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MORBIDADE DA CERATOCONJUNTIVITE SECA ASSOCIADA AO VÍRUS LINFOTRÓPICO DE CÉLULAS T HUMANA TIPO 1 (HTLV-1): ESTUDO DE COORTE RETROSPECTIVA

TESE DE DOUTORADO

Salvador-Bahia
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Tese apresentada ao Programa de Pós-graduação Stricto Sensu em Medicina e Saúde Humana da Escola Bahiana de Medicina e Saúde Pública como requisito parcial para obtenção do Título de Doutora em Medicina e Saúde Humana.

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Salvador, 26 de junho de 2018.

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Mestres da Minha Vida:

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André Luiz e Chico Xavier

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RESUMO

Fundamento: A ceratoconjuntivite seca (CCS) é uma doença que atinge cerca de 37% dos indivíduos com o vírus linfotrópico de células T humana tipo 1 (HTLV-1). No entanto, a incidência da doença em indivíduos com HTLV-1 é pouco estudada, bem como a influência de fatores de risco e a detalhada descrição de suas manifestações clínicas. **Objetivo:** Estudar a morbidade da CCS em pacientes portadores de HTLV-1, e descrever suas manifestações clinicas em uma grande coorte de indivíduos infectados, residentes na Bahia, Brasil. **Métodos:** No período entre junho de 2004 a setembro de 2017, no Centro de HTLV da Escola Bahiana de Medicina (CHTLV) em Salvador, Bahia-Brasil, foi realizada uma coorte retrospectiva em que dados referentes ao exame oftalmológico de pacientes com HTLV-1 foram avaliados com relação à detecção de CCS. Os dados utilizados para análise foram obtidos a partir de exames oftalmológicos completos incluindo medida da acuidade visual, biomicroscopia anterior e posterior, pressão intraocular e avaliação do filme lacrimal incluindo os testes de Tempo de ruptura do filme lacrimal (TBUT), Rosa Bengala e Schirmer I. O diagnóstico de CCS foi considerado na presença de pelo menos dois dos três testes positivos. A análise estatística foi realizada utilizando o software Stata v. 13.0. **Resultados:** Foram coletados dados de 1137 pacientes com HTLV-1, matriculados no CHTLV e que concordassem em participar do estudo. Destes, 379 foram excluídos por terem menos de 18 anos, não terem exame oftalmológico completo, serem portadores de doenças sistêmicas ou oftalmológicas associadas ou terem diagnostico prévio de CCS. Foram então, avaliados os dados de 758 pacientes para o cálculo de prevalência e 628 para incidência. Dos 628, 210 realizaram consultas subsequentes, porém 27 destas estavam incompletas. A incidência total foi de 44,3% (81/183) e a densidade de incidência foi de 76 / 1000 pessoas / ano, com mediana (25 - 75) de tempo de seguimento de 4,3 (2,2 – 8,0) anos. Uma análise bivariada ajustada reforçou a associação entre a incidência de CCS em portadores de HTLV-1 independentemente da idade, sexo e outras condições, com HAM / TSP sendo fator de risco para o desenvolvimento de CCS em portadores de HTLV-1. Notou-se uma prevalência geral de CCS de 31,7%, com maiores taxas observadas em pacientes com HAM / TSP mesmo após ajuste para idade, sexo, tempo de diagnóstico e escolaridade (RP ajustada: 1,63; IC95%: 1,31-2,02). Acuidade visual menor que 20/30, dor, ardor e prurido foram significativamente maiores em pacientes com CCS. **Conclusão:** A incidência de CCS em pacientes com HTLV-1 mostrou-se mais alta do que a média da população soronegativa para a doença. Dor e/ou ardor, prurido e acuidade visual menor que 20/30, foram as alterações mais comuns entre os portadores de HTLV-1 e CCS. Desta forma, recomenda-se que os pacientes com HTLV-1 sejam submetidos a exames oftalmológicos periódicos para promover o diagnóstico precoce da CCS e prevenir as consequências associadas à Doença do Olho Seco.

Palavras-chave: HTLV-1. Ceratoconjuntivite seca. Incidência. Prevalência.

ABSTRACT

Background: Dry keratoconjunctivitis (KCS) is a disease that affects about 37% of individuals with human T-cell lymphotropic virus type 1 (HTLV-1). However, the incidence of the disease in individuals with HTLV-1 has not yet been well studied, as well as the influence of risk factors and the detailed description of their clinical manifestations. **Purpose:** To study the morbidity of KCS in patients with HTLV-1 and to describe their clinical manifestations in a large cohort of infected individuals living in Bahia, Brazil. **Methods:** A retrospective cohort study was performed between June 2004 and September 2017 at the HTLV Center of the Bahia School of Medicine (CHTLV) in Salvador, Bahia, Brazil, in which data referring to the ophthalmological examination of patients with HTLV-1 were evaluated in relation to KCS detection. The data used for analysis were obtained from complete ophthalmologic examinations including measurement of visual acuity, anterior and posterior biomicroscopy, intraocular pressure and lacrimal film evaluation including Tear breakup time (TBUT), Rose Bengal and Schirmer I tests. The diagnosis of KCS was considered in the presence of at least two of the three positive tests. Statistical analysis was performed using Stata v. 13.0. **Results:** Data from 1137 patients with HTLV-1 enrolled in CHTLV and who agreed to participate in the study were collected. Of these, 379 were excluded because they were under 18 years of age, did not have a complete eye exam, had associated systemic diseases, or had a previous diagnosis of KCS. The data of 758 patients were then evaluated for the calculation of prevalence and 628 for incidence. Of the 628, 210 had subsequent consultations but 27 of these were incomplete. The total incidence was 44.3% (81/183) and the incidence density was 76/1000 people / year, with a median (25-75) follow - up time of 4.3 (2.2 – 8.0) years. An adjusted bivariate analysis reinforced the association between the incidence of KCS in HTLV-1 carriers regardless of age, gender and other conditions, with HAM / TSP being a risk factor for the development of KCS in HTLV-1 carriers. There was a general prevalence of KCS of 31.7%, with higher rates observed in patients with HAM / TSP even after adjusting for age, sex, diagnosis time and schooling (adjusted PR: 1.63, 95% CI: 1, 31-2.02). Visual acuity <20/30, pain, burning and pruritus were significantly greater in patients with KCS. **Conclusion:** The incidence of KCS in patients with HTLV-1 was higher than the mean of the seronegative population for the disease. Pain and / or burning, pruritus and visual acuity <20/30, were the most common changes among HTLV-1 and KCS patients. Thus, it is recommended that patients with HTLV-1 undergo periodic eye exams to promote the early diagnosis of KCS and to prevent the consequences associated with Dry Eye Disease.

Keywords: HTLV-1. Keratoconjunctivitis Sicca. Incidence. Prevalence.

LISTA DE ABREVIATURAS

ATL	*Adult T-cell Leukemia* (Leucemia de células T do Adulto)
CCS	Ceratoconjuntivite Seca
CHTLV	Centro Integrativo e Multidisciplinar de HTLV da EBMSP / FBDC / FIOCRUZ
DNA	Ácido Desoxirribonucléico
EBMSP	Escola Bahiana de Medicina e Saúde Pública
ELISA	Ensaio imuno enzimático
FBDC	Fundação Bahiana para o Desenvolvimento das Ciências
FR	Fator Reumatóide
HAM	*HTLV Associated Myelopathy* (Mielopatia Associada ao HTLV)
HAU	Uveíte associada ao HTLV
HIV	*Human Immunodeficiency Virus* (Vírus da Imunodeficiência Humana)
HTLV	*Human T Lymphotropic Virus* (Vírus Linfotrópico de Células T humana)
IC	Intervalo de Confiança
IBOPC	Instituto Brasileiro de Oftalmologia e Prevenção da Cegueira
IL	Interleucina
FIOCRUZ	Fundação Oswaldo Cruz
LLcTA	Leucemia/ Linfoma de células T do Adulto
MMP	*Matrix metallopeptidase*
OD	Olho Direito
OE	Olho Esquerdo
OSDI	*Ocular Surface Disease Index* (Índice de Doença da Superfície Ocular)
PCR	*Polimerase Chain Reaction*
PIO	Pressão intraocular
RP	Razão de Prevalência
RNA	Ácido Ribonucléico
UAH	Uveíte Associada ao HTLV-1
TBUT	*Tears Break Up Time* (Tempo de Ruptura do Filme Lacrimal)
TCLE	Termo de Consentimento Livre e Esclarecido
TNF-α	*Tumor Necrosis Factor alpha* (Fator de Necrose Tumoral alfa)
Treg	Células T regulatórias
TSP	*Tropical Spastic Paraparesias* (Paraparesia Espástica Tropical)

SUMÁRIO

1 INTRODUÇÃO

A infecção pelo vírus HTLV-1 está presente em todo o mundo. Em algumas regiões existe alta endemicidade onde a soroprevalência atinge 20-40% das pessoas com mais de 50 anos. No mundo, as principais áreas estão localizadas no Japão, Caribe, América do Sul, África intertropical, Irã e a Romênia[1]. No Brasil, o maior número de casos de infectados está presente nas regiões Norte e Nordeste do pais. Salvador, a capital do estado da Bahia, localizado no Nordeste do Brasil, tem a maior prevalência de HTLV-1 do país[2,3]. Um estudo populacional em Salvador, estimou a prevalência de HTLV-1 em 1,7% em 2003, ou seja, cerca de 40.000 pessoas poderiam estar infectadas com HTLV-1[4].

O vírus T-linfotrópico humano tipo 1 (HTLV-1) foi identificado como retrovírus oncogênico humano há mais de 30 anos[1,5] estando etiologicamente ligado à leucemia de células T adultas (ATL)[6,7], paraparesia espástica tropical / mielopatia associada ao HTLV-1 (HAM / TSP)[8-10], dermatite infecciosa[11] e uveíte[12,13]. Muitas outras doenças foram associadas ao HTLV-1, como polimiosite[14], pneumonia alveolar brônquica[15], bronquiectasias[16] e Síndrome de Sjögren[17,18], indicando envolvimento multissistêmico desta infecção. Além disso, outras lesões oculares também foram associadas ao HTLV-1, tais como, lesões da córnea[19], vasculites retinianas[20] e a ceratoconjuntivite seca (CCS)[21-26]. Embora as repercussões da infecção pelo HTLV-1 estejam claramente demonstradas, essa infecção e suas doenças relacionadas ainda são pouco estudadas[27].

A CCS, ou doença do olho seco, é uma doença multifatorial da superfície ocular caracterizada pela perda da homeostase do filme lacrimal e acompanhada por sintomas oculares, nos quais a instabilidade e hiperosmolaridade do filme lacrimal, inflamação e dano da superfície ocular e anormalidades neurosensoriais desempenham papéis etiológicos [28].

A prevalência de CCS associada ao HTLV-1 varia em diferentes partes do mundo. Na Martinica, a prevalência de CCS associada ao HTLV-1 foi de 37,0%[23], enquanto no Japão foi de 15,4%[29]. O Brasil, um país com mais de 200 milhões de habitantes,

representa uma das maiores áreas endêmicas para o HTLV-1 e doenças associadas no mundo[1]. Investigações sobre a prevalência de CCS em associação com o HTLV-1 demonstraram discrepâncias, com variância de 3,9% a 36,4%[21,24,25]. Um estudo realizado no estado de São Paulo, localizado na região sudeste, encontrou uma prevalência muito baixa (3,9%)[21], o que contrasta com outros realizados nos estados da Bahia e Minas Gerais, localizados no nordeste e regiões sudeste, onde a prevalência variou entre 27,5% a 36,4%[24,25]. Além disso, o número de pacientes estudados também foi variável, oscilando de 52 a 262[21,23-25], e as manifestações clínicas associadas à CCS nesses pacientes foram pouco descritas. Até o momento não temos estudos longitudinais com grandes amostras que descrevam bem os pacientes com CCS e HTLV-1, sendo este o primeiro trabalho de incidência de CCS em portadores de HTLV-1, realizado em uma coorte de 1137 pacientes.

Portanto, o presente estudo buscou determinar a morbidade da CCS associada ao HTLV-1 em pacientes portadores de HTLV-1, utilizando uma amostra substancialmente maior do que a estimativa anterior, além de identificar manifestações clínicas e fatores associados para estimar com maior precisão a magnitude desse problema de saúde, em Salvador.

2 OBJETIVOS

2.1 Objetivos primários

- Determinar a incidência e prevalência da CCS em pacientes portadores de HTLV-1.
- Determinar os fatores associados e de risco para CCS em pacientes portadores de HTLV-1.
- Identificar a prevalência e fatores associados de CCS em pacientes portadores de HTLV-1.

2.2 Objetivo secundário

- Descrever as manifestações clinicas associados a CCS do HTLV-1.

3 REVISÃO DE LITERATURA

3.1 O HTLV-1

3.1.1 Histórico

O HTLV-1 pertence à família *Retroviridae*, subfamília *Orthoretroviridae* e ao gênero Deltaretrovirus. Foi primeiro identificado em 1980, de uma linhagem de células linfoblastóides provenientes de um paciente com linfoma cutâneo de células T[30]. Logo em seguida, em 1982, o HTLV-2 foi isolado de células obtidas de paciente com tricoleucemia[31]. Em 1986, o HTLV-1 foi detectado pela primeira vez no Brasil em imigrantes japoneses em Campo Grande, Mato Grosso do Sul[32].

3.1.2 Epidemiologia

O HTLV-1 está presente em todo o mundo e estima-se que 20 milhões de pessoas estejam infectadas[33]. No entanto, a prevalência varia de acordo com a região geográfica, os padrões sócio-comportamentais e étnicos das populações. Esta infecção caracteriza-se, também, por ser mais frequente no gênero feminino e aumentar com a idade[34,35].

A prevalência é elevada no sudoeste do Japão, Caribe, Melanésia (Oceania) e em países da África localizados abaixo do Saara. Por outro lado, nos Estados Unidos da América do Norte, Europa, Ásia exceto Japão e no norte da África a prevalência é muito baixa[36]. O HTLV-1 é também endêmico em vários países da América do Sul e Central, incluindo Argentina, Bolívia, Brasil, Chile, Colômbia, Honduras, Panamá, Peru e Venezuela[36].

No Brasil, observou-se uma prevalência de 13% nos imigrantes japoneses e de 8% nos seus descendentes. Posteriormente, vários estudos demonstraram a presença do HTLV-1 em outros grupos populacionais[37].

Na cidade de Salvador, Moreira Jr e colaboradores em 1993 demonstraram uma prevalência de 1,8% em doadores de sangue[38]. No Brasil, foi realizado um estudo, sob a coordenação do Ministério da Saúde que envolveu cerca de 5.000 doadores de

sangue em cinco cidades de quatro regiões geográficas (Manaus, Recife, Salvador, Rio de Janeiro e Florianópolis), verificando-se que a prevalência variava nas diferentes regiões, sendo 0,1% em Manaus e Florianópolis, 0,33% em Recife e Rio de Janeiro e 1,35% em Salvador[2]. Esta cidade, por apresentar a mais elevada taxa de prevalência, foi considerada o epicentro da epidemia no país. Um estudo em 1998 demonstrou uma prevalência de 1,76% na população geral, através de amostra constituída por 1385 indivíduos sendo 42% do gênero masculino e 58% do feminino, de várias faixas etárias[4]. A prevalência nos gêneros masculino e feminino foi de 1,2% e de 2%, respectivamente. Observou-se uma elevação significativa da prevalência com o aumento da idade, atingindo um valor de 9% em mulheres acima de 51 anos. Baseado nestes dados estimou-se que 50.000 indivíduos desta cidade estão infectados por este vírus[4].

3.1.3 Modos de Transmissão

A transmissão inter-humana do HTLV-1 depende essencialmente da veiculação de linfócitos infectados, pois o plasma livre de células é incapaz de promover a infecção. São três as formas de transmissão: via perinatal ou vertical (mãe para o filho); via sexual e via parenteral (transfusão de componentes celulares de sangue contaminado e compartilhamento de seringas e agulhas contaminadas).

