Universidade do Estado do Rio de Janeiro
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Faculdade de Ciências Médicas

Laura Gomes Nunes de Melo

Prevalência da retinopatia diabética e seus fatores de risco em pacientes com diabetes tipo 1 no Brasil

Rio de Janeiro
2019

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Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Fisiopatologia Clínica e Experimental, da Universidade do Estado do Rio de Janeiro.

Orientadora: Prof.ª Dra. Marilia de Brito Gomes

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Laura Gomes Nunes de Melo

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A persistência é o caminho do êxito.

Charles Chaplin

RESUMO

MELO, Laura Gomes Nunes. *Prevalência da retinopatia diabética e seus fatores de risco em pacientes com diabetes tipo 1 no Brasil.* 2019. 100 f. Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2019.

Este estudo tem como objetivo avaliar a prevalência da retinopatia diabética, seus principais fatores de risco e sua relação com a doença cardiovascular e com fatores inflamatórios, em pacientes com diabetes tipo 1, no Brasil. A tese é baseada no estudo multicêntrico que envolveu 1760 pacientes, submetidos a um questionário padronizado, exames laboratoriais e clínicos, incluindo rastreamento da retinopatia diabética com oftalmoscopia binocular indireta e posterior classificação em retinopatia diabética não proliferativa leve, moderada, severa e retinopatia diabética proliferativa. A retinopatia foi considerada como ameaça `a visão nas formas não proliferativa severa, proliferativa e edema macular, e como referenciável nas formas não proliferativa moderada, severa, proliferativa e presença de edema macular. Os resultados da tese foram apresentados em forma de artigos científicos. O primeiro artigo é uma carta ao editor, ambos publicados, demonstraram uma prevalência da retinopatia diabética em 35%, das formas mais graves da retinopatia, que ameaçam a visão, em 12% e da retinopatia referenciável em 18%. Os principais fatores de risco relacionados com a retinopatia diabética, independente do grupamento pela gravidade, foram: o tempo de duração do diabetes, os níveis de hemoglobina glicada e os níveis séricos elevados de ácido úrico. A hipertensão arterial foi também relacionada `a presença de retinopatia diabética e com a forma referenciável. A doença renal crônica foi associada com as formas mais graves da retinopatia, que ameaçam a visão, e com a forma referenciável. O terceiro artigo publicado demonstrou que a presença da retinopatia diabética pode ser considerada um indicador de risco cardiovascular. O quarto artigo, submetido, revelou uma associação entre os níveis séricos da proteína C reativa com a retinopatia diabética proliferativa, porém não houve relação com os outros fatores inflamatórios pesquisados (interleucina-6, fator de crescimento endotelial vascular e o fator de necrose tumoral-α). Concluímos que a prevalência da retinopatia diabética no Brasil se assemelha a prevalência mundial, assim como os principais fatores de risco que foram semelhantes aos já estabelecidos, exceto pelo ácido úrico que provavelmente pelas suas características pró-oxidantes, foi também relacionado a presença da retinopatia diabética. A associação da retinopatia diabética com a doença cardiovascular sugere que os pacientes com diabetes tipo 1 e retinopatia diabética sejam investigados para a doença cardiovascular, podendo a fundoscopia auxiliar na identificação de pacientes sobre risco dessa importante causa de mortalidade principalmente nos pacientes com diabetes. Em relação aos fatores inflamatórios, novos estudos são necessários para avaliar o papel dessas citocinas na fisiopatologia da retinopatia diabética proliferativa.

Palavras-chave: Retinopatia diabética. Diabetes tipo 1. Prevalência. Fatores de risco. Fatores inflamatórios.

ABSTRACT

MELO, Laura Gomes Nunes. *Prevalence of diabetic retinopathy and its risk fators in patients with type 1 diabetes in Brazil.* 2019. 100 f. Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2019.

This study aims to evaluate the prevalence of diabetic retinopathy, its main risk factors and its relationship with cardiovascular disease and inflammatory factors in patients with type 1 diabetes in Brazil. This thesis is based on a multicenter study involving 1760 patients submitted to a questionnaire, laboratory and clinical examinations, including screening for diabetic retinopathy with indirect ophthalmoscopy and further classification into mild, moderate, severe nonproliferative diabetic retinopathy and proliferative diabetic retinopathy. Retinopathy was considered as vision threatening in severe nonproliferative, proliferative, and macular edema, and as referable in moderate, severe, proliferative, and macular edema. The results of the thesis were presented in scientific articles. The first article and a letter to the editor, both published, showed a 35% prevalence of diabetic retinopathy, the vision-threatening retinopathy in 12% and referable retinopathy in 18%. The main risk factors related to diabetic retinopathy, regardless of severity grouping were: duration of diabetes, glycated hemoglobin levels and elevated serum uric acid levels. Arterial hypertension was also related to the presence of diabetic retinopathy and the referable form. Chronic kidney disease has been associated with the vision-threatening diabetic retinopathy and the referable form. The third published article demonstrated that the presence of diabetic retinopathy can be considered an indicator of cardiovascular risk. The fourth article submitted demonstrated an association between serum C-reactive protein levels and proliferative diabetic retinopathy, but there was no relationship with the other inflammatory factors investigated (interleukin-6, vascular endothelial growth factor and tumor necrosis factor-α). We conclude that the prevalence of diabetic retinopathy in Brazil resembles the worldwide prevalence, as well as the main risk factors that were similar to those already established, except for uric acid, which probably due to its pro-oxidant characteristics, was also related to the presence of diabetic retinopathy. The association of diabetic retinopathy with cardiovascular disease suggests that patients with type 1 diabetes and diabetic retinopathy should be investigated for cardiovascular disease, and fundoscopy can help in identifying patients at risk for this important cause of mortality, especially in patients with diabetes. Regarding inflammatory factors, further studies are needed to evaluate the role of these cytokines in the pathophysiology of proliferative diabetic retinopathy.

Keywords: Diabetic retinopathy. Type 1 diabetes mellitus. Prevalence. Risk factors. Inflammation mediators.

LISTA DE ABREVIATURAS E SIGLAS

ABEP	Associação Brasileira de Estudos Populacionais
ACU	Ácido Úrico Sérico
ADA	*American diabetes association*
AGE	Produtos finais da glicação avançada
ARIC	*Atherosclerosis Risk in Communities*
CKD-EPI	*Chronic Kidney Disease Epidemiology Collaboration*
CRIC	*Chronic Renal Insufficiency Cohort*
DCV	Doença Cardiovascular
DCCT	*Diabetes Control and Complications Trial*
DCCT/EDIC	*Diabetes Control and Complications Trial/Epidemiology of diabetes Interventions and Complications Study*
DM	*Diabetes Mellitus*
DM1	*Diabetes Mellitus* tipo 1
DNA	Ácido desoxirribonucleico
ERO	Espécie reativa de oxigênio
ETDRS	*Early Treatment of Diabetic Retinopathy Study*
FC	Frequencia Cardíaca
HbA1c	Hemoglobina glicada
HDL	*High Density Lipoprotein*
HPLC	*High Performance Liquid Cromatography*
IBGE	Intituto Brasileiro de Geografia e Estatística
IDF	*International Diabetes Federation*
IECA	Inibidor da enzima conversora de angiotensina
IL-6	Interleucina-6
IMC	Índice de Massa Corporal
IRC	Insuficiência Renal Crônica
IRMA	Dilatações segmentares irregulares dos capilares retinianos.
ISO	*International Standards Organization*
LASER	*Light Amplification by Stimulated Emission*
LDL	*Low density Lipoprotein*

LMW-PTP	*Low Molecular Weight Protein Tyrosine Phosphatase*
MCP-1	Proteína Quimiotática de Monócitos.
NAC	Neuropatia Autonômica Cardiovascular
NF-kB	Fator Nuclear de Transcrição
OCT	Tomografia de Coerência Óptica
OMS	Organização mundial de saúde
OR	Odds Ratio
PA	Pressão Arterial
PCR	Proteína C Reativa
PKC	Proteina Kinase C
RAGE	Receptor de AGE
RD	Retinopatia Diabética
RDNP	Retinopatia Diabética não Proliferativa
SPSS	Statistical Package for the Social Science
SUS	Sistema Único de Saúde
TFG	Taxa de Filtração Glomerular
TNF-α	Fator de necrose tumoral alfa
UERJ	Universidade do Estado do Rio de Janeiro
VEGF	Fator de Crescimento Endotelial Vascular
VEGFR	Receptor do Fator de Crescimento Endotelial Vascular
VTDR	*Vision threatening diabetic retinopathy*
WESDR	*Wisconsin Epidemiologic Study of Diabetic Retinopathy*

LISTA DE SÍMBOLOS

%	Porcentagem
α	Alfa
±	Mais ou menos
cm	Centímetro
cm^3	Centímetros cúbicos
dL	Decilitro
Kg	Quilograma
L	Litro
m	Metro
m^2	Metro quadrado
min	Minuto
mg	Miligrama
ml	Mililitro
mmol	Milimol

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INTRODUÇÃO

O diabetes mellitus é considerado mundialmente um importante problema de saúde pública, tanto pela morbidade de suas complicações como pelos crescentes níveis de incidência e prevalência (1). Estima-se que o número de pacientes acometidos com diabetes no mundo, seja de aproximadamente 425 milhões de adultos (20-79 anos), uma prevalência de 8,8% em 2017, com perspectiva de um aumento para 9,9% em 2045, alcançando aproximadamente 629 milhões de pessoas, segundo a *International Diabetes Federation* (IDF) (2). No Brasil, dados do Ministério da Saúde revelaram uma prevalência de pacientes com diabetes (30-69 anos), de 7,6% em 2016 (3,4).

O diabetes tipo 1 (DM1) é uma doença autoimune, responsável por aproximadamente 7-12% dos casos de diabetes no mundo e é caracterizada pela destruição das células beta no pâncreas, que leva a uma inabilidade do organismo em produzir insulina (2,5). A ausência deste hormônio, que é responsável pela absorção da glicose circulante no nosso sangue, resulta em hiperglicemia crônica. Este estado hiperglicêmico desencadeia uma série de alterações metabólicas, resultando em complicações microvasculares e macrovasculares. As alterações microvasculares causam o desenvolvimento de retinopatia, nefropatia e neuropatia diabética. Nos vasos maiores, os danos ocasionados pelo diabetes geram a doença cardiovascular (DCV), que inclui a doença arterial coronariana, a doença cerebrovascular e a doença arterial periférica (1).

A retinopatia diabética é uma complicações microvasculares debilitante e responsável pela principal causa de cegueira na população economicamente ativa (20-65 anos), no mundo (2).

De acordo com a Organização Mundial de Saúde (OMS), a prevalência de retinopatia diabética no mundo encontra-se em aproximadamente 35% e das formas mais graves, que ameaçam a visão, entre 7 a 10% (1).

O mecanismo da fisiopatologia relacionado a retinopatia diabética ainda não está completamente esclarecido, porém, existem evidências científicas de que a hiperglicemia crônica desencadeia o estresse oxidativo e a liberação de citocinas inflamatórias que geram dano no endotélio da microvasculatura retiniana (6).

Os principais fatores de risco relacionados ao desenvolvimento da retinopatia diabética, segundo a Associação Americana de Diabetes (ADA) são o tempo de duração do diabetes, o controle glicêmico e a hipertensão arterial. Outros fatores como a nefropatia e a dislipidemia

também estão relacionados com o aumento do risco de desenvolvimento da retinopatia diabética (7,8).

Apesar da importância do impacto da retinopatia diabética na vida de cada paciente acometido e na saúde pública como um todo, os dados de prevalência de retinopatia diabética e seus fatores de risco, são escassos no Brasil.

Esperamos que com esse estudo, possamos adicionar mais conhecimentos sobre as principais características da retinopatia diabética nos pacientes com diabetes tipo 1 no Brasil, incluindo dados sobre prevalência e fatores de risco sistêmicos. Dessa forma, contribuindo para um melhor acompanhamento clínico desses pacientes e auxiliando em possíveis futuras medidas públicas no intuito de identificar e prevenir esta debilitante comorbidade.

Nos próximos capítulos, serão apresentados uma revisão bibliográfica e os artigos científicos originais, publicados em língua inglesa, como resultados deste estudo. O primeiro artigo e uma carta ao editor, referem-se principalmente a epidemiologia na retinopatia diabética nos pacientes com diabetes tipo 1 e seus fatores de risco na população estudada. O segundo artigo publicado demonstra a relação da retinopatia diabética com a doença cardiovascular, ressaltando a importância da retinopatia diabética na identificação dos pacientes sob risco cardiovascular. Um terceiro artigo, já submetido, porém ainda não publicado, refere-se à relação dos fatores inflamatórios com a retinopatia diabética proliferativa. E finalmente, será apresentado uma discussão e conclusão geral do estudo.

1 **REVISÃO BIBLIOGRÁFICA**

1.1 **Epidemiologia**

O diabetes tipo 1 acomete aproximadamente 7-12% dos pacientes com diabetes e a retinopatia diabética acomete aproximadamente 1/3 dos pacientes com diabetes, sendo considerada a princial causa de cegueira na população economicamente ativa (1,2,5).

A prevalência mundial de diabetes e de retinopatia diabética varia de acordo com características geográficas, étnicas, socioeconômicas e genética de cada população, entretanto, até o presente momento, não existem dados sobre prevalência de retinopatia diabética em pacientes com diabetes tipo 1, na população brasileira (9).

As formas mais graves da retinopatia diabética, que ameaçam a visão também conhecidas como *vision threatening diabetic retinopathy* (VTDR), inclui as formas de retinopatia não proliferativa severa, retinopatia proliferativa e o edema macular, acometem de 7 a 10 % dos pacientes com retinopatia diabética, segundo a OMS (1). A retinopatia diabética proliferativa é a forma da retinopatia diabética que mais ameaça a visão no diabetes tipo 1 e o edema macular é a principal lesão responsável pela perda visual em pacientes com diabetes tipo 2 (10).

1.2 **Fisiopatologia da retinopatia diabética**

A retinopatia diabética é uma complicação microvascular do diabetes caracterizada por desordem estrutural com comprometimento anatômico e funcional da retina (11).

A complexa estrutura anatômica da retina inclui neurônios fotorreceptores responsáveis pela transdução e transmissão do impulso luminoso por sinapses; células de Müller, responsáveis pela estabilização estrutural da retina e modulação das respostas imunológicas e inflamatórias, além dos capilares retinianos. A troca de nutrientes e metabólitos ocorre através das barreiras hemato-retinianas interna e externa. A interna é formada pelas junções apertadas do endotélio dos capilares retinianos e a externa, pelas junções celulares do epitélio pigmentado retiniano (12,13).

Histologicamente, as alterações mais precoces que ocorrem na retinopatia diabética são o espessamento da membrana basal capilar e a redução no número de pericitos. Pericitos são células mesoteliais que envolvem as células endoteliais capilares e são responsáveis pela integridade estrutural do vaso (14). A distensão da parede vascular causada pela perda dos pericitos, forma os microaneurismas e a proliferação de células endoteliais. Além disso, ocorre também na retinopatia diabética, lesão na barreira hemato-retiniana interna, causando aumento da permeabilidade vascular e consequente edema intra-retiniano (15). A hipóxia progressiva devido à oclusão das arteríolas pré-capilares causa infartos na camada de fibras nervosas, que resulta em edema destas fibras e bloqueio do fluxo axoplasmático, formando as manchas algodonosas, além de dilatações segmentares e irregulares do leito capilar retiniano (IRMA)(12,16). A progressão da isquemia retiniana pode resultar na formação de neovasos que inicialmente são finos, delicados e de baixa resistência, associado ao surgimento de proliferação intensa com formação de tecido conjuntivo. Na fase terminal do processo ocorre regressão do sistema vascular com contratura do tecido conjuntivo, desenvolvendo espessamento na parte posterior do vítreo, podendo resultar em descolamento tracional da retina, retinosquise e/ou hemorragia vítrea (6,12).

A hiperglicemia desencadeia eventos metabólicos que culminam em hiperprodução de espécies reativas de oxigênio (ERO), gerando o estresse oxidativo que danifica todos os tipos de estrutura celular, desde lipídios da membrana até o DNA (17). A produção dos EROs promove ativação do fator nuclear de transcrição NF-kB que resulta em um aumento no fluxo da via de hexosamina e vias do polyol, aumentando a formação de produtos finais da glicação avançada (AGE) e ativação da proteína kinase C (PKC) (18–21). A relação da hiperglicemia com o NF-kB foi demonstrada por El-Osta e col. no estudo experimental com cultura de células endoteliais de aorta de humanos e de ratos sem diabetes, submetidas a exposição de hiperglicemia, levando a produção de EROs e consequente aumento da expressão do NF-kB (22).

A produção dos AGEs ocorre naturalmente com o processo de envelhecimento, porém encontra-se exacerbada em algumas situações patológicas, como no diabetes (23). Na retina, os receptores dos AGEs (RAGE) estão presentes em quase todas as células, sendo mais numerosos nas células de Muller, e quando ativados pelos AGEs, levam a produção do fator de crescimento endotelial vascular (VEGF) (24). Stitt e col. demonstraram em estudo experimental, que níveis elevados de AGEs estão relacionados com quebra da barreira hemato-retiniana interna e aumento da permeabilidade vascular na retina, por possível interação com os RAGEs nas células endoteliais (25). Os pericitos também expressam receptores de AGEs

que, ao interagirem com seus ligantes, desencadeiam o aumento na transdução de sinais celulares causando apoptose dessas células (26).

A PKC é uma família de 10 enzimas, das quais, as isoformas beta 1 e 2 estão diretamente relacionada com o desenvolvimento da RD (27). A ativação dessas enzimas ocorre por aumento do diacilglicerol gerado pela glicólise em situação de hiperglicemia e desencadeia um efeito em cascata que resultam em aumento da permeabilidade vascular, expressão de VEGF no tecido retiniano e leucostasia (28).

