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CCR5Δ32 polymorphism analysis of lipodystrophy syndrome development in HIV/AIDS patients

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ABSTRACT. The HIV/AIDS associated lipodystrophy syndrome is characterized by alterations in body fat and metabolism. We investigated whether CCR5Δ32 polymorphisms are associated with development of lipodystrophy. We made a case-control study of 248 HIV/AIDS patients who used antiretroviral therapy; they were divided into a group of patients (n = 148) who had lipodystrophy and a control group (n = 100) that did not present this syndrome. Both groups answered a questionnaire concerning age, gender and level of physical activity. DNA was extracted from blood samples using the mini salting out technique and PCR to identify polymorphisms of the CCR5Δ32 gene. Among lipodystrophy patients, most were men (64.8%) and 56.2% were sedentary. We found no significant association of the CCR5Δ32 allele with prevalence of lipodystrophy. However, male homozygotes were more susceptible to lipodystrophy (OR 1.78, P = 0.0350). We observed that the highest incidence of

lipodystrophy was in sedentary men. More studies would be needed, using other markers, to allow precise use of genetic polymorphisms as a diagnostic parameter for lipodystrophy syndrome.

Key words: HIV/AIDS; Lipodystrophy syndrome; CCR5Δ32

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is an infectious disease caused by the human immunodeficiency virus (HIV); it is considered a major issue for public health due to its pandemic character, its gravity and high mortality rate, when untreated (Sharp and Hahn, 2011). HIV causes a dysfunction in the immune system, acting particularly on CD4 T-cells facilitating opportunistic infections, cancer and central nervous system degeneration (Lundgren et al., 2018). Despite advances in the prevention of infection, HIV continues to cause a big impact on global public health, mainly in developing countries.

However, Highly Active Antiretroviral Therapy (HAART) has turned a fatal into a chronic disease; but it has numerous side effects, including lipodystrophy syndrome (Montessori et al., 2004; Merck Manual, 2018), which is characterized by abnormal fat deposition and metabolic changes (Paton et al., 2002). Changes in fat deposition may result in lipoatrophy, reduction in facial, gluteal and member fat; lipohipertrophy and fat accumulation in the abdomen, neck and thorax, and mixed lipodystrophy, which is a combination of both (Justina et al., 2014). Metabolic changes can include an increase in triglycerides, total cholesterol, LDL-cholesterol, hyperglycemia and reduction in HDL-cholesterol (Simha, 2014).

Besides being characterized as a side effect of HAART, genetic factors are important for lipodystrophy development, such as Dunnigan family partial lipodystrophy, a rare autosomal dominant disease and Berardinelli-Seip syndrome, an autosomal recessive disorder (Foss-Freitas et al., 2018). Other genetic factors such as coreceptors related to inflammation may be associated with lipodystrophy, , including the coreceptor CC-chemokine 5 (CCR5), a 40.6 kDa protein made of 352 amino acids, expressed by the CCR5 gene, mapped on chromosome 3 p21.3 region (Liu et al., 1996).

CCR5 is a chemokine coreceptor with seven transmembrane dominions that bind to chemokines, macrophages and regulates T cell activation, being the cofactor that allows HIV strains to enter the host cells (Grove et al., 2014; Tam et al., 2016). Interaction between T cells and macrophages, including that occur in adipose tissue, lead to a modification to a proinflammatory state, contributing to lipidic, glycemic and fat deposition changes (Egaña-Gorroño et al., 2014; Castilhos et al., 2015), that are typical changes of lipodystrophy.

However, HIV does not successfully invade host cells in all individuals exposed to HIV; this resistance is caused by a mutation in the gene that codes CCR5. A 32 base pair deletion, known as CCR5 delta 32 mutation (CCR5Δ32), causes a change in translation, from 193 bases in the wild allele to 161 in the mutant allele, creating a trunked protein that cannot be detected by HIC on the cell surface (Liu et al., 1996). This is a recessive autosomal mutation; so CCR5Δ32 heterozygotes still express, to a lesser extent, the receptors, causing a slower progression of HIV (Samson et al., 1996). Thus, the

inflammatory levels can be influenced by the deletion CCR5Δ32, which can be a precursor to lipodystrophy development.

Based on these premises, we looked for a possible association between CCR5Δ32 polymorphisms and the development of lipodystrophy, in order to determine if genetic markers can be used for early diagnosis of this syndrome.

MATERIAL AND METHODS

Study Design and Sample

This was a case-control study in which the case group was formed by patients with HIV who had developed lipodystrophy, while the control group was made up of patients with HIV but without lipodystrophy. This study was approved by the Human Research Ethics Committee of the Federal University of Pernambuco (UFPE), number 516.093. All participants signed informed consent forms.