A transmissão vertical pode ocorrer por três vias: transplacentária ou hematogênica, durante o trabalho de parto e pelo aleitamento materno. Tem sido demonstrado que este último é o fator principal de contaminação dos recém-nascidos[39-42]. A taxa de soroconversão pela amamentação é de cerca de 20% e está relacionado com o tempo de aleitamento[43]. No Japão, observou-se uma redução de 80% da transmissão do vírus com a suspensão do aleitamento materno[43,44].

Outra possibilidade de transmissão se dá durante o parto. No trabalho de parto, as contrações uterinas podem romper a barreira placentária resultando em microtransfusões da mãe para o filho ou pelo contato direto com o sangue contaminado. Por outro lado, pode ocorrer também a migração ascendente destes microorganismos, pela vagina durante o parto natural[39,44,42]. Mesmo com valores

mais baixos, não se pode excluir a transmissão vertical durante a gravidez por via transplacentária ou hematogênica.

Evidências biológicas e epidemiológicas demonstram que a transmissão heterossexual é uma importante via de contaminação pelo HTLV-1, principalmente para as mulheres. Estudos têm demonstrado que num período de 10 anos de relacionamento conjugal, a eficiência de transmissão é 60% do homem para a mulher, enquanto no sentido inverso é apenas de 0,4%[45-47].

A transmissão parenteral do HTLV-1 pode ocorrer durante a transfusão de células do sangue infectadas e no compartilhamento de seringas ou agulhas contaminadas pelo vírus[34]. No Japão, a taxa de soroconversão foi de 63% em recipientes de produtos celulares de sangue contaminado. Demonstrou-se também que a média de soroconversão ocorreu entre 20 a 50 dias após a transfusão[48]. A triagem sorológica obrigatória de sangue é uma medida extremamente eficaz na redução de transmissão pela via sanguínea. A prática do compartilhamento de seringas e agulhas contaminadas entre usuários de drogas injetáveis é uma via importante de transmissão parenteral e tem contribuído para a disseminação deste vírus neste grupo de indivíduos.

3.1.4 Diagnóstico Laboratorial

O diagnóstico de uma infecção tem como objetivo a detecção do agente infeccioso. Devido ao fato dos métodos utilizados para detecção direta destes agentes consumirem muito tempo para diagnóstico, serem de custo elevado e trabalhosos, utiliza-se, rotineiramente, a detecção indireta dos agentes por meio da demonstração de anticorpos específicos. Este método de medida é conhecido como sorologia ou diagnóstico sorológico. Devido a problemas de sensibilidade e especificidade dos testes adota-se, como regra geral, que qualquer amostra testada com resultado reagente deve ser retestada por um outro teste com antígeno e/ou princípio diferente do primeiro. Portanto, o diagnóstico sorológico da infecção causada pelo HTLV baseia-se na detecção de anticorpos específicos aos vírus[37].

Os métodos diagnósticos podem ser divididos em testes de triagem e de confirmação. O primeiro se caracteriza por apresentar elevada sensibilidade evitando resultados falso negativos. No entanto, por apresentarem baixa especificidade podem resultar em reações falso positivas. Consequentemente, as amostras devem ser reavaliadas por testes mais específicos.

Em relação ao HTLV, os testes de triagem mais utilizados são o ensaio imunoenzimático (Elisa) e as reações de aglutinação. Estes testes não discriminam o HTLV-1 do HTLV-2 devido à elevada homologia entre estes vírus. Os Elisa são de fácil execução, podem ser automatizados e realizados em larga escala e, por isto, são preferencialmente utilizados na triagem de doadores de sangue. Os testes confirmatórios mais utilizados são o Western Blot e a imunofluorescência. Estes, além se serem muito específicos, discriminam na maioria das vezes, o HTLV-1 do HTLV-2. Esta discriminação ocorre no Western Blot, pois os antígenos virais são separados conforme seu peso molecular por meio da eletroforese. Algumas amostras não são discriminadas entre HTLV-1 e HTLV-2 e são consideradas simplesmente como HTLV. Outras, não preenchem o critério de diagnóstico positivo e são rotuladas como indeterminadas. Nestes casos, para o esclarecimento do diagnóstico laboratorial se lança mão de técnicas moleculares.

O método molecular comumente empregado é a detecção do DNA proviral, devido à ausência de viremia plasmática, por meio da Reação em Cadeia da Polimerase (PCR). Este teste apresenta alta sensibilidade e especificidade. A técnica de PCR em tempo real, desenvolvida mais recentemente, tem sido de grande valia porque quantifica o número de cópias de DNA proviral na amostra e, portanto, determina a carga proviral. Há crescentes evidências de que a carga proviral elevada esteja associada à progressão da doença[49].

3.1.5 Doenças Associadas

As patologias incontestavelmente associadas com o HTLV-1 são a Paraparesia Espástica Tropical ou Mielopatia Associada ao HTLV-1 (HAM-TSP), a Leucemia/ Linfoma de células T do Adulto (LLTA)[7] e a Uveíte associada ao HTLV (UAH)[12].

Além das uveítes, outras alterações oftalmológicas vêm sendo associadas ao HTLV-1, como a ceratite intersticial e a ceratoconjuntivite seca, tema dessa tese[21-26].

3.1.6 Alterações Oftalmológicas

A maior parte do conhecimento sobre alterações oculares foi obtida em trabalhos realizados no Japão[50]. A primeira evidência de associação entre HTLV e uveítes idiopáticas ocorreu em 1992, no Japão com a demonstração de DNA pro-viral e RNA do HTLV-1 em tecidos oculares e em clones de células T derivadas do humor aquoso de pacientes portadores[12]. Posteriormente, o mesmo grupo de pesquisadores demonstraram, por meio de estudos sorológicos, na região de Kyushu (Miyakonodo), uma forte associação de uveítes e a infecção causada pelo HTLV-1[49,51].

Merle e colaboradores em 2002 demonstraram elevada prevalência de uveítes (14,5%) em pacientes assintomáticos e portadores de TSP/HAM ou LLcTA na Martinica[23].

No Brasil, em 1999, Yamamoto e colaboradores observaram prevalência de 2,8% e 3,9% de uveítes e de ceratoconjuntivites seca respectivamente em portadores de HTLV-1[21]. Em 1995, Pinheiro e colaboradores estudaram 55 pacientes com uveítes idiopáticas evidenciando uma soroprevalência para HTLV-1 de 3,7%[52].

Soares e Moraes Junior, em 2000, analisaram 17 pacientes portadores e constataram 11,8% de uveítes, 11,8% de vasculites retinianas, 5,9% de opacidades vítreas e 55 assintomáticos, ausência de uveítes, 1,8% de vasculites retinianas e 1,8% de exsudatos algodonosos[53].

Em 2006, Pinheiro e colaboradores realizaram estudo para avaliar a ocorrência de uveítes em três grupos distintos, sendo eles: portadores assintomáticos, portadores com TSP/HAM e controles. Foi evidenciada uma prevalência de 1,82% nos portadores com TSP/HAM e 1,93% nos portadores assintomáticos[24]. Mais recentemente Rathsam-Pinheiro e colaboradores evidenciaram 2,8% de uveítes em portadores de HTLV[25].

Outra moléstia ocular de grande importância nos portadores de HTLV-1 é a ceratoconjuntivite seca a qual ocorre por uma deficiência na lubrificação da superfície ocular. A Síndrome de Sjogren, caracterizada por uma diminuição da secreção salivar e lacrimal, também têm sido relatada[54,55].

3.2 A Ceratoconjuntivite Seca

3.2.1 O Filme Lacrimal e suas Alterações

O filme lacrimal e a superfície ocular formam um sistema complexo e estável do qual o olho depende para manter uma boa visão. As funções do filme lacrimal incluem limpeza da superfície ocular com ação antimicrobiana, lubrificação e transporte de oxigênio e nutrientes para o epitélio corneano, além de formar a superfície óptica mais anterior do olho. As camadas do filme lacrimal são subdivididas em camadas aquosa, lipídica e mucosa[56].

A ceratoconjuntivite seca resulta da perda qualitativa ou quantitativa de qualquer uma das camadas do filme lacrimal. Sendo assim, é necessária uma quantidade suficiente de lágrima, a sua composição equilibrada, o fechamento normal das pálpebras, além do ato de piscar regular. Outros fatores, como alterações patológicas das pálpebras, córnea ou conjuntiva, também podem levar a um distúrbio da função normal do filme lacrimal[57].

Cada vez mais a ceratoconjuntivite seca vem sendo considerada uma doença inflamatória. A hiperosmolaridade do filme lacrimal causa dano no epitélio da superfície ocular através da ativação de uma cascata de eventos inflamatórios envolvendo citocinas inflamatórias como IL-1, TNF e MMP-9[58]. Tanto a diminuição na produção aquosa (olho seco por deficiência aquosa) quanto um aumento na sua evaporação (olho seco evaporativo) podem levar ao aumento da osmolaridade da lágrima e, consequentemente, a inflamação da superfície ocular. A deficiência aquosa é secundária a uma falência na secreção lacrimal, e pode estar relacionada ou não à síndrome de Sjögren. A diminuição da produção aquosa pela glândula lacrimal principal geralmente resulta de doenças infiltrativas que levam à fibrose do tecido glandular. Quando a disfunção lacrimal é devido à inflamação e infiltração da glândula,

mediadores inflamatórios produzidos nela vão através da lágrima ser depositados na superfície ocular[59]. A deficiência aquosa não-Sjögren geralmente se apresenta como olho seco relacionado à idade ou pode ser secundária a infiltração glandular por linfomas, neurofibromas, infecções virais e sarcoidose[60].

3.2.2 Prevalência de CCS

Em estudo de base populacional na cidade de Salisbury, nos Estados Unidos, realizado em 2.520 pacientes acima de 65 anos, foi identificada uma prevalência de 14,6% de pacientes com sintomas frequentes de olho seco[61].

O estudo de coorte Beaver Eye Study acompanhou 2.414 indivíduos, de 48 a 91 anos, por 05 anos, identificando uma incidência de 13,3% de CCS. A incidência aumentou com a idade, passando de 10,7% em pacientes de 48 a 59 anos para 17.9% em pacientes com 80 anos ou mais. A incidência de CCS também foi maior em mulheres (14,7%) quando comparada aos homens (11,7%), porém após o ajuste para a idade essa diferença não apresentou significância estatística ($p = 0,06$)[62].

Em 2002, foi realizado estudo com 39.876 mulheres americanas, no qual foi observada uma prevalência geral de 6,7% de CCS. A prevalência foi maior com o aumento da idade, passando de 5,7% nas mulheres com menos de 50 anos para 9,8% em mulheres com 75 anos ou mais[63].

No Brasil, e particularmente em Salvador, a escassez de dados de prevalência e incidência da CCS na população geral dificulta o real dimensionamento dessa patologia em nosso meio.

3.2.3 Diagnóstico e Quadro Clínico

O olho seco pode ser diagnosticado através dos seguintes critérios: 1) sintomatologia; 2) anormalidades na dinâmica lacrimal, através do teste de Schirmer e do tempo de ruptura do filme lacrimal; 3) dano na superfície ocular, pelos testes de coloração pela fluoresceína e com o corante Rosa Bengala; e 4) medida da osmolaridade da lágrima. Embora estes elementos estejam presentes na maioria dos casos de olho seco, na

prática clínica observa-se que há pacientes com sintomas, porém com mínimo dano ocular, ou sinais de dano na ausência de sintomas.[64-67]

Os testes diagnósticos utilizados nesse estudo foram: TBUT, Teste de Schirmer I e a coloração pelo corante Rosa Bengala. O TBUT é ideal para avaliação da instabilidade do filme lacrimal e é feito contando o intervalo de tempo entre o último piscar e o aparecimento de um ponto seco no filme lacrimal, sendo um intervalo menor que 10 segundos considerado sugestivo de olho seco. No teste de coloração pela fluoresceína o corante irá se depositar nas áreas onde o epitélio corneano encontra-se lesionado, sendo analisadas cinco regiões da córnea (superior, inferior, nasal, temporal e central) e quatro regiões da conjuntiva (superior, inferior, nasal e temporal). Cada região é graduada em uma escala de 0 a 4, e determinado o escore total através do somatório de todas as regiões. É considerado anormal um escore maior ou igual a 3. Esse teste normalmente revela uma cloração característica e está confinada a área interpalpebral exposta da superfície ocular, porém em casos mais severos, a coloração pode se estender a áreas não expostas, particularmente na conjuntiva bulbar superior[64,65].

O teste de Schirmer I é o teste mais simples para avaliar produção lacrimal, embora possa estar afetado por condições ambientais, como temperatura e umidade. Pode ser realizado com ou sem a instilação de colírio anestésico, analisando respectivamente a secreção lacrimal basal e reflexa[66]. Para realização do exame coloca-se um filtro milimetrado (Papel de Whartman número 41, 5mm x 35mm); no fornix inferior do olho e observa-se a umidificação do mesmo por um intervalo de tempo de 5 minutos. O teste é considerado alterado se a secreção observada após 5 minutos, for menor ou igual a 5 mm para o Schirmer I com anestésico e menor ou igual a 10 mm sem anestésico[64,65].O Dews 2017 preconiza positividade com um exame menor ou igual a 10 mm de umidificação após 5 minutos de observação[28].

A Rosa Bengala é um corante derivado da fluoresceína e cora células lesadas e mortas, assim como o muco, sem, no entanto, penetrar em defeitos epiteliais. A solução a 1% é instilada no fórnice conjuntival inferior, precedida de anestesia tópica. Posteriormente, o número de pontos vermelhos presentes na conjuntiva nasal e lateral

e na córnea são contados e a somatória das três áreas avaliadas for superior ou igual a 4+ o teste é considerado positivo[66-68].

3.3 Ceratoconjuntivite seca e HTLV-1

A associação entre o vírus HTLV-1 e a CCS começou a ser descrita no final dos anos 80, em pacientes com uma síndrome tipo-Sjögren[69,70]. Em um grupo de pacientes com mielopatia associada ao HTLV foi descrito um infiltrado de células mononucleares na glândula salivar, demonstrando que o HTLV-1 está envolvido na patogênese da CCS em pacientes de áreas endêmicas[54]. Porém, a ausência de alterações imunológicas relacionadas a doenças reumatológicas faz da CCS associada ao HTLV-1 uma entidade diferente da síndrome de Sjögren primária[71,72].

Estudo de prevalência de CCS em pacientes com HTLV vem sendo realizados no Brasil e em outras regiões do mundo. Em 1999, Yamamoto e colaboradores, em São Paulo observaram uma prevalência 3,9% de CCS em pacientes infectados pelo HTLV-1, porém esse estudo foi realizado em pacientes sem HAM-TSP[21]. Em 2002, estudo realizado na Martinica avaliando 200 indivíduos infectados pelo HTLV-1, encontrou uma prevalência de 37% de CCS[23]. Em Minas Gerais, a prevalência de CCS foi de 54,5% em pacientes com HAM-TSP e 20,3% em portadores assintomáticos. Nos indivíduos-controle a prevalência foi de 12,7%[24]. Na Bahia Rathsam-Pinheiro em 2009 e Vargens-Sena em 2001 evidenciaram respectivamente, uma prevalência de 36,4% e 44,2% em 140 pacientes portadores de HTLV [25,26].

4 MÉTODOS

4.1 Desenho de estudo

Coorte retrospectiva realizada no período de junho de 2004 a setembro de 2017. Para o estudo de prevalência foi realizado um corte transversal compreendido no mesmo período da coorte.

4.2 Local e população do estudo

Pacientes HTLV-1 positivos acompanhados no Centro Integrativo e Multidisciplinar de HTLV da Escola Bahiana de Medicina e Saúde Pública (CHTLV/EBMSP), diagnosticados através de sorologia pelo método ELISA, e Western Blot para confirmação.