Esses produtos resultantes do estresse oxidativo podem ativar os macrófagos e a síntese de citocinas inflamatórias, que estão relacionados ao aumento de algumas proteínas de fase aguda e do fator de crescimento vascular (VEGF) (29). As características dos principais fatores inflamatórios e sua relação com a retinopatia diabética estão abaixo descritas:

a)Interleucina-6 (IL-6): É uma citocina pró-inflamatória e um potente mediador da inflamação intraocular, agindo como mediador da produção de PCR, além de estar indiretamente relacionada com a neovascularização por estimular produção de VEGF (30). Na retina, a IL-6 é produzida pelas células de Müller e pelo epitélio pigmentado da retina (EPR) (31). A produção da IL-6 é ativada pela hipóxia, pela circulação dos AGE e pela PKC (15,32). Um estudo recente de metanálise demonstrou relação dos níveis de IL-6 com a retinopatia diabética, sugerindo relação desses níveis com a gravidade da retinopatia (33);

b) Fator de necrose tumoral alfa (TNF-α): É um mediador de citotoxicidade, produzido por macrófagos e monócitos. Na retina, as células de Muller também apresentam capacidade de produzir TNF-α sob estímulo de hipóxia e hiperglicemia (34). Além de fazer parte do processo inflamatório, também tem um importante papel na neovascularização. TNF-α induz a síntese de outras citocinas, incluindo o VEGF e também influencia na expressão de moléculas de adesão nos vasos retinianos, promovendo leucostasia (adesão de leucócitos nos capilares), e quebra da barreira hemato-retiniana (15,35,36). Estudos em pacientes com diabetes tipo 1, têm demonstrado relação do TNF-α com a presença de retinopatia diabética, sugerindo que a inflamação tem um papel importante na fisiopatologia da retinopatia diabética (37,38). Um estudo de revisão e metanálise também demonstrou associação entre os níveis de TNF-α e RD, em pacientes com DM tipo 1 e tipo 2 (39).

c) Fator de Crescimento Endotelial Vascular (VEGF): É o principal fator que estimula a angiogênese. Os efeitos danosos da angiogênese, responsáveis pela neovascularização da retina, característica da forma proliferativa da RD, ocorrem quando existe uma expressão exacerbada do VEGF por um estímulo patológico como a hipóxia ou hiperglicemia (40,41). O VEGF é produzido por diferentes tipos celulares, dentre eles: células endoteliais, macrófagos,

linfócitos TCD4, miócitos e megacariócitos. As células gliais da retina (células de Muller e astrócitos) também tem a capacidade de produzir VEGF (13,34). Essas células têm a função juntamente com os pericitos de auxiliar na estrutura dos dos vasos retinianos, assim como de regular a barreira hemato-retiniana interna, participando da comunicação entre células neuronais e endoteliais (13). A produção do VEGF pelas células gliais (principalmente pela célula de Muller) encontra-se exacerbada no diabetes, devido a hipóxia e a hiperglicemia, que estimulam a produção dos AGEs e estes interagem com os RAGEs presentes nestas células (34).

Além do estímulo angiogênico, o VEGF também age como um importante indutor do processo inflamatório, promovendo aumento da permeabilidade vascular. Ao se ligar ao seu receptor na membrana das células endoteliais (VEGFR), desencadeia cascatas de sinalização intracelular que em última instância, leva ao aumento da produção de óxido nítrico, potente agente vasodilatador (42). O VEGF promove também, ativação dos fatores de transcrição NFκB, induzindo a produção da proteína quimiotáxica de monócitos (MCP-1), que regula migração e infiltração de monócitos e macrófagos, causando alteração vasculares na retina (43,44). Esse efeito pró-inflamatório está relacionado com aumento da produção de moléculas de adesão celular 1 (ICAM-1) que é um receptor de adesão para leucócitos, promovendo leucostasia, resultando em perda da barreira funcional e aumentando a permeabilidade vascular. (29,45).

Um dos estudos que avaliou a relação dos níveis de VEGF no soro de pacientes com diabetes tipo 1, demonstrou que os níveis desse fator de crescimento endotelial era maior nos pacientes com diabetes tipo 1 do que nos controles (pacientes sem diabetes), e era ainda mais elevado no grupo de pacientes com DM1 e retinopatia diabética (46).

A relevância do papel do VEGF na fisiopatologia da RD, pode ser também evidenciada pela eficácia no tratamento do edema macular diabético com medicamentos anti-VEGF intra-vítreo (47);

d) Proteína C reativa (PCR) é uma proteína de fase aguda, considerada um marcador de doença inflamatória sistêmica e também associada ao risco de doença cardiovascular (48). A síntese de PCR ocorre no fígado ou no tecido adiposo e é regulada em parte, pela IL-6 (49). Devido ao fato da PCR estar relacionada com a resposta de fase aguda, ela é uma resposta inespecífica ao estímulo gerado por dano tecidual ou inflamatório (50). Alguns estudos tem demonstrado um aumento na concentração de PCR em pacientes com DM1, mesmo na ausência de complicações vasculares, sugerindo um estado inflamação crônica de baixo grau, justificado possivelmente pela ativação de macrófagos, aumento do estresse oxidativo e indução de

citocinas inflamatórias (50,51). A relação da PCR com a retinopatia diabética em pacientes com DM1 tem sido demonstrada em alguns estudos (52,53). Entretanto, a maioria dos estudos que analisam essa relação da PCR com a RD, envolve pacientes com diabetes tipo 2 como demonstrado em uma recente metanálise (54).

Apesar das evidências científicas da relação dos marcadores inflamatórios com a retinopatia diabética em pacientes com DM1, a fisiopatologia da retinopatia diabética não está completamente esclarecida e os resultados dos estudos são controversos. Alguns estudos demonstram essa relação (55–57), enquanto outros estudos não ratificam essa associação (58,59).

Além dos mecanismos acima descritos, atualmente tem sido evidenciado uma lesão neuronal na retina, relacionada ao diabetes, antecedendo os danos vasculares e as alterações inflamatórias, de acordo com recente artigo de revisão sobre o assunto (60). As primeiras células neuronais a sofrerem morte celular, são as células ganglionares, afetando diretamente a camada de fibras nervosas (61). Em pacientes com diabetes e ausência de retinopatia diabética ou RD leve, foi demonstrado progressiva redução na espessura da camada de fibras nervosas, na camada de células ganglionares e na camada plexiforme interna, medidas pela tomografia de coerência óptica, sugerindo a existência de uma neuropatia diabética retiniana (62). O efeito clínico consequente a redução na camada de células ganglionares, parece estar relacionado com e redução da sensibilidade ao contraste em pacientes com diabetes mesmo sem sinais de RD (63).

1.3 **Classificação da retinopatia diabética**

Com base nas alterações fundoscópicas, a Classificação Internacional da Retinopatia Diabética (*International Clinical Diabetic Retinopathy Severity Scale*) busca uniformizar e facilitar a comunicação entre oftalmologistas, endocrinologistas e clínicos gerais. O sistema atual de classificação é baseado nos estudos Early Treatment of Diabetic Retinopathy Study (ETDRS) e no Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) (64–66).

A retinopatia diabética pode ser classificada em:

a) Retinopatia ausente;

b) Retinopatia diabética não proliferativa (RDNP) leve: presença apenas de microaneurismas;

c) RDNP moderada: mais alterações do que microaneurismas, podendo apresentar hemorragias intra-retinianas, exsudatos duros, manchas algodonosas, porém, com menos alterações do que a retinopatia diabética não proliferativa severa;

d) RDNP severa: mais de 20 hemorragias nos 4 quadrantes da retina, perolização venosa definitiva (*beading* venoso) em 2 ou mais quadrantes ou presença de IRMA em 1 ou mais quadrantes;

e) Retinopatia diabética proliferativa: presença de neovasos, hemorragia vítrea ou hemorragia pré-retiniana.

O edema macular pode se apresentar em qualquer estágio da retinopatia diabética.

Além da classificação tradicional, podemos agrupar a retinopatia nas formas mais graves, que ameaçam a visão e podem levar a cegueira se não tratadas, também conhecida como *vision threatening diabetic retinopathy* (VTDR), que inclui as formas de retinopatia não proliferativa severa, retinopatia proliferativa e o edema macular (67–69). Alguns estudos agrupam a retinopatia como não referenciável e referenciável, que quando diagnosticada, indica que o paciente deve ser encaminhado para o oftalmologista. Esta forma inclui a retinopatia diabética não proliferativa moderada, a retinopatia não proliferativa severa, a retinopatia proliferativa e o edema macular, excluindo a RDNP leve. (70,71).

1.4 **Fatores de risco**

A identificação dos principais fatores de risco para o desenvolvimento da retinopatia diabética é importante para que possamos agir preventivamente nessa debilitante comorbidade.

De acordo com a *american diabetes association* (ADA), os principais fatores de risco já estabelecidos, relacionados com desenvolvimento de retinopatia diabética são o tempo de duração da doença, a hiperglicemia crônica e a hipertensão arterial. Outros fatores de risco também relacionados são a dislipidemia e a nefropatia (7).

1.4.1 Duração da doença

A duração da doença é um fator de risco determinante para o desenvolvimento da retinopatia diabética. Segundo a Organização Mundial de Saúde, 75% a 90% dos pacientes com diabetes tipo 1 irão apresentar algum grau de retinopatia diabética após 20 anos de doença (72). No WESDR, também foi demonstrado que o tempo de duração do DM1 está fortemente associado a frequência e a severidade da retinopatia. Neste estudo, a frequência da RD nos pacientes com mais de 15 anos de doença foi de 97,5 % (73).

1.4.2 Hiperglicemia

A hiperglicemia crônica, avaliada clinicamente pelo valor da hemoglobina glicada (HbA1c), é um fator de risco bem estabelecido, relacionado tanto com o desenvolvimento quanto com a progressão da retinopatia diabética. Esta relação está bem estabelecida por vários estudos epidemiológicos. O estudo *Diabetes Control and Complications Trial* (DCCT) demonstrou que o tratamento intensivo do diabetes tipo 1 reduz o risco de desenvolvimento da retinopatia em 54%, além de reduzir a progressão da retinopatia diabética, comparado com a terapia convencional (74–76). Hiperglicemia é um dos fatores de risco mais importantes para desenvolvimento da retinopatia diabética tanto para o diabetes tipo 1 como para o tipo 2. Entretanto o benefício do controle glicêmico intensivo parece ser mais evidente para os pacientes com diabetes tipo 1 (77).

1.4.3 Hipertensão arterial

A hipertensão arterial tem sido consistentemente relacionada como um importante fator de risco para a retinopatia diabética (68,78). De acordo com a ADA, o controle pressórico, reduz o risco de progressão da retinopatia diabética (7).

O WESDR, após acompanhar 14 anos de incidência e progressão da retinopatia diabética em pacientes com DM1, observou que a pressão arterial diastólica foi um preditor de progressão

da retinopatia e a hipertensão arterial foi um preditor de progressão para retinopatia diabética proliferativa (79).

1.4.4 Dislipidemia

Níveis elevados de triglicerídeos, colesterol total e LDL colesterol, estão relacionados, com progressão da retinopatia diabética, desenvolvimento de retinopatia diabética proliferativa e edema macular em pacientes com diabetes tipo 1 (80–82).

Em pacientes com diabetes tipo 2 já foi demonstrado inclusive, que o uso de fenofibrato e estatinas reduz a progressão da RD e consequentemente, a necessidade de tratamento da retinopatia com fotocoagulação a laser, independente do efeito das drogas no controle dos níveis do colesterol (83,84). Em estudo experimental recente, foi demonstrado que em ratos com diabetes tipo 1, o uso de fenofibratos inibiu a expressão de citocinas inflamatórias, atenuando o estresse oxidativo e consequentemente reduzindo o extravasamento vascular dos vasos retinianos, demonstrando uma proteção contra a quebra da barreira hemato-retiniana interna (85).

Existem ainda, na literatura, evidências da associação entre obesidade e a retinopatia diabética em pacientes com DM1.O mecanismo fisiopatológico dessa relação ainda não está completamente estabelecido, mas acredita-se que possivelmente esteja associado ao aumento do estresse oxidativo e da inflamação em ambas as condições (86). O tecido adiposo é considerado atualmente um órgão endócrino ativo e pró-inflamatório, que secreta adipocinas e moléculas bioativas como IL-6 e TNF-α (86,87). Além disso, a dislipidemia e a hipertensão arterial que comumente estão associadas a obesidade são importantes fatores de risco para a retinopatia diabética (88)

1.4.5 Nefropatia

A associação da nefropatia com a retinopatia diabética é atribuída a semelhança entre ambas nos mecanismos fisiopatológicos. Assim como na retina, os capilares dos rins sofrem danos na membrana basal, desencadeados pela liberação de marcadores inflamatórios e estresse

oxidativo, consequentemente a hiperglicemia crônica (89,90). A similaridade dos mecanismos patogênicos entre a nefropatia e a retinopatia foi sugerida no estudo *Cronic Renal Insufficiency Cohort* (CRIC), em que foram avaliadas as retinografias dos pacientes com doença renal crônica e encontraram a associação entre a menor a taxa de filtração glomerular (TFG) e o maior o percentual de pacientes com retinopatia (91).

Além dos mecanismos fisiopatológicos, ambas as comorbidades, compartilham fatores de risco como duração do diabetes, hipertensão arterial e dislipidemia. Quando se avalia essa relação, observa-se algumas características particulares. Os pacientes com nefropatia apresentam mais chance de terem retinopatia diabética, sendo essa relação mais evidente em pacientes com DM1 do que pacientes com diabetes tipo 2 (92,93). Os pacientes com retinopatia diabética proliferativa têm mais chance de apresentar nefropatia, do que os pacientes com formas mais leves da retinopatia (90,94,95)

1.5 **Doença cardiovascular e retinopatia diabética**

A doença cardiovascular (DCV) é a principal causa mundial de morte, segundo a OMS, representando aproximadamente 30% de todas as causas mortis (96). No diabetes tipo 1, a DCV também representa a principal causa de morte e de redução na expectativa de vida. A doença cardiovascular representa a complicação macrovascular do diabetes e inclui a doença arterial coronariana, a doença arterial periférica e a doença cerebrovascular. O diabetes confere um risco duas vezes maior de desenvolvimento de DCV, independente de outros fatores de risco (97,98).

A relação da hiperglicemia crônica com o desenvolvimento da DCV no diabetes, foi evidenciado pelo estudo DCCT/EDIC com 17 anos de acompanhamento. Neste estudo foi demonstrado uma redução de 42% de qualquer evento cardiovascular (doença arterial coronariana, doença cerebrovascular e doença arterial periférica) e de 57% o risco de um evento clínico severo, como infarto agudo do miocárdio, acidente vascular cerebral e morte por doença cardiovascular no grupo dos pacientes tratados com terapia intensiva, comparado com o grupo dos pacientes de terapia hipoglicemiante convencional (75).

Além da hiperglicemia, outros fatores de risco também estão relacionados com o desenvolvimento da DCV, dentre eles: dislipidemia, hipertensão, doença renal crônica e

obesidade (99). Estes fatores de risco também estão relacionados com o desenvolvimento da retinopatia diabética (96,99,100).

O compartilhamento de alguns fatores de risco, assim como a semelhança em alguns aspectos da fisiopatologia envolvendo resposta inflamatória e dano endotelial, sugere que exista uma associação entre DCV e a retinopatia diabética. Azzam e col. demonstraram em pacientes com diabetes tipo 2, a evidência de uma variante genética em comum, no gene *CEP 162,* que estaria relacionada tanto com a RD quanto com a DCV (101). Um estudo em adolescentes, com diabetes tipo 1, sem retinopatia diabética, demonstrou uma associação entre sinais precoces de aterosclerose com alterações microvasculares na retina, sugerindo uma fisiopatologia em comum entre ambas as patologias (102).

A associação entre a retina e a doença cardiovascular foi também demonstrada pelo estudo *Atherosclerosis Risk in Communities* (ARIC), que analisou a retinografia de 10.470 pacientes e correlacionou com a incidência de eventos cardiovasculares durante um acompanhamento de 16 anos. Observaram que o estreitamento arteriolar e o alargamento das vênulas retinianas estavam relacionados com maior risco de morte, de infarto e de doença coronariana, assim como maior chance de ter idade mais avançada, apresentar diabetes, maiores níveis pressóricos e de colesterol (103). Farrah e col. demonstraram também que alterações retinianas como afinamento da coroide e redução da densidade e da perfusão vascular, avaliadas com tomografia de coerência óptica (OCT) e OCT *angiography*, estavam associadas com maior risco do paciente apresentar doença cardiovascular (104)

Esta relação entre a RD e a DCV tem sido evidenciada tanto no diabetes tipo 1 (105–108) quanto no diabetes tipo 2 (109,110). Entretanto, como demonstrado em recente estudo de metanálise, observamos uma quantidade maior de estudos envolvendo pacientes com diabetes tipo 2 (111).

2 **OBJETIVOS**

Os objetivos principais do nosso estudo são:

a) Definir a prevalência da retinopatia diabética em pacientes com diabetes tipo 1 de um estudo multicêntrico no Brasil;

b) Identificar os principais fatores de risco para o desenvolvimento da retinopatia diabética em pacientes com diabetes tipo 1 de um estudo multicêntrico no Brasil.

Os objetivos secundários são:

a) Analisar a relação da retinopatia diabética com a doença cardiovascular em pacientes com diabetes tipo 1 de um estudo multicêntrico no Brasil;

b) Analisar a relação da retinopatia diabética proliferativa com os níveis séricos de fatores inflamatórios e de crescimento vascular, associados a fisiopatologia desta complicação microvascular em pacientes com diabetes tipo 1 de um estudo multicêntrico no Brasil.

3 **METODOLOGIA**

O estudo apresentado nesta tese é baseado nos dados do estudo multicêntrico desenvolvido no Brasil, em pacientes com diabetes tipo 1. Cada artigo desenvolvido a partir da análise do banco de dados gerado nesse trabalho, será apresentado nos capítulos a seguir.

O estudo transversal, multicêntrico "Estratégias de rastreamento e diagnóstico da retinopatia e neuropatia diabética e identificação de biomarcadores de complicações crônicas em pacientes com Diabetes Mellitus tipo 1 associado a análise econômica do tratamento" teve como objetivo principal, avaliar a prevalência de complicações crônicas (retinopatia, nefropatia e neuropatia sensitivo motora e autonômica cardíaca) em pacientes com diabetes tipo 1. Foi realizado entre 2011-2014, em 14 centros de 5 regiões do Brasil (Sul, Sudeste, Norte, Nordeste e Centro-Oeste), sob coordenação da Dra. Marília de Brito Gomes, e incluiu 1760 pacientes. Os pacientes eram atendidos em centros secundários e terciários de assistência médica da rede pública de saúde conveniadas ao sistema único de saúde (SUS). Cada centro forneceu os dados de pelo menos 50 pacientes consecutivos regularmente atendidos.

O protocolo do estudo foi aprovado pelo comitê de ética (CAAE:0214.0.228.000-10) e o consentimento informado foi obtido de cada paciente ou seu representante legal.

3.1 **Critérios de inclusão**

Foram incluídos no estudo, pacientes com diagnóstico de diabetes tipo 1, feito por médico endocrinologista, baseado em critérios clínicos (hiperglicemia, poliúria, perda de peso, polidipsia, polifagia e necessidade de uso de insulina desde o diagnóstico), idade maior que 13 anos, em acompanhamento da doença no referido centro, há pelo menos 6 meses.

