosome loss and the degree of cognitive impairment (MMSE test) was found in AD patients. There was also no association between chromosomal loss and age or time to diagnosis, which emphasizes the need for complementary studies.

Several authors have suggested that the relatively high frequency of chromosomal alterations was not necessarily related to AD itself, but rather to aging[7,26]. Alzheimer's disease is a systematic disease that accelerates aging. It has been proposed that the X chromosome may underlie the instability of the AD cell genome, leading to a significant level of aneuploidy and a high percentage of premature centromere division[27,28]. Premature centromere division is one of the cellular events that causes loss of the control of the segregation of the centromeres, contributing to the emergence of chromosomal alterations[28,29]. Furthermore, the concomitant loss of one of the sex chromosomes (X chromosome) and one of the autosomal chromosomes (one of the homologues of the chromosome pairs 10, 13 and 21) has been described in AD patients for the first time. Double aneuploidies (gain or loss of two or more chromosomes) have been rarely reported in the literature. The mechanism underlying the origin of double aneuploidy is unclear. It has been hypothesized that double aneuploidy results either from two nondisjunctional events in gametogenesis or from a single nondisjunctional event in a trisomic zygote[30,31]. Regarding the autosomal chromosomes, one of the main genes involved in AD, the *amyloid precursor protein* gene, is located on chromosome 21[32]. A*myloid precursor protein* is directly linked to AD pathogenesis, and it was one of the first genes to be associated with chromosomal alterations. Down syndrome patients (chromosome 21 trisomy), who usually manifest this neuropathology around the age of 40 years, have a greater expression of the *amyloid precursor protein* gene[33,34,35]. It is worth mentioning that aneuploidy of other types of autosomes (1, 7, 11, 14, 17 and 18) has also been reported in AD patients, indicating a significant increase in aneuploidies of autosomal chromosomes[9,17].

In conclusion, the results of the present study, together with literature data, indicate that the karyotypes of patients diagnosed with AD go through different levels of chromosomal alterations, reinforcing the importance of the use of cytogenetic analyses (cytogenetic markers) in medical trials. Additional studies are needed to understand the implications of the loss of X chromosomes involved in patients with Alzheimer's disease in Manaus, Amazonas, Brazil.

Acknowledgments

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