4.3 Seleção da amostra

Todos os pacientes matriculados no CHTLV/EBMSP em junho de 2004 foram convidados por telefone e telegrama; e pacientes de primeira consulta ou que frequentavam regularmente o centro, eram convidados durante o seu atendimento, para consulta oftalmológica.

4.4 Critérios de inclusão

Todos os pacientes matriculados no CHTLV/EBMSP com sorologia positiva para HTLV-1 e que concordassem em participar do estudo.

4.5 Critérios de exclusão

Pacientes menores de 18 anos, consultas incompletas, portadores de HIV/AIDS, Hepatite C, Hepatite B, HAM/TSP possíveis ou prováveis e pacientes portadores de doenças diagnosticadas previamente como alterações palpebrais, infecções oculares crônicas, cirurgias refrativas, uso de lente de contato, uso de antidepressivos e doenças sistêmicas. Para a avaliação de incidência foram também excluídos

pacientes com diagnóstico prévio de CCS, pacientes sem consultas subsequentes e com essas incompletas.

4.6 Termo de Consentimento

Os pacientes inclusos, compareceram para consulta oftalmológica, foram informados sobre o projeto e assinaram o termo de consentimento livre e esclarecido (*vide* modelo Apêndice A).

4.7 Avaliação Oftalmológica

Todos os pacientes realizaram exame oftalmológico completo no Centro de HTLV e quando necessário, exames complementares foram realizados no IBOPC.

- Medida da Acuidade Visual com tabelas de Snellen
- Exame Ocular Externo
- Refratometria com prescrição de lentes corretoras quando necessário
- Biomicroscopia anterior
- Fundoscopia direta e Biomicroscopia posterior
- Tonometria de Aplanação
- Testes específicos para diagnóstico de CCS:

Tempo de Ruptura do Filme Lacrimal (*TBUT – Tears Break Up Time*): instila-se 25µl de solução de fluoresceína a 1% no fórnice inferior de cada olho, solicitando ao paciente que pisque e contando o intervalo de tempo entre o piscar e o aparecimento de um ponto seco no filme lacrimal, considerando positivo um intervalo menor que 10 segundos[67,73].

Teste de Schirmer I sem anestesia: realizado com a introdução de uma das extremidades de uma tira milimetrada de papel de Whartman número 41 (5mm de largura por 35mm de comprimento) no fórnice inferior da conjuntiva, observando-se em 5 minutos quantos milímetros do papel foram umidificados pela secreção lacrimal[67,73].

Teste de coloração com Rosa Bengala: A solução a 1% é instilada no fórnice conjuntival inferior, precedida de anestesia tópica. Posteriormente, o número de pontos vermelhos presentes na conjuntiva nasal e lateral e na córnea são contados e graduados: 1+, cora esparsamente; 2+ cora densamente; e 3+ cora intensamente e de forma confluente. Se a somatória das três áreas avaliadas for superior ou igual a 4+ o teste é considerado positivo segundo os critérios propostos por van Bijsterveld[67,73].

Questionário de Sintomatologia: OSDI (*Ocular Surface Disease Index*): questionário que consiste em 12 perguntas relacionadas à presença e intensidade de sintomas de olho seco (**Anexo B**). Para cada pergunta é estipulado um valor de 0 a 4. Posteriormente todos os valores são somados e esse subtotal é multiplicado por 25 e dividido pelo número de questões respondidas. O valor final desse cálculo é lançado em uma escala de cores que representa a intensidade dos sintomas: normal, leve, moderado e severo[67,73].

4.8 Critérios Diagnósticos

O diagnóstico de ceratoconjuntivite seca foi definido com a positividade de pelo menos dois dos seguintes exames: Tempo de Ruptura do Filme Lacrimal (*TBUT*), Teste de Schirmer e coloração com Rosa Bengala[67,73-75].

O diagnóstico de HTLV-1 seguiu o algoritmo recomendado pelo Ministério da Saúde do Brasil: amostras de plasma repetidamente positivas em duplicata pelo ELISA (HTLV-1/HTLV-2 Ab-Capture ELISA Test System, Ortho. Clinical Diagnostic Inc. Raritan, New Jersey, USA) foram confirmadas e discriminadas entre HTLV-1 e HTLV-2 usando Western Blot (HTLV Blot 2.4; Genelabs, Singapore)[76]. Reação em Cadeia de Polimerase (PCR) foi realizada em amostras com resultados indeterminados de acordo com a técnica descrita por Kashima[77].

O diagnóstico de HAM/TSP foi feito pelo médico neurologista do Centro de HTLV, de acordo com os critérios estabelecidos por Castro-Costa o qual se baseia em níveis de consciência de HAM/TSP, e o divide em HAM/TSP Definido, Provável e Possivel[78].

HAM/TSP Definido:

1. Uma paraparesia espástica progressiva não remitente com marcha suficientemente prejudicada para ser percebida pelo paciente. Sensorial sintomas ou sinais podem ou não estar presentes. Quando presentes, eles permanecem sutis e sem um nível sensorial claro. Sinais ou sintomas do esfíncter urinário e anal podem ou não estar presentes.

2. Presença de anticorpos contra HTLV-I no soro e no LCR confirmados por Western blot e / ou PCR positivo para HTLV-I no sangue e / ou CSF.

3. Exclusão de outros distúrbios que podem se assemelhar a TSP / HAM.

HAM/TSP Provável:

4. Apresentação monossintomática: espasticidade ou hiperreflexia nos membros inferiores ou sinal de Babinski isolado com ou sem sinais ou sintomas sensoriais sutis, ou bexiga neurogênica apenas confirmada por testes urodinâmicos.

5. Presença de anticorpos anti-HTLV-I no soro e / ou no LCR confirmados por Western blot e / ou PCR positivo para o HTLV-I sangue e / ou CSF.

6. Exclusão de outros distúrbios que podem se assemelhar a TSP / HAM.

HAM/TSP Possível:

1. Apresentação clínica completa ou incompleta.

2. Presença de anticorpos anti-HTLV-I no soro e / ou no LCR confirmados por Western blot e / ou PCR positivo para o HTLV-I sangue e / ou CSF.

3. Desordens que podem se assemelhar a TSP / HAM não foram excluídas.

4.9 Analise Estatística

A análise estatística foi realizada utilizando o software Stata v. 13.0. Dados ausentes foram excluídos da análise. A medida de incidência global foi calculada pela razão entre o número total de casos novos diagnosticados com CCS e o número total de pacientes avaliados no período do estudo, e expresso em porcentagem. Para considerar o tempo entre as avaliações, a densidade de incidência foi obtida e expressa em pessoas/ano. A prevalência global foi calculada dividindo-se o número de casos de CCS pelo número de pacientes incluídos avaliados, expresso em porcentagem. Idade e tempo de diagnóstico sorológico foram considerados como variável contínua. A presença de CCS foi avaliada de acordo com o status sociodemográfico, clínico e tempo de diagnóstico do HTLV, utilizando-se o teste do qui-quadrado de Pearson ou o teste exato de Fisher, quando apropriado. As taxas de prevalência brutas e ajustadas (RP) e os riscos relativos brutos e ajustados com seus respectivos intervalos de confiança de 95% (IC 95%) foram estimadas usando regressão de Poisson multivariada com variância de erro robusta. Valores de $p < 0,05$ foram considerados significativos.

5 ARTIGOS CIENTÍFICOS

5.1 "Incidence of Keratoconjunctivitis sicca associated with Human T-Cell Lymphotropic Virus Type 1"

Incidence of Keratoconjunctivitis Sicca associated with Human T-Cell Lymphotropic Virus Type 1

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Keywords: HTLV-1 infections; keratoconjunctivitis sicca; incidence; dry eye syndromes.

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Descritores: ceratoconjuntivite seca; HTLV-1; vírus 1 linfotrópico T humano; síndrome do olho seco; incidência.

ABSTRACT

Background: Keratoconjunctivitis sicca is an important ophthalmological disease often observed in patients infected with HTLV-1 virus. The prevalence of this association may reach 30-40%. However, this is the first study that evaluates incidence of KCS in HTLV-1 positive individuals and describes risk factors and clinical manifestations.

Purpose: Determine the incidence of Keratoconjunctivitis sicca in HTLV-1 infected patients in Salvador, Bahia**.**

Methods: This is a longitudinal study performed at the HTLV Multidisciplinary Center (CHTLV) of the EBMSP, in Salvador - Bahia. Patients with HTLV-1 infection who attended an ophthalmological appointment for a complete eye exam from June 2004 to September 2017 were included. To evaluate the quality of the tear film, patients were submitted to Tear breakup time (TBUT), Schirmer I test and Rose Bengal staining. Dry eye disease was diagnosed when at least two of these three tests were abnormal. The overall incidence of dry keratoconjunctivitis in patients with HTLV-1 was determined.

Results: Data from 1137 patients with HTLV-1, enrolled in CHTLV and who agreed to participate in the study were collected. Of these, 207 were excluded because they had an incomplete ophthalmological assessment, 302 had a positive diagnosis for KCS at the first ophthalmological exam and 377 did not have a subsequent evaluation. Of the remaining 251 patients, nine were under 18 years old, six had associated systemic diseases, six had HIV or HCV and 20 did not have a definite diagnosis of HAM/TSP. The data of 210 were then evaluated, but 27 did not have a second complete ophthalmological evaluation and were also excluded. The total incidence was 44.0% (81/183) and the

incidence density was 76 / 1000 people / year, with a median (p25 - p75) follow-up time of 4.3 years (2.2 - 8). An adjusted bivariate analysis reinforced the association between the incidence of KCS in HTLV-1 carriers regardless of age, sex and other conditions, with HAM / TSP and time of diagnosis being risk factors for the development of KCS in HTLV-1 carriers.

Conclusion: The incidence of KCS in patients with HTLV-1 was higher than the mean of the seronegative population for the disease. Pain and / or burning, pruritus and visual acuity <20/30, were the most common changes among HTLV-1 and KCS patients. Thus, it is recommended that patients with HTLV-1 undergo periodic eye exams to promote the early diagnosis of KCS and to prevent the consequences associated with Dry Eye Disease.

RESUMO

Fundamentação: A ceratoconjuntivite seca é uma importante doença oftalmológica frequentemente observada em pacientes infectados pelo vírus HTLV-1. A prevalência dessa associação pode chegar a 30-40%. No entanto, este é o primeiro estudo que avalia a incidência de CCS em indivíduos positivos para o HTLV-1 e descreve fatores de risco e manifestações clínicas.

Objetivo: Determinar a incidência de ceratoconjuntivite seca em pacientes infectados pelo HTLV-1 em Salvador, Bahia.

Métodos: Trata-se de um estudo longitudinal realizado no Centro Multidisciplinar do HTLV (CHTLV) da EBMSP, em Salvador - Bahia. Pacientes com infecção por HTLV-1 que compareceram a uma consulta oftalmológica para um exame oftalmológico completo de junho de 2004 a setembro de 2017 foram incluídos. Para avaliar a qualidade do filme lacrimal, os pacientes foram submetidos ao tempo de ruptura do filme lacrimal (TBUT),

teste de Schirmer I e coloração de Rosa Bengala. A doença do olho seco foi diagnosticada quando pelo menos dois destes três testes foram anormais. A incidência global de ceratoconjuntivite seca em pacientes com HTLV-1 foi determinada.

Resultados: Foram coletados dados de 1137 pacientes com HTLV-1, inscritos no CHTLV e que concordaram em participar do estudo. Destes, 207 foram excluídos por terem uma avaliação oftalmológica incompleta, 302 tiveram diagnóstico positivo para CCS no primeiro exame oftalmológico e 377 não tiveram avaliação subsequente. Dos restantes 251 pacientes, nove tinham menos de 18 anos, seis tinham doenças sistêmicas associadas, seis eram portadores de HIV ou HCV e 20 não tinham diagnóstico definitivo de HAM / TSP. Os dados de 210 foram então avaliados, mas 27 não tiveram a segunda avaliação oftalmológica completa e também foram excluídos. A incidência total foi de 44,0% (81/183) e a densidade de incidência foi de 76/1000 pessoas / ano, com mediana (25 a 75) de tempo de seguimento de 4,3 anos (2,2 - 8). Uma análise bivariada ajustada reforçou a associação entre a incidência de CCS em portadores de HTLV-1 independentemente da idade, sexo e outras condições, com HAM / TSP e tempo de diagnóstico como fatores de risco para o desenvolvimento de CCS em portadores de HTLV-1.

Conclusão: A incidência de CCS em pacientes com HTLV-1 foi maior que a média da população soronegativa para a doença. Dor e / ou ardor, prurido e acuidade visual <20/30, foram as alterações mais comuns entre os pacientes com HTLV-1 e CCS. Assim, recomenda-se que pacientes com HTLV-1 sejam submetidos a exames oftalmológicos periódicos para promover o diagnóstico precoce da CCS e prevenir as consequências associadas à Ceratoconjuntivite Seca.

Author Summary

The infection with HTLV-1 is endemic worldwide with a high prevalence in cities like Salvador, Brasil. For the first time we determined the incidence of HTLV-1 associated KCS, their clinical manifestations and associated factors, using a cohort of 1137 subjects, to more precisely estimate the magnitude of this neglected health problem, in Salvador. A longitudinal study was conducted between June 2004 and September 2017 and during

this period data from 1137 HTLV-1-infected patients was collected. A complete ophthalmologic examination and specific lacrimal evaluation tests such as Tears breakup time (TBUT), Schirmer I and Rose Bengal stain were realized. KCS was diagnosed when two of these three tests were positive and the overall incidence was determined. This study shows the importance of more assiduous serological screening of HTLV-1 and recommends a periodic ophthalmologic examination to promote the early diagnosis of KCS and prevent the consequences associated with Dry Eye Disease.

INTRODUCTION

Identified in 1980[1], human T-cell lymphotropic virus type 1 (HTLV-1) was the first retrovirus linked to human disease. The HTLV-1 is a delta retrovirus of worldwide distribution and it is estimated that at least 5 to 10 million people are carriers of the virus[2]. Transmission occurs through contaminated blood or tissue, from mother to child (predominantly through breastfeeding), as well as through sexual contact[2]. Many diseases have been associated with HTLV-1 infection, such as adult T-cell leukemia (ATL), tropical spastic paraparesis / HTLV-1 associated myelopathy (TSP / HAM) and ophthalmologic diseases, indicating multisystem involvement[3,4,5]. Ocular diseases such as HTLV-I-associated uveitis (HAU), corneal lesions, retinal vasculitis and dry keratoconjunctivitis (KCS) or dry eye disease (DED) [6,7,8,9] have also been associated with HTLV- 1. KCS is characterized by bilateral and chronic discomfort of the conjunctiva and cornea due to insufficient and poor quality tears with a Tears breakup time test of less than 10 seconds[10]. Yamamoto et al[11] reported that the prevalence of reduced Tears breakup time was significantly higher in HTLV-1 blood donors than in age and sex matched noncarrier blood donors.

Brazil, Japan and Iran are endemic countries with high virus prevalence[12]. Salvador, the capital of Bahia, in northeastern Brazil, has the highest prevalence of HTLV-1 in the country. About 40,000 people are infected with HTLV-1 in the city[13,14].

The prevalence of KCS HTLV-1 infected patients may reach 30-40% [6,15,16,17], especially in symptomatic patients with HAM / TSP. However, there have been no studies concerning the incidence of KCS associated with HTLV-1 infection.

SUBJECTS AND METHODS

A longitudinal study carried out at the Reference Center for HTLV at the Bahia School of Medicine and Public Health, in Salvador-Bahia, Brazil, from June 2004 to September 2017 and approved by the Ethics and Research Committee of this institution, was conducted. Those with a positive serological diagnosis of HTLV-1 (ELISA and Western Blot) were eligible to participate in the study and were submitted to routine ophthalmologic evaluation.

Exclusionary criteria were: patients under 18 years of age, without definite HAM/TSP, with associated HCV, HIV, systemic disorders, previous eyelid disorders, intraocular surgery and nasolacrimal duct obstruction, and those with incomplete ophthalmologic evaluation, without a subsequent exam or with CCS diagnosis at the first evaluation.

Ophthalmologic examination and measurements

Patients underwent detailed ophthalmologic evaluation, including corrected visual acuity through the Snellen chart, intraocular pressure measurement with aplanation tonometer, anterior segment evaluation, eye fundus examination through indirect ophthalmoscopy and evaluation of lacrimal function through three tests: TBUT, Schirmer I and Rose Bengal. Dry eye disease was diagnosed when at least two of these three tests were abnormal.[18]

TBUT was performed by the instillation of 1% of fluorescein solution (Fluorescein® eye drops, Ophtalmos®, São Paulo, Brazil) and the time count necessary for dry spots to appear on the corneal surface after blinking. The Schirmer I test used 5 mm x 35 mm Whatman strips (Schirmer® test, Opthalmos®, São Paulo, Brazil) placed in the lower fornix near the lateral canthus of both eyes. After 5 minutes the strips were removed and the moist portion was measured. Rose Bengal was performed by instilling 1% of the solution of coloring of Rose Bengal (Rose Bengal®, Ophthalmos®, São Paulo, Brazil). Abnormal results were: TBUT under 10 seconds, Schirmer I less than 5mm, and Rose Bengal greater than 3.[18]

Patients without a complete evaluation were excluded from the study.

Statistical Analysis: Statistical analysis was performed using Stata v. 13.0. Missing data were excluded from the analysis. The overall incidence measure was calculated by the ratio between the total number of cases diagnosed with CCS and the total number of patients assessed in the study period, and expressed as a percentage. To consider the time between evaluations, incidence density was obtained and expressed in person / year. Age and time of serological diagnosis were considered as continuous variable. The presence of KCS was evaluated according to the sociodemographic, clinical status and time of diagnosis of HTLV using Pearson's chi-square test or Fisher's exact test, when appropriate. The crude and adjusted relative risks with their respective 95% confidence intervals (95% CI) were estimated using multivariate Poisson regression with robust error variance. Values of $p < 0.05$ were considered significant.

RESULTS

A total of 1137 HTLV-1 infected patients were evaluated between June 2004 and September 2017 (Figure 1). Of these, 207 were excluded because they had an incomplete ophthalmological assessment. After the first ophthalmologic exam, 302 (32.5%) patients had a positive diagnosis of KCS. Among the 628 patients without KCS, nine subjects under 18 years of age, 20 without definite HAM/TSP, 6 with HIV or HCV and 6 with systemic disorders were excluded from the study. Two hundred and ten (33.0%) individuals were reexamined, on average 2.8 (SD=2.0) visits, with time interval between the first and the last visit of 5.1 (SD=3.5) years.

With regards to patients without KCS, table 1 compares patients with and without follow-up. Females outnumber males in both groups, with a discrete increase, but not significant, of the proportion of women in the group with follow-up (71.2% versus 77.6%, p = 0.085). In addition, patients with follow-up had a larger proportion of definite HAM/TSP (21.9% versus 11.4%, p=0.005) and low visual accountability during ophthalmological examination (30.2% versus 40.95%, p=0.034), . For the other variables, there was no significant statistical difference.

Among the 210 patients who had more than one ophthalmologic consultation, 81 (38.6%) had KCS diagnostic (incident cases), while 102 (48.6%) remained without KCS (non-incident cases). Twenty-seven (12.9%) individuals did not complete ophthalmologic assessment and were excluded from the analysis. The overall incidence was 44.3% (81/183) and the incidence density was 76/1000 persons per year in a median (25^{th}-75^{th}) follow-up time of 4.3 (2.2 – 8.0) years.

Table 2 shows the comparison between non-incident and incident cases in relation to socio-demographic, clinical, laboratory, and ophthalmological baseline data. There were no statistically significant differences in age (43.9 versus 44.7, p = 0.679) or sex (females outnumbered males ,81.5% versus 18.5%, p = 0.411). Race pardo (62.8% versus 44.4%, p = 0.025); time of diagnosis of HTLV (4.7 versus 4.3; p 0.013); follow-up time (4.0 versus 6.7, p <0.001) and number of ophthalmologic visits (2.1 versus 3.9; p <0.001) were all statistically significant.

On bivariate analyses follow-up time and time of serological HTLV-1 diagnosis. in baseline, were risk factors for developing KCS. HTLV-1 (table 3). Pardo skin color was a protective factor to KCS incidence (RR:0.55; CI95%: 0.37-0.84). After adjusting for sex, age, time of follow-up, time of serological HTLV-1 diagnostic and skin color,

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definite HAM/TSP becomes an independent risk factor for KCS (RR_{adj}=1.53; CI95%:1.07-2.18) as shown in table 3.

FIGURE 1. Flowchart for study of incidence of Keratoconjunctivitis sicca (KCS) in Human T-Cell Lymphotropic Virus (HTLV) infected persons, in Salvador, Bahia, 2004-2017.

Keratoconjunctivitis sicca (KCS); Human T-cell Lymphotropic Virus type 1 (HTLV-1)

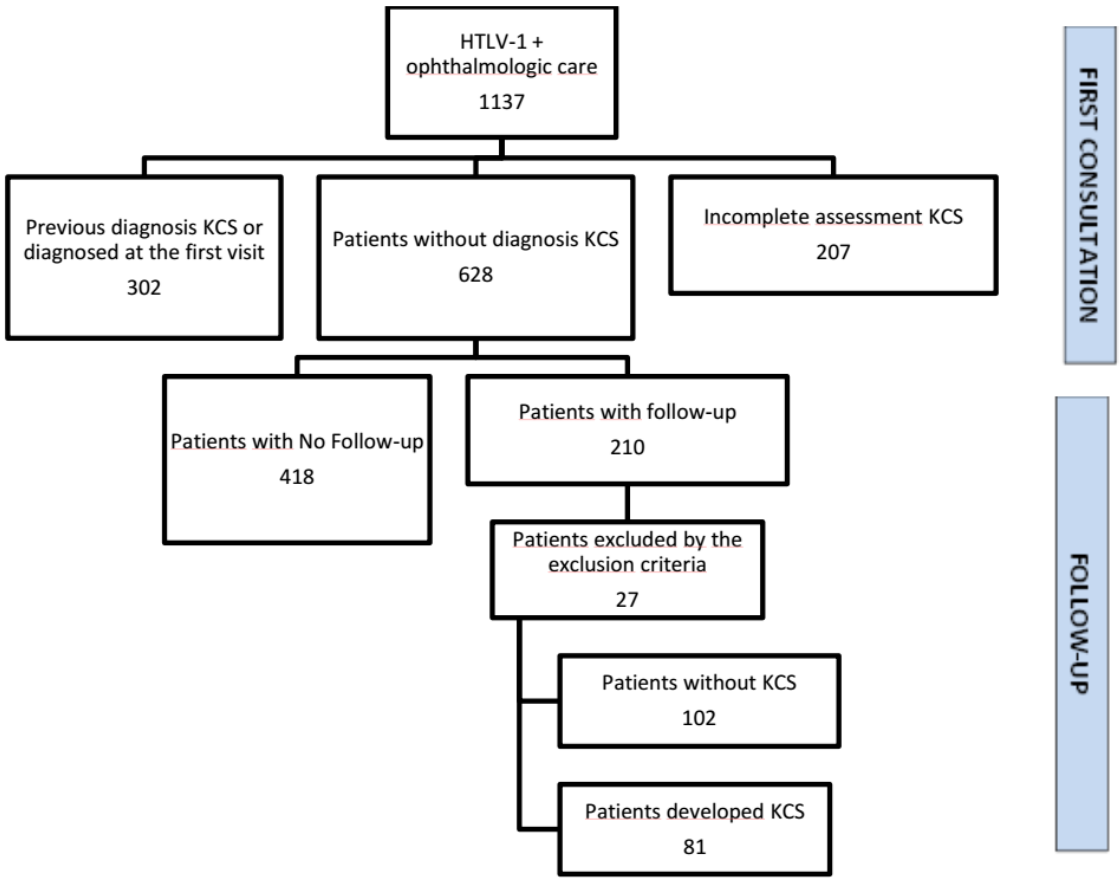

TABLE 1 – Sociodemographic, clinical and laboratorial characteristics of the HTLV-1 infected patients KCS negative on baseline with and without ophthalmologic follow-up accompanied in a reference center, Salvador, Bahia, 2004-2017.

VARIABLES	No follow-up (n=418)	With follow-up (n=210)	P value
Sociodemographic Variables	**n (%)**	**n (%)**	
Age (years)*	44.5 (15.8)	44.3 (13.4)	0.869
Sex			0.074
Male	121 (28.8)	47 (22.4)	
Female	299 (71.2)	163 (77.6)	
Skin color[i]			0.085
White	57 (13.6)	24 (11.4)	
Pardo	195 (46.4)	114 (54.3)	
Black	158 (37.6)	72 (34.3)	
Yellow	2 (0.5)	-	
No registry	08 (1.9)	-	
Education (Years)			0.787
<8	139 (33.3)	74 (35.2)	
8-11	122 (29.3)	56 (27.1)	
≥11	156 (37.4)	75 (37.6)	
No registry	1 (0.7)	-	
Clinical Variables			
HAM/TSP			0.005
Asymptomatic	299 (71.2)	164 (78.1)	
Definite	48 (11.4)	46 (21.9)	
Misclassification	73 (17.4)	0	
HTLV-1 diagnostic time (years)**	1.0 (0.0 – 3.0)	1.0 (0.0 – 3.0)	0.385[a]
Ophthalmologic Variables			
Complaints			
Low visual acuity	68 (16.2)	24 (11.4)	0.299
Burning or ocular pain	115 (27.4)	68 (32.4)	0.331
Hyperemia	20 (4.8)	06 (2.9)	0.477
Pruritus	70 (16.7)	46 (21.9)	0.218
Foreign Body Sensation	20 (4.8)	11 (5.2)	0.842
Tearing	34 (8.1)	25 (11.9)	0.246
Flying Flies	14 (3.3)	06 (2.9)	0.841
No complaints	136 (32.4)	54 (25.7)	0.233
Low visual acuity (<20/30)	127 (30.2)	86 (40.95)	0.034

Keratoconjunctivitis sicca (KCS); Human T-cell Lymphotropic Virus type 1 (HTLV-1); HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis)

*Mean(SD); **Median; [a]Mann_Whitney test; [b]missing values used to calculate chi-square test; [c] chi-square for trend;

[ii] This article uses official Brazilian census "color" labels as a proxy for the socio-demographic category of "race". Patients self-identify as one of five census color options: White, Black, Pardo (mixed-race), Yellow (corresponding to those of Asian descent). Together, Black and Pardo make up the Afro-descendant population. A fifth official color category, Indigenous, was not selected.

TABLE 2 – Sociodemographic and clinical characteristics of the incident and nonincident patients for KCS after ophthalmologic follow-up at a reference center, Salvador, Bahia, 2004-2017.

VARIABLES	Non incident (n=102)	Incident (n=81)	P value
Sociodemographic Variables	**n (%)**	**n (%)**	
Age (years)*	43.9 (14.1)	44.7 (11.6)	0.679
Sex-			0.411
Male	24 (23.5)	15 (18.5)	
Female	78 (76.5)	66 (81.5)	
Skin color			0.025
White	7 (6.9)	13 (16.1)	
Pardo	64 (62.8)	36 (44.4)	
Black	31 (30.4)	32 (39.5)	
No registry	1 (0.8)	-	
Education (Years)			0.684[b]
<8	35 (34.3)	27 (33.3)	
8-11	30 (29.4)	20 (24.7)	
≥11	37 (36.3)	34 (42.0)	
Marital status			0.998
Single	38 (37.3)	30 (37.0)	
Married	48 (47.1)	38 (46.9)	
Divorced/ Widow	16 (15.7)	13 (16.1)	
CLINICAL VARIABLES			
HAM/TSP			0.174
Asymptomatic	84 (82.4)	60 (74.1)	
Definite	18 (16.4)	21 (25.9)	
HTLV-1 diagnostic time (years)*	6.8 (4.7)	8.3 (4.3)	0.013 [a]
OPHTHALMOLOGIC VARIABLES			
Follow-up time	4.0 (3.2)	6.7 (3.6)	<0.001
Ophthalmologic Visits (n)	2.1 (1.6)	3.9 (2.3)	<0.001
Complaints			
Low visual acuity	13 (12.8)	05 (6.2)	0.256
Burning or ocular pain	35 (34.3)	22 (27.2)	0.373
Hyperemia	03 (2.9)	01 (1.2)	0.794 [c]
Pruritus	23 (22.6)	18 (22.2)	0.668
Foreign Body Sensation	04 (3.9)	06 (7.4)	0.420 [c]
Tearing	11 (10.8)	13 (16.0)	0.398
Flying Flies	04 (3.9)	02 (2.5)	0.829 [c]
No complaints	27 (26.5)	19 (23.5)	0.590
Low visual acuity (<20/30)	40 (39.2)	37 (45.7)	0.477

Keratoconjunctivitis sicca (KCS); Human T-cell Lymphotropic Virus type 1 (HTLV-1); HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis)

*Mean(SD); **Median (25^{th}-75^{th}). [a] Mann-Whitney test; [b] Chi-square for trend; [c] Fisher's Exact Test

TABLE 3 – Crude and adjusted prevalence ratios of association between variables and incidence of KCS, Salvador, Bahia, 2004-2017.

VARIABLES	RR Crude (IC 95%)	RR Adjusted (IC 95%)
Sex-		
Male	1.00	1.00
Female	1.19 (0.78-1.69)	1.31 (0.90-1.91)
Age (Years)	1.00 (0.99-1.01)	1.01 (0.99-1.02)
Follow-up time	1.12 (1.07-1.16)	1.20 (1.12-1.29)
HTLV-1 diagnostic time (years)	1.04 (1.01-1.07)	0.92 (0.86-0.99)
Marital status		
Single	1.0	
Married	1.00 (0.70-1.43)	
Divorced/ Widow	1.02 (0.63-1.65)	
Education (Years)		
<8	0.91 (0.63-1.32)	
8-11	0.83 (0.55-1.27)	
≥11	1.0	
Skin color		
White	1.00	1.00
Mulatto	0.55 (0.37-0.84)	0.59 (0.39-0.89)
Black	0.78 (0.52-1.17)	0.92 (0.62-1.39)
HAM/TSP		
Absent	1.00	1.00
Definite	1.14 (0.95-1.36)	1.53 (1.07-2.18)
Ophtalmologic complaints		
Any complaint		1.00
No	1.00	
Yes	0.91 (0.61 – 1.34)	
Burning/Ocular pain		
No	1.00	
Yes	0.82 (0.56 – 1.19)	
Hyperemia		
No	1.00	
Yes	0.56 (0.10 – 3.07)	
Pruritus		
No	1.00	
Yes	0.98 (0.66 – 1.46)	
Foreign Body sensation		
No	1.0	
Yes	1.37 (0.81 – 2.35)	
Tearing		
No	1.0	
Yes	1.26 (0.83 – 1.90)	
Low Visual Acuity		
No	1.0	
Yes	1.15 (0.83 – 1.59)	
Visual Acuity < 20/30		
No	1.0	
Yes	0.61 (0.28 – 1.31)	

HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis); Keratoconjunctivitis sicca (KCS); prevalence ratios (PR).

DISCUSSION

The present study had a 13-year follow-up, with a total of 1137 patients infected by HTLV, the only study to date that demonstrates the incidence rate of dry keratoconjunctivitis and correlated factors in patients infected with the virus in Brazil and, to our knowledge, in the world. Despite a large number of potential subjects, many were excluded due to a number of reasons, such as a previously established diagnosis of KCS; a positive diagnosis at first visit; one-time patient visit; and a lack of supplies to establish the diagnosis. This limited the group of infected individuals included in the analysis, which may have underestimated the evidence.