3.2 **Critérios de exclusão**

Os critérios de exclusão foram gestação, lactação, insuficiência cardíaca congestiva, arritmias cardíacas, insuficiência respiratória aguda ou doença pulmonar obstrutiva grave. Além de história de quadros infecciosos agudos ou cetoacidose diabética nos três meses anteriores à avaliação.

3.3 **Cálculo da amostra**

O cálculo da amostra foi realizado com base no levantamento de dados prévios de estimativa de prevalência de DM1, visando manter a proporção da distribuição da população brasileira em cada centro, de acordo com o senso do ano 2000, realizado pelo Instituto Brasileiro de Geografia e Estatística (IBGE) (112).

3.4 **Avaliação clínica e laboratorial**

Cada paciente foi submetido a um inquérito clínico-demográfico através de um questionário padronizado, aplicado por entrevistadores treinados, no qual foram coletados dados relativos a gênero, idade (anos), etnia autodeclarada, idade ao diagnóstico (anos), tempo de duração do diabetes (anos), dieta (características e adesão), nível de atividade física, tabagismo, etilismo, dose diária de insulina, uso de outras medicações, doenças associadas e uso atual de medicamentos. O nível social foi avaliado de acordo com a Associação Brasileira de Estudos Populacionais (ABEP) (113).

Variáveis clínicas foram avaliadas, dentre elas: peso (kg), altura (cm), índice de massa corporal (IMC) considerando o peso (Kg) divido pelo quadrado da altura (m), pressão arterial (PA), frequência cardíaca (FC), circunferência abdominal (determinada no ponto médio entre a crista ilíaca ântero-superior e o último arco costal), além de pesquisa de sinais e sintomas de neuropatia periférica sensitivo motora, neuropatia autonômica cardiovascular (NAC), pesquisa de retinopatia e nefropatia diabética.

Obesidade foi considerada na presença de um resultado do IMC maior ou igual que 30 Kg/m^2 e sobrepeso em caso de IMC maior ou igual a 25 Kg/m^2 .

Hipertensão arterial foi definida pela presença, reportada pelo paciente, de pelo menos dois episódios de pressão arterial maior que 140x90mmHg, realizado por um profissional de saúde.

A doença cardiovascular foi definida pela presença, segundo dados do prontuário, de uma ou mais das seguintes condições: doença arterial coronariana (angina, angioplastia coronariana, cirurgia de *bypass* coronariano ou infarto agudo do miocárdio), doença vascular periférica e/ou acidente vascular cerebral.

Os dados laboratoriais foram dosados em um laboratório central, da disciplina de Diabetes da Universidade do Estado do Rio de Janeiro (UERJ), e os resultados foram avaliados baseados nos valores de normalidade recomendados pela ADA para um bom controle clínico e metabólico (7). A análise da hemoglobina glicada (HbA1c) foi realizada por *High Performance Liquid Cromatography* (HPLC, Bio-Rad Laboratories, Hercules, California, USA), técnicas enzimáticas foram usadas para dosagem da glicemia de jejum e do colesterol (total, HDL e LDL e triglicerídeos). As variáveis laboratoriais e os valores de normalidade a seguir foram considerados: bom controle glicêmico se HbA1c <7,0% (53 mmol/mol) para adultos e <7,5% (53 mmol/mol) para adolescentes; bom controle do colesterol se colesterol total <200mg/dl (5,2 mmol/L), LDL colesterol <100mg/dl (2,6 mmol/L), HDL colesterol >50 mg/dl (1,3 mmol/L) para mulher e >40mg/dl para homens (1,1 mmol/L) e triglicerídeos <150 mg/dl (1,7 mmol/L). O kit comercial baseado em uréase (BioSystem) foi usado para medir o ácido úrico sérico (ACU) e os seguintes valores normais foram considerados: 2,6 – 6,0 mg/dl em mulheres e 3,5-7,2mg/dl em homens.

A concentração urinária de albumina foi determinada através da coleta de amostra isolada de urina no período da manhã, com intervalo mínimo de uma semana e intervalo máximo de 6 meses entre cada coleta. Os valores da albumina urinária foram representados em média (mg/dl) e a dosagem foi realizada por imunoturbidimetria. A função renal foi estimada pela equação *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI) (114) em adultos e pela fórmula de Scwartz em adolescentes (115), e a expressamos com a taxa de filtração glomerular (TFG) por unidades por minuto por 1,73 m^2 (ml/min). Definimos a insuficiência renal crônica (IRC) quando a albuminúria ≥ 30mg/dl e taxa de filtração glomerular <60 ml/min.

A dosagem de marcadores inflamatórios (IL-6 e TNF-α) e do fator de crescimento endotelial vascular (VEGF) foi realizada para o estudo caso-controle sobre a relação destes

marcadores com a retinopatia diabética proliferativa. Após a seleção dos referidos casos e controles, acessamos as amostras dos soros destes pacientes que se encontravam armazenadas sob refrigeração -80^0 C. Utilizamos o sistema de análise Multiplex (Milliplex® x-MAP, Merk, Millipore Corp., Billerica, MA, USA). Após a incubação dos soros, foi realizado a medida de cada biomarcador pelo equipamento Luminex (Luminex, Austin, TX). A quantificação foi realizada de acordo com as especificações do kit do fabricante.

3.5 **Avaliação da retinopatia diabética**

O exame de fundo de olho foi realizado por um oftalmologista retinólogo do serviço de oftalmologia de cada centro. Um treinamento coletivo foi realizado em um único centro universitário para padronizar a avaliação e a classificação da RD. O exame foi realizado sob midríase com colírio de Tropicamida 1%, por oftalmoscopia indireta com oftalmoscópio Eyetec (Eyetec- São Carlos- SP, Brasil) e lente de 20 dioptrias (Volk Optical, Mentor, OH, EUA). O fundo de olho foi classificado, de acordo como o olho mais grave, em: retinopatia diabética ausente, retinopatia diabética não proliferativa leve, moderada e severa, retinopatia diabética proliferativa e avaliado quanto a presença de edema macular diabético, conforme consenso internacional de retinopatia diabética apresentado pela Academia Americana de Oftalmologia em 2003 (64).

3.6 **Análise estatística**

Para a estatística descritiva, as variáveis quantitativas foram inicialmente avaliadas para definir se suas distribuições eram paramétricas. Os dados foram submetidos ao teste Kolmogorov Smirnof, caso o valor de p se revelasse maior que 0,05 a distribuição foi considerada paramétrica. Para as variáveis quantitativas paramétricas foi definido como estatística de localização a média e para os não paramétricos foi considerada a mediana. As medidas de dispersão foram determinadas pelo desvio padrão para variáveis paramétricas e o intervalo inerquartil para as não paramétricas. As variáveis qualitativas foram analisadas a partir da construção de tabelas de frequências com percentagem.

Na estatística descritiva, para os dados quantitativos com distribuição paramétrica foram utilizados o teste-t de *Student* para comparação de dois grupos e a ANOVA para comparação de 3 ou mais grupos. Foi avaliado a significância entre grupos e se revelado com valores significativos ($p<0,05$), foram realizadas as comparações par a par com uma pós-hoc com correção pelo teste de Sidak. Na comparação par a par foram consideradas as variâncias pelo teste de Levene. Para as variáveis que a análise descritiva revelou uma distribuição não-paramétrica, utilizamos o teste de Mann-Whitney para análise para a par e o de Kruskal-Wallis quando a comparação foi entre 3 ou mais grupos. Para as variáveis qualitativas, foi realizada a estatística do qui-quadrado, considerando no coeficiente de Pearson, a significância ou p-valor $<0,05$.

Na regressão logística binária, o valor do Odds Ratio (OR) foi considerado como medida de força de associação entre a variável dependente e a independente. Foi aplicado o método de *stepwise* para obter um modelo reduzido com as principais variáveis relacionadas com a análise em questão. Foi considerado o intervalo de confiança de 95% e o nível de significância adotado foi de 5%.

As análises estatísticas foram realizadas pelo programa Statistical Package for the Social Science (SPSS v.20, IBM, Armonk, NY).

4 RESULTADOS

4.1 Current epidemiology of diabetic retinopathy in patients with type 1 diabetes: a national multicenter study in Brazil (artigo publicado)

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Current epidemiology of diabetic retinopathy in patients with type 1 diabetes: a national multicenter study in Brazil

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Abstract

Background: Diabetic retinopathy is the leading cause of blindness in economically active populations. The aims of this study were to estimate the prevalence and to identify risk factors for diabetic retinopathy in patients with type 1 diabetes in Brazil.

Methods: This was a nationwide, cross-sectional study conducted between August 2010 and August 2014. The study included 1760 patients with type 1 diabetes. Patients underwent a standard questionnaire, clinical and laboratory analyses and were screened for diabetic retinopathy. To analyze the risk factors related to diabetic retinopathy, two models of logistic regression models were performed, one considering vision-threatening cases and the other with any diabetic retinopathy cases as dependent variables. The group with vision-threatening included patients with severe non-proliferative diabetic retinopathy, proliferative diabetic retinopathy and macular edema.

Results: In total, 1644 patients (mean age, 30.1± 12.0 years; duration of diabetes, 15.3 ± 9.3 years; female, 55.8%) were studied. 35.7% presented diabetic retinopathy and 12% presented vision-threatening diabetic retinopathy. Three risk factors associated with diabetic retinopathy were in common to both groups: longer diabetes duration (OR 1.07; 95% CI, 1.05–1.09), higher levels of HbA1c (OR 1.24; CI, 1.17–1.32) and higher levels of serum uric acid (OR 1.22; CI, 1.13–1.31) ($p < 0.001$ for all comparisons).

Conclusion: The higher rate of vision-threatening retinopathy found in our study highlights the need to improve access to eye care and screening programs for diabetic retinopathy in Brazil. In addition to traditional risk factors, we found an association between serum uric acid levels and diabetic retinopathy. Further studies are needed to address this association.

Keywords: Retinopathy, Epidemiology, Microvascular disease, Type1 diabetes, Vision-threatening, Risk factors, Uric acid

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Background

Type 1 diabetes mellitus (T1D) is a chronic metabolic disease that is characterized by sustained hyperglycemia that leads to macro and microvascular complications, such as retinopathy, nephropathy and neuropathy [1]. Diabetic retinopathy (DR) is considered the leading cause of blindness in economically active populations worldwide and represents a significant social and financial burden for the patients and healthcare systems [2]. The combination of an increasing incidence of T1D worldwide [1, 3] and the aging of populations is resulting in an increase in diabetes-related complications, including DR. The worldwide prevalence of DR has been estimated to be 35%, and the prevalence of vision-threatening DR (VTDR) has been estimated to be 10% [4, 5]. VTDR may soon result in vision loss if left untreated.

DR represents a retinal pathology that may closely reflect microvascular damage in other vascular beds and is considered an important indicator of microvascular complications [6]. Furthermore, fundus examination is an inexpensive and non-invasive opportunity to access the microvascular bed.

The duration of diabetes and poor glycemic control are the most important risk factors associated with the development of DR and the majority of diabetes-related chronic complications. Important clinical trials, such as *The Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications* (DCCT/EDIC) have demonstrated that intensive treatment of hyperglycemia effectively delays the onset and slows the progression of complications in T1D, including DR [7]. However, other risk factors have been associated with DR, including nephropathy, hypertension and dyslipidemia [2].

Screening for DR is an important tool for detecting VTDR and preventing blindness. Currently, approximately only 60% of patients with diabetes have yearly screenings for DR [5]. However, recent studies have shown a tendency towards individualized retinal screening intervals based on glycated hemoglobin levels, type and the duration of diabetes [8].

Systematic screening for DR is important to identify patients who need referral to a specialist, especially those with VTDR thereby preventing blindness and visual impairment [9–11]. However, Brazil, along with several other developing countries, does not have a national screening program for DR, neither, as far as we know, data on its prevalence.

The purpose of this study was to estimate the prevalence of and the risk factors for DR based on a nationwide study of T1D in Brazil.

Methods

Study design

This report describes a multicenter, nationwide, cross-sectional study including 1760 patients that was conducted between August 2010 and August 2014 in 14 secondary public centers (ambulatory outpatient clinics) and tertiary care centers (ambulatory outpatient clinics in university hospitals) located in the urban areas of 10 cities, representing all Brazilian geographic regions. The detailed methodology has been described previously [12]. Briefly, all patients received public healthcare from the National Brazilian Health Care System (NBHCS), and each clinic reported data for at least 50 consecutive type 1 diabetes patients who regularly attended the clinic.

The diagnosis of T1D was done by an endocrinologist in the presence of characteristics signs of TD1, such as polydipsia, weight loss, polyuria, variable degrees of hyperglycemia and the need for continuous insulin use since the diagnosis. Inclusion criteria were patients older than 13 years, followed at each center for more than 6 months. Patients who had had diabetic ketoacidosis or infectious diseases in the last 3 months, as well as pregnant and lactating women, were excluded. The ethics committees of each participating center, in addition to the ethics committee of the coordinating center, at the Pedro Ernesto University Hospital, at the State University of Rio de Janeiro, approved the study. The informed consent term was signed by patients or their legal guardians. The research was conducted in accordance with the Helsinki Declaration.

The quality of the study was assessed using the checklist "Strengthening the Observational Report on Epidemiology" (STROBE) [13].

Data collection

Clinical data

The patients were submitted to a questionnaire standardized by a trained physician. Patients characteristics included height (m), age at diagnosis, age, diabetes duration, weight (kg), abdominal circumference, adherence to diet, insulin therapeutic regimen, smoking status, personal and family medical histories, economic status and number of years of formal education.

The definition of smoking was based on the current use of more than one cigarette per day.

Hypertension in adults was self-reported. We consider hypertensive patients who report a previous diagnosis of hypertension on at least two different occasions, by a health professional.

For the definition of obesity, we considered the body mass index (BMI) ≥ 30 kg / m^2 and overweight a BMI ≥ 25 kg / m^2.

The Macrovascular disease was defined by the presence in the patient medical record of one or more of the following conditions: coronary artery bypass surgery, angina, coronary angioplasty, myocardial infarction, peripheral vascular disease or stroke.

Laboratorial data

To analyze the blood samples, high-performance liquid chromatography (HPLC, Bio-Rad Laboratories, Hercules, California, USA) was used for glycemia and for glycated hemoglobin (HbA1c), enzymatic techniques was used for cholesterol (total, HDL and LDL and triglycerides). We adopted the following ADA parameters for adequate clinical and metabolic control [2, 14]: good glycemic control was defined as HbA1c < 7.0% (53 mmol/mol) for adult and < 7.5% (58 mmol/mol) for adolescents and poor glycemic control was defined as HbA1c ≥ 9.0% (75 mmol/mol), for a good control of cholesterol were considered the values of total cholesterol < 200 mg/dl (5.2 mmol/L), LDL cholesterol < 100 mg/dL (2.6 mmol/L), HDL cholesterol > 50 mg/dL (1.3 mmol/L) for women and > 40 mg/dL for men (1, 1 mmol/L), and triglycerides < 150 mg/dL (1.7 mmol/L).

A commercial urease-based kit (BioSystem) was used to measure serum uric acid (SUA) and the following normal values were considered: 2.6–6.0 mg/dl in women and 3.5–7.2 mg/dl in men.

Albuminuria was measured in two morning urine samples with a minimum interval of 1 week and a maximum of 6 months. The values of the urinary albumin were expressed in means (mg/dl) and the dosage was performed by immunoturbidimetry. We used the CKD-EPI equation [15] to estimate renal function and expressed it as a glomerular filtration rate (GFR) in units per milliliters per minute per 1.73 m^2 (ml/min). We defined chronic kidney disease (CKD) when albuminuria ≥30 mg/dl and GFR < 60 ml/min [2].

Diabetic retinopathy data

DR screening was accessed by mydriatic binocular indirect ophthalmoscopy (BIO), which was performed in each center, by an ophthalmologist specialized in retina, who was trained before the beginning of the study in an ophthalmologic university center. The stage of retinopathy for each patient was considered by the eye with more severe retinopathy. Each eye was classified based on the absence or presence of DR. Patients with DR are classified considering the international classification of DR as: non-proliferative diabetic retinopathy (NPDR) mild, moderate or severe; proliferative diabetic retinopathy (PDR) and macular edema [16]. Additionally, DR was categorized as VTDR (severe NPDR, PDR, and macular edema) or non-VTDR (absent of DR, mild NPDR and moderate NPDR, without macular edema).

Sample data calculation and economic status

In order to determine the sample size of this study, the distribution of the Brazilian population in the different geographic regions was taken into account, according to the population distribution reported by the 2000 Brazilian Institute of Geography and Statistics Census (IBGE) [17]. The calculation of this sample is already described previously in the Brazilian Multicenter Type 1 Diabetes Study [12].

Centers included in our study were all from urban areas because the vast majority of type 1 diabetes patients are treated on reference centers located in urban areas. It is important to note that, based on the IBGE census, the majority of the Brazilian population (approximately 85%) resides in urban areas.

The economic status was stratified in the categories of high, medium, low and very low income, according to the Brazilian Economic Classification Criteria [18].

Statistical analysis

We conducted descriptive analyzes to evaluate the associations between demographic, clinical and laboratory data with DR. The Student t test or the ANOVA with Sidak correction were used for continuous variables. The chi-square test was used for the categorical variables. We present the data as frequencies (percentages) for the categorical variables and as the standard deviation of the means (SD) for the continuous variables.

Univariate analysis was performed to evaluate the relationship between RD and its risk factors. We included in the multivariate analysis the variables with $p < 0.1$ in the univariate and some variables of interest like gender and economic status. Two models of logistic regression were performed: one model for each considered category of DR as the dependent variable. First, we considered any DR vs. the absence of DR; secondly, we considered VTDR vs. non-VTDR (absent, mild NPDR and moderate NPDR). For each model, we tested the same covariates, including gender, age, duration of diabetes, economic status, years of formal education, HbA1C, CKD (yes/no), hypertension (yes/no), use of angiotensin-converting enzyme (ACE) inhibitor (yes/no), serum uric acid (SUA), HDL cholesterol, LDL cholesterol, triglycerides, BMI, macrovascular disease (yes/no), and smoking status (yes/no). The exploratory Forward-Wald stepwise regression was performed, and it was determined which variables contributed more to the discrimination between groups. The Nagelkerke R-squared value was also calculated.

All analyses were performed using the Statistical Package for the Social Sciences (SPSS version 20, SPSS, Inc., Chicago, IL, USA). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated when indicated. A two-sided $p < 0.05$ was considered statistically significant.