Two hundred and forty-eight patients were evaluated, randomly invited from the Infectoparasitic Disease Sector of the Clinics Hospital of Universidade Federal de Pernambuco (HC-UFPE). The inclusion criteria were positive serology for HIV, both sexes, 18 years old as a minimum age and use of HAART.

After selection, the patients were submitted to the physical examination proposed by Sutinen and Yki-Jarvinen (2007) and Santos et al. (2013), to diagnose lipodystrophy, in which a self report and the researcher's report from physical examination of the arms, legs, face, abdomen and neck are used to diagnose the syndrome. Patients who had a positive diagnosis for lipodystrophy made up the case group; those who had a negative diagnosis were the control group. Afterwards, the patients answered a questionnaire for age, sex and physical activity level and 5 mL of blood collected in an EDTA tube. The DNA was extracted by the Mini Salting Out technique (Miller et al., 1988), then PCR was used to identify the CCR5 Δ32 gene.

PCR

After quantification with a spectrophotometer, DNA samples were diluted to 50 ng concentration and tested for the 32 bp deletion in the amplified sequence of CCR5Δ32 gene, using conventional PCR, conducted in a thermocycler "Peltier PTC-Cycler- térmica 200 - MJ Reseach", using the primers: 5'- ATCACTTGGGTGGTGGCTGTGTTTGCGTC-3' (forward) and 5'- AGTAGCAGATGACCATGACAAGCAGCGG -3' (reverse).

The final volume of 25 µL was composed of: 12.5 µL 200 nM Go Taq Master Mix (Promega), 9.5 µL of nuclease free water, 1 µL of each initiator at 10 pmol and 1 uL of DNA mold at 50 ng. The PCR parameters were 1 cycle at 94°C for 5 min initial denaturation, 35 cycles of 94°C for 1 min, 56°C for 30 s, and 72°C for 1 min and a final extension at 72°C for 7 min

The PCR products that were 193 bp long corresponded to wild type allele (WT), while the Δ32 product was 161 bp long. The presence of both fragments indicated a heterozygote genotype. The amplicons were separated using agarose gel at 3%, stained with ethidium bromide and visualized under ultraviolet light.

Data Analysis

Data were submitted to descriptive statistical analysis using Microsoft Excel to characterize the sample. SNPStat software was used to compare the allelic and genotype frequencies, to evaluate Hardy-Weinberg equilibrium (HWE), and to associate the polymorphism with susceptibility for the disease, adopting 5% as a level of significance.

RESULTS

This study used 248 patients as a sample, more than half were men, less than half were physically active, and over 90% were homozygotes for the wild type CCR5 allele (Tables 1 and 2).

Table 1. Characterization of the sample(case and control group) regarding age, physical activity level, diagnosis and incidence of lipodystrophy and CCR5 genotype found in the HIV/AIDS patients.

	Overall (n = 248)	**Men (n = 144)**	**Women (n = 104)**
Mean age (minimum and maximum)	46 (22-86)	48 (25-76)	45 (22-86)
Physically active, n (%)	40.3	43.7	49.6
Non active (%)	59.7	56.2	50.3
Patients with lipodystrophy (%)	59.7	66.7	50.0
Patients without lipodystrophy (%)	40.3	33.3	55.0
Lipohipertrophy (%)	30.4	51.1	48.9
Lipoatrophy (%)	37.8	75.0	25.0
Mixed lipodystrophy (%)	31.8	65.9	34.1
CCR5 – wt/wt (%)	92.7	53.6	46.4
CCR5 – wt/A32 (%)	7.3	4.4	2.9
CCR5 - Δ32/Δ32 (%)	0	0	0

Table 2. Genotypic and allelic frequencies for CCR5 gene polymorphism in patients with HIV/AIDS from case (HIV+ with lipodystrophy) and control (HIV+ without lipodystrophy) groups.

Genotype	**Total patients (n=248)**	**HIV (+) with lipodystrophy (n=148)**	**HIV (+) without lipodystrophy (n=100)**	**OR (CI 95%)**	**P-value**
GENOTYPIC FREQUENCY					
Wt/wt, %	92.7	91.9	94.0	1.00	-
wt/Δ32 %	7.3	8.1	6.0	1.36 (0.49-3.75)	0.55
Δ32/Δ32	0	0	0	-	-
ALLELIC FREQUENCY					
Wt	0.96	0.96	0.97		
Δ32	0.04	0.04	0.03		

The relation between sex and CCR5Δ32 polymorphism in both case and control case did not show protection or susceptibility to lipodystrophy development; however in male patients who presented a wt/wt genotype (193/193), we found a higher susceptibility to develop lipodystrophy associated to HIV (Table 3).