Salvador has one of the highest prevalence rates of HTLV-1 in country[19]. In 2003, Dourado et al.[14] found a prevalence of 1.70% in our city, corresponding to approximately 40,000 people infected with the virus. Romanelli et al.[20] cite in their study the various systemic manifestations associated with HTLV-1, leaving well documented the most common ophthalmological alterations, especially dry keratoconjunctivitis. Giozza et al.[19] demonstrated dry eye syndrome in 75% of patients with HAM / TSP and in 22% of patients without this manifestation. Thus, Salvador is an endemic area for HTLV-1 and given the strong association between this infection and KCS, it is possible to justify the high incidence of KCS found in our study.

An adjusted bivariate analysis reinforced the association between the incidence of KCS in HTLV-1 carriers regardless of age, gender and other conditions, with HAM / TSP, follow-up time and diagnostic time, being multifactor and risk factors.

It is worth noting that HAM / TSP patients were significantly lower in those excluded due to lack of follow-up. Race color (pardo) was a protective factor for KCS in HTLV-1 patients, with race being a protective factor in baseline and after adjustment. Previous studies have documented the appearance of ophthalmological symptoms and higher KCS rates in patients with HAM / TSP[16,17,20].

The sample of individuals with follow-up and without follow-up was similar in terms of age (mean: 44 years), sex ratio and race (Afro-descendants). The same sociodemographic characteristics were found when we compared the incident and nonincident groups and are in agreement with the findings described in other literature[16,21]. This is expected given

the age of onset of ophthalmological diseases, the fact that women are more likely to seek medical attention, and that the majority of the population is black or mixed-race.

Comparing the incident and non-incident groups, patients with a longer diagnosis (8.3 years, $p = 0.013$) and greater ophthalmologic follow-up (3.9 visits, $p < 0.001$) presented more frequent ophthalmological complaints and were more likely to present KCS during follow-up. These data suggest that more frequent follow-ups in patients with longer duration of disease may be indicated. It has been described that, over the years, multiple seroconversions occur in patients with HTLV-1, culminating in the deterioration of sebaceous gland function and the appearance of KCS symptoms [12,22].

Despite the peculiarities typical of a specific Brazilian population and the difficulties common to developing countries, such as the lack of supplies in ophthalmologic evaluations, we believe that the strong correlation between KCS, female sex, disease time and HAM / TSP can be demonstrated in other communities and helps prevent and treat comorbidities related to a poorly understood virus. Thus, additional studies and scientific interest are needed from institutions in other endemic regions, such as Japan, Iran, and Martinique.

Therefore, the absence of similar studies and a control group that allowed comparing the incidence of KCS in the seronegative population for HTLV-1 made some analyzes difficult.

Finally, this study shows the importance of a more assiduous serological screening of HTLV-1 in patients with several systemic manifestations, especially those cited in this study.

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5.2 “Revisiting the prevalence of Keratoconjunctivitis sicca (KCS) associated with Human T-Cell Lymphotropic Virus Type 1(HTLV-1) in Salvador, Bahia: the city with highest prevalence of HTLV-1 in Brazil.

PLOS Neglected Tropical Diseases

Revisiting the prevalence of Keratoconjunctivitis sicca (KCS) associated with Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) in Salvador, Bahia: the city with highest prevalence of HTLV-1 in Brazil

--Manuscript Draft--

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Abstract:	Background: The prevalence of KCS associated with HTLV-1(HTLV-1/KCS) has been estimated at around 37%, but its clinical manifestations are poorly described. Purpose: To determine the prevalence and associated factors of HTLV-1/KCS in a large cohort of HTLV-1-infected individuals living in Salvador, Brazil. Methods: A cross-sectional study was conducted between June 2004 and September 2017 at CHTLV-Bahiana School of Medicine, Salvador, Bahia-Brazil. Data from 758 HTLV-1-infected patients was collected. A complete ophthalmologic examination was performed in both eyes. Lacrimal function was evaluated by breakup time (BUT), Rose Bengal and Schirmer I Tests. KCS diagnosis was considered in the presence of at least two out of three positive tests. Crude and Adjusted Prevalence Rates (PR) with 95% Confidence Intervals (95%CI) were estimated using multivariate Poisson Regression with robust error variance. Results: The overall prevalence of KCS was 31.7%, with higher rates observed in HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients (crude PR: 1.84; CI95%:1.50-2.26) even after adjusting for age, sex, time of HTLV-1 diagnosis and schooling (adjusted PR: 1.63; CI95%:1.31-2.02). Low visual acuity, burning and/or pain and itching were significantly higher in patients with KCS. Conclusion: Burning and/or pain and itching and low visual acuity were the most

	common alterations of HTLV-1/KCS. It is strongly recommended that HTLV-1 patients undergo periodic ophthalmologic examination to promote the early diagnosis of KCS and prevent the consequences associated with Dry Eye Disease.
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Cover Letter

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Editor of PLOS Neglected Tropical Diseases
Salvador, 15 May 2018.

Dear Editor,

Please find attached our article entitled "Revisiting the prevalence of Keratoconjunctivitis sicca (KCS) associated with Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) in Salvador, Bahia: the city with highest prevalence of HTLV-1 in Brazil", which we are submitting for publication in PLOS Neglected Tropical Diseases.

As you are aware, in spite of the fact that the burden of HTLV-1 infection it is clearly demonstrated, this infection and its related diseases are still neglected.

This infection is disseminated worldwide, but most infected individuals reside in developing countries. Infected people besides develop debilitating neurological disease may present fatal proliferative disorders changes. Many other diseases have been associated with HTLV-1 including, ocular lesions have also been associated with HTLV-1, such as uveitis, corneal lesions and retinal vasculitis as well as KCS, indicating a multisystemic involvement in this infection. The prevalence of KCS or dry eye disease associated with HTLV-1 varies in different parts of the world. Investigations regarding the prevalence of HTLV-1/KCS in Brazil have demonstrated discrepancies, with variance from 3.9% to 36.4% and the clinical manifestations associated with KCS in these patients were poorly described.

This article determines the prevalence of HTLV-1-associated KCS using a substantially larger sample than the previous estimation, in addition to identifying clinical manifestations as well as associated factors to more precisely estimate the magnitude of this neglected health problem

It is our sincere hope that you will find this article suitable for publication in the PLOS Neglected Tropical Diseases.

Sincerely yours,

Prof. Dr. Bernardo Galvão-Castro.
Coordenador do Centro Integrativo e Multidisciplinar de HTLV.
Escola Bahiana de Medicina e Saúde Pública.

Title: Revisiting the prevalence of Keratoconjunctivitis sicca (KCS) associated with Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) in Salvador, Bahia: the city with highest prevalence of HTLV-1 in Brazil.

Short title: Prevalence of KCS associated with HTLV-1 in Salvador, Bahia, Brazil.

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Keywords: HTLV-1; keratoconjunctivitis sicca; prevalence; dry eye syndromes.

Abstract

Background: The prevalence of KCS associated with HTLV-1(HTLV-1/KCS) has been estimated at around 37%, but its clinical manifestations are poorly described.

Purpose: To determine the prevalence and associated factors of HTLV-1/KCS in a large cohort of HTLV-1-infected individuals living in Salvador, Brazil.

Methods: A cross-sectional study was conducted between June 2004 and September 2017 at CHTLV-Bahiana School of Medicine, Salvador, Bahia-Brazil. Data from 758 HTLV-1-infected patients was collected. A complete ophthalmologic examination was performed in both eyes. Lacrimal function was evaluated by breakup time (BUT), Rose Bengal and Schirmer I Tests. KCS diagnosis was considered in the presence of at least two out of three positive tests. Crude and Adjusted Prevalence Rates (PR) with 95% Confidence Intervals (95%CI) were estimated using multivariate Poisson Regression with robust error variance.

Results: The overall prevalence of KCS was 31.7%, with higher rates observed in HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients (crude PR: 1.84; CI95%:1.50-2.26) even after adjusting for age, sex, time of HTLV-1 diagnosis and schooling (adjusted PR: 1.63; CI95%:1.31-2.02). Low visual acuity, burning and/or pain and itching were significantly higher in patients with KCS.

Conclusion: Burning and/or pain and itching and low visual acuity were the most common alterations of HTLV-1/KCS. It is strongly recommended that HTLV-1 patients undergo periodic ophthalmologic examination to promote the early diagnosis of KCS and prevent the consequences associated with Dry Eye Disease.

Author Summary

HTLV-1 infection is disseminated worldwide and KCS is a disease associated with HTLV-1. Salvador has the highest prevalence of HTLV-1 in Brazil. We determine the prevalence of HTLV-1-associated KCS using a substantially larger sample than the previous estimation, in addition to identifying clinical manifestations as well as associated factors to more precisely estimate the magnitude of this neglected health problem, in Salvador. A cross-sectional study was conducted between June 2004 and September 2017 Data from 758 HTLV-1-infected patients was collected. A complete ophthalmologic examination was performed in both eyes. Lacrimal function was evaluated by breakup time (BUT), Rose Bengal and Schirmer I Tests. KCS diagnosis was considered in the presence of at least two out of three positive tests. The overall prevalence of KCS was 31.7%, with higher rates observed in HTLV-1-associated HAM/TSP patients (crude PR: 1.84; CI95%:1.50-2.26) even after adjusting for age, sex, time of HTLV-1 diagnosis and schooling (adjusted PR: 1.63; CI95%:1.31-2.02). Burning and/or pain and itching and low visual acuity were the most common alterations of HTLV-1/KCS. It is strongly recommended that HTLV-1 patients undergo periodic ophthalmologic examination to promote the early diagnosis of KCS and prevent the consequences associated with Dry Eye Disease.

Introduction

Human T-lymphotropic virus type 1 (HTLV-1) is etiologically linked with adult T cell leukemia (ATL) [1], tropical spastic paraparesis/HTLV-1-associated myelopathy (HAM/TSP) [2, 3], infective dermatitis [4] and uveitis [5] . Many other diseases have been associated with HTLV-1, such as polymyositis [6] , bronchial alveolar pneumonia [7], bronchiectasis [8], and Sjörgren's Syndrome [9-11], indicating multisystemic involvement in this infection. Moreover, other ocular lesions have also been associated with HTLV-1, such as corneal lesions [12], retinal vasculitis [13] and keratoconjunctivitis sicca (KCS) [14-19] . Although, the burden of HTLV-1 infection it is clearly demonstrated, this infection and its related diseases are still neglected [20].

KCS, or dry eye disease (DED), is a multifactorial disease of the lacrimal film resulting in eye discomfort, visual disturbances, and tear film instability that could potentially damage the ocular surface [21]. It is accompanied by increased osmolality of the tear film and inflammation of the ocular surface [22, 23].

The prevalence of KCS associated with HTLV-1 varies in different parts of the world. In Martinique, the prevalence of KCS was 37.0% [15], while in Japan this was 15.4% [16]. Brazil, a country of more than 200 million inhabitants, represents one of the largest endemic areas for HTLV-1 and associated diseases in the world [24]. Investigations regarding the prevalence of KCS in association with HTLV-1 have demonstrated discrepancies, with variance from 3.9% to 36.4% [17-19]. A study carried out in the state of São Paulo, located in the southeastern region, found a very low prevalence (3.9%) [19], which stands in contrast to others conducted in the states of Bahia and Minas Gerais, located in northeastern and southeastern regions respectively, where prevalence varied between 27.5% to 36.4% [17, 18]. In addition, the number of

studied patients also varied greatly, ranging from 52 to 262 [15, 17-19] , and the clinical manifestations associated with KCS in these patients were poorly described.

Salvador, the capital of the State of Bahia, has the highest prevalence of HTLV-1 in the country [25, 26]. A population-based study estimated the prevalence of HTLV-1 at 1.7% in 2003, i.e. potentially 40,000 people could have been infected with HTLV-1 at that time [27].

Therefore, the present study sought to determine the prevalence of HTLV-1-associated KCS using a substantially larger sample than the previous estimation, in addition to identifying clinical manifestations as well as associated factors to more precisely estimate the magnitude of this health problem, in Salvador.

Subjects and methods

Study design and population.

An outpatient cross-sectional study was conducted between June 2004 and September 2017 at the Integrative and Multidisciplinary Center for HTLV (CHTLV), Bahia School of Medicine and Public Health Salvador (EBMSP), Bahia-Brazil. CHTLV is an outpatient clinic open to the public that provides inter-disciplinary care and services, including general medical treatment, laboratory diagnosis, psychological counseling and physical therapy. Data from non-probabilistic sample, comprised 1137 HTLV-1-infected patients was collected from medical records, including sociodemographic profile, date of HTLV serology and clinical status. All study volunteers provided written informed consent prior to inclusion in the present research protocol. For children informed consent were obtained from their parents or guardian. The Institutional Research Board of EBMSP approved this study (protocol no. 71/2006).

The eligible population comprised 1,137 HTLV-1-infected individuals of which 72.1% were females; age ranged from to 4 and 93 years. The majority (38.4 %) had less than 8 years of education.

Laboratory, clinical and ophthalmologic evaluation.

HTLV-1 infection was diagnosed using several commercially available enzyme-linked immunosorbent (ELISA) (HTLV, enhanced, EIA, Cambridge Biotech Corporation, Worcester, MA) followed by Western Blot 2.4 (Genelabs Diagnostics GLD, Science Park Drive, Singapore) for confirmation and discrimination between HTLV-1 and HTLV-2. The clinical status of HTLV-1 patients with respect to HAM /TSP was

116 determined according to the classification criteria established by De Castro-Costa et al,
117 [28] were obtained from medical records.

118 A complete ophthalmologic examination was performed in both eyes, including visual
119 acuity as measured by the Snellen eye chart, in addition to optical correction, optical
120 motility, applanation tonometry, biomicroscopy of the anterior and posterior chambers,
121 and binocular indirect ophthalmoscopy. Lacrimal function was evaluated by break-up
122 time (BUT), Rose Bengal and Schirmer I Tests. Tear BUT was performed via the
123 instillation of 1% fluorescein solution (Fluorescein eye drops Ophtalmos®, São Paulo,
124 Brazil), measuring the time required for dry spots to appear on the corneal surface after
125 blinking. For Schirmer I test, 5mm x 35mm Whatman strips (Schirmer's tear test,
126 Opthalmos®, São Paulo, Brazil) were placed in the lower fornix near the lateral canthus
127 of both eyes for 5 minutes. The Rose Bengal test was performed using 1% Rose Bengal
128 staining solution (Rose Bengal eye drops; Ophthalmos®, São Paulo, Brazil), with
129 results considered abnormal when a total Van-Bijsterveld score higher than three points
130 was obtained. BUT scores <10 seconds and <5 mm for the Schirmer I test were
131 considered abnormal [29]. KCS diagnosis was considered in the presence of at least two
132 out of three positive lacrimal function tests [30].

133 All patients without the three lacrimal function tests required to diagnose KCS, and
134 those presenting any previous palpebral disorders or nasolacrimal duct obstruction, as
135 well as those who underwent intraocular surgery, were excluded. In addition, patients
136 <18 years of age, a positive serology for Hepatitis B or C and HIV, diagnosis of
137 Diabetes Mellitus [31], as well as the diagnosis of HAM/TSP possible and probable
138 were excluded. In sum, only patients infected by HTLV-1, ≥ 18years of age and those
139 considered as definite HAM/TSP were included.

Data Analysis.

Statistical analysis was performed using Stata software v. 13.0. Missing data was excluded from the analysis. Overall prevalence was calculated by dividing the number of KCS cases by the number of included patients assessed, expressed as a percentage. Age and time of serological diagnosis were considering as a continuous variable. The presence of KCS was evaluated according to sociodemographic, clinical status and time of HTLV diagnosis using the Pearson chi-square test or Fisher's exact test, where appropriate. Crude and Adjusted Prevalence Rates (PR) with 95% Confidence Intervals (95%CI) were estimated using multivariate Poisson Regression with robust error variance. P values < 0.05 were considered significant.

Results

A total of 1,137 HTLV-1 infected individuals were evaluated and a total of 379 (33.4%) were excluded as follows: <18 years=17; incomplete ophthalmologic evaluation for KCS =207; with positive serology for Hepatitis B or C=12 or HIV=3; HCV/HIV=1; diagnosis of diabetes=18; diagnosis of possible HAM/TSP=111, probable HAM/TSP=10. Accordingly, the resulting study sample included 758 individuals (1,516 eyes).

A comparison between the excluded and included patients revealed differences with respect to age (48.5[17.2] vs. 46.5[14.2]; p=0.041, respectively) but not as regards sex (p=0.061). Furthermore, the number of individuals classified as definite HAM/TSP was greater in the excluded patients than in those enlisted (33.5% vs. 22.3%; p= 0.001).

161 Ophthalmologic examinations revealed a diagnosis of KCS in 240 out of 758 patients,
162 with a resulting overall prevalence of 31.7% (CI95%: 28.4-35.1). In contrast, the
163 prevalence of KCS in patients with definite HAM-TSP was 49.1%.

164 Table 1 comparatively lists the sociodemographic variables among KCS-positive
165 patients and those without KCS. Concerning this sociodemographic data, while the KCS
166 group was older (50.7 [13.3] vs. 44.5 [14.5] years; p<0.001) and more patients with
167 KCS attended school for fewer than eight years (42.4% vs. 32.8%; p=0.04), no
168 significant differences were detected with regard to sex, skin color or marital status.

169 Table 2 comparatively lists the clinical and ophthalmological variables among the KCS-
170 positive patients and those without KCS. More patients with KCS were classified as
171 definite HAM/TSP (34.6% vs. 16.6%; p<0.001). Table 3 details the crude and adjusted
172 prevalence ratios of KCS in association with sociodemographic and clinical variables.
173 Age and the presence of HAM/TSP remained statistically significant, even after
174 adjusting for sex, time of HTLV-1 diagnosis and duration of schooling. For every one-
175 year increase in age, the prevalence of KCS increases, on average, by 2%. A diagnosis
176 of definite HAM/TSP was associated with a 63% increase in the probability of
177 presenting KCS in comparison to those without HAM/TSP. Concerning the frequency
178 of symptoms, burning and/or ocular pain (38.9% vs. 29.7%; p=0.012) and itching
179 (29.7% vs. 19.4%; p=0.002) were significantly higher in the KCS group as compared to
180 those without KCS. In addition, low visual acuity on ophthalmologic examination was
181 significantly higher (24.6%) versus individuals without KCS (13.7%; p<0.001).

182 Table 4 describes the association between the presence of KCS and ophthalmic signs
183 and symptoms. Even after adjusting for age and sex, patients with KCS presented a
184 higher frequency of low acuity, burning and/or ocular pain and itching.

TABLE 1 – Sociodemographic parameters of 758 HTLV-1 infected patients.

VARIABLES	KCS		P value
	Presence	Absence	
Sociodemographic Variables	**N (%)**	**N (%)**	
Age (years)*	50.7 (±13.3)	44.5 (±14.5))	<0.001
Sex			0.956
Male	63 (26.3)	135 (26.1)	
Female	177 (73.7)	383 (73.9)	
Skin color			0.627[a]
Mulatto	123 (51.3)	261 (50.4)	
Black	84 (35.0)	179 (34.6)	
White	27 (11.2)	70 (13.5)	
Yellow	2 (0.8)	1 (0.2)	
No registry	4 (1.7)	7 (1.4)	
Schooling (Years)			0.036[b]
<8	100 (41.7)	169 (32.6)	
8-11	55 (22.9)	138 (26.6)	
≥11	81 (33.7)	208 (40.2)	
No registry	4 (1.7)	3 (0.6)	
Marital status			0.070[a]
Single	86 (35.8)	194 (37.4)	
Married/Stable union	110 (45.8)	257 (49.6)	
Divorced	12 (5.0)	27 (5.2)	
Widowed	30 (12.5)	40 (7.7)	
No registry	2 (0.8)	-	

KCS: Keratoconjunctivitis sicca; *Mean (Standard Deviation); [a] Fisher's Exact Test; [b] Chi-square for trend analysis

188 **TABLE 2 - Clinical parameters of 758 HTLV-1 infected patients**

VARIABLES	KCS		P value
	Presence	Absence	
Clinical Variables	**N (%)**	**N (%)**	
HAM/TSP			<0.001
Absent	157 (65.4)	432 (83.4)	
Present	83 (34.6)	86 (16. 6)	
Time of HTLV-1 diagnosis (years)**	2.0 (0.0 – 4.0)	1.0 (0.0 – 3.0)	0.006[a]
Ophthalmic Variables			
Complaints			
Burning and/or ocular pain	93 (38.9)	153 (29.7)	0.012
Visual blurring	88 (36.8)	168 (32.6)	0.250
Itching	71 (29.7)	100 (19.4)	0.002
Tear flow	29 (12.1)	47 (9.1)	0.199
Foreign Body Sensation	18 (7.6)	24 (4.7)	0.105
Hyperemia	10 (4.2)	18 (3.5)	0.638
Flying Flies	08 (3.4)	15 (2.9)	0.743
No complaints	38 (15.9)	164 (31.8)	<0.001
Low visual acuity (<20/30)	58 (24.6)	70 (13.7)	<0.001

189 KCS: Keratoconjunctivitis sicca; HTLV-1: Human T-cell Lymphotropic Virus type 1; HAM/TSP:
190 HTLV-1-associated myelopathy/tropical spastic paraparesis; *Mean(SD); **Median (Interquartile
191 range) or Median (25^{th} – 75^{th}); [a]Mann-Whitney test.

TABLE 3 – Crude and adjusted prevalence ratios of KCS in association with sociodemographic variables, time of HTLV-1 diagnosis and definite HAM/TSP status

Variables	PR Crude (95% CI)	PR Adjusted (95% CI)
Sex		
Male	1.00	1.00
Female	0.99 (0.78-1.26)	1.10 (0.86-1.39)
Age (Years)	**1.02 (1.01-1.03)**	**1.02 (1.01-1.03)**
Schooling (Years)		
<8	1.00	1.00
8-11	0.75 (0.59 – 0.96)	0.91 (0.72 – 1.16)
≥11	0.77 (0.58 – 1.01)	0.84 (0.64 – 1.10)
Time of HTLV-1 diagnosis (years)	1.02 (1.00 - 1.04)	1.01 (0.98 - 1.03)
HAM/TSP status		
Absent	1.00	1.00
Present	**1.84 (1.50-2.26)**	**1.63 (1.31-2.02)**

HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis; KCS: Keratoconjunctivitis sicca; PR: prevalence ratio.

198 **TABLE 4 - Crude and adjusted prevalence ratios of KCS in association with**

199 **selected ophthalmic signs and symptoms**

Signs and Symptoms	PR Crude (95% CI)	PR Adjusted (95% CI)[1]	PR Adjusted (95% CI)[2]
Low visual acuity (<20/30)	1.79 (1.31-2.45)	1.41 (1.06-1.90)	1.40 (1.05-1.88)
Itching	1.53 (1.18-1.99)	1.46 (1.12-1.91)	1.45 (1.11-1.90)
Burning and/or ocular pain	1.31 (1.07-1.61)	1.34 (1.08-1.65)	1.33 (1.08-1.65)
Foreign Body Sensation	1.62 (0.90-2.94)	1.53 (0.85-2.77)	1.53 (0.85-2.77)
Tear flow	1.33 (0.86-2.06)	1.28 (0.83-1.99)	1.28 (0.82-1.98)
Hyperemia	1.20 (0.56-2.56)	1.19 (0.55-2.57)	1.18 (0.54-2.57)
Flying Flies	1.15(0.49-2.68)–	0.92 (0.41 – 2.11)	0.91 (0.40 – 2.06)
No complaints	0.50(0.37-0.69)–	0.52 (0.38 – 0.72)	0.53 (0.38 – 0.72)

200 KCS: Keratoconjunctivitis sicca; [1] Adjusted for age; [2] Adjusted for age and sex

201

Discussion

To the best of our knowledge, the present study represents the first attempt to investigate the prevalence of KCS in association with HTLV-1 in a large cohort consisting of 758 patients. In addition, we present comprehensively described clinical findings regarding significantly inferior corrected visual acuity, burning and/or pain, and itching in HTLV-1-positive patients with KCS. In addition, we confirmed a higher prevalence of KCS in definite HAM/TSP patients.

The overall prevalence of HTLV-1/KCS found in the present study was 31.7% (CI95%: 28.5-34.6), similar to estimates previously reported in two cities from Brazil, Salvador (36.4%; 39/107) in 2009 [18] and Belo Horizonte (27.5%; 72/262 in 2006 [17], as well as in Martinique (37%; 74/200) in 2002 [15]. These rates were much higher than the prevalence observed in asymptomatic carriers in São Paulo, Brazil (3.9%; 2/52) [19] and in HTLV-1 patients with ocular disorders in Japan (15.4%) [16]. This discrepancy could be due to the absence of inclusion of individuals with HAM/TSP [16, 19] (or to the criteria employed for diagnosing KCS. In contrast to Yamamoto et al, all other studies based a diagnosis of KCS on the presence of symptoms together with at least two out of three positive test results, namely BUT, Rose Bengal or Schirmer I. However, Yamamoto and colleagues diagnosed KCS exclusively on clinical findings consistent with dry eye, i.e. when abnormal results from all three tests were obtained, which could have led to an underestimation of the actual prevalence rate [14].

The prevalence of KCS in patients with HAM/TSP (49.1%) found herein was sharply higher than in asymptomatic individuals, which is consistent with previous reports [15, 17, 18] . Furthermore, we confirmed a positive association between KCS and HAM/TSP, which was approximately twice as common in individuals with definite

HAM/TSP than in asymptomatic carriers, even after adjusting for age, sex, time of HTLV-1 diagnosis and schooling [15, 17, 18].

Our study demonstrated that ocular burning and/or pain and itching were significantly more frequent in HTLV-1-patients with KCS, even after adjusting for sex and age. Moreover, the present data provide new evidence that HTLV-1/KCS patients had significantly lower corrected visual acuity compared to HTLV-1 individuals without KCS. The frequencies of clinical complaints, such as burning/pain, itching and VA, were similar in individuals with KCS, regardless of HAM/TSP diagnosis (data not shown). In contrast to this finding, higher frequencies of burning and/or pain, as well as eye redness, have been described in HAM/TSP patients compared to both asymptomatic and uninfected individuals, while visual acuity and eye pressure were normal across all studied groups [17]. In 2002, Merle et al. reported that the presence of ocular symptoms in HTLV-1-infected individuals is rare, and that ocular pruritus was the most commonly reported complaint [15].

However, due to the fact that the previous articles did not describe the frequencies of ophthalmological symptoms, a more robust comparison was not possible.

Based on epidemiological, clinical and experimental evidence, Sjögren's syndrome has been associated with KCS in association with HTLV-1 infection [9, 10, 32-34]. This syndrome is an autoimmune disorder that evolves with lymphocytic infiltration in the exocrine glands, generally leading to xerostomia and/or KCS [35]. However, the pathogenesis of HTLV-1-associated KCS might differ from what is observed in Sjogren’s syndrome [34, 36-38]. Indeed, in a previous study conducted in patients with KCS diagnosis from the CHTLV cohort, no patients tested positive for ANA, anti-SSA/Ro, anti-SSB/La or RF autoantibodies [36], which was similar to previous reports

250 [15, 33, 38]. Thus, the mechanism underlying HTLV-1-associated KCS could be either
251 directly or indirectly related to the virus itself [34]. Recently, it was demonstrated that
252 patients with KCS had significantly higher HTLV-1 proviral load than patients without
253 the disease. In addition, HTLV-1 proviral load > 100,000 copies/10^6 PBMC was shown
254 to be significantly associated with KCS [39]. Also, higher production of
255 proinflammatory cytokines (IFN-γ and TNF-α) were found in HTLV-1 infected
256 patients with KCS than in HTLV-1 infected subjects without dry eye syndrome,
257 indicating that an exacerbated proinflammatory response may play a role in the
258 destruction of the salivary and lacrimal glands observed during HTLV-1 infection [37].

259 While the absence of an uninfected control group was a relevant limitation, previous
260 controlled studies carried out in the Brazilian state of Minas Gerais and Martinique [15,
261 17] reported a significantly higher prevalence of KCS in HTLV-1-infected patients than
262 in uninfected individuals. In addition, the patients who were excluded herein were older
263 and had a higher proportion of HAM/TSP. These aspects may have reduced the
264 magnitude of the strength of association between HAM/TSP and KCS. Of note,
265 burning/pain, itching and low visual acuity, the most common symptoms of KCS found
266 herein, could interfere greatly in patient quality of life [40]. Therefore, it is important to
267 perform early KCS diagnosis and treatment to avoid chronic clinical changes in the
268 ocular surface, including palpebral and corneal disorders, such as keratitis,
269 neovascularization, opacification and a predisposition to ulcer formation [21, 41, 42].
270 Finally, based on the results obtained herein, it is strongly recommended that HTLV-I
271 patients undergo periodic ophthalmologic examination.

272

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Supporting Information Legends

S1. STROBE Checklist

6 DISCUSSÃO

Este é um estudo longitudinal em uma população endêmica de HTLV-1 no Brasil[28-30] representando a primeira tentativa de investigar a morbidade da CCS em associação com o HTLV-1 em uma grande coorte composta por 1137 pacientes.

A prevalência geral de CCS em portadores de HTLV-1 encontrada foi de 31,7% (IC95%: 28,5-34,6), semelhante a estimativas previamente relatadas em duas cidades do Brasil, Salvador (36,4%; 39/107) em 2009[23] e Belo Horizonte (27,5%; 72/262) em 2006[22], assim como na Martinica (37%; 74/200) em 2002[21]. Índices muito inferiores de prevalência foram observadas em portadores assintomáticos em São Paulo, Brasil (3,9%; 2/52)[19] e em pacientes com HTLV-1 com alterações oculares no Japão (15,4%)[27], discrepância esta que pode ser devido à ausência de inclusão de indivíduos com HAM/TSP[19,27] ou para os critérios utilizados para o diagnóstico de CCS. Em contraste com Yamamoto et al, todos os outros estudos basearam o diagnóstico da CCS na presença de sintomas[78], juntamente com a positividade de dois dos três testes realizados[21-23]. No entanto, Yamamoto e colaboradores diagnosticaram CCS exclusivamente quando os resultados de todos os três testes foram obtidos, o que poderia ter levado a uma subestimação da taxa de prevalência real[19].

A prevalência de CCS em pacientes com HAM/TSP (49,1%) encontrada no presente estudo foi nitidamente maior do que em indivíduos assintomáticos, o que é consistente com relatos anteriores[21-23]. Além disso, confirmamos uma associação positiva entre CCS e HAM/TSP, que foi aproximadamente duas vezes mais comum em indivíduos com HAM/TSP definida do que em portadores assintomáticos, mesmo após ajuste para idade, sexo, tempo de diagnóstico e escolaridade do HTLV-1[21-23].

Neste estudo demonstrou-se que a ardência ocular e/ou dor e prurido foram significativamente mais frequentes em pacientes HTLV-1 com CCS, mesmo após o ajuste para sexo e idade. Além disso, os dados presentes fornecem novas evidências de que pacientes com CCS e HTLV-1 tiveram acuidade visual corrigida significativamente menor em comparação com indivíduos HTLV-1 sem CCS. As frequências de queixas clínicas, como ardor/dor, prurido e acuidade visual foram

semelhantes em indivíduos com CCS, independentemente do diagnóstico HAM/TSP. Em contraste com este achado, maiores frequências de queimação e/ou dor, bem como vermelhidão ocular, foram descritas em pacientes com HAM/TSP em comparação com indivíduos assintomáticos e não infectados, enquanto a acuidade visual e pressão ocular foram normais em todos os grupos estudados[22]. Em 2002, Merle et al. relataram que a presença de sintomas oculares em indivíduos infectados pelo HTLV-1 é rara e que o prurido ocular foi a queixa mais comumente relatada[21]. No entanto, devido ao fato de os artigos anteriores não descreverem as frequências dos sintomas oftalmológicos, uma comparação mais robusta não foi possível.

Enquanto a ausência de um grupo controle não infectado foi uma limitação relevante, estudos prévios controlados realizados no estado brasileiro de Minas Gerais e Martinica[21,22] relataram uma prevalência significativamente maior de CCS em pacientes infectados pelo HTLV-1 do que em indivíduos não infectados. Além disso, os pacientes excluídos durante a análise de prevalência, foram mais velhos e tiveram maior proporção de HAM/TSP. Esses aspectos podem ter reduzido a magnitude da força de associação entre HAM/TSP e CCS.

Comparando-se os pacientes sem CCS e com consultas de acompanhamento na linha de base, com os pacientes excluídos por falta de consultas de acompanhamento, observamos diferença significativa apenas na presença de HAM/TSP definido podendo desta forma sobrestimar a incidência de CCS em portadores de HTLV-1. As demais diferenças nas características sociodemográficas, clínicas e laboratoriais não foram significativas. Mesmos as queixas mais frequentes de dor/ardor, baixa acuidade visual e prurido, não apresentaram diferenças significativas entre os grupos.

Comparando-se os grupos incidente e não-incidente, pacientes com diagnóstico mais longo (8,3 anos, p = 0,013) e maior acompanhamento oftalmológico (3,9 consultas, p <0,001) apresentaram queixas oftalmológicas mais frequentes e maior probabilidade de apresentarem KCS durante o seguimento -acima. Esses dados sugerem que acompanhamentos mais frequentes em pacientes com maior tempo de doença podem ser indicados. Tem sido descrito que, ao longo dos anos, múltiplas soroconversões ocorrem em pacientes com HTLV-1, culminando com a deterioração da função das glândulas sebáceas e o aparecimento de sintomas de KCS

Vale ressaltar que os pacientes que se autodenominaram de cor parda, foram mais predominantes no grupo de não incidentes, sendo fator de proteção para CCS. Após ajuste para sexo, idade e cor da pele; tempo de diagnostico e cor parda, mantiveram-se como fator de proteção para o desenvolvimento de CCS em portadores de HTLV-1, e em contraste, a presença de HAM/TSP definido, evidenciou-se como fator de risco.

É digno de nota que a queimação / dor, prurido e baixa acuidade visual, são os sintomas mais comuns da CCS, podendo interferir muito na qualidade de vida do paciente[79]. Portanto, é importante realizar o diagnóstico e tratamento precoce da CCS para evitar alterações clínicas crônicas na superfície ocular, incluindo desordens palpebrais e corneanas, como ceratite, neovascularização, opacificação e uma predisposição à formação de úlcera[26,80,81]. Finalmente, com base nos resultados obtidos, é altamente recomendável que os pacientes com HTLV-1 sejam submetidos a exames oftalmológicos periódicos.

7 LIMITAÇÕES E PERSPECTIVAS

Uma das limitações deste estudo foi a ausência de um grupo controle composto por pacientes não infectados pelo HTLV-1, já que não há conhecimento da real prevalência de CCS na população geral de Salvador, o que impossibilita uma comparação entre os pacientes com HTLV. Pretendemos, ainda em 2018, iniciar uma pesquisa sobre a incidência de CCS na população geral de Salvador, através de uma parceria com o Hospital Humberto Castro Lima, importante hospital oftalmológico em Salvador-Bahia.

O diagnóstico foi realizado com base nos testes de Tempo de ruptura do filme lacrimal, Schirmer I e Rosa Bengala. Durante o período do estudo, observamos que os pacientes tinham queixas durante a instilação do colírio de Rosa Bengala e propusemos no CHTLV, a inclusão do questionário OSDI propondo um fluxograma para diagnóstico da CCS. Desta forma, minimizamos as queixas dos pacientes e reduzimos custos, proporcionando assim uma redução na exclusão de pacientes da amostra (*vide* modelo apendice B).

Pretendemos, futuramente:

- Comparar a incidência de CCS nos pacientes portadores de HTLV com a encontrada em grupo controle.
- Avaliar sensibilidade e especificidade da lisamina verde para possível inclusão nos métodos diagnósticos de CCS.
- Avaliar a associação da carga proviral em pacientes incidentes e não incidentes
- Avaliar o fator protetor da raça mista contra o desenvolvimento de CCS entre pacientes com HTLV1
- Prevenção do CCS em portadores de HTLV-1
- Eficácia comparativa dos protocolos de diagnóstico do CCS no HTLV-1
- Carga Proviral no incidente CCS
- Comparação da prevalência de CCS entre HTLV-1 e pacientes com doença autoimune
- Descrever os mecanismos patogénicos da CCS por HTLV-1

8 CONCLUSÕES

Esse estudo reafirma, através de uma ampliação importante do número de pacientes avaliados, a importante prevalência da CCS em pacientes portadores de HTLV-1, e uma associação positiva com a presença de HAM-TSP. Além disso, é pioneiro na avaliação da incidência de CCS nesta população, bem como na detalhada avaliação de sinais e sintomas em pacientes com CCS. Destaque para dor/ardor, prurido e baixa acuidade visual, os quais estão mais fortemente associados a CCS. Portanto, comprova a importância do acompanhamento oftalmológico periódico ao paciente portador do vírus HTLV-1 e enfatiza quais sinais e sintomas estão mais frequentemente associados à CCS.

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APÊNDICES

Apêndice A – Termo de Consentimento Livre e Esclarecido

Incidência da Ceratoconjuntivite Seca (CCS) associada ao Vírus Linfotrópico de Células T humana tipo 1 (HTLV-1)

TERMO DE CONSENTIMENTO INFORMADO

RESOLUÇÃO CNS Nº 466, DE 12 DE DEZEMBRO DE 2012

Você é portador do Vírus Linfotrópico de Células T-humanas (HTLV) e está sendo convidado a participar da pesquisa acima citada. As informações que seguem estão sendo fornecidas para o seu esclarecimento e participação voluntária.

A Ceratoconjuntivite Seca é uma doença que leva a uma diminuição de lágrimas, ocasionando ressecamento nos olhos. A Ceratoconjuntivite Seca tem se mostrado mais comum em pacientes com HTLV. Nosso objetivo com esse estudo é determinar quais são os sintomas da ceratoconjuntivite seca nas pessoas que tem esse vírus e como o sistema imune (as células) se defendem do vírus. Você será submetido a um exame oftalmológico, por médicos treinados para esse fim. Neste exame serão realizados 4 testes onde vamos pingar corantes chamados de “fluoresceína” , “lisamina verde” e “rosa bengala” e um colírio anestésico para colocação de uma pequena faixa de papel de 05 mm de largura no canto dos olhos. Durante esses procedimentos é comum a sensação de ardor ocular pela maioria dos pacientes. Se for identificado qualquer alteração, você receberá tratamento e orientação adequados . Colocaremos colírios lubrificantes que melhorarão a sensação de ardor e desconforto nos olhos. Além disso, nós pedimos que você forneça 20 ml de sangue (mais ou menos duas colheres de sopa) para estudar suas células no laboratório. Esta coleta de sangue será realizado por técnico capacitado. O risco associado à coleta de sangue é limitado a um pequeno desconforto no local da punção sanguínea e sangramento A equipe que acompanhará os pacientes é treinada e estará disponível para fornecer qualquer esclarecimento relacionado ao estudo. Os riscos da pesquisa são de desconforto e ardor nos olhos que podem ser melhorados com o uso de colírios lubrificantes .

Você terá o direito de ser mantido atualizado sobre os resultados parciais da pesquisa e terá total liberdade de se recusar a participar, ou retirar seu consentimento, em qualquer momento da pesquisa, sem que isso leve a qualquer prejuízo no seu acompanhamento. É garantido sigilo quanto aos seus dados, pois as informações obtidas serão analisadas em conjunto com outros pacientes, não sendo divulgada a identificação de nenhum paciente. Você não terá qualquer despesa com a pesquisa, incluindo exames e consultas. Também não há nenhuma compensação financeira relativa à sua participação. É um compromisso do pesquisador utilizar os dados e o material coletado somente para essa pesquisa.

Em qualquer momento do estudo você poderá entrar em contato com os profissionais responsáveis através de ligação telefônica ou pessoalmente, para esclarecimento de eventuais dúvidas. Os pesquisadores responsáveis sao: Dr Bernardo Galvão, que pode ser encontrado na Av. Dom João VI, 275 , Brotas Telefone: 3276-8281 e Dra. Regina Pinheiro, que pode ser encontrada na Rua Pedro Lessa, 118, Canela. Telefone: 71 3173-8218.

Este termo é composto de duas vias de igual conteúdo sendo a primeira para arquivamento pelos pesquisadores, e a segunda para o paciente ou seu responsável legal.

Em caso de duvida ou denuncia contatar o Comitê de Ética em Pesquisa em Seres Humanos (CEP) da Escola Bahiana de Medicina e Saúde Pública - Fundação Bahiana para o Desenvolvimento das Ciências localizado na Av D. Joao VI, 275, Brotas, CEP: 40.290.000, Salvador, Bahia . Tel: (71) 3276-8225. Email: cep@bahiana.edu.br

Acredito ter sido suficientemente esclarecido a respeito das informações que li ou que foram lidas para mim, descrevendo a pesquisa: **Incidência da Ceratoconjuntivite Seca (CCS) associada ao Vírus Linfotrópico de Células T humana tipo 1 (HTLV-1)**

_____

Assinatura do paciente ou representante legal

_____

Assinatura da testemunha

Impressão datiloscópica

Apêndice B – Questionário de Sintomatologia Ocular

Teve alguma das experiências a seguir durante a ÚLTIMA SEMANA:

1- Olhos mais sensíveis na claridade?
a. Todo o tempo
b. A maior parte do tempo
c. Em alguns momentos
d. Em poucos momentos
e. Em nenhum momento

2- Sente como se houvesse areia nos olhos?
a. Todo o tempo
b. A maior parte do tempo
c. Em alguns momentos
d. Em poucos momentos
e. Em nenhum momento

3- Os olhos estão dolorosos ou ardendo?
a. Todo o tempo
b. A maior parte do tempo
c. Em alguns momentos
d. Em poucos momentos
e. Em nenhum momento

4- Visão borrada?
a. Todo o tempo
b. A maior parte do tempo
c. Em alguns momentos
d. Em poucos momentos
e. Em nenhum momento

5- Visão ruim?
a. Todo o tempo
b. A maior parte do tempo
c. Em alguns momentos
d. Em poucos momentos
e. Em nenhum momento

Problemas com seus olhos limitaram sua capacidade em realizar alguma destas atividades durante a última semana:

1- Ler?
2- dirigir à noite?
3- trabalhar com o computador?
4- assistir TV?

Seus olhos ficaram desconfortáveis em alguma das situações seguintes durante a última semana:

1- Quando expostos ao vento?
2- Locais muito secos (com pouca umidade)?
3- Locais com ar condicionado

ANEXO

Anexo A – Artigo publicado

ORIGINAL ARTICLE

Algorithm for dry eye disease diagnosis in individuals infected with human T-cell lymphotropic virus type 1

Algoritmo para o diagnóstico da doença do olho seco em indivíduos infectados com vírus linfotrópico de células-T humanas tipo 1

CRISTINA CASTRO-LIMA-VARGENS[1,2], MARIA FERNANDA RIOS GRASSI[1,3], NEY BOA-SORTE[1], REGINA HELENA RATHSAM-PINHEIRO[2], PAULA CAROLINE MATOS ALMEIDA[2], BERNARDO GALVÃO-CASTRO[1,3]

ABSTRACT

Purpose: To evaluate the accuracy of lacrimal film tests and propose an algorithm for the diagnosis of dry eye disease in individuals infected with human T-cell lymphotropic virus type 1.

Methods: Ninety-six patients infected with human T-cell lymphotropic virus type 1 were enrolled in the study. To assess clinical complaints, patients completed the Ocular Surface Disease Index questionnaire. To evaluate lacrimal film quality, patients underwent the tear breakup time test, Schirmer I test, and Rose Bengal staining. Dry eye disease was diagnosed when at least two of the three test results were abnormal. The sensitivity, specificity, positive and negative predictive values, and overall accuracy of the questionnaire as well as of each test alone and combined in parallel and in series were determined.

Results: The most sensitive test was the tear breakup time test (98%), whereas the most specific was the Schirmer I test (100%). Rose Bengal staining had the highest overall accuracy (88.64%), whereas the Ocular Surface Disease Index had the lowest overall accuracy (62.65%). The tear breakup time test, Schirmer I test, and Ocular Surface Disease Index combined in parallel showed increased sensitivity and decreased specificity for all tests. In contrast, when combined in series, these tests demonstrated increased specificity and decreased sensitivity.

Conclusion: This study shows the need to use multiple tests to evaluate tear film quality and include a symptom questionnaire as part of the diagnostic algorithm for dry eye disease.

Keywords: Keratoconjunctivitis sicca; Human T-cell lymphotropic virus 1; Dry eye syndromes/diagnosis

RESUMO

Objetivo: *Avaliar a precisão da propedêutica do filme lacrimal e propor um algoritmo para o diagnóstico da doença do olho seco em indivíduos infectados com Vírus linfotrópico de células-T humanas tipo 1.*

Métodos: *Noventa e seis pacientes infectados com o vírus linfotrópico de células T humana tipo 1 foram incluídos no estudo. Para avaliar sintomatologia, os pacientes responderam o questionário Índice para Doenças da Superfície Ocular. A fim de avaliar a qualidade do filme lacrimal, os pacientes foram submetidos ao teste de ruptura do filme lacrimal, teste de Schirmer I e coloração com Rosa Bengala. A doença do olho seco foi diagnosticada quando, pelo menos, dois dos testes ruptura do filme lacrimal, teste de Schirmer I e coloração com Rosa Bengala) eram anormais. Foram determinados sensibilidade, especificidade, valor preditivo positivo e negativo e acurácia do questionário e de cada teste sozinho e combinados em paralelo e em série.*

Resultados: *O teste de ruptura do filme lacrimal foi o mais sensível (98%) e o teste de Schirmer I foi o mais específico (100%). A maior acurácia foi encontrada no teste de coloração com Rosa Bengala (88,64%), enquanto sintomas avaliados usando o questionário Índice para Doenças da Superfície Ocular teve a menor acurácia geral (62,65%). O teste de ruptura do filme lacrimal, teste de Schirmer I e Questionário de Doença da Superfície Ocular quando combinados em paralelo mostraram um aumento da sensibilidade e uma diminuição na especificidade de todos os testes. Por outro lado, combinados em série, teste de ruptura do filme lacrimal, Schirmer I e questionário Índice para Doenças da Superfície Ocular tiveram um aumento na especificidade e sensibilidade diminuída.*

Conclusão: *Este estudo apontou a necessidade de utilizar mais do que um teste para avaliar a qualidade do filme lacrimal, bem como utilizar um questionário de sintomas como parte do algoritmo de diagnóstico para doença do olho seco.*

Descritores: *Ceratoconjuntivite seca; Vírus 1 linfotrópico T humano; Síndromes do olho seco/diagnóstico*

INTRODUCTION

Human T-cell lymphotropic virus type 1 (HTLV-1) is the etiologic agent of adult T-cell leukemia[1], HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[2], and infective dermatitis in children[3]. It is estimated that 5-10 million people worldwide are infected with HTLV-1[4]. HTLV-1-associated uveitis (HAU), an ophthalmologic disease, is also linked to HTLV-1 infection[5,6]. In Japan, HAU has a prevalence of 9.5%-44.8%[7,8]. Other ophthalmologic alterations such as corneal lesions, retinal vasculitis, and keratoconjunctivitis sicca (KCS) or dry eye disease (DED) are also associated with HTLV-1[9-12]. In individuals with HTLV-1, the prevalence of DED may reach 30%-40%[13-16], especially in symptomatic patients with HAM/TSP.

DED is an ocular surface disease that causes eye discomfort, visual disturbance, and tear film instability[17]. Aging, medications, eyelid problems, and environmental factors may be associated with DED. Diseases such as Sjögren's syndrome and rheumatoid arthritis can

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also cause DED[18]. In these autoimmune diseases, the major finding is the destruction of lacrimal gland ducts by autoantibodies[19].

The mechanism leading to DED in HTLV-1-infected persons remains unclear. It is reported that antinuclear antibodies, such as rheumatoid factor, anti-SSA/Ro, and anti-SSB/La, which are present in autoimmune diseases, are absent in patients with HTLV-1-associated DED[20]. Conversely, there is an association between a high HTLV-1 proviral load and DED in infected patients[20,21].

Complaints suggestive of DED are blurred vision, dryness, foreign body sensation, and burning eyes. To confirm the diagnosis, it is mandatory to measure tear volume and evaluate tear quality[17]. Three widely used tests for the assessment of DED are the Schirmer I test, tear breakup time test (TBUT), and Rose Bengal staining[22]. First described in 1903, the Schirmer I test is still being used to measure basal and reflex tear secretions[23]. TBUT is used to assess tear film stability[17]. Rose Bengal staining evaluates the conjunctiva and cornea for the absence of membrane-associated mucins[24]. However, this test has the disadvantage of being toxic, and it typically causes a burning sensation[25]. Patient complaints can also be evaluated using the Ocular Surface Disease Index (OSDI)[26], a specific questionnaire that rapidly assesses dry eye symptoms and their impact on vision-related functioning[27].

Studies to date have generally combined symptoms questionnaires and two or three tests to evaluate tear volume and quality. However, no definite protocol for DED diagnosis has been proposed, and there is a poor relationship between symptoms and diagnostic tests[22,27]. The aim of the present study was to evaluate the accuracy of the TBUT, Schirmer I test, Rose Bengal staining, and OSDI alone or combined for diagnosing DED in HTLV-1-infected individuals and to propose an algorithm for diagnosing DED using low-cost and minimally invasive procedures.

METHODS

This prospective study was conducted at the Bahiana School of Medicine and Public Health reference center for HTLV, Salvador, Bahia, Brazil, between February and November 2013. Patients were sequentially invited to the ophthalmology clinic during routine medical visits. Individuals were eligible to participate if they had a positive serological diagnosis of HTLV-1 on enzyme-linked immunosorbent assay and western blotting. Patients presenting with any previous palpebral and conjunctival disorders, history of ocular surgery, active eye infection, nasolacrimal duct obstruction, contact lens use, chemical or thermal ocular burn, or pregnancy were excluded. The Institutional Research Board of Bahiana School of Medicine approved the study and all patients signed an informed consent form.

To calculate the sample size, we considered the KCS prevalence in patients with HTLV-1 to be equal to 35.0%[13-16] and confidence limits as 10.0%. Considering an alpha error of 5%, the estimated minimum sample obtained was 83 individuals.

Ophthalmologic examination and measurements

Patients underwent a detailed ophthalmic examination, including best-corrected visual acuity and intraocular pressure measurement with an applanation tonometer. The presence of uveitis was evaluated by an anterior segment and fundus examination with a slit-lamp biomicroscope.

All patients were evaluated for clinical symptoms using the OSDI. This questionnaire has been validated in Brazil[28] and provides a rapid assessment of the symptoms of ocular irritation consistent with DED. Patients were classified on a dry eye intensity scale as normal, mild, moderate, or severe according to the OSDI score. The test was considered positive if the patient's final OSDI score was >20 (moderate or severe).

Tear secretions for both eyes were evaluated using the TBUT, Schirmer I test, and Rose Bengal staining. TBUT was performed by the instillation of a 1% fluorescein solution (Colírio de Fluoresceína®; Ophthalmos®, São Paulo, Brazil), and the time required for dry spots to appear on the corneal surface after blinking was recorded. Dry spots that appeared in <10 s were considered abnormal.

To perform the Schirmer I test, a Whatman filter paper strip (Teste de Schirmer®; Ophthalmos®) with a dimension of 5 × 35 mm was placed into the lower fornix near the lateral canthus of each eye as the anesthetic was administered. After 5 min, the strips were removed and the wet portion was measured. The result was considered abnormal if <5 mm of moisture was present on the filter paper.

The Rose Bengal test was performed with 1% Rose Bengal staining solution (Colírio de Rosa Bengala®; Ophthalmos®). The nasal conjunctiva, cornea, and temporal conjunctiva were evaluated and each was scored 0-3 points. The exam was considered abnormal when the total score was >3 points[29].

DED was diagnosed when the results of at least two of the three tests were abnormal.

Statistical analysis

Age is expressed as mean (SD), while sex and the presence of DED are expressed as relative frequency. The Kolmogorov-Smirnov test was used to assess the presence of a normal age distribution. The means were compared using t-tests or the Mann-Whitney tests according to Gaussian or non-Gaussian statistical distribution. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA) of each test alone were calculated using the OpenEpi software program version 3.01. OA was calculated as the sum of the true positives plus true negatives divided by the total number of individuals tested. Sensitivity was obtained by the ratio of the number of true positive assessments and the number of all positive assessments. Specificity was obtained by the ratio of the number of true negative assessments and the number of all negative assessments. PPV was defined as the proportion of patients with positive test results who were correctly diagnosed, while NPV was defined as the proportion of patients with negative test results who were correctly diagnosed. Two tests at a time were combined in parallel and in series to evaluate their ability to differentiate persons with DED from normal individuals. When combined in parallel, only one positive result was sufficient, while when combined in series, both tests must be positive. Global algorithm accuracy, sensitivity, specificity, PPV, and NPV were determined. The MS Excel software program was used to calculate accuracy. All statistical analyses were performed using SPSS/PC Statistical Software Program version 18.0 (SPSS, Chicago, IL, USA).

RESULTS

Ninety-six subjects were included in the study; of them, 71 (74%) were females. Fifty patients (52.1%) had a diagnosis of DED. The mean age for patients with the DED diagnosis was 53.6 years, while that for patients without DED was 47.4 years; the difference was statistically significant (p=0.017). The age for all patients was 23-78 years.

The sensitivity, specificity, PPV, NPV, and OA of each test alone and combined in parallel or in series are presented in table 1. When evaluated alone, the most sensitive test was TBUT (98%), presenting a false-negative rate of 3.0% and specificity of 69.6%. The Schirmer I test was the most specific (100%), with no false-positives cases and a sensitivity of 44%. Rose Bengal staining had the highest OA for sensitivity and specificity (88.6%), while OSDI had the lowest OA (62.7%).

Combined in parallel, the TBUT and OSDI were the most sensitive (99.5%), followed by the combination of the TBUT and Schirmer I test (98.9% sensitivity), with false-positive rates of 37.3% and 22.1%, respectively. The specificities of both combinations were 35.6% and 69.6%, respectively. The combination of the TBUT, Schirmer I test, and OSDI showed the highest sensitivity (35.6%).

Table 1. Effectiveness of tests used to evaluate KCS in HTLV-1 patients

TEST	SENS (%)	SPEC (%)	False-Neg	False-Pos	PPV (%)	NPV (%)	OA (%)
OSDI	75.0	51.2	31.3	41.2	58.8	68.8	62.7
TBUT	98.0	69.5	3.0	22.2	77.8	97.0	84.4
SCH	44.0	100.0	37.8	0.0	100.0	62.2	70.8
RB	87.8	89.7	14.6	8.1	91.5	85.4	88.6
Combined tests (Parallel)							
OSDI or TBUT	99.5	35.6	1.5	37.3	62.7	98.5	
OSDI or SCH	86.0	51.2	22.9	62.8	37.3	77.1	
TBUT or SCH	98.9	69.6	1.7	22.1	78.0	98.3	
OSDI or TBUT or SCH	99.7	35.6	0.8	37.3	62.7	99.2	
Combined tests (Series)							
OSDI and TBUT	73.5	85.1	25.3	15.7	84.3	74.7	
OSDI and SCH	33.0	1.0	42.2	0.0	1.0	57.9	
TBUT and SCH	43.1	1.0	38.2	0.0	1.0	61.8	
OSDI and TBUT and SCH	37.8	100.0	40.3	0.0	100.0	59.7	

KCS= keratoconjunctivitis sicca; HTLV-1= human T-cell lymphotropic virus type 1; SENS= sensitivity; SPEC= specificity; False-Neg= false-negative result; False-Pos= false-positive result; PPV= positive predictive value; NPV= negative predictive value; OA= overall accuracy; TBUT= tear breakup time test; SCH= Schirmer I test; RB= Rose Bengal staining; OSDI= Ocular Surface Disease Index.

Combined in series, a positive Schirmer I test combined with a positive TBUT or OSDI test reached a specificity of 100%, with 43.1% and 33.0% sensitivity, respectively. The TBUT, Schirmer I test, and OSDI combined showed 100% specificity and 37.8% sensitivity. Global algorithm accuracy, sensitivity, specificity, PPV, and NPV are shown in figure 1. Four patients did not complete all of the ophthalmologic evaluations. For the 92 individuals tested, the algorithm's OA, sensitivity, and specificity were 95.7% (95% confidence interval [CI], 89.4-98.3), 98.0% (95% CI, 89.5-99.7), and 92.9% (95% CI, 81.0-97.5), respectively. PPV was 94.2% (95% CI, 84.4-98.0) and NPV was 97.5% (95% CI, 87.1-99.6).

DISCUSSION

The results presented herein refer to the assessment of the efficacy of low-cost tests that are widely used to diagnose DED applied to a group of HTLV-1-infected patients. Although DED is more prevalent in HAM/TSP patients[14,15], the patients in this series were subjected to the same protocol as asymptomatic patients.

When used alone, the TBUT was the best screening test, featuring the highest sensitivity and NPV. Similar results were found in patients with a diagnosis of Sjögren's syndrome but not HTLV-1[30]. However, to confirm the diagnosis, additional tests were required. In the present study, the Schirmer I test showed a low sensitivity (<50%) but had the highest specificity (100%) similar to rates described in the literature[30,31]. Rose Bengal staining presented the best OA. However, when evaluated alone, Rose Bengal did not provide a high PPV or NPV. Therefore, the use of this test alone is unsuitable for confirming or excluding the diagnosis of DED. Although Rose Bengal staining seems to be an efficient test for making the ophthalmic differential diagnosis of Sjögren's syndrome in KCS patients[32], it is best used as an adjunct due to its lack of sensitivity and specificity[33]. Moreover, patients usually complained of itching and redness or even severe ocular inflammation after application of the Rose Bengal stain. Despite the stain being a derivative of fluorescein, which is harmless, it has a dose-dependent toxic effect on human corneal epithelial cells *in vitro*[24]. Due to its side effects, the use of Rose Bengal stain should be limited to patients for whom the DED diagnosis is inconclusive. The Rose Bengal stain can also be replaced with lissamine green, a stain that is well tolerated and as effective as Rose Bengal for evaluating the ocular surface[34].

To confirm the diagnosis of DED, symptoms of discomfort and visual disturbance should be evaluated[35]. Regarding the effectiveness of the OSDI, the accuracy was very low when the questionnaire alone was used to evaluate for DED. This demonstrated a weak correlation between the patient's symptoms and clinical signs[36].

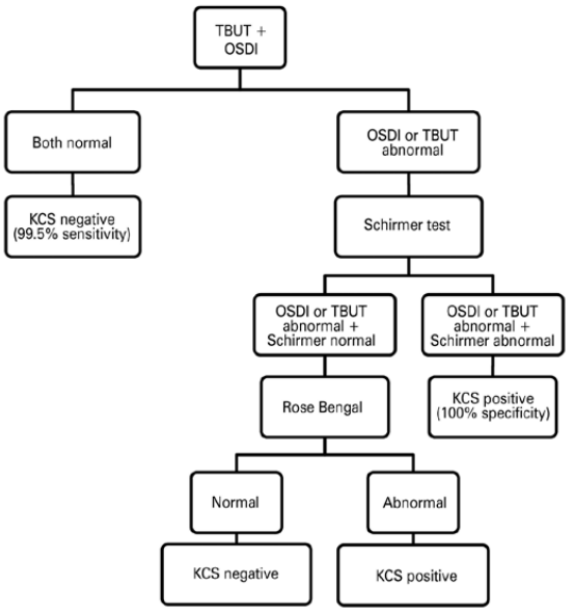
Serial testing maximizes specificity and the PPV but decreases sensitivity and the NPV. Multiple tests combined in parallel increase the sensitivity and, therefore, the NPV. Instead of performing all tests for all patients, the diagnostic algorithm proposed in the present study follows a sequence that involves performing the tests in three stages. Based on the results obtained herein, we suggest an algorithm for screening HTLV-1-infected patients and making a reliable diagnosis of KCS (Figure 1). First, all infected patients would be screened using the OSDI questionnaire and the TBUT. If both tests are normal, a diagnosis of DED can be excluded. These results corroborate those of recent studies indicating that OSDI and TBUT are important tests for diagnosing DED[35,37].

Second, if the OSDI questionnaire and/or TBUT results are abnormal, the Schirmer I test must be performed for all patients. A positive Schirmer I test confirms the KCS diagnosis. In distinct well-defined conditions, the combination use of the OSDI, TBUT, and Schirmer test was the best combination to detect DED[30].

Third, in those patients for whom the diagnosis remains indeterminate (Schirmer I test negative), Rose Bengal staining must be performed. The Japanese Diagnostic Criteria for Dry Eye considers a simultaneous positive test for OSDI, TBUT, and Rose Bengal as a definite DED diagnosis[38].

Although a 100% PPV was found for several test combinations evaluated in this study, none of the tests was sufficiently effective to completely exclude the occurrence of false negatives. Therefore, in patients with a HAM/TSP diagnosis, with an HTLV-1 proviral load of >10% of infected cells, or those >45 years of age, Rose Bengal staining should be considered, even when TBUT, OSDI, and Schirmer test results are negative.

One limitation of the present study is that the proposed algorithm cannot further distinguish DED as aqueous deficient dry eye (ADDE) or evaporative dry eye (EDE). However, it is probable that DED in patients infected with HTLV-1 is mainly due to viral damage to the lacrimal gland, resulting in decreased tear production[39,40]. In addition, Meibomian gland disease, the most common form of EDE, can be easily excluded by examination of the eye structures using a biomicroscope.



KCS= keratoconjunctivitis sicca; HTLV-1= human T-cell lymphotropic virus type 1; TBUT= tear break up time; OSDI= Ocular Surface Disease Index. Overall accuracy: 95.7% (95% confidence interval [CI], 89.4-98.3); sensitivity: 98.0% (95% CI, 89.5-99.7); specificity: 92.9% (95% CI: 81.0-97.5); positive predictive value: 94.2% (95% CI, 84.4-98.0); negative predictive value: 97.5% (95% CI, 87.1-99.6).
Figure 1. DED diagnostic algorithm for HTLV-1-infected patients.

In summary, this study demonstrated that a reliable DED diagnosis can be made in HTLV-1 patients using tests to evaluate DED along with a validated questionnaire, not only to obtain information about the patients' complaints but also as part of the diagnostic algorithm. The described algorithm might be useful for diagnosing moderate to severe ADDE. This would enable the exclusion of patients in whom it is possible to confirm or exclude the DED diagnosis before the next step. Therefore, this algorithm can contribute to reducing cost, discomfort, and time without compromising diagnostic efficacy.

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