Results

Prevalence of diabetic retinopathy

Of the 1760 patients recruited at baseline, a total of 1644 (93.4%) were screened for DR with fundoscopy and were included in this study. Of these patients, 1055

(64.2%) had no retinopathy and 589 (35.7%) presented with DR as follows: 298 (18.1%) had mild NPDR, 108 (6.6%) had moderate NPDR, 11 (0.6%) had severe NPDR, and 172 (10.4%) had proliferative DR. Among those patients with DR, 44 (2.7%) presented macular edema. The clinical and demographic characteristics of the studied population are shown in Table 1.

The distribution of patients in the sub-group of VTDR was as follows: 198 (12%) VTDR and 1446 (88%) non-VTDR (Fig. 1).

Social-demographic, clinical and laboratory risk factors for DR

The descriptive analysis has shown that patients with DR, were older, had longer diabetes duration, less years of formal education, higher BMI, SUA, HbA1c and triglycerides, were more likely to be users of angiotensin-converting enzyme (ACE) inhibitors, and had a higher prevalence of hypertension, macrovascular disease and CKD ($p < 0.001$ for all comparisons) than patients without DR. Levels of serum uric acid were higher in men than in women (mean 5.57 vs. 4.69 mg/dl; $p < 0.001$). Retinopathy was also not associated with gender, economic status, smoking status, HDL or LDL cholesterol levels (Table 2).

Descriptive analysis of clinical and laboratory features of individuals, stratified by traditional classification of DR (non proliferative mild, moderate, severe and proliferative), are shown as an Additional file 1: Table S1.

The final adjusted model of multivariate binomial logistic regression is described in Table 3. The independent variables included in the model explained 32.5% (Nagelkerke R^2) of the variance for the presence of any type of DR. The highest odds were 2.26 for macrovascular disease (CI95% 1.08–4.71; $p = 0.03$) and 1.68 for arterial hypertension (CI95% 1.13–2.5; $p = 0.01$). Age, duration of DM, HbA1c and serum uric acid were also significant predisposing factors to DR.

The crude odds ratio (OR) at univariate level can be accessed as Additional file 2: Table S2 and Additional file 3: Table S3.

Social-demographic, clinical and laboratory risk factors for VTDR DR (severe NPDR, proliferative DR and macular edema)

Descriptive analysis showed that patients with VTDR were older ($p < 0.001$), with a longer diabetes duration ($p < 0.001$), had higher levels of SUA ($p < 0.001$), triglycerides ($p = 0,02$) and BMI ($p = 0.01$), were more likely to be users of an ACE inhibitor ($p < 0.001$), to have hypertension ($p < 0.001$), macrovascular disease ($p < 0.001$) and CKD ($p < 0.001$), than patients without VTDR. We found no association with gender, number of years of formal education, economic status, levels of HbA1c, HDL or LDL cholesterol, or smoking status (Table 2).

Table 1 Demographic and clinical data of the study population

Variable	
N	1644
Demographic characteristics	
Gender, female, n (%)	917 (55.8)
Age, years	30.1 ± 12.0
Age of diagnosis, years	14.6 ± 8.9
Duration of diabetes, years	15.3 ± 9.3
Years of formal education, years	12.3 ± 3.8
Economic status, n (%)	
High	49 (3.0)
Medium	745 (45.3)
Low	795 (48.4)
Very low	55 (3.3)
Clinical data	
HbA1c (%)	9.0 ± 2.1
HbA1c, mean (SD), mmol/mol	74.5 ± 23.1
Diet adherence > 80%, n (%)	846 (58.4)
Insulin regimens, n (%)	
Intermediate or long acting	80 (4.9)
Intermediate/long plus short acting	1.510 (91.8)
CSII	54 (3.3)
Serum uric acid, mean (SD), mg/dL	5.1 ± 1.8
HDL cholesterol, mean (SD), mg/dL	56.8 ± 19.1
LDL cholesterol, mean (SD), mg/dL	109.8 ± 40.8
Triglycerides, mean (SD), mg/dL	109.8 ± 84.6
BMI, mean (SD), kg/m^2	24.2 ± 4.2
Arterial hypertension, yes, n (%)	288 (17.5)
Macrovascular disease, yes n (%)	57 (3.5)
Use of an angiotensin-converting enzyme (ACE) inhibitor, yes, n (%)	437 (26.6)
Current smoker, yes, n (%)	86 (5.6)
Chronic kidney disease, yes, n (%)	289 (16.4)
Diabetic retinopathy, n (%)	
Absent	1055 (64.2)
Mild non-proliferative	298 (18.1)
Moderate non-proliferative	108 (6.6)
Severe non-proliferative	11 (0.6)
Proliferative	172 (10.4)
Macular edema, yes, n (%)[†]	44 (2.7)

Notes: Data are presented as numbers (percentages) or means ± SD (standard deviation)
[†]Of the total patiens with DR (mild, moderate, severe and proliferative), 44 presented macular edema
Abbreviations: *BMI* body mass index, *HbA1c* glycated hemoglobin, *CSII* continuous subcutaneous insulin infusion, *LDL-c* low density lipoprotein cholesterol, *HDL* high density lipoprotein cholesterol

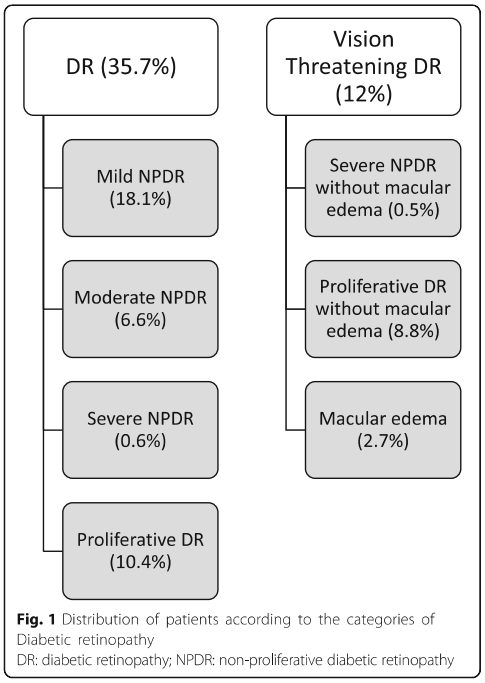

Fig. 1 Distribution of patients according to the categories of Diabetic retinopathy
DR: diabetic retinopathy; NPDR: non-proliferative diabetic retinopathy

The independent variables included in the final adjusted multivariate binomial logistic regression model explained 27.3% (Nagelkerke R^2) of the variance for the presence of VTDR. The highest odds were 2.52 for CKD (CI95% 1.69–3.77; $p < 0.001$) and 2.17 for use of an ACE inhibitor (CI95% 1.51–3.11; $p < 0.001$). Duration of DM, HbA1c and serum uric acid were also significant predisposing factors to VTDR. High levels of LDL cholesterol is related to low risk of VTDR. Data are shown in Table 3.

Discussion

This was the first multicenter study in Brazil to estimate the prevalence of DR in patients with T1D. We also analyzed risk factors for the development of any DR or VTDR in this population. The prevalence of any DR was 35%, of which 12% presented VTDR that required immediate referral for treatment evaluation.

This result is consistent with the worldwide estimates of the overall prevalence of DR (34.6%) but we found a slightly higher prevalence of VTDR (12% vs. 10%) [1, 2, 19]. However, it is important to emphasize that the prevalence of DR varies with geography and ethnicity worldwide [4]. Socioeconomic and genetic factors may influence this variability. In Brazil, we found only one study that described the prevalence of diabetic retinopathy in patients with T1D [20]. However, this was a small study conducted in a selected population limited to a single hospital and showed a higher DR prevalence than in our sample (40.4% vs 35.7%, respectively).

Although in our sample the majority of patients were using intensive insulin therapeutic regimens with less than 5% using continuous insulin infusion, we found high levels of HbA1c, which could be explained by the lack of adherence to insulin therapeutic regimens [12] as well as low adherence to diet [21]. However, other factors such as socioeconomic status and educational level could be related to the observed higher levels of HbA1c as recently demonstrated [22, 23].

In our study, three variables were frequently associated with retinopathy regardless of DR group: longer duration of diabetes, higher levels of HbA1c and SUA. Arterial hypertension was associated with DR in patients with any kind of DR. Multiple studies have consistently shown that a longer duration of diabetes, poor glycemic control and hypertension are positively associated with the development of DR [11, 24]. The finding of a significant association between HbA1c and DR is consistent with the results found on DCCT [7] and the Wisconsin epidemiologic study of DR (WESDR) [25]. The use of ACE inhibitors was also associated with DR in both groups. We believe that the use of ACE inhibitors was found to be significant because of its frequent use regarding the treatment of arterial hypertension.

Another interesting finding was the association of DR, independent of its severity, with SUA levels. This result is consistent with observations from a number of studies suggesting an association between SUA levels and vascular complications in patients with T1D, including DR [26, 27]. Some cross-sectional and prospective studies, conducted in Thailand and Japan [28, 29] have also shown association between uric acid serum levels and DR in patients with type 2 diabetes. However, in Japan this association was only observed in men [29]. Although our study found higher levels of SUA in men, gender was not a significant variable for DR or VTDR. Possible mechanisms that may explain this association include the pro-inflammatory and pro-oxidant effects of SUA, which can lead to endothelial damage. Uric acid, a product of purine metabolism, usually acts as an antioxidant. However, paradoxically, it can also act as a pro-oxidant because reactive oxygen species are generated during its production [30, 31]. A previous experimental study suggests that higher levels of SUA may stimulate the production of inflammatory cytokines [32].

In this current study, the presence of VTDR showed a significant association with CKD. A number of studies provide evidence that the presence of DR may indicate patients at risk of diabetic nephropathy [33, 34].

Table 2 Demographic, clinical and laboratory data stratified by diabetic retinopathy type

Variables	Present	Absent	*p*-Value	VTDR	Non VTDR	*p*-Value
N, (%)	589 (35.8)	1055 (64.2)		198 (12.1)	1446 (87.9)	
Demographic data						
Gender, female, n (%)	343 (58.2)	574 (54.4)	0.1	114 (57.6)	803 (55.5)	0.5
Age, mean (SD), years	35.77 ± 11.56	26.86 ± 11.11	< 0.001	37.35 ± 11.07	29.05 ± 11.84	< 0.001
Duration of diabetes, mean (SD), years	20.26 ± 9.18	12.62 ± 8.15	< 0.001	23.28 ± 9.02	14.25 ± 8.77	< 0.001
Years of formal education, mean (SD), years	11.92 ± 4.22	12.43 ± 3.55	0.01	12.05 ± 4.22	12.27 ± 3.75	0.4
Economic status, n (%)						
High	14 (2.4)	35 (3.3)	0.3	5 (2.5)	44 (3.0)	0.1
Medium	261 (44.3)	484 (45.9)		86 (43.4)	659 (45.6)	
Low	289 (49.1)	506 (48.0)		95 (48.0)	700 (48.4)	
Very low	25 (4.2)	30 (2.8)		12 (6.1)	43 (3.0)	
Clinical data						
HbA1c mg/dl (%)	9.27 (2.22)	8.86 (2.04)	< 0.001	9.10 (2.06)	8.99 (2.12)	0.4
HbA1c, mean (SD), mmol/mol	77.84 ± 24.27	73.34 ± 22.33	< 0.001	76.03 ± 22.53	74.8 ± 23.22	0.4
Serum uric acid, mean (SD), mg/dL	5.58 ± 2.09	4.81 ± 1.63	< 0.001	6.09 ± 2.04	4.95 ± 1.77	< 0.001
Hypertension, yes, n (%)	197 (33.4)	91 (8.6)	< 0.001	85 (42.9)	203 (14.1)	< 0.001
Triglycerides, mean (SD), mg/dL	102.99 ± 76.59	121.98 ± 96.30	< 0.001	122.46 ± 88.96	108.07	0.02
HDL cholesterol, mean (SD), mg/dL	57.84 ± 19.89	56.21 ± 18.63	0.1	54.50 ± 17.53	57.11 ± 19.29	0.56
LDL cholesterol, mean (SD), md/dL	111.90 ± 43.91	108.31 ± 38.98	0.09	113.37 ± 38.46	110.56 ± 41.28	0.3
BMI, mean (SD), kg/m2	25.09 ± 4.69	23.65 ± 3.81	< 0.001	25.00 ± 4.90	24.05 ± 4.08	0.01
Macrovascular disease, yes n (%)	39 (6.6)	18 (1.7)	< 0.001	13 (6.6)	44 (3.1)	0.01
Use of an angiotensin converting enzyme (ACE) inhibitor, yes, n (%)	269 (45.7)	168 (16.0)	< 0.001	112 (22.6)	325 (56.6)	< 0.001
Current smoker, yes, n (%)	38 (6.5)	48 (4.5)	0.09	14 (7.1)	72 (5.0)	0.2
Chronic kidney disease, yes, n (%)	159 (27.4)	105 (10.1)	< 0.001	83 (42.8)	181 (12.7)	< 0.001

The data are presented as numbers (percentages) or means ± SD (standard deviation). The *p* value compares differences between the groups using Student's t-test. *BMI* body mass index, *HbA1c* glycated hemoglobin, *LDL-c* low density lipoprotein cholesterol, high density lipoprotein cholesterol, *VTDR* vision-threatening diabetic retinopathy

Retinopathy and nephropathy are microvascular complications that may reflect similar vascular changes with common mechanisms. Hyperglycemia activates intracellular signaling pathways, leading to oxidative stress, endothelial injury and the overproduction of inflammatory markers [35]. Increased vascular permeability, thickening of the basement membrane and muscular layers are common in both retinopathy and nephropathy [33].

As in other studies, our results have shown a significant association between any type of DR and macrovascular disease. This association may be related to the fact that both conditions share similar risk factors, such as poor glycemic control and hypertension [36, 37].

Patients with higher levels of LDL-cholesterol showed lower risk of VTDR. We believe that patients with a high level of LDL-cholesterol use more frequently statins or fibrates and these drugs could have a protector effect regarding DR. The impact of statins and fibrates on reducing DR is still a matter of debate. However, studies on patients with type 2 diabetes have shown, that the use of fibrates and statins can prevent the progression of DR and reduce the requirements of laser therapy, independent of the effect on cholesterol control or levels [38, 39].

The strengths of our study are as follows: first, to the best of our knowledge, this present large and multicenter study with a multi-ethnic population is the first to determine the prevalence of DR in patients with T1D in Brazil. Second, the fact that T1D patients are younger and have a less comorbidities than those with type 2 diabetes, allows a more reliable assessment of the risk factors for DR. Third, we categorized DR as VTDR, consistent with the epidemiological literature, thus allowing an analysis of patients at greater risk of visual impairment.

Our study has also some limitations. First, this was a cross-sectional study; hence, it is not possible to establish a true cause and effect relationship based on the data. Second, we used the self-reported diagnosis of arterial hypertension; thus, the prevalence of this disease may have been underestimated. However, we consider

Table 3 Final adjusted logistic regression model

	b	OR	CI 95%		*p* value
Present vs. Absent					
Age, years	0.028	1.03	1.02	1.04	< 0.001
Duration of DM, years	0.067	1.07	1.05	1.09	< 0.001
HbA1c (%)	0.216	1.24	1.17	1.32	< 0.001
Serum uric acid, mg/dL	0.196	1.22	1.13	1.31	< 0.001
Use of an angiotensin-converting enzyme (ACE) inhibitor, yes	0.563	1.78	1.26	2.43	< 0.001
Macrovascular disease, yes	0.813	2.26	1.08	4.71	0.03
Arterial hypertension, yes	0.521	1.68	1.13	2.5	0.01
VTDR vs. Non-VTDR					
Duration of DM, years	0.075	1.08	1.06	1.1	< 0.001
HbA1c %	0.105	1.11	1.02	1.21	0.014
Serum uric acid, mg/dL	0.219	1.25	1.13	1.37	< 0.001
Use of an angiotensin-converting enzyme (ACE) inhibitor yes	0.774	2.17	1.51	3.11	< 0.001
LDL cholesterol, mg/dL	−0.01	0.99	0.99	1	0.019
Chronic kidney disease, yes	0.925	2.52	1.69	3.77	< 0.001

VTDR vision-threatening diabetic retinopathy, *HbA1c* glycated hemoglobin, *LDL-c* low density lipoprotein cholesterol, *b* coefficient for logistic regression; *OR* odds ratio, *CI 95%* 95% confidence interval

that this type of self-reported information exhibits substantial agreement between the questionnaire responses and medical records regarding information for hypertension (approximately 82% sensitivity and 92.2% specificity) [40]. Third, T1D was diagnosed according to the clinical definition assigned by a physician without evaluation of islet cell antibodies and c-peptide as well as genetic evaluation for MODY. However, clinical definition of T1D is used in most epidemiological studies. Fourth, although the gold standard methodology for diagnosis of DR is the 7-field stereoscopic photographs established by ETDRS [41], both BIO and retinography are valid strategies for the screening of DR. Previous study from our group has shown significant agreement between the two methods for DR classification [9]. Due to logistic issues, assessment of inter observer variability on screening of DR was not conducted. Fifth, we have included patients with less than 5 years of DM, of whom eight had DR, and seven of these with mild NPDR. Although ADA consensus recommends screening for DR in patients with more than 5 years of T1D, some studies, as DCCT, revealed that patients with T1D and less than 5 years of disease could present DR [42].

Conclusion

In summary, our data have shown a high prevalence of DR among Brazilian T1D patients consistent with the worldwide prevalence of this condition. We found a high proportion of patients with VTDR. This highlights the need for improving access to eye care and DR screening programs in Brazil, which would be beneficial for the prevention of visual impairment and loss. In addition, our data have shown that besides the traditional risk factors (duration of diabetes, poor glycemic control and hypertension) SUA levels has emerged as a potential marker of DR. This emphasizes the need to continuously seek the best clinical control for patients with T1D to prevent this disabling complication. Furthermore, future studies addressing the relationship between DR and SUA levels are needed.

Additional files

Additional file 1: Table S1. Demographic, clinical and laboratory data stratified by diabetic retinopathy type. (DOCX 20 kb)

Additional file 2: Table S2. Multivariate analysis of diabetic retinopathy (Present vs. Absent). (DOCX 21 kb)

Additional file 3: Table S3. Multivariate analysis of diabetic retinopathy (Vision threatening vs. no vision threatening). (DOCX 102 kb)

Abbreviations

ACE: Angiotensin-converting enzyme; ADA: American diabetes association; BIO: Binocular indirect ophthalmoscopy; BMI: Body mass index; CKD: Chronic kidney disease; CSII: Continuous subcutaneous insulin infusion; DCCT: Diabetes Control and Complications Trial; DR: Diabetic retinopathy; GFR: Glomerular filtration rate; HbA1c: Glycated hemoglobin; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; SUA: Serum uric acid; T1D: Type 1 diabetes; TG: Triglycerides; VTDR: Vision threatening diabetic retinopathy

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
M.B.G. was the principal investigator of the study, designed the study and the survey questionnaire, supervised the project and reviewed the manuscript; L.G.N.M. conducted statistical analysis, conducted the literature review and drafted the manuscript; D.C.S, M.H.P and B.S.V.P. helped to conduct statistical analysis and reviewed the manuscript; P.H.M., K.R.G.D, T.C.L.M.,A.A.P., F.M., F.S.L.L., F.K.M., were responsible for collecting data on diabetic retinopathy in each center. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Pedro Ernesto University Hospital, at State University of Rio de Janeiro (protocol: CEP/HUPE 2769/2010), which was the coordinator center, and by the follow local ethics committee of each center: Ethics Committee of Unifesp (Federal University of São Paulo); Ethics Committee of Clementino Fraga Filho University Hospital; Ethics Committee of CEDEBA (Centro de Endocrinologia e Diabetes do Estado da Bahia); Ethics Committee of UFRGS (Federal University of Rio Grande do Sul); Ethics Committee of UNICAMP (University of Campinas); Ethics Committee of Hospital Regional de Taguatinga; Ethics Committee of Bauru's diabetics association; Ethics Committee of João de Barros Barreto University Hospital; Ethics Committee of USP (University of Sao Paulo); Ethics Committee of Walter Cantidio University Hospital and Ethics Committee of UFPR (Ferderal University of Paraná). All participants or their guardians signed informed consent.

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Competing interests
All authors declare that they have no conflicts of interest.

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Table S1. Demographic, clinical and laboratory data stratified by diabetic retinopathy type

Variables	Absent	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	P-Value
N (%)	1055 (64.2)	298 (18.1)	108 (6.6)	11 (0.7)	172 (10.4)	
Demographic data						
Gender, female n(%)	574 (54.4)	176 (59.1)	62 (57.4)	9 (81.8)	96 (55.8)	0.2
Age, mean (SD), years	26.86 ± 11.11	34.33 ± 11.84	36.11 ± 11.23	28.64 ± 8.22	38.5 ± 10.89	<0.001
Duration of diabetes, mean (SD), years	12.62 ± 8.15	18.13 ± 8.79	19.96 ± 8.60	19.73 ± 8.23	24.05 ± 9.10	<0.001
Years of formal education, mean (SD), years	12.43 ± 3.55	12.12 ± 4.26	11.27 ± 4.14	12.27 ± 3.03	11.95 ± 4.26	0.02
Economic status, n (%)						
High	35 (3.3)	5 (1.7)	4 (3.7)	0	5 (2.9)	
Medium	484 (45.9)	139 (46.6)	41 (38.0)	4 (36.4)	77 (44.8)	0.09
Low	506 (48.0)	147 (49.3)	56 (51.9)	5 (45.5)	81 (47.1)	
Very low	30 (2.8)	7 (2.3)	7 (6.5)	2 (18.2)	9 (5.2)	
Clinical data						
HbA1c mg/dL, %	8.86 ± 2.04	9.21 ± 2.17	9.97 ± 2.70	9.5 ± 2.52	8.91 ± 1.81	<0,001
HbA1c, mean (SD), mmol/mol	73.34 ± 22.33	77.23 ± 23.82	85.47 ± 29.59	80.42 ± 27.64	73.9 ± 19.80	<0.001
Serum uric acid, mean (SD), mg/dL	4.81 ± 1.63	5.24 ± 1.99	5.62 ± 2.15	6.27 ± 1.92	6.1 ± 2.11	<0.001
Hypertension, n(%)	91 (8.6)	75 (25.2)	41 (38.0)	3 (27.3)	78 (45.3)	<0.001
Triglycerides, mean (SD), mg/dL	102.99 ± 76.59	121.52 ± 106.01	127.36 ± 82.00	158.1 ± 148.13	117.3 ± 82.49	<0.001
HDL cholesterol, mean (SD), mg/dL	56.21 ± 18.63	59.54 ± 20.56	59.28 ± 20.97	57.07 ± 20.10	54.02 ± 17.50	0.01
LDL cholesterol, mean (SD), mg/dL	110.2 ± 42.26	112.96 ± 36.77	110.67 ± 41.90	125.18 ± 79.78	110.91 ± 33.92	0.6
BMI, mean (SD), kg/m^2	23.65 ± 38.81	24.99 ± 4.55	25.41 ± 4.44	23.1 ± 4.53	25.19 ± 5.06	<0.001
Macrovascular disease, yes, n (%)	18 (1.7)	15 (5.0)	11 (10.2)	1 (9.1)	12 (7.0)	<0.001
Use of an angiotensin-converting enzyme (ACE) inhibitor, yes, n(%)	168 (16.0)	108 (36.4)	54 (50.0)	9 (81.8)	98 (57.0)	<0.001
Current smoker, yes, n(%)	48 (4.5)	15 (5.1)	10 (9.3)	1 (9.1)	12 (7.0)	0.2
Chronic kidney disease, yes, n (%)	105 (10.1)	54 (18.2)	27 (25.7)	4 (40.0)	74 (43.8)	<0.001

The data are presented as numbers (percentages), means ± SD (standard deviation). The p value compares differences between the groups using one-way ANOVA. BMI: body mass index; HbA1c: glycated hemoglobin; LDL-c: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; NPDR = Non-proliferative diabetic retinopathy. PDR = Proliferative diabetic retinopathy

Table S2: Multivariate analysis of diabetic retinopathy (Present *vs.* Absent)

	Unadjusted	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
Duration of DM, years	1.070 (1.051-1.089)	1.101 (1.086-1.117)	1.084 (1.069-1.100)	1.096 (1.080-1.113)	1.095 (1.079-1.112)	1.070 (1.052-1.089)	1.070 (1.051-1.089)	1.069 (1.051-1.088)
Use of an angiotensin-converting enzyme (ACE) inhibitor	1.782 (1.280-2.480)		2.983 (2.306-3.858)	2.857 (2.198-3.714)	2.620 (2.007-3.419)	2.248 (1.708-2.957)	1.793 (1.295-2.482)	1.755 (1.266-2.433)
HbA1c (%)	1.227 (1.152-1.308)			1.213 (1.144-1.285)	1.212 (1.143-1.285)	1.238 (1.165-1.314)	1.240 (1.167 -1.316)	1.241 (1.168-1.317)
Serum uric acid, mg/dL	1.210 (1.108-1.321)				1.213 (1.131-1.301)	1.228 (1.144-1.318)	1.218 (1.134-1.309)	1.217 (1.132-1.307)
Age, years	1.025 (1.011-1.039)					1.033 (1.020-1.46)	1.029 (1.016-1.043)	1.028 (1.015-1.042)
Arterial hypertension, yes	1.526 (1.013-2.298)						1.678 (1.131-2.488)	1.684 (1.134-2.502)
Macrovascular disease, yes	2.231 (1.061-4.692)							2.256 (1.081-4.709
Gender	0.860 (0.655-1.128)							
Years of formal education	0.990 (0.956-1.026)							
BMI, kg/m^2	1.013 (0.981-1.046)							
Current smoker	1.231 (0.722-2.100)							
LDL cholesterol, mean (SD), mg/dL	0.999 (0.996-1.002)							
HDL cholesterol, mg/dL	1.004 (0.997-1.010)							
Triglycerides, mg/dL	1.001 (0.998-1.003)							
Chronic kidney disease	1.262 (0.873-1.824)							
Economic status, n (%)								
High	0.878 (0.316-2.441)							
Medium	1.056 (0.522-2.137)							
Low	1.106 (0.559-2.189)							

Data are presented as: odds ratio and CI 95% (95% confidence interval). Abbreviations: DM, diabetes Mellitus; ACE, angiotensin-converting enzyme; BMI, body mass index; HbA1c, glycated hemoglobin; LDL-c, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol.

Unadjusted: non-adjusted (forward:wald model)

Model 1: after adjustment for duration of DM, use of ACE inhibitor, HbA1c, serum uric acid, age, arterial hypertension, macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.

Model 2: after adjustment for duration of DM + use of ACE inhibitor, HbA1c, serum uric acid, age, arterial hypertension, macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.
Model 3: after adjustment for duration of DM + use of ACE inhibitor + HbA1c, serum uric acid, age, arterial hypertension, macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.
Model 4: after adjustment for duration of DM + use of ACE inhibitor + HbA1c + serum uric acid, age, arterial hypertension, macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.
Model 5: after adjustment for duration of DM + use of ACE inhibitor + HbA1c + serum uric acid + age, arterial hypertension, macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.
Model 6: after adjustment for duration of DM + use of ACE inhibitor + HbA1c + serum uric acid + age + arterial hypertension, macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.
Model 7: after adjustment for duration of DM + use of ACE inhibitor + HbA1c + serum uric acid + age + arterial hypertension + macrovascuar disease , chronic kidney disease,LDL, gender, years of formal education, BMI, current smoker, HDL, triglycerides, and economic status, did not persisted in the model.

Table S3: Multivariate analysis of diabetic retinopathy (Vision threatening *vs.* no vision threatening)

	Unadjusted	Model1	Model2	Model3	Model4	Model5	Model6
Duration of DM, years	1.084 (1.058-1.111)	1.095 (1.078-1.113)	1.093 (1.075-1.112)	1.080 (1.061-1.099)	1.073 (1.054-1.092)	1,078 (1,058-1,098)	1,078 (1,058-1,098)
Serum uric acid, mg/dL	1.304 (1.164-1.460)		1.351 (1.241-1.470)	1.314 (1.205-1.433)	1.223 (1.115-1.341)	1.222 (1.115-1.340)	1.245 (1.113-1.367)
Use of an angiotensin-converting enzyme (ACE) inhibitor	1.883 (1.216-2.918)			2.451 (1.726-3.481)	2.317 (1.624-3.306)	2.271 (1.591-3.242)	2.169 (1.513-3.108)
Chronic kidney disease	2.314 (1.499-3.572)				2.361 (1.591-3.505)	2.353 (1.585-3.493)	2.521 (1.687-3.767)
HbA1c (%)	1.121 (1.026-1.225)					1.090 (1.004-1.184)	1.111 (1.022-1.207)
LDL cholesterol, mg/dL	0.995 (0.990-1.000)						0.994 (0.990-0.999)
Gender	0.849 (0.576-1.249)						
Age, years	0.996 (0.976-1.016)						
Years of formal education	1.004 (0.957-1.053)						
BMI, kg/m^2	0.979 (0.938-1.022)						
Current smoker	1.902 (0.970-3.728)						
HDL cholesterol, mg/dL	0.991 (0.981-1.001)						
Triglycerides, mg/dL	0.998 (0.994-1.001)						
Arterial hypertension, yes	1.460 (0.898-2.374)						
Macrovascular disease, yes	0.694 (0.305-1.577)						
Economic status, n (%)							
High	0.719 (0.179-2.891)						
Medium	0.652 (0.267-1.589)						
Low	0.706 (0.300-1.661)						

Data are presented as: odds ratio and CI 95% (95% confidence interval). Abbreviations: DM, diabetes Mellitus; ACE, angiotensin-converting enzyme; BMI, body mass index; HbA1c, glycated hemoglobin; LDL-c, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol.

Unadjusted: non-adjusted (forward:wald model)

Model 1: after adjustment for duration of DM, serum uric acid, use of ACE inhibitor, chronic kidney disease, HbA1c,LDL, gender, age, years of formal education, BMI, current smoker, HDL, triglycerides, arterial hypertension, macrovascuar disease and economic status, did not persisted in the model.
Model 2: after adjustment for duration of DM + serum uric acid, use of ACE inhibitor, chronic kidney disease, HbA1c, LDL, gender, age, years of formal education, BMI, current smoker, HDL, triglycerides, arterial hypertension, macrovascuar disease and economic status, did not persisted in the model.
Model 3: after adjustment for duration of DM + serum uric acid + use of ACE inhibitor, chronic kidney disease, HbA1c, LDL, gender, age, years of formal education, BMI, current smoker, HDL, triglycerides, arterial hypertension, macrovascuar disease and economic status, did not persisted in the model.
Model 4: after adjustment for duration of DM +serum uric acid + use of ACE inhibitor + chronic kidney disease, HbA1c, LDL, gender, age, years of formal education, BMI, current smoker, HDL, triglycerides, arterial hypertension, macrovascuar disease and economic status, did not persisted in the model.
Model 5: after adjustment for duration of DM + serum uric acid + use of ACE inhibitor + chronic kidney disease + HbA1c, LDL, gender, age, years of formal education, BMI, current smoker, HDL, triglycerides, arterial hypertension, macrovascuar disease and economic status, did not persisted in the model.
Model 6: after adjustment for duration of DM + serum uric acid + use of ACE inhibitor + chronic kidney disease + HbA1c + LDL, gender, age, years of formal education, BMI, current smoker, HDL, triglycerides, arterial hypertension, macrovascuar disease and economic status, did not persisted in the model.

4.2 **Prevalence and risk factors for referable diabetic retinopathy in patients with type 1 diabetes: a nationwide study in Brazil (artigo publicado)**

Acta Ophthalmologica

Acta Ophthalmologica 2018

Letter to the Editor

Prevalence and risk factors for referable diabetic retinopathy in patients with type 1 diabetes: a nationwide study in Brazil

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Editor,

Nowadays, there is a tendency to individualize the screening for diabetic retinopathy (DR), based on clinical data such as glycated haemoglobin and duration of diabetes (DCCT/EDIC 2017). These methods allow the identification of patients that require referral to a specialist. Currently, in the epidemiological literature, DR has also been graded as referable and nonreferable (Abràmoff et al. 2013). This letter reports a multicentre, nationwide study, conducted in Brazil, a country with a highly admixed population. The study included 1760 patients with type 1 diabetes that were submitted to a standard questionnaire, clinical and laboratorial tests.

The screening for DR was assessed by mydriatic binocular indirect ophthalmoscopy and then categorized as referable (moderate nonproliferative DR, severe nonproliferative DR, proliferative DR and macular oedema) and nonreferable (DR absent and mild DR).

Of the 1760 patients recruited at baseline, a total of 1644 (93.4%) performed fundoscopy and were included in the study. The distribution of patients by subgroups according to DR categories was as follows: 296 (18%) referable DR and 1348 (82%) nonreferable DR.

The exploratory analyses were performed by t-test or chi-square test and showed that patients with referable DR were older ($p < 0.001$), had fewer years of formal education ($p = 0.01$), lower socioeconomic status ($p = 0.03$), longer duration of diabetes ($p < 0.001$), higher HbA1c levels ($p = 0.005$), BMI ($p < 0.001$), serum uric acid (SUA) ($p < 0.001$) and triglycerides ($p = 0.008$) and were more likely to be users of an ACE inhibitor ($p < 0.001$), current smokers ($p = 0.03$) and hypertensive ($p < 0.001$); they also presented more frequently macrovascular disease ($p < 0.001$) or chronic kidney disease (CKD) ($p < 0.001$) than patients without referable DR. There were no associations with gender, HDL cholesterol or LDL cholesterol.

The final adjusted multivariate binomial logistic regression model was statistically significant ($p < 0.001$). The model explained 31.2% (Nagelkerke R^2) of the variance for the presence of referable DR. The highest odds were 2.09 for the use of ACE inhibitor (CI 95% 1.44–3.04; $p < 0.001$), 1.95 for current smoking (CI 95% 1.09–3.48; $p = 0.025$) and 1.44 for chronic kidney disease (CI 95% 1.84–1.27; $p = 0.001$) (Table 1).

Longer diabetes duration, poor glycemic control and hypertension are well-stablished risk factors for the development of DR as has been shown in several studies (ADA 2017).

One result that needs to be highlighted and discussed is the role of SUA, which has been shown to be a potential marker of diabetic microvascular disease (Krizova et al. 2015). Possible mechanisms that may explain this association include the pro-inflammatory and pro-oxidant effects of SUA, which can lead to endothelial damage.

Our results show an association between referable patients with cigarette smoking. Despite the systemic effects of cigarette smoking, which are related to coagulation and inflammatory pathways and lead to hypoxia and endothelial damage, data regarding the relationship between smoking and DR are conflicting. Some studies have shown no association or a negative association between smoking and DR (Thorlund et al. 2013), whereas other studies have shown a positive association. However, our sample included only a small number of smokers (5.6%), and this might have influenced our results.

In the current study, the presence of referable DR showed a significant association with CKD. Retinopathy and nephropathy are microvascular

Table 1. Final adjusted logistic regression model.

	b	OR	CI 95%		p value
Referable versus Nonreferable					
Duration of DM, years	0.08	1.08	1.07	1.1	<0.001
HbA1c %	0.181	1.2	1.12	1.29	<0.001
Serum uric acid, mg/dl	0.202	1.22	1.12	1.33	<0.001
Use of an angiotensin-converting enzyme (ACE) inhibitor, yes	0.737	2.09	1.44	3.04	<0.001
Current smoking, yes	0.665	1.95	1.09	3.48	0.025
Arterial hypertension, yes	0.462	1.59	1.05	2.4	0.029
Chronic kidney disease, yes	0.607	1.84	1.84	1.27	0.001

HbA1c = glycated haemoglobin.

complications that may reflect similar vascular changes sharing common baseline mechanisms.

In conclusion, our data show a high prevalence of referable retinopathy. Referable retinopathy was associated with traditional risk factors such as duration of diabetes, levels of HbA1c, use of ACE inhibitor, current smokers, and presence of hypertension and CKD but also with serum uric acid. Further studies are needed to confirm and explain this association. It is important to highlight the urgent need for screening programs aiming the prevention of this incapacitating comorbidity.

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4.3 **Diabetic retinopathy may indicate na increased risk of cardiovascular disease in patients with type 1 diabetes- a nested case-control study in Brazil (artigo publicado)**

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Diabetic Retinopathy May Indicate an Increased Risk of Cardiovascular Disease in Patients With Type 1 Diabetes—A Nested Case-Control Study in Brazil

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Objective: Cardiovascular disease, the leading cause of death worldwide, and diabetic retinopathy, the main cause of blindness in economically active populations, share clinical risk factors, and pathophysiological features. The aim of this study is to examine the association between diabetic retinopathy, cardiovascular disease, and common risk factors in patients with type 1 diabetes.

Design and Methods: This nested case-control study was performed in patients from the Brazilian Type 1 Diabetes Study Group, a nationwide survey that was conducted in Brazil and enrolled 1,760 patients with type 1 diabetes. A total of 342 patients were selected (57 cases with macrovascular disease and 285 controls who were matched for duration of diabetes and gender).

Results: In the exploratory analysis, stratified by cardiovascular disease, the following variables were statistically significant: age ($p = 0.037$), hypertension ($p = 0.035$), high BMI ($p = 0.046$), diabetic retinopathy ($p = 0.003$), and chronic kidney disease ($p = 0.026$). By multivariate logistic regression, patients with diabetic retinopathy were more likely to develop cardiovascular disease (OR 2.16, 95% CI 1.16–4.02, $p = 0.015$). Although to a lesser extent than diabetic retinopathy, higher BMI levels were also related to an increase in the risk of cardiovascular disease of 1.08 (95% CI 1.01–1.15, $p = 0.024$).

Conclusion: The presence of diabetic retinopathy indicates a greater risk for cardiovascular disease in Brazilian patients with type 1 diabetes. Further studies are warranted to determine whether a noninvasive exam, such as fundoscopy, could help identify patients who show an increased risk for cardiovascular disease.

Keywords: cardiovascular disease, diabetic retinopathy, diabetes, type 1, risk factors, microvascular

INTRODUCTION

According to the World Health Organization (WHO), cardiovascular disease (CVD) is the main cause of death worldwide, accounting for nearly 31% of all mortalities (1). Today, diabetes is the seventh leading cause of death globally and a major cause of blindness, chronic kidney disease, heart attacks, and stroke (2). In adults, diabetes, independent of other risk factors, confers an excess risk of CVD of approximately 2-fold, representing a macrovascular complication of diabetes and including coronary artery disease, peripheral arterial disease, and cerebrovascular disease (3, 4).

Hyperglycemia has injurious effects that are related to macrovascular complications (as reflected by CVD) and microvascular disease (diabetic retinopathy, neuropathy, and nephropathy). Intensive diabetes treatment reduces the risk of any cardiovascular disease event by 42%, compared with traditional therapy, as shown by the Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC), a study with 17 years of follow-up (5). In addition to hyperglycemia, many clinical risk factors are associated with CVD in patients with type 1 diabetes, such as hypertension, dyslipidemia, obesity, and diabetic kidney disease (1, 5, 6). Nonetheless, other risk factors that are related to lifestyle, such as smoking, unhealthy diet, stress, and physical inactivity, could also be important. All of these risk factors are associated with macrovascular and microvascular complications, such as diabetic retinopathy (DR) (6–8). Hyperglycemia is one of the most important risk factor for DR for both type 1 and type 2 diabetes. However, benefits of intensive glycemic control were more evident in type 1 patients (9). There are also, evidences of common genetic variance that increases the risk of DR associated with CVD (10, 11). Recent study in adolescents with type 1 diabetes and without DR showed that early signs of atherosclerosis in this population were associated with retinal microvascular changes, suggesting a common pathophysiology between the two conditions (12).

Diabetic retinopathy is the most common microvascular chronic complication and remains the leading cause of blindness in the working-age population (13). Due to the similarity in their risk factors and pathophysiology, involving inflammatory factors and endothelial damage, an association between DR and cardiovascular events is expected. Several studies have reported this link in type 1 and type 2 diabetes, as we can see in a meta-analysis published in 2011 (14). However, the number of such studies in type 1 diabetes is lower (15–18).

Despite the significance of CVD as the major cause of death and reduction in life expectancy in patients with type 1 diabetes, the routine screening of asymptomatic patients is not recommended according to the American Diabetes Association (ADA) statement. The advanced cardiac test such as: calcium score, pharmacologic stress echocardiography and exercise ECG testing with or without echocardiography are not cost-effective. Therefore, any benefit of non-invasive screening methods to identify patient subgroup at risk of CVD is interesting (19).

The aim of our study was to examine the association between DR with CVD and its risk factors in patients with type 1 diabetes.

MATERIALS AND METHODS

Study Design

This case-control study included 342 patients with type 1 diabetes (57 cases and 285 controls with and without macrovascular disease, respectively), nested in the Brazilian Type 1 Diabetes Study Group (BrazDiab1SG). Detailed information on the main study has been published elsewhere (20). Briefly, this cross-sectional, multicenter study was conducted between August 2011 and August 2014 in 14 public clinics, comprising 1,760 patients who had been diagnosed by an endocrinologist, based on the typical clinical presentation.

A standardized questionnaire was administered, and clinical and demographic data were collected, such as age, gender, duration of diabetes, years of formal education, smoking (defined as the current use of 1 or more cigarettes per day), physical activity, alcohol consumption, self-reported ethnicity, diet, and hospitalization due to any cause in the past year. Economic status was stratified into high-, middle-, low-, and very-low-income, according to the Brazilian Economic Classification Criteria (21). The participants were also subjected to a clinical examination that included height (centimeters), weight (kilograms), BMI [weight (kg) divided by the square of the height (m^2)], and blood pressure.

Data from medical records were also analyzed, and hypertension was defined, as reported by the patient, as at least 2 previous episodes of blood pressure $\geq$140/80 mm Hg, measured by a health professional. CVD was defined as positive information in the patient's medical records on 1 or more of the following conditions: coronary artery disease (angina, coronary artery bypass surgery, coronary angioplasty, myocardial infarction), peripheral vascular disease, and cerebrovascular disease.

Laboratory data were measured in a central laboratory, and the following ADA goals were adopted with regard to achieving adequate clinical and metabolic control (19): Good glycemic control was defined as HbA1c <7.5% for adolescents and <7% for adults. Poor glycemic control was defined as HbA1c $\geq$9%. HbA1c was measured by high-performance liquid chromatography (HPLC, Bio-Rad Laboratories, Hercules, California, US). For lipid management, we considered the following values as normal: triglycerides <150 mg/dL (1.7 mmol/L), HDL cholesterol >50 mg/dL (1.3 mmol/L) for women, and >40 mg/dL for men (1.1 mmol/L).

Renal function was assessed using the CKD-EPI equation (22) in adults and the Schwartz formula in adolescents (23) and was expressed as glomerular filtration rate (GFR) in milliliters per minute per 1.73 m^2 (ml/min/1.73 m^2).

Chronic kidney disease (CKD) was defined as the presence of a low estimated glomerular filtration rate (GFR < 60 ml/min/1.73 m^2) or elevated urinary albumin excretion (albuminuria $\geq$ 30 mg/dl). Creatinine was measured using a colorimetric assay kit (Biosystems).

Of the 1,760 patients from the original sample, 1,644 were screened for DR by mydriatic binocular indirect ophthalmoscopy by an ophthalmologist who specialized in the retina and had trained before the beginning of the study at an ophthalmological

university center. DR was categorized per the international classification (24), but in this study we considered only the presence or absence of DR.

Of the 1,644 patients, 57 had macrovascular disease and were defined as "cases." The group of cases was initially divided in the following duration of diabetes ranges: 0–5 years, 6–10 years, 11–15 years, 16–20 years, more than 20 years of disease. The proportion of gender in each range was analyzed. The 1,583 patients who did not present with cardiovascular disease, were random selected to match with the case group according to gender and duration of diabetes ranges. We set a ratio of 1 case to 5 controls, resulting in 285 controls. Ultimately, 342 patients, including cases and controls were analyzed.

The study protocol was approved by the coordinating center of the Pedro Ernesto University Hospital Ethics Committee (protocol: CEP/HUPE 2769/2010) and the ethics committee of each participating center. Informed consent forms were signed by the patient or his legal guardians. The research was conducted in accordance with the Helsinki Declaration.

The quality of the study was assessed using the checklist "Strengthening the Observational Report on Epidemiology" (STROBE) (25).

Statistical Analysis

Initially, an exploratory analysis was performed to evaluate the relationship between CVD, DR, and certain clinical and social risk factors. For continuous variables, we performed the parametric test and those that did not present normal distribution were submitted to the Mann-Whitney test. Median and interquartile range [IQR] data were used for these variables. The variables with nonparametric distribution were: HbA1c, HbA1c mmol, triglycerides, HDL cholesterol, LDL cholesterol, and years of formal education. We performed the student's *t*-test and presented the data as standard deviation (SD) of the means for the others continuous variables that presented normal distribution. For categorical variables, we used chi-square test and presented the data as frequencies (percentage). We used Fisher's exact test for variables that presented cells with <5 elements, which occurred only in the smoking variable. Binary logistic regression was performed to assess the predisposing factors to CVD, considering variables with $p < 0.2$ in the univariate analysis and several clinically relevant variables. The following covariates were included: LDL cholesterol, hypertension (yes/no), BMI, smoking status (yes/no), HbA1c, DR (present/absent), age, economic status, years of formal education, and self-reported ethnicity (white/not white).

Exploratory Forward-Wald stepwise logistic regression was performed to determine the variables that contributed more to the discrimination between groups. Nagelkerke R-squared values were also calculated. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated when indicated. Two-sided $p < 0.05$ was considered to be statistically significant.

All analyses were performed using SPSS, version 20 (SPSS, Inc., Chicago, IL, US).

TABLE 1 | Demographic and clinical data of the study population.

Variable	
N	1,644
Demographic Characteristics	
Gender, female, *n* (%)	917 (55.8)
Age, years	30.1 ± 12.0
Age of diagnosis, years	14.6 ± 8.9
Duration of diabetes, years	15.3 ± 9.3
Years of formal education, years	12.3 ± 3.8
Economic status, *n* (%)	
High	49 (3.0)
Middle	745 (45.3)
Low	795 (48.4)
Very low	55 (3.3)
Insulin regimens, *n* (%)	
Intermediate or long acting	80 (4.9)
Intermediate/long plus short acting	1.510 (91.8)
CSII	54 (3.3)
Clinical Data	
HbA1c (%)	9.0 ± 2.1
HbA1c, mean (SD), mmol/mol	74.5 ± 23.1
Use of angiotensin-converting enzyme (ACE) inhibitor, yes, *n* (%)	356 (20.2)
Use of Statin, yes *n* (%)	382 (21.7)
BMI, mean (SD), kg/m^2	24.2 ± 4.2
Arterial hypertension, yes, *n* (%)	288 (17.5)
Macrovascular disease, yes *n* (%)	57 (3.5)
Current smoker, yes, *n* (%)	86 (5.6)
Chronic kidney disease, yes, *n* (%)	404 (24.6)
Diabetic retinopathy, yes, *n* (%)	589 (35.8)

Data are presented as numbers (percentages) or means ± SD (standard deviation). BMI, body mass index; HbA1c, glycated hemoglobin; LDL-c, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; CSII, Continuous Subcutaneos Insulin Infusion.

RESULTS

Overview of the Study Population

Of the 1,644 patients, 57 presented with macrovascular disease and were considered "cases," constituting 3.46% of all patients. The 285 controls were matched for gender and duration of diabetes. The clinical and demographic characteristics of the study population are shown in **Table 1**.

Overview of the Study Population Stratified by Cardiovascular Disease (CVD)

In the exploratory analysis, patients with CVD were older ($p = 0.037$); had a greater prevalence of hypertension ($p = 0.035$), DR ($p = 0.003$), and CKD ($p = 0.026$); had a higher BMI ($p = 0.046$) and were more likely to be users of statins compared with patients without CVD. CVD was not associated in the exploratory analysis with years of formal education, economic status, self-reported ethnicity, use of angiotensin-converting enzyme (ACE) inhibitor, HbA1c, triglyceride levels, HDL cholesterol, LDL cholesterol, or smoking status. The results are shown in **Table 2**.

TABLE 2 | Demographic, clinical and laboratory data stratified by Cardiovascular disease.

	Cardiovascular disease		
Variables	**Present**	**Absent**	***p*-value**
N (%)	57 (16.7)	285 (83.3)	
Demographic Data			
Gender, female, *n* (%)	26 (45.6)	130 (45.6)	1.0
Age, mean (SD), years	38.91± 15.01	34.42 ± 12.41	0.037
Duration of diabetes, mean (SD), years	22.59 ± 12.90	20.56 ± 10.31	0.27
Years of formal education, mean (SD), years	12.00 (3.00)	12.00 (4.00)	0.85
Economic status, *n* (%)			
High	1 (1.8)	8 (2.8)	0.28
Middle	22 (38.6)	146 (51.2)	
Low	31(54.4)	122 (42.8)	
Very low	3 (5.3)	9 (3.2)	
Clinical Data			
HbA1c mg/dl (%)	8.25 (2.62)	8.2 (2.30)	0.64
HbA1c, mean (SD), mmol/mol	66.66 (28.69)	66.12 (25.14)	0.64
Hypertension, yes, *n* (%)	24 (42.1)	80 (28.1)	0.035
Triglycerides, mean (SD), mg/dL	92 (59.75)	85 (58)	0.48
HDL cholesterol, mean (SD), mg/dL	49.85 (25.85)	54.70 (20.25)	0.18
LDL cholesterol, mean (SD), md/dL	99.40 (45)	101.20 (41.40)	0.16
BMI, mean (SD), kg/m2	26.09± 5.23	24.59 ± 4.20	0.046
Diabetic retinopathy, yes *n* (%)	39 (68.4)	133 (46.7)	0.003
Current smoker, yes, *n* (%)	3 (5.3)	18 (6.3)	1.00
Chronic kidney disease, yes, *n* (%)	28 (50.9)	97 (35)	0.026
Use of angiotensin-converting enzyme (ACE) inhibitor, yes, *n* (%)	23 (65.7)	78 (69.6)	0.66
Use of Statin, yes *n* (%)	33 (57.9)	85 (29.8)	0.00
Color race, white, yes (%)	29 (50.9)	168 (58.9)	0.26

The data are presented as numbers (percentages), means ± SD (standard deviation) or median [IQR] (interquartile range). The p-value compares differences between the groups using Student's t-test. BMI, body mass index; HbA1c, glycated hemoglobin; LDL-c, low density lipoprotein cholesterol; high density lipoprotein cholesterol.

Multivariate logistic regression was performed to determine the effects of social and clinical risk factors for CVD, wherein patients with DR were 2.16 times (95% CI 1.16–4.02, p-0.015) more likely to present with CVD. BMI was also associated with 1.08-fold (95% CI 1.01–1.15, $p = 0.024$) greater odds of having CVD. The independent variable included in the final adjusted multivariate binomial logistic regression model, explained 66% (Nagelkerke R) of the variance for the presence of CVD. The results of crude odds ratio and unadjusted p-value are described in **Table 3**.

DISCUSSION

Patients with DR have a greater likelihood of presenting with CVD, even after controlling for the main risk factors. In our sample, the overall prevalence of CVD was 3.46% which is consistent with a report of the Wisconsin Epidemiologic Study of Diabetic Retinopathy that have shown rates of CVD of between 1.0 and 3.1% (17). The International Diabetes Federation (IDF) showed a prevalence of CVD, in patients with type 1 diabetes, ranging from 2.6 to 16.2% in high income countries. However, there is a lack of data from low-income countries (26). Although CVD is still an important cause of mortality and reduced life expectancy in patients with type 1 diabetes, a decrease in mortality on this population has been related both with a decrease in late diabetes complications and cardiovascular disease (6, 27).

The prevalence of DR in our population was 35.7% as we described earlier (28). This result is consistent with worldwide prevalence DR. However, although DR is still the leading cause of visual impairment among patients with type 1 diabetes, a recent review suggested that there is a declining trend in the prevalence of DR due to public health efforts (9).

The association between DR and CVD can be explained by the similarity in risk factors between both complications and by the evidence that microvascular and macrovascular complications of diabetes share pathophysiological mechanisms that are related to hyperglycemia (5). Hyperglycemia activates several intracellular signaling pathways that lead to oxidative stress and the overproduction of inflammatory markers. These occurrences contribute to retinal endothelial dysfunction, increasing vascular permeability—the chief event in the development of DR (29). There is evidence that tight glycemic control tends to be more benefit to prevent DR in patients with type 1 diabetes than in patients with type 2 diabetes, as shown in DCCT and in the United Kingdom Prospective Diabetes Study (UKPDS). The DCCT study, included only patients with type 1 diabetes and demonstrated in a mean follow up of 6.5 years, a reduced risk of 76% in DR and 54% in the progression of DR at the intensive glycemic control group (30).The UKPDS study, included patients with type 2 diabetes and the impact of tight glycemic control was a reduction in 25% the risk of microvascular endpoints, including the need of retinal photocoagulation (31).

There is also evidence of overactivation of the renin-angiotensin-aldosterone system as consequence of the hyperglycemia, impacting the pathogenesis of the microvascular complications in type 1 diabetes, affecting retina, nerves, and kidney (32).

The primary pathological mechanism in CVD is atherosclerosis, which is associated with endothelial injury and chronic inflammation (33).

CVD and DR share several risk factors, such as age, duration of diabetes, hypertension, higher BMI, and CKD. (6, 8) We also noted these variables in the descriptive analysis of this study and a separate report by our group that evaluated the risk factors that are associated with DR. (28).

Of these common risk factors, duration of diabetes could not be considered in our study, because it was used as a matching criterion in our population. Hypertension is another major risk factor for CVD and microvascular complications (5, 17). Hypertension has a direct correlation with CVD, because it is linked to arterial stiffness and thickness (6). Several studies, such as DCCT/EDIC, have shown that high levels of arterial pressure

TABLE 3 | Multivariate logistic regression of cardiovascular disease (Present vs. Absent).

	Unadjusted		Model 1		Model 2	
	Odds ratio	***p*-value**	**OR (CI 95%)**	***p*-value**	**OR (CI 95%)**	***p*-value**
Diabetic retinopathy, yes	1.813 (0.897–3.665)	0.097	2.349 (1.274–4.332)	0.006	2.163 (1.164–4.020)	0.015
BMI, kg/m2	1.097 (1.022–1.177)	0.01			1.078 (1.010–1.150)	0.024
Age, years	1.015 (0.986–1.044)	0.322				
Arterial hypertension, yes	0.933 (0.436–1.994)	0.857				
Years of formal education	1.051 (0.961–1.149)	0.276				
Current smoker	1.254 (0.338–4.652)	0.735				
LDL cholesterol, mean (SD), mg/dL	0.993 (0.984–1.002)	0.134				
Chronic kidney disease	0.621 (0.318–1.213)	0.163				
Economic Status, *n* (%)						
High	0.380 (0.022–6.434)	0.503				
Medium	0.638 (0.109–3.734)	0.618				
Low	1.236 (0.231–6.622)	0.804				
Color race, white, yes	1.635 (0.854–3.131)	0.138				

Data are presented as: odds ratio and CI 95% (95% confidence interval). BMI, body mass index; Model 1: after adjustment for presence of diabetic retinopathy; BMI, age, arterial hypertension, years of formal education, current smoker, LDL cholesterol, chronic kidney disease, economic status and self-reported ethnicity did not persist in the model. Model 2: after adjustment for duration of DM + BMI, age, arterial hypertension, years of formal education, current smoker, LDL cholesterol, chronic kidney disease, economic status, and self-reported ethnicity did not persist in the model.

are strongly positively associated with the progression of DR (34). CKD, a result of diabetic microangiopathy, is also related to CVD, due primarily to its association with high fibrinogen levels, blood viscosity, lipoprotein levels, and platelet aggregation (17).

In our study, in addition to DR, BMI was a significant risk factor for CVD. Our population had a high prevalence of metabolic syndrome (35). These patients presented with clinical and laboratory indications that have been suggested to increase the risk for non-alcoholic fatty liver disease (NAFLD), which has also been associated with the development of CVD. There is evidence that obesity, an established risk factor for CVD, has a significant function in the endothelial damage in DR (36). Adipose tissue releases proinflammatory cytokines that induce insulin resistance and endothelial dysfunction, leading to diabetic angiopathy (37, 38).

We did not find association between smoking and CVD in our exploratory analyze. However, our sample included only a small number of smokers in cases (5.3%) and control (6.3%) groups and this might have influenced our results. This small number could be related to the fact that the percentage of adult smokers in Brazil has been significantly decreasing and according to the most recent data, it has a national average of 14.7% (39).

The strength of our study is that it included patients with type 1 diabetes which in general is not as studied as type 2 diabetes.

The study has some limitations. It was a cross-sectional study and so we cannot confirm causal associations between DR and its risk factors. Further, we evaluated DR by indirect ophthalmoscopy. Seven-field stereoscopic photography is considered the standard approach by the Early Treatment Diabetic Retinopathy Study (ETDRS) (40), but studies show significant agreement between the 2 methods (41). Also, retinopathy was not screened by the same physician, and for logistic reasons, inter-observer variability was not assessed. However, all exams were performed by an ophthalmologist who specialized in the retina and had been trained before the beginning of the study at an ophthalmologic university center. Further, our number of patients with CVD was small and data on CVD were based on medical records, no active screening was performed, according with ADA recommendations, that suggests systematic assessment of cardiovascular risk factors annually and does not suggest routine screening for coronary artery disease in asymptomatic patients with a high risk of CVD, such as our population (19). Nevertheless, the prevalence of CVD in our study was similar to that in other studies of patients with type 1 diabetes (17).

CONCLUSION

The strong association between DR and CVD showed in our study suggests that presence of DR may be indicative of an increased risk for CVD. The presence of this ocular complication can identify those patients with type 1 diabetes who should be screened for CVD.

Cardiovascular disease is the leading cause of morbidity and mortality in patients with diabetes; thus, any effort toward its prevention is valuable. Fundoscopy could help to identify patients who are at higher risk of CVD. However, further studies are needed to understand the association between DR with CVD and the function of DR as a predictor of this significant and fatal comorbidity.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

MG was the principal investigator of the study, designed the study and the survey questionnaire, supervised the project, and reviewed the manuscript. LGNM conducted statistical analysis, conducted the literature review, and drafted the manuscript. DS, MP, BB, and LHM helped to conduct statistical analysis and reviewed the manuscript. PM, KD, TM, AP, FM, FL, and FKM were responsible for collecting data on diabetic retinopathy in each center. All authors read and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.1 **Relationship between proliferative diabetic retinopathy and inflammatory markers in patients with type 1 diabetes in Brazil: a nested case control study (artigo submetido)**

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Abstract

Purpose

This study examined the relationship between proliferative diabetic retinopathy (PDR) and serum levels of C-reactive protein, VEGF, TNF-α, and IL-6 inflammatory biomarkers, related to the pathophysiology of diabetic retinopathy.

Methods

This cross-sectional, case control study comprised 240 patients with type 1 diabetes (80 cases with PDR and 160 controls without diabetic retinopathy) who were matched for gender and duration of diabetes.

Results

C-reactive protein was the only inflammatory biomarker that was positive related to PDR (OR 1.96; 95% CI 1.01-3.78, p= 0.0045). We also noted an association between high glycated hemoglobin levels, the use of angiotensin-converting enzyme inhibitor, low glomerular filtration rate and PDR.

Conclusion

Patients with higher levels of C-reactive protein are more likely to present with PDR. We did not find link between serum levels of VEGF, TNF-α or IL-6 and PDR. The function of inflammatory biomarkers in PDR must be addressed in further studies.

Key Word: Diabetic retinopathy; Type1 diabetes, inflammatory markers

Introduction

Diabetic retinopathy (DR) is a microvascular complication of diabetes, that is present in nearly 90% of patients with type 1 diabetes (T1D) who have had the disease for over 20 years and is one of the main causes of blindness worldwide, representing a significant burden on each patient and the public health (1).

The mechanisms in the pathophysiology of diabetic retinopathy are related to the activation of several intracellular signaling pathways by hyperglycemia, such as sorbitol, advanced glycation end products (AGEs), polyol accumulation, activation of protein kinase C, hexosamine, and NFkB (2,3). The latter is stimulated by the overproduction of reactive oxygen species (ROS) due to hyperglycemia, increasing the transcription of proinflammatory cytokines, chemokines, and consequently endothelial injury, leading to damage to the retinal capillaries (4,5).

Certain cytokines are related to the triggering and maintenance of DR, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (6). IL-6 is an important mediator of intraocular inflammation, induces the final differentiation of B cells to antibody-producing cells, and mediates C-reactive protein (CRP) expression (7). IL-6 also indirectly stimulates angiogenesis through the induction of vascular endothelial growth factor (VEGF) (8). TNF-α is an inflammatory cytokine that is involved in leukocyte adhesion in endothelial cells and neovascularization, upregulating VEGF (3,9).

VEGF is the most potent factor in angiogenesis and is also a proinflammatory molecule. Hypoxia and hyperglycemia are the major stimuli for VEGF overproduction (10,11). VEGF has a significant function in the pathogenesis of DR—chiefly in proliferative DR (PDR)—stimulating endothelial cell growth and neovascularization (10,12,13). However, the evidence of elevated levels of intravitreous VEGF in the earlier stages of DR suggests that it acts as an inflammatory mediator, resulting in breakdown of the blood-retina barrier and increased retinal vascular permeability (12). Clinical data indicate that intravitreal injections of corticosteroids and anti-VEGF agents impede the development of DR, supporting the link between inflammatory biomarkers and DR (14).

CRP is an acute phase protein that is secreted predominantly by the liver and adipose tissue in response to inflammatory stress and is regulated primarily by IL-6 (15). CRP has been associated with DR, as shown in a systematic review and meta-analysis that implicates CRP as a biomarker of the severity of retinopathy (16). Although most of the studies in this meta-analysis were based on patients with type 2 diabetes, there is evidence of this relationship in patients with T1D (17,18).

The association between DR and IL-6, TNF-α, CRP, and VEGF has been reported in several studies, but the results are conflicting, based on the characteristics of the study population (17,19–28). However, the specific mechanism of the pathophysiology of DR is not fully understood.

This multicenter study evaluated the association between PDR and serum levels of IL-6, TNF-α, and VEGF in patients with T1D in Brazil.

Methods

This nested case-control study is part of the Brazilian Type 1 Diabetes Study Group (BrazDiab 1SG). The original cohort consisted of 1760 patients who participated in a T1D multicenter study in 14 centers in Brazil between 2010 and 2014. Information about the methods of the main study have been detailed (29). Baseline measurements were made using a

questionnaire and laboratory exams. Each clinic provided data for at least 50 outpatients with type 1 diabetes, age ≥ 13 years, who had attended these centers for at least 6 months. The exclusion criteria were lactation, pregnancy, recent history of diabetic ketoacidosis, and acute infection in the 3 months that preceded the evaluation.

The local ethics committee approved the study protocol (CAAE:0214.0.228.000-10), and written informed consent was obtained from all patients or their legal representative.

Clinical and laboratorial data

During the clinical visit, the following variables were assessed: age, duration of diabetes, age at diagnosis, height (m), weight (kg), lifestyle variables (ie, smoking status, physical activity, and alcohol consumption), years of school attendance, and blood pressure. Data from medical records were also analyzed; and hypertension, as reported by the patient, was defined as at least 2 previous episodes of blood pressure > 140/90 mm Hg, measured by a health professional. Smoking was defined as the current use of more than 1 cigarette per day. Body mass index (BMI) was determined by dividing weight (Kg) by the square of the height (m^2).

Blood samples from each patient were collected for clinical laboratory analysis, and the serum was stored at -80°C for future assay. The baseline analyses and the respective goals per the American Diabetes Association (ADA) regarding clinical and metabolic control measured several parameters (30): Glycated hemoglobin (HbA1c) levels (by high-performance liquid chromatography- HPLC, Bio-Rad Laboratories, Hercules, California, USA). Good glycemic control was defined as HbA1c <7.0% (53 mmol/mol) for adults and <7.5% (58 mmol/mol) for adolescents; poor glycemic control was considered HbA1c ≥ 9.0% (75 mmol/mol). For lipid management, we considered the following values to be normal: triglycerides < 150 mg/dL (1.7 mmol/L) and HDL cholesterol > 50 mg/dL (1.3 mmol/L) for women and > 40 mg/dL for men (1.1 mmol/L).

Renal function was assessed using the CKD-EPI equation in adults (31) and the Schwartz formula in adolescents (32) and was expressed as glomerular filtration rate (GFR) in ml/min/1.73 m^2.

C-reactive protein (CRP) was measured using by high-sensitivity enzyme immune assay and was included in the baseline analyses.

Measurements of inflammatory biomarkers

The baseline serum samples from each patient that were stored at -80°C were accessed. Based on a literature review and their potential function in the pathogenesis of DR, we analyzed IL-6, TNF-α, VEGF.

These analyses were performed on a multiplex bead analysis system (Milliplex® x-MAP, Merk, Millipore Corp., Billerica, MA, USA). After overnight incubation of the serum in the plates, the specific fluorescence for each set of biomarkers was measured on a Luminex® (Luminex, Austin, TX) instrument and quantified per the manufacturer's instructions. The minimum detectable concentration for the biomarkers, as determined by the manufacturer, was 0.5 pg/ml for IL-6, 26.3 pg/ml for VEGF, and 0.7 pg/ml for TNF-α. For analysis purposes, biomarkers that were below the lowest concentration in the standard curve were given a predefined minimum value.

Diabetic retinopathy data

Diabetic retinopathy was assessed by funduscopic evaluation with mydriatic indirect ophthalmoscopy by a retina specialist at each center. To standardize the procedure, each ophthalmologist in the study was trained at an ophthalmological university center. Diabetic retinopathy was categorized, based on the international classification: absent, mild- no PDR, moderate- no PDR, severe- no PDR, and PDR (33). The severity of DR in each patient was determined, based on the level of severity in the worse eye.

Of the 1760 patients, 1640 were screened for diabetic retinopathy, 240 of whom 1640 were selected for this case-control study. Case subjects (n=80) were those with PDR, and control subjects (n=160) had no evidence of DR. The groups (cases and controls) were matched for gender and duration of diabetes. The case group was initially divided into the following durations of disease: 0-5 years, 6-10 years, 11-15 years, 15-20 years, and more than 20 years. The control patients were selected randomly in SPSS to maintain the same ratio between males and females in each group of duration.

Statistical analysis

Variables with a normal distribution were expressed as mean ± standard deviation (SD). Variables that were not distributed normally, as by with Kolmogorov-Smirnov test ($p<0.05$), were expressed as median and interquartile range [IQR]. Differences between groups were

calculated by student t-test or nonparametric Mann-Whitney U test. Chi-square test was used to compare categorical variables and present the data as frequencies (percentage).

The backward stepwise binary logistic regression method was used to progressively eliminate variables until the model included only potential predictors of PDR. Variables with a significance level of $p < 0.2$ in the descriptive analysis were included as descriptive variables. The inflammatory biomarkers (IL-6, TNF- α, VEGF, CRP) were also included in the logistic regression analysis as control variables, based on their clinical relevance. Risk for PDR was estimated by odds ratios (ORs) with 95% confidence intervals (CIs). Nagelkerke R-squared values were also calculated.

Statistical analyses were performed SPSS, v20 (IBM, Armonk, NY).

Results

Overview of study population

A total of 240 patients were enrolled, comprising 80 cases and 160 controls. The clinical information and baseline characteristics of the patients are shown in Table 1.

Overview of study population stratified by proliferative diabetic retinopathy

Cases were older and more frequently experienced hypertension than controls. There was no difference between groups regarding gender or duration of diabetes, both of which were deliberately matched. The mean levels of HbA1c and CRP were higher in the case versus control group ($p<0.001$), and mean GFR levels were lower in the former. More patients in the case group used angiotensin-converting enzyme (ACE) inhibitors and statins than control subjects.

There was no significant difference in IL-6, TNF-α, or VEGF between groups. The following variables did differ significantly between groups: BMI, smoking status, HDL, LDL cholesterol, and triglycerides. The comparison between the case and control groups is shown in Table 2.

Multivariate analysis

Multivariate logistic regression was performed with PDR as the dependent variable. The variables that had p-values < 0.2 in the descriptive analysis (HbA1c, hypertension, BMI, triglycerides, GFR, use of ACE inhibitor, use of statin, and age) and the inflammatory biomarkers (IL-6, TNF- α, VEGF, CRP) were included as independent variables. The use of ACE inhibitor, high glycated hemoglobin levels, low GFR levels, and high serum levels of CRP

were associated with PDR. None of the other inflammatory biomarkers was significantly linked to PDR.

The final model was statistically significant [X2(8)= 61.997 p <.0001]. The model explained 31.6% (Nagelkerke R2) of the variance and correctly classified 66.7% of cases. The results are shown in Table 3.

Discussion

In this nested case control study of patients with T1D, CRP was the main inflammatory marker that was related to PDR. There was no positive association with the serum levels of other inflammatory markers, such as IL-6, TNF-α, and VEGF.

In this study, the main clinical factors that were related to PDR were the use of ACE inhibitors, high levels of glycated hemoglobin, and low GFR levels. The importance of intensive glycemic therapy in the incidence and progression of DR has been demonstrated in the Diabetes Control and Complications Trial (DCCT) (34). In contrast to our result, ACE inhibitors have a protective effect against DR in type 1 diabetes, according to several prospective and randomized trials (35,36). There is also evidence that ACE inhibitors protect against deterioration in renal function in T1D (37). The relationship between the severity of DR and lower estimated GFR has also been reported, justifying the more frequent use of ACE inhibitors in our patients. (38).

The function of inflammation and endothelial damage is recognized as a mechanism of the development of DR. Previous studies have demonstrated the relationship between serum levels of inflammatory biomarkers and the presence of DR, implicating these cytokines in the pathophysiology of DR (26). However, the results are controversial. The heterogeneity between studies is attributed to the characteristics of the study populations, such as type of diabetes, duration of disease, level of glycated hemoglobin, and the source of the biomarkers (serum, plasma, or vitreous).

The association between CRP and the severity of DR that we observed is consistent with Sasongko et al., who reported this link in patients with type 1 and type 2 diabetes, finding that serum CRP level correlated with vision-threatening —more prominently in patients with BMI $\geq$ 30 kg/m^2 (17). Obesity is strongly related to chronic subacute inflammation and the upregulation of inflammatory cytokines, such as CRP and TNF-α (39); however, in our study, there was no difference between case and control groups regarding BMI.

A meta-analysis in patients with diabetes compared CRP levels between those with and without DR and showed that CRP levels were higher in patients with any DR and with PDR,

regardless of the type diabetes (16). As separate study of patients with T1D reported a link between CRP levels and the severity of DR, but the significance was lost after adjustments for other variables, such as age, duration of DM, and BMI (28).

CRP is an acute-phase protein and a marker of nonspecific inflammation that is synthesized in the liver and stimulated by adipocytes, which might explain the association of this protein with obesity (17). High plasma concentrations of CRP have been seen in patients with T1D, even without clinical complications (24,40). Scholin et al. demonstrated that in patients with T1D, elevated CRP concentration correlated with insulin resistance and poor glycemic control (41). That our patients with PDR also had elevated glycated hemoglobin levels might justify the higher serum of CRP levels in these patients.

Our results showed that IL-6 level was not associated with PDR. IL-6 is an early mediator of acute phase inflammatory proteins, such as CRP, and is an indirect angiogenic factor, upregulating VEGF under hypoxic conditions (8,42). A meta-analysis by Yao et al. of 31 studies showed that IL-6 levels were higher in patients with any DR, including PDR, than in those without DR. Two studies in this meta-analysis did not show a significant difference between case (children with T1D) and control (healthy children) groups regarding serum IL-6 levels (43,44).

Other studies have also shown a link between IL-6 and DR (26,45). In 1992, El Asrar et al. found that vitreous but not serum IL-6 levels correlated positively with the severity of DR (46). Consistent with our result, Ozturk et al. found that serum levels of IL-6 did not diff between controls, patients without DR, no-PDR patients, and PDR patients (47). Recent studies have shown that patients with T1D have higher serum levels of IL-6, regardless of the presence of microvascular or macrovascular complications due to low-grade chronic inflammation (12,48).

We did not find an association between TNF-α and PDR. TNF-α is an angiogenic and proinflammatory cytokine that induces leukocyte adhesion in endothelial cells of retinal vessels, increasing vascular permeability and neovascularization in diabetic retinopathy (3,49). Studies have shown a link between serum levels of TNF-α and DR (20,44,50). Gustavsson et al. compared the serum levels of inflammatory biomarkers (TNF- α, IL-1, IL-6, and CRP) in T1D patients with and without PDR, of which TNF-α was the only inflammatory biomarker that was related to PDR (51). However, unlike our study, their study population was not matched for duration or gender. Roy et al. demonstrated that TNF-α was a significant predictor of the incidence of PDR (22). In contrast, Cheung et al. examined inflammatory biomarkers in the aqueous humor of diabetic patients with and without DR but did not find any association

with TNF-α; but, IL-6 and VEGF were higher in patients with any DR. Nevertheless, in this study, the sample was small and was not matched for duration of diabetes (45). Loukovaara et al. did not find any difference in vitreous TNF-α levels between patients with and without PDR (52).

In our study, the serum level of VEGF was not significantly associated with PDR, consistent with Crosby-Nwaobi et al. (50). Despite the importance of the function of VEGF in the pathophysiology of DR, which increases vascular permeability and angiogenesis, the data on vitreous, plasma, and serum VEGF levels and DR are conflicting. A recent meta-analysis showed that serum but not plasma VEGF correlates with the presence and severity of DR (10), which was attributed to the use of anticoagulants to keep blood samples from clotting and the fact that platelets are a rich source of VEGF. Baharivand et al. found that serum and vitreous VEGF levels were significantly higher in patients with PDR versus without PDR, but it was a small study that did not match for duration of diabetes (53). Other studies have also shown evidence that vitreous VEGF levels correlate with severity of DR (54–56).

Considering these data, we conclude that there is no consensus on the function of inflammatory markers in the pathophysiology of DR or the ideal source of biological material for marker analysis.

The strengths of our study include the assessment of serum markers of inflammation and that we only included patients with T1D who were matched for duration of disease and gender. Several limitations of the study should be addressed. First, its cross-sectional design did not allow us to establish a causal relationship between systemic CRP level and clinical risk factors with proliferative DR. Second, the wide variations in the concentrations of cytokines could be related to the lack of significance between TNF-α, IL-6, and VEGF with PDR.

Conclusion

Only the serum level of CRP is associated with PDR. Further, glycemic control, the use of ACEs inhibitor, and renal function were the clinical variables that were most significantly related as risk factors for PDR. Because CRP is a nonspecific marker of inflammation and because IL-6, TNF-α, and VEGF were not positively associated with PDR, necessitating further studies to better understand the mechanisms of the development of PDR.

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Table 1. Clinical characteristics of the 240 patients with type 1 diabetes

Variable	
Gender, female, n (%)	134 (55.8)
Duration of type 1 diabetes (years)	23.34 ± 8.63
Age (years)	36.54 ± 11.74
Body mass index (Kg/m)	24.45 ± 4.04
Smoking status, yes (%)	16 (6.7)
Hypertension yes (%)	59 (24.6)
HbA1c (%)	8.10 [2.27]
GFR (ml per min per 1.73 m 2)	77.36 ± 26.21
HDL cholesterol (mmol/l)	55.44 ± 15.90
LDL cholesterol (mmol/l)	106.72 ± 32.20
Triglycerides (mmol/l)	85.00 [53.00]
IL-6 (pg/ml)	0.540 [1.82]
TNF-α (pg/ml)	8.77 [7.88]
VEGF (pg/ml)	93.03 [2.27]
CRP (mg/dl)	0.195 [0.388]

Data are presente as number, percentage or mean ± SD (Standard Deviation), except for IL-6, TNF-α, VEGF, HbA1c, Triglycerides and CPR, wich are shown as median [interquartile range]. BMI: body mass index; HbA1c: glycated hemoglobin; LDL: low density lipoprotein cholesterol; HDL:high density lipoprotein cholesterol; IL-6: interleucine-6; TNF-α: Tumor necrosis fator -α; VEGF: vascular endotelial growth fator; CRP: C-reactive protein.

Table 2. Characteristics of 240 patients with type 1 diabetes by retinopathy status

Variable	Individuals with proliferative diabetic retinopathy (*n*=80)	Individuals without diabetic retinopathy (*n*=160)	*p* Value
Gender, female, n (%)	45 (56.2)	89 (55.6)	0.927
Duration of type 1 diabetes (years)	24.10 ± 8.66	22.96 ± 8.62	0.335
Age (years)	39.48 ± 10.53	35.08 ± 12.06	0.006
BMI (Kg/m^2)	24.90 ± 4.46	24.23 ± 24.23	0.224
Smoking status, yes (%)	6 (7.5)	10 (6.2)	0.714
Hypertension yes (%)	34 (42.5)	25 (15.6)	<0.001
Use of ACE inhibitor , yes (%)	44 (55.0)	37 (23.1)	<0.001
HbA1c (%)	8.55 [2.35]	7.90 [1.77]	<0.001
GFR (ml per min per 1.73 m^2)	66.09 ± 24.38	82.99 ± 25.32	<0.001
HDL cholesterol (mmol/l)	55.81 ± 17.26	55.26 ± 15.22	0.799
LDL cholesterol (mmol/l)	106.78 ± 30.72	106.69 ± 33.01	0.985
Triglycerides (mmol/l)	92.00 [42.00]	82.50 [55.75]	0.109
Use of Statin, yes (%)	39 (48.8)	43 (26.9)	0.001
IL-6 (pg/ml)	0.455 [1.89]	0.620 [1.78]	0.055
TNF-α (pg/ml)	8.270 [11.46]	8.90 [6.87]	0.373
VEGF (pg/ml)	88.47 [169.06]	93.03 [165.89]	0.897
CRP (mg/dl)	0.325 [0.530]	0.165 [0.255]	<0.001

Data are presente as number, percentage or mean ± SD (Standard Deviation), except for IL-6, TNF-α, VEGF, HbA1c, Triglycerides and CPR, wich are shown as median [interquartile range]. The p value compares differences between the groups using Student's t-test or Mann-Whitney test. BMI: body mass index; HbA1c: glycated hemoglobin; ACE: angiotensin-converting enzyme; LDL: low density lipoprotein cholesterol; HDL:high density lipoprotein cholesterol; IL-6: interleucine-6; TNF-α: Tumor necrosis fator -α; VEGF: vascular endotelial growth fator; CRP: C-reactive protein.

Table 3. Final adjusted logistic regression model

	b	OR	CI 95%		P value
Proliferative DR *vs.* DR Absent					
HbA1c (%)	0.339	1.40	1.17	1.69	<0.001
CRP mg/dl	0.672	1.96	1.01	3.78	0.045
GFR (ml per min per 1.73 m^2)	-0,26	0.97	0.96	0.98	<0.001
Use of ACE inhibitor, yes	0.672	3.15	1.67	5.94	<0.001

HbA1c: glycated hemoglobin; GFR: glomerular filtration rate; CRP: C-reactive protein; ACE:angiotensin-converting enzyme ; b: coefficient of logistic regression; OR: odds ratio; CI 95%: coeficient interval.

5 **DISCUSSÃO**

Este é um estudo multicêntrico, pioneiro no Brasil, um país de dimensão continental e população miscigenada, envolvendo um número expressivo de pacientes com diabetes tipo 1. Com os resultados do estudo, foi possível estimar a prevalência da retinopatia diabética nos pacientes com diabetes tipo 1 e avaliar quais os possíveis fatores de risco relacionados com o desenvolvimento desta debilitante comorbidade. Avaliamos ainda a relação da retinopatia diabética com a doença cardiovascular e com os fatores inflamatórios.

Na população estudada, a prevalência de retinopatia diabética foi de 35%. Desses pacientes com retinopatia, 12% apresentavam a forma que ameaça a visão (VTDR- *vision threatening diabetic retinopathy*) e 18% dos pacientes eram considerados referenciáveis para avaliação especializada com um oftalmologista.

A média da prevalência mundial da retinopatia diabética, segundo a OMS encontra-se em aproximadamente 35% e das formas que ameaçam a visão (VTDR) de 7 a 10% (1). Um estudo de metanálise, também demonstrou uma prevalência mundial média de 34,6%, variando de acordo com características geográficas, étnicas, socioeconômicas e genética. Esta mesma metanálise revelou também uma taxa mundial de VTDR de aproximadamente 10% (9). Porém, até o nosso conhecimento, não existem ainda dados de estudos multicêntricos sobre prevalência de retinopatia diabética no Brasil. Um estudo realizado na região sul do Brasil, envolvendo pacientes com diabetes tipo 1, revelou uma prevalência de retinopatia diabética de 40% (116).

Os resultados do nosso estudo estão consistentes com a estimativa mundial de prevalência mundial de retinopatia diabética, entretanto com uma prevalência maior de VTDR (12%, sendo 0,8% de retinopatia não proliferativa severa sem edema macular; 8,8% de retinopatia diabética proliferativa sem edema macular e 2,7% de edema macular). Esses pacientes, por apresentarem formas mais graves da retinopatia, requerem encaminhamento imediato para avaliar tratamento.

O nosso percentual de edema macular, de 2,7% encontra-se abaixo da média que em pacientes com diabetes tipo 1, varia de 4,9% a 7,9% (10). O fato da técnica empregada em nosso estudo ter sido de oftalmoscopia binocular indireta, pode ter afetado a acurácia na detecção do edema macular.

Apesar da retinopatia diabética ainda ser a principal causa de cegueira e de comprometimento visual em pacientes com diabetes tipo 1, estudos recentes sugerem uma tendência da redução da prevalência desta comorbidade em países desenvolvidos, em função

de maiores esforços na saúde pública na prevenção desta comorbidade e consciência em relação aos fatores de risco (68,77).

No nosso estudo, avaliamos também fatores clínicos, demográficos e laboratoriais relacionados com retinopatia diabética. As principais variáveis que se mostraram estatisticamente significativas, após ajuste pela regressão multivariada, com a presença da retinopatia diabética, foram: idade, tempo de duração do diabetes, HbA1c, hipertensão arterial sistêmica, níveis séricos de ácido úrico, uso de inibidor da enzima conversora de angiotensina (IECA) e presença de doença macrovasular. Os fatores de risco que foram significativos nas formas da RD que ameaçam a visão (VTDR) foram: duração do diabetes, HbA1c, níveis séricos de ácido úrico, uso de IECA, colesterol LDL e doença renal crônica. Na retinopatia diabética referenciável, os fatores de risco relacionados foram: tempo de duração do diabetes, HbA1c, hipertensão arterial sistêmica, níveis séricos de ácido úrico, uso de IECA, tabagismo e doença renal crônica. Portanto, os fatores de risco em comum `as análises dos três grupos (presença de qualquer forma de RD, VTDR e RD referenciável) foram: duração do diabetes, níveis de hemoglobina glicada, nível sérico de ácido úrico e uso de IECA (medicamento frequentemente utilizado no tratamento da hipertensão arterial sistêmica).

Nos pacientes com retinopatia diabética, a média de duração da doença foi de 20 anos, enquanto nos pacientes com ausência de retinopatia, a média foi de 12 anos. Esse resultado é consistente com a literatura, que demonstra um forte relação da duração do diabetes tipo 1 com a retinopatia diabética, independente do seu estadiamento, mesmo após controle para outros importantes fatores de risco (73,117).

A hemoglobina glicada média dos pacientes que apresentavam retinopatia diabética era de 9,2 mg/dl enquanto a dos pacientes que não apresentavam a retinopatia diabética era de 8,8 mg/dl. O nosso estudo também demonstrou que quanto maior o nível da hemoglobina glicada, maior a chance do paciente apresentar a retinopatia diabética. A relação da hemoglobina glicada com o desenvolvimento da retinopatia diabética é consistente com os achado do DCCT, do WESDR dentre outros (68,75,76,79,117)

O nosso estudo revelou que tanto a hipertensão arterial quanto o uso da droga hipotensora, inibidor da enzima conversora da angiotensina (IECA), foram relacionadas com a presença da retinopatia diabética. Nos pacientes com presença de retinopatia diabética 33% apresentavam hipertensão arterial e 45% usavam IECA. Enquanto que nos pacientes com ausência de retinopatia diabética, 8% apresentaram hipertensão arterial e 16% faziam uso do IECA. Essa relação da hipertensão arterial sistêmica com a RD já é bem estabelecida, tendo sido demonstrada em outros estudos incluindo tanto pacientes com diabetes tipo 1 como

diabetes tipo 2 (68,79,89). Contrariamente ao nosso resultado, estudos prospectivos e randomizados demonstram uma redução na incidência e na progressão da RD em pacientes com DM 1 em uso de IECA, sugerindo um benefício dessas drogas em reduzir a progressão da RD independente do efeito hipotensor (118,119). Em pacientes com RD existe um aumento da expressão do sistema renina-angiotensina, assim como dos receptores de angiotensina, que estão presentes nas células gliais de Muller e nos vasos retinianos (28,120). Apesar do mecanismo do sistema renina-angiotensina na fisiopatologia da RD não estar completamente esclarecido, um estudo *in vitro* sugere que a angiotensina II estaria relacionada com a ativação da PKC e consequentemente com a expressão de VEGF (121). A ação do IECA inibindo esse sistema estaria relacionado com o efeito protetivo dessa medicação em relação incidência e progressão da RD. O fato dos pacientes com retinopatia diabética envolvidos no nosso estudo apresentarem uma alta frequência de hipertensão arterial e de nefropatia, poderia justificar a associação do uso de IECA com a retinopatia diabética em nossos resultados.

Observamos uma associação positiva na nossa população entre doença renal crônica, presença de VTDR e retinopatia diabética referenciável, em concordância com o resultados de outros estudos que também encontraram associação entre as formas mais graves da RD e a doença renal crônica (90,92,95,122). A semelhança do mecanismo fisiopatológico entre essas duas complicações microvasculares pode justificar essa associação. A hiperglicemia ativa vias de sinalização intracelular, gerando estresse oxidativo e consequente produção de marcadores inflamatórios e dano endotelial. Essas alterações causam o aumento da permeabilidade vascular e espessamento de membrana basal tanto nos capilares retinianos como nos renais, levando a retinopatia e a nefropatia diabética (90).

Apesar da relação já estabelecida em outros estudos entre níveis elevados de colesterol total, LDL colesterol e triglicerídeos com retinopatia diabética, no nosso estudo, os pacientes com níveis mais altos de LDL colesterol apresentavam menos chance de apresentar retinopatia diabética nas formas ameaçadoras da visão (VTDR). Atribuímos esse resultado ao fato de que esses pacientes com níveis altos de LDL colesterol usarem mais frequentemente drogas como fenofibrato e estatinas, drogas que podem apresentar efeito protetor na retinopatia diabética (83,85). O estudo *"The Singapore Malay Eye Study"*, sobre prevalência e fatores de risco para retinopatia diabética, envolvendo 757 pacientes com diabetes (incluindo tipo 1 e tipo 2), também encontrou uma relação inversa entre os níveis de LDL colesterol e retinopatia diabética, semelhante ao nosso resultado, porém ele sugere que novos estudos sejam realizados visando obter uma explicação (123).

Encontramos uma relação entre tabagismo e a retinopatia diabética referenciável. Apesar dos efeitos sistêmicos do tabagismo, relacionado com coagulação e ativação de vias inflamatórias, levando a hipóxia e dano endotelial, os dados na literatura sobre a associação entre tabagismo e retinopatia diabética são conflitantes. Alguns estudos, realizados com pacientes com DM1, revelaram não haver associação (124,125), enquanto outros revelaram uma associação positiva (126,127). O estudo de Magrini 2002 (128), realizado em puérperas com DM1, sugere que a relação do tabagismo com a retinopatia teria uma influência genética. A proteína *low-molecular-weight protein tyrosine phosphatase* (LMW- PTP ou ACP1) estaria relacionada com a proliferação endotelial e com as complicações vasculares do diabetes. As substâncias alcaloides e as espécies reativas de oxigênio produzidas pelo efeito do tabagismo, teriam um efeito danoso na transcrição dessa proteína. Na conclusão do estudo, as pacientes com uma expressão fenotípica maior da proteína ACP1 apresentaram uma associação negativa da retinopatia com o tabagismo. No nosso estudo, o número de pacientes tabagistas foi reduzido (5,6%), e talvez isso pode ter afetado o resultado da nossa análise.

Uma variável que se mostrou relacionada com a retinopatia diabética no nosso estudo, em as análises, independente da classificação, foi o nível sérico de ácido úrico. A relação do ácido úrico com complicações vasculares, incluindo a nefropatia e a retinopatia diabética em pacientes com diabetes tipo 1 já foi demonstrada em outros estudos e o mecanismo que poderia explicar essa relação está relacionado com os efeitos pró-inflamatórios do ácido úrico, levando a estresse oxidativo e consequente dano endotelial (129). Krizova et al. demonstrou uma concentração elevada de ácido úrico intra-vítreo e correlacionou com a concentração de VEGF intra-vítreo, em pacientes com diabetes tipo 2, sugerindo um papel do ácido úrico na fisiopatologia da RD provavelmente relacionado ao potencial oxidativo desta molécula, podendo agravar o dano endotelial (130). A ação oxidante do ácido úrico estaria relacionada com a produção das espécies reativas de oxigênio geradas durante a sua produção, entretanto estudos experimentais sugerem também que o ácido úrico, como produto final do metabolismo das purinas, poderia agir como um antioxidante (131,132). A ação pró-oxidante do ácido úrico é corroborada por estudos que demonstram que o aumento dos níveis séricos do ácido úrico promove disfunção endotelial, hipertensão arterial e síndrome metabólica (133–135).

No estudo caso-controle sobre a relação da doença cardiovascular com a retinopatia diabética, consideramos os casos, os pacientes com DCV. A prevalência de pacientes com doença cardiovascular, na nossa amostra, foi de 3,46%, compatível com a prevalência descrita em outros estudos, que foi entre 1,0 a 3,1% (107). A IDF demonstrou uma prevalência de doença cardiovascular em pacientes com diabetes, variando de 2,6% a 16,2% em países

desenvolvidos, entretanto, não existem dados suficientes, referentes a países em desenvolvimento (136).

No presente estudo, ratificamos a relação da retinopatia diabética com a doença cardiovascular, já descrita em outros estudos tanto no diabetes tipo 1 (105,107) quanto no diabetes tipo 2 (137,138). Van Hecke et al. demonstrou no estudo EURODIAB, que a presença de RD proliferativa e não proliferativa em pacientes com DM1 foram preditores de todas as causas de mortalidade e eventos cardiovasculares (105). O estudo WESDR realizou o acompanhamento de pacientes com DM1 durante 20 anos e demonstrou que a gravidade da RD estava associada com maior chance de mortalidade por doença cardiovascular (107). De acordo com nossos resultados, pacientes com retinopatia diabética têm chance maior de apresentar doença cardiovascular. O fato de compartilharem mecanismos fisiopatológicos e fatores de risco semelhantes (como duração do diabetes, hiperglicemia, doença renal crônica e hipertensão arterial), pode justificar a relação entre ambas as comorbidades. O principal mecanismo envolvido na fisiopatologia da DCV é a aterosclerose, que está associada com inflamação crônica e injuria endotelial, resultando na formação de placas de ateromas na parede arterial e a ruptura dessa lesão, pode causar um infarto vascular agudo (139). O mecanismo de inflamação crônica desencadeando dano endotelial também está relacionado com o desenvolvimento da retinopatia diabética.

A relação dos níveis séricos de fatores inflamatórios com a severidade da retinopatia diabética em pacientes com DM1, corroboram o papel dessas citocinas na fisiopatologia da RD (56,57). Entretanto, os resultados dos estudos são controversos, havendo heterogeneidade entre os estudos pelas características da população, como tipo do diabetes, duração da doença, níveis de hemoglobina glicada da população em estudo e fonte do fluido tecidual, se soro, plasma ou vítreo. No nosso estudo caso-controle, comparando pacientes com retinopatia diabética proliferativa com pacientes com ausência de retinopatia diabética, encontramos associação positiva entre os níveis séricos de PCR e a RD proliferativa. Entretanto, não encontramos associação entre os outros marcadores (IL-6, TNF-α e VEGF).

A associação que encontramos entre a PCR e a RD é consistente com outros estudos como demonstrado em uma metanálise incluindo pacientes com diabetes tipo 1 e tipo 2 (54). Neste estudo de metanálise, foi demonstrado que os níveis de PCR eram elevados nos pacientes com RD comparado com os pacientes com ausência de RD, assim como nos pacientