Evaluation of tear film function, ocular surface and tear film in HIV-seropositive patients

Avaliação da função lacrimal, da superfície ocular e do filme lacrimal em pacientes soropositivos para o HIV

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ABSTRACT

Objective: Evaluate tear function, tear film and ocular surface in patients with positive serology for HIV. **Methods:** Observational, cross-sectional, quantitative and analytical study, performed between june and october 2011, in the HSPE-FMO, SP, Ophthalmology Department, including 32 patients. Sixteen were HIV-positive patients and 16 HIV-negative. **Results:** There was no significant statistical difference in the middle age between both groups (p=0.083). The ferning test was statistically different in HIV group (with predominance of III and IV) compared to the control group (in which predominated the patterns I and II), both in the right and the left eye (p = 0.019 and p=0.001, respectively). Other parameters were not statistically significant between the groups. **Conclusion:** HIV-positive patients had no changes in lacrimal function and ocular surface, however, samples of tears showed differences considered statistically significant in the crystallization test, compared with samples obtained from controls.

Keywords: keratoconjunctivitis sicca; HIV; Dry eye

RESUMO

*Objetivo: Avaliar a função lacrimal, a superfície ocular e o filme lacrimal de pacientes com sorologia positiva para o vírus HIV. Métodos: Estudo observacional, transversal, quantitativo e analítico, realizado entre junho e outubro de 2011, no Departamento de Oftalmologia do Hospital do Servidor Público Estadual "Francisco Morato de Oliveira", com 32 pacientes no total, sendo 16 soropositivos para o HIV e 16 soronegativos. Resultados: Não houve diferença estatística significante na média de idade entre os grupos estudados (p = 0,083). O padrão do teste de cristalização da lágrima foi estatisticamente diferente no grupo de pacientes HIV (com predomínio dos padrões III e IV) em relação ao grupo controle (no qual predominaram os padrões I e II), tanto no olho direito como no esquerdo (p = 0,019 e p < 0,001, respectivamente). As demais variáveis estudadas não mostraram-se estatisticamente relevantes entre os grupos. Conclusão: Os pacientes soropositivos para o HIV não apresentaram alterações da função lacrimal e da superfície ocular, porém amostras de lágrimas evidenciaram diferenças consideradas estatisticamente significantes nos padrões dos testes de cristalização do filme lacrimal, quando comparadas com amostras obtidas de pacientes controles.

Descritores: Ceratoconjuntivite seca; HIV; Olho seco*

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) was first recognized in the United States in the eighties, and it is assumed that the human immunodeficiency virus (HIV) originates from primates of Africa[1,2]. The transmission of the retrovirus to humans may be due to bites, scratches or any kind of contact with the blood of these animals. However, the AIDS pandemic has followed the use in a large-scale of primates for biological experiments, including xenotransplantation[3].

According to the World Health Organization (WHO), it is estimated that there are 33.3 million people infected with HIV in the world, and in Brazil more than 590,000 cases have been identified from 1980, when HIV was discovered, until June 2010[4].

The systematic classification for the AIDS definition was proposed in 1982 by the *Centers for Disease Control* (CDC), and revised in 1997. Currently, the classification is based on the quantification of CD4+ T-lymphocytes, since the drop in the levels of these lymphocytes is a major characteristic of the disease[5,6].

Regarding the ocular changes of the anterior segment in AIDS, before the era of combined antiretroviral therapy (HAART), Kaposi's sarcoma, Burkitt's lymphoma, conjunctival microvasculopathy, non-specific conjunctivitis, keratoconjunctivitis sicca and iridocyclitis were frequent. The main changes of the posterior segment were the cotton wool spots, cytomegalovirus retinitis, herpes simplex and varicella zoster, HIV, toxoplasmosis, syphilitic retinitis and infectious endophthalmitis[7,8].

Among the external eye diseases, several studies in patients with positive HIV serology report a significant prevalence of dry eye in patients with AIDS in the period before HAART [9,10].

Dry eye syndrome refers to a disease of the ocular surface with different etiologies which often coexist. The precise prevalence of dry eye syndrome in a certain population is difficult to be established precisely due to the lack of accurate diagnostic criteria and the subjectivity of symptoms. To make the diagnosis more objective, clinical tests are established, such as assessment of the tear film breakup time (TFBUT), surface staining with vital dyes (fluorescein, rose bengal and lissamine green), Schirmer's test and tear crystallization test (*ferning test*). Other tests used, such as osmolarity of the tear film, measurement of protein concentration in the film, interferometry and evaporimetry, are of little use in the daily practice due to the low practicality and the high cost of implementation.

In 2007, the results of the *International Dry Eye Workshop (DEWS* [11] were published, wherein dry eye was defined as a multifactorial disease of the tears and the ocular surface, resulting in discomfort, visual blurring and tear film instability with potential damage to the ocular surface, and associated to an increase in tear osmolarity and ocular surface inflammation.

The etiology of the dry eye associated with HIV is not well established yet, but the tear reduction may be associated with lymphocytic infiltration and eventual destruction of the acini and ducts of the lacrimal gland, generating the Sjogren-*like* syndrome, not just due to the clinical appearance, but also the histopathological one[12]. The prevalence of dry eye in HIV-infected patients in previous studies ranged from 7.79% to 38.8%, and the symptoms of dry eye have an important impact on the quality of life of these patients[13-17]. Early in the HIV epidemic, SJ cases were reported as secondary to the infection[18]. However, a few years later, the *sicca* syndrome associated with HIV was defined as a clinical entity that was called diffuse infiltrative lymphocytosis syndrome (DILS). Present in 0.85 to 3% of HIV-positive patients, the DILS is characterized by lymphocytic infiltration in various organs and peripheral lymphocytosis at CD8+ costs, clinically manifested as *sicca* syndrome and increased parotid, thus simulating a condition similar to Sjogren's syndrome (*Sjogren-like*). Tear reduction may be associated with this lymphocytic infiltration and eventual destruction of acinar and ducts of the lacrimal gland[18]. In SJ lymphocytic infiltration is by CD4+ and serological tests (anti-RO and anti-LA) are positive[19, 20].

OBJECTIVES

Assess the lacrimal function, the ocular surface and the tear film in patients with positive serology for HIV.

METHODS

This is an observational, cross-sectional, quantitative and analytical study carried out between June and October 2011, on the premises of the Department of Ophthalmology of the State Public Health Service Hospital of São Paulo. The study protocol was approved by the Ethics and Research Committee of the institution under number 079/11.

Two groups named "study group" and "control group" were defined.

Study group

Inclusion criteria: positive serology for human immunodeficiency virus (HIV), legal majority and signature of the consent form.

Exclusion criteria: positive serology for hepatitis B or C, use of ocular medication or contact lenses during the seven days prior to the assessment, patients with previously diagnosed eye diseases that compromise the lacrimal production or drainage (Sjögren's syndrome, Stevens-Johnson syndrome, ocular pemphigoid, ocular chemical burns, trachoma, peripheral facial paralysis), continuous use of medication with anticholinergic effect, pregnant and lactating women.

Control group

Inclusion criteria: negative serology for human immunodeficiency virus (HIV), legal majority and signature of the consent form.

Exclusion criteria: positive serology for hepatitis B or C, use of ocular medication or contact lenses during the seven days prior to the assessment, patients with previously diagnosed eye diseases that compromise the lacrimal production or drainage (Sjögren's syndrome, Stevens-Johnson syndrome, ocular pemphigoid, ocular chemical burns, trachoma, peripheral facial paralysis), continuous use of medication with anticholinergic effect, pregnant and lactating women.

Sixteen patients with HIV and 16 patients without the virus infection were selected according to the proposed criteria.

In the Study Group, 5 patients were male and 11 were female. The mean age was 44.94 years (± 10.33). In the Control Group, 5 patients were male and 11 were female, and the mean age was 55.50 years (± 20.81). Tables 1 and 2 summarize the data, demonstrating no statistically significant differences between both groups with respect to age (p = 0.083) and sex (p> 0.999), respectively. Table *3* summarizes the data of patients in the study group, including the period of HIV infection.

Sequence of exams

After consulting the relevant literature, we did not find consensus regarding the optimal sequence for the realization of dry eye tests, so that the proposal in this study was created to avoid the maximum that the realization of a test would influence the performance of the next one.

Both patients in the study group and in the control group underwent the same assessment sequence. Initially, a standardized questionnaire was presented about the known duration of the infection and the likely form of contagion, as well as about the antiretroviral therapy in use, when present.

After obtaining said information, the parameters studied and the interval between each exam are as follows:

Table 1

Assessment of the groups regarding sex

| Variable | Group | | | | | | p-Value |
| | Control | | HIV+ | | Total | | |
	n	%	n	%	N	%	
Sex							>0.999#
Female	11	68.8	11	68.8	22	68.8	
Male	5	31.3	5	31.3	10	31.3	
Total	**16**	**100**	**16**	**100**	**32**	**100**	

Table 2

Assessment of the groups regarding age

Variable	Group	Mean	SD	Median	Minimum	Maximum	N	p-Value
Age	Control	55.50	20.81	55.5	18	85	16	0.083
(years)	HIV+	44.94	10.33	44	18	72	16	

Table 3

Study Group Data (HIV+)

Patient	Sex	Age	Δt Infection
1	F	46	132
2	M	41	7
3	F	44	96
4	M	43	96
5	M	41	120
6	M	51	168
7	F	50	168
8	M	50	24
9	F	45	156
10	F	72	228
11	F	43	144
12	F	42	108
13	F	44	168
14	F	18	84
15	F	43	84
16	F	46	168

Legend: F = female; M= male; Age = in years;
Δt infection = in months

The questionnaire "dry eye - specific" was initially applied: Ocular Surface Disease Index® (OSDI); immediately after, a tear sample was collected to perform the tear film crystallization test; afterwards, the observation of the tear film breakup time was observed, and the cornea was assessed with fluorescein dye 1%. After at least thirty minutes of the previous exam, the Schirmer's test I was performed. After that, the ocular surface was studied once again, but this time using the rose bengal dye 1%. Finally, after at least thirty minutes from the previous test, the sensitivity of the cornea was measured with a Cochet-Bonet esthesiometer.

Location of exams

The questionnaires, measurements of tear film breakup time, ocular surface staining with fluorescein dye 1%, Schirmer I test, ocular surface staining with rose bengal dye 1%, esthesiometry, and the collection of tear samples were performed in the same room of the Cornea and External Diseases Ambulatory of the Department of Ophthalmology of IAMSPE, with doors and windows closed.

In times of collections, the temperature and relative humidity in the room were recorded with a digital thermo-hygrometer (Barigo®, Barometerfabrik, Villingen-Schwenningen, Germany). The apparatus records the minimum and maximum temperature in a given period of time, as well as minimum and maximum relative humidity. Table 4 summarizes the measures recorded on days when the patients were assessed.

Table 4

Record of temperature (°C) and relative humidity (%) values

	T. mín° C	T. máx° C	R.H. min %	R.H.max %
Minimum	20.7	21.9	43	49
Maximum	23.2	25.8	68	73
Média	22.05	23.8	53.5	60.6
DP	0.96	1.54	7.81	7.52

Caption: T = temperature; R.H. = relative humidity; min. = minimum; max. = maximum; SD = Standard deviation.

Parameter Description

• *Questionnaire OSDI*

All patients underwent a dry eye questionnaire - specific "Ocular Surface Disease Index" (OSDI®, Allergan, Irvine, California, USA), composed of twelve questions asked by the

researcher to the respondents. To answer them, they should consider the seven days preceding the interview. The questions cover three areas: ocular symptoms, possible environmental stimuli causing ocular discomfort, and finally limitations in daily activities of patients according to the ocular clinical profile. In this questionnaire, in order to measure the intensity of the answers provided the patients refer to each question one answer on a scale ranging from 0 to 4 points, with 0 being minimum commitment and 4 maximum commitment. In the end, an overall score is generated, taking into account both the indexes provided in each response and the total number of questions that could be answered. This score ranges from 0 to 100, with 0 being absence of eye discomfort, and 100 maximum eye discomfort. The questionnaire was administered to patients at all stages, always by the same researcher (CM).

• *Tear film crystallization test*

In such assessment, also known as *ferning test*, the patients were accommodated in a conventional way at the slit lamp and with non-heparinized capillary glass tube, and the tear sample was collected from the right eye fornix. A droplet with diameter ranging between two and three millimeters was deposited on the surface of a clean glass slide in the center of a circle previously marked with blue or red pen. The tear was allowed to dry at room temperature for about ten minutes before being stored in the appropriate box for transportation. The analysis of the slides was made later, with the help of a light microscope Zeiss Axistar, with which digital pictures of the location corresponding to dry tear droplet were obtained, with the camera attached to the microscope (*Sony Cybershot^O model DSC-W120*) in zooms of 5, 10 and 40 times and resolution of 3 megapixels. The material was collected and the pictures were taken by the same researcher (CM). The pictures obtained were examined in a separate moment and in an independent way by another researcher with previous experience in test reading (SF).

For the classification, the findings of the tear film crystallization test were characterized in four patterns, according to the model proposed by Rolando, with patterns I and II being considered normal and patterns III and IV abnormal[21].

• *Tear film breakup time (TFBUT)*

The exam was performed with a slit lamp, and lighting being cobalt blue. One droplet of 1% fluorescein was instilled in the lower fornix of both eyes of the patients. The patients were asked to blink a few times, and then stop blinking when the timer was immediately triggered. The time to the appearance of the first tear film breakup point was observed on the cornea surface. Three measures were recorded so that the average was obtained.

The test was conducted at all stages by the same researcher (CM).

• *Cornea exam with fluorescein dye 1%*

Taking advantage of the same volume of fluorescein instilled for TRFL checking, immediately afterwards the cornea of each eye was assessed according to the following score proposed by the researchers:

Score 0: cornea without changes; does not stain with fluorescein.

Score 1: punctate keratitis, with sparse points evidenced by the dye.

Score 2: punctate keratitis, with nearby points evidenced by the dye.

Score 3: severe keratitis, with confluent points evidenced by the dye.

The test was conducted at all stages by the same researcher (CM).

• *Schirmer's Test I*

The Schirmer's test I, also called a Schirmer's test without topical anesthesia, was performed simultaneously on both eyes by placing a standardized and millimetric strip of Wathmann filter paper number 41 (Ophthalmos^ã, São Paulo, Brazil) to the side third of each one of the lower eyelids. The patients were instructed to remain with their eyes closed for five minutes. Soon after, the value corresponding to the wetting of the paper in each eye was observed and recorded.

The test was conducted at all stages by the same researcher (CM).

• *Exam of the ocular surface with rose bengal dye 1%*

The assessment of damage to the ocular surface was analyzed with rose bengal dye 1%. A droplet of the dye was instilled in the upper bulbar conjunctiva of both eyes of the patients. Then they were assessed at the slit lamp with lighting and green filter. The eyes were classified according to the proposal of van Bjesterveld[22], in which each eye has the ocular surface area exposed by the naturally open slit divided into three thirds: side bulbar conjunctiva, cornea and medial bulbar conjunctiva. Each third receives from the examiner a score ranging from 0 to 3, in which:

Score 0: does not stain with rose bengal.

Score 1: dyes slightly, with sparse points.

Score 2: dyes moderately, with nearby points.

Score 3: dyes intensely, with confluent points.

The scores of the three thirds are summed to generate a final score ranging from 0 to 9 points, with 0 being considered the absence of damage to the surface and 9 the maximum damage. The exam was conducted at all stages by the same researcher (CM).

• *Esthesiometry*

In the assessment of the central cornea sensitivity, the patient reported a minimum sensitivity to touch when stimulated in the central region of the cornea by the Cochet-Bonnet esthesiometer (Luneau Ophthalmologie, Paris-France), according to standardization proposed by Norn[23]. The exam was performed in both eyes, always by the same examiner (CM), which initiated the test in the right eye, touching the central cornea with a nylon thread in maximum exposure (level 6). If the touch was not perceived, the thread was reduced to level 5.5 and so on, 0.5 by 0.5, to level 0. If the touch was perceived, the amount corresponding to the level of exposure of the wire was registered.

Statistical analysis

To meet the goals of the study, first the measures assessed were described by group using a summary of measures (mean, standard deviation, median, minimum and maximum), then the values were compared between the groups using the Mann-Whitney tests, except for ages, which were compared using the with Student's t-test.

Sex, the OSDI and crystallization classification were described according to the groups using absolute and relative frequencies, and the existance of an association between the gender and the groups was verified using the chi-square test, and the scale degree among the groups was compared using tests Mann-Whitney tests.

The tests were conducted at a significance level of 5%.

RESULTS

Table 5 shows that the mean age of the control group is statistically equals to the HIV+ group (p = 0.083), and the tests that take scores (fluorescein and rose bengal) also did not differ between the control and the HIV+ groups (p > 0, 05).

Table 6 shows that the standard of tear crystallization test is statistically different in the group of HIV+ patients (with a predominance of patterns III and IV) in relation to the control group (in which the predominant patterns are I and II), both in the right and the left eyes (p = 0.019 and p < 0.001, respectively).

Charts 1 and 2 illustrate the results in *Table 5*, but the tear crystallization scores in both eyes were statistically higher in HIV+ patients.

DISCUSSION

The data presented in this study showed that assessments of the lacrimal function tests (Schirmer test and tear film breakup time) and the status of the ocular surface (tests with the dyes fluorescein and rose bengal and sensitivity of the cornea) of HIV seropositive patients did not show significant differences compared with the control patients in the conditions studied.

Factors that limit the interpretation of the data are the small number of patients in the study group, the heterogeneity of time between viral infection and ocular assessment, and individual characteristics of both antiretroviral treatment and the consequent clinical spectrum of HIV infection. Initially we planned to describe the findings using the absolute number of eyes (64 eyes); however, as the disease studied involves both eyes similarly, we chose to use the number of patients and not eyes, although both have undergone all tests. Thus, the fact that we found no changes that characterize keratoconjunctivitis sicca in the study group allows us to infer only that, unlike the findings of other researchers, the group of selected patients did not present dry eye at the very moment that our assessment was made.

If we assume that the clinical profile of ocular dryness associated with HIV infection observed by other authors is in fact similar to that observed in the Sjögren's syndrome, it is possible to conclude that the damage caused in the secretory exocrine tissue occur in the first group in a gradual way as well, with slow and gradual installation of the symptoms. In this regard, it is not possible to ignore the possibility of the group studied herein develop further changes of the lacrimal function and consequently the ocular surface, and is therefore recommended to evaluate it periodically.

Table 5

Description of the numerical scale values of the second groups and the results of the comparative tests

Variable	Group	Mean	SD	Median	Minimum	Maximum	N	p-Value
Age (years)	Controle	55.50	20.81	55.5	18	85	16	0.083*
	HIV+	44.94	10.33	44	18	72	16	
OSDI absolute	Controle	13.79	17.13	7.15	0	70	16	0.926
	HIV+	13.18	13.16	7.67	0	41,6	16	
BUT RE (seconds)	Controle	7.94	2.67	10	3	10	16	0.224
	HIV+	6.56	3.27	7	2	10	16	
BUT LE (seconds)	Controle	7.81	2.59	9	3	10	16	0.224
	HIV+	6.31	3.26	5	2	10	16	
Schirmer RE (mm)	Controle	14.81	9.84	15	2	35	16	0.838
	HIV+	15.56	9.75	14.5	1	35	16	
Schirmer LE (mm)	Controle	15.25	8.24	13.5	6	35	16	0.809
	HIV+	15.25	9.83	11.5	3	35	16	
Esthesio RE	Controle	5.13	0.81	5.5	3,5	6	16	0.171
	HIV+	4.34	1.55	4.5	1	6	16	
Esthesio LE	Controle	5.25	0.71	5.5	3,5	6	16	0.210
	HIV+	4.41	1.54	4.75	1,5	6	16	
Fluorine RE	Controle	0.19	0.54	0	0	2	16	0.752
	HIV+	0.06	0.25	0	0	1	16	
Fluorine LE	Controle	0.19	0.54	0	0	2	16	0.780
	HIV+	0.25	0.58	0	0	2	16	
Rose RE	Controle	0.13	0.34	0	0	1	16	>0.999
	HIV+	0.13	0.34	0	0	1	16	
Rose LE	Controle	0.19	0.40	0	0	1	16	0.780
	HIV+	0.25	0.45	0	0	1	16	

Results of the Mann-Whitney test
(*) Result of *t-Student test*

Table 6

Description of qualitative scales and according to groups and results of the association test and comparative tests of scales.

Variable	Control n	Control %	HIV+ n	HIV+ %	Total N	Total %	p-Value
OSDI classification							**0.809**
Normal	11	68.8	12	75.0	23	71.9	
Mild	2	12.5	1	6.3	3	9.4	
Moderate	3	18.8	3	18.8	6	18.8	
Cristal RE							**0.019**
I	6	37.5	2	12.5	8	25.0	
II	6	37.5	3	18.8	9	28.1	
III	3	18.8	7	43.8	10	31.3	
IV	1	6.3	4	25.0	5	15.6	
Cristal LE							**<0.001**
I	10	62.5	2	12.5	12	37.5	
II	4	25.0	1	6.3	5	15.6	
III	2	12.5	5	31.3	7	21.9	
IV	0	0.0	8	50.0	8	25.0	
Total	**16**	**100**	**16**	**100**	**32**	**100**	

Chart 1

Percentage of crystallization in the right eye observed according to groups.

Chart 2

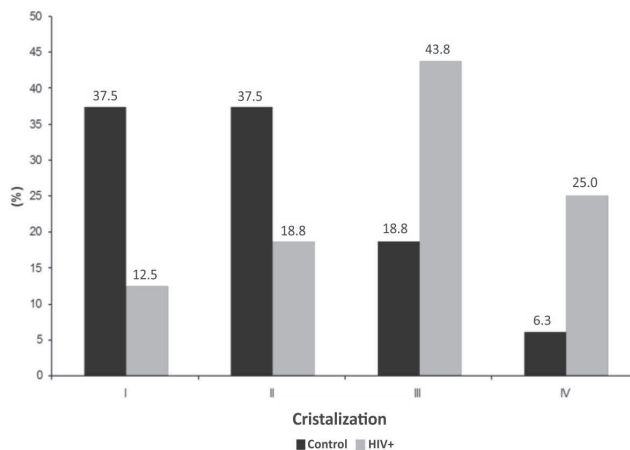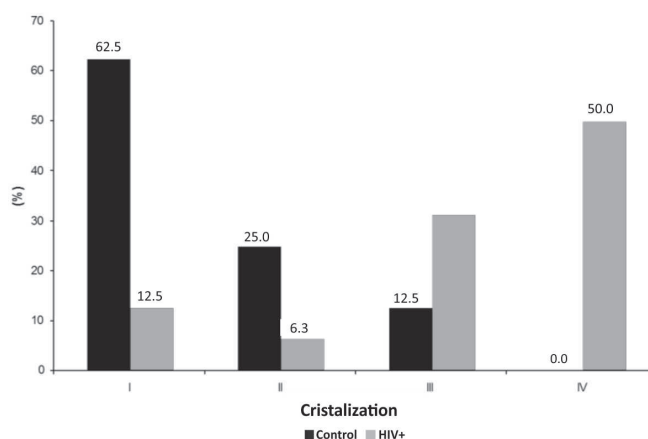**Percentage of crystallization in the left eye observed according to groups**

The etiology of keratoconjunctivitis sicca related to HIV infection is not well established yet. Evidence derived from studies of patients with Sjögren's syndrome shows the presence of a cross-sectional reaction of serum antibodies with retroviral proteins, and the occurrence of reverse transcriptase activity in the salivary glands[18]. Furthermore, the detection of retroviral antigens, retrovirus-like particles or retroviral sequences in the salivary glands of patients with systemic retrovirus infection (EBV, HIV and HTLV) corroborate the theory that the infection could represent the trigger for the development of manifestations caused by underactivity of the exocrine glands, although to date no study has evidenced that these findings present in the salivary glands are reproduces in the lacrimal ones, but clinical evidence suggest that the pathogenic mechanism is similar[24].

That is, both the presence of the virus live in glandular microenvironment and the immune dysfunction leading the defense system to consider *non-self* proteins of the ducts and glandular acini in a cross-section reaction with viral proteins can lead to inflammation and local destruction, justifying the abnormalities of the lacrimal function described above and observed by other authors in HIV-positive patients. This means, therefore, that some HIV-infected patients who develop dry eye are in fact genetically predisposed to this cross-section reaction, and it is unclear exactly in which percentage they represent the group of patients developing dry eye by this mechanism in the universe of seropositive. It is possible to believe that in such cases keratoconjunctivitis sicca is installed later than in those determined solely by the presence of the virus in the acinar tissue. The sample reduced from patients in the present study favors the selection bias, and the group may have been composed of patients predisposed to long-term changes.

Rodrigues et. al. observed a significant increase in the frequency of the dry eye syndrome in patients with the disease developed for over four years, especially among those who were on HAART, but the decrease in tear production was not related to the severity or time of infection[25]. In the group studied, all patients were using HAART, and the time of infection was not taken into account; however, we did not consider the relevant fact due to not observing differences between them and the controls. If changes were detected in the infected group in the performance of the tests, there would be a doubt whether the use of systemic medication might account for the findings. Presumably, HAART scheme can eliminate the manifestations of the *Sjögren-like* syndrome; however, no study has clearly shown the action of antiretroviral drugs alone or in combination, or if they may have a deleterious effect on some of the tear film components, regardless of the infection. On the other hand, in order for the influence of antiretroviral therapy be concluded as a protective factor in the prevention of keratoconjunctivitis sicca, an event that may have occurred to the patients studied herein, a new study would be needed comparing HIV-positive patients under treatment with other without any treatment or systemic comorbidities.

Again, the samples will tend to be reduced because of the frequent association of HIV infection with other viruses potentially triggering dry eye, including the HCV and HVB (considered as an exclusion criteria of the present study, being one of the responsible for the restriction on the size of the sample that was formed).

Colombo et. al. described the dissociation between signs and symptoms of dry eye in patients with Sjögren's syndrome, i.e., the weak relation between the intensity of complaints (also assessed by questionnaire of dry eye - specific - OSDI) and the *status* of the ocular surface, especially in chronic and severe cases[26]. One explanation proposed by the authors was that chronic inflammation caused by dry eye and the local release of inflammatory mediators would lead to cornea hypoesthesia, with subsequent reduction of perception of nuisance, despite a significantly changed ocular surface. We believe, however, that the normal values observed in OSDI questionnaire in our study and that indicate the absence of ocular discomfort represent the absence of complaints due to the lack of eye damage, verified by the sequence of the other tests and confirmed by the sensitivity of the normal corneas assessed.

It is possible to consider that biochemical changes of the tear film responsible for the mucous component under certain conditions can be earlier signs of lacrimal dysfunction than checking the deficit in water production or the presence of damage to the ocular surface. This justifies the inaltered Schirmer's test. The test of crystallization in the tear samples collected showed that HIV-infected patients tend to have a worse quality than normal patients. Seropositive patients present patterns III and IV of Rolando more often than individuals without the infection. In these, the patterns I and II were more prevalent. The relative humidity above 50% can reduce the test reproducibility; however, the pioneer studies describing this test do not measure the humidity and not to mention it as an influence parameter[27], while other studies in order to assess only this test did it with humidity between 48-69%[28]. The classification system of the crystallization test works as an evaluation index of the overall quality of the tear film, since so as to form each of the paterns considered in the classification the mucus, glycoproteins, and electrolytes concentrations are fully determinant.

Another important test, although not conducted in our study, is the osmolarity measure of the tear film. The role of hyperosmolarity is known as a key mechanism in the physiopathogenesis of dry eye, as demonstrated in numerous studies and increasingly discussed[29]. Hyperosmolarity occurs as a consequence of the reduction in aqueous production and/ or the increase of tear evaporation. It is also responsible for reducing the density of goblet cells of the bulbar conjunctiva. The loss of goblet cells may be responsible for the unstable tear film and the reduction of mucin[30], showing changes in the biochemical component of the tear. As the test and crystallization, due to evaluating the first affected component in the Sjögren-*like* cases, probably the measure of osmolality was also changed in our study.

This finding confirms the need for the present study follow-up, with new assessments of the patients observed under the same conditions described here, in addition to its development with different tests, such as the tear osmolarity measure itself in this group.

CONCLUSION

Under the conditions studied, the HIV seropositive patients showed no changes in lacrimal function nor in the ocular surface; however, the tear samples showed differences considered statistically significant in the patterns of the tear film crystallization tests when compared with the samples obtained from control patients.

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