Table 3. Lipodystrophy prevalence between wild homozygote wt/wt (193/193) individuals and heterozygotes wt/Δ32 (193/161).

Gender	HIV (+) with lipodystrophy (n=148)	HIV (+) without lipodystrophy (n=100)	OR (95% CI)	P-value
		Wt/wt		
Female, % (n)	94.2 (49)	92.3 (48)	1.00	
Male, % (n)	90.6 (87)	95.8 (46)	*1.78 (1.04-3.04)	0.0350
		wt/Δ32		
Female, % (n)	7.7 (4)	5.8 (3)	1.00	
Male, % (n)	4.2 (2)	9.4 (9)	6.00 (0.70-51.10)	0.1011
Δ32/Δ32	0	0		

DISCUSSION

Lipodystrophy can affect 80% of people with HIV/AIDS using HAART (Paton et al., 2002). In our study the frequency of lipodystrophy was 59.7%. Brazilian studies estimated lipodystrophy prevalence in people with HIV range from 32.4 to 55% (Diehl et al., 2008; Ceccato et al., 2011).

Contrary to Guzman and Aboud (2018), who found a higher incidence of lipodystrophy in women, 64.8% of the patients with lipodystrophy were male, even though men appear to be less susceptible to lipodystrophy due to testosterone concentrations and their correlation with body fat distribution (Ponte et al., 2009).

The higher incidence of lipodystrophy in men may be justified by most of the men not practicing physical activity, since 56% were not active. According to Vancampfort et al. (2016), physically active people have a lower risk of developing lipodystrophy. These data support the conclusions of Segatto et al. (2011) and Trevisol et al. (2012), who reported an association between lipodystrophy and physical activity level, as they found that physically active individuals were 79% less likely to present lipodystrophy than sedentary patients.

Regarding the type of lipodystrophy, we observed that lipoatrophy was present in 37.8% of the patients, mainly in men (75%), followed by mixed lipodystrophy in 31.8% of the patients, 65.9% were men and the least frequent was lipohypertrophy, present in 30.4% of the patients, most of whom were male (65.9%). Our results are different from those reported by Justina et al. (2014), who found greater prevalence of lipoatrophy (62.5%), and a lower frequency of lipohypertrophy (8.3%) and mixed lipodystrophy (29.2%). The variation in the frequency of lipodystrophy may also be due to a lack of standards to determine it, and the use of autoperception by the subject; also the diagnosis by the researcher could be subjective, even though it has been described in a publication (Loonam and Mullen, 2012).

In the genetic analysis of patients, there were no homozygotes for the CCR5Δ32 mutant allele, 92.7% of them were homozygous for the wild genotype and 7.3% heterozygotes. Analyzing the groups separately, we observed that the case group presented 8.1% heterozygous patients, while the control group had 6%. Our study was in the same region where Lopes et al. (2014) found lower frequencies, 5.8 and 2.9%, respectively.

The mutant allele is common in Caucasians from Europe. Its frequency varies worldwide; it is less frequent in East Asia, Oceania, Africa and Native Americans (Novembre et al., 2005). In Brazil, its frequency varies in the various regions of the country,

due to the complex miscegenation that occurred during the colonial period (Vieira et al., 2011).

The higher susceptibility to lipodystrophy seen in male patients with wt/wt (193/193) genotype may be due to the fact that CCR5 codes a chemokine receptor that is present in immune cells that, when infected by HIV, can lead to a local inflammation in the adipose tissue, resulting in changes in adipocyte differentiation and apoptosis (Hammond and Nolan, 2007); when CCR5 had its function reduced by CCR5Δ32 polymorphism, no association was found with a possible protection against lipodystrophy development. A possible explanation to that may be that, though the receptor has a limited function due to this mutation, other receptors may recognize and bind to CCR5 chemokines, resulting in the normal inflammatory response.

The findings demonstrate that the frequency of the CCR5Δ32 allele is variable in in Brazilians with HIV/AIDS, reflecting their intense miscegenation to the mutant allele of the polymorphism was not significantly with lipodystrophy development. However, male wild type homozygotes were more susceptible to this syndrome. More studies are needed, using other markers, to obtain a more precise understanding of genetic polymorphism as a diagnostic parameter for lipodystrophy syndrome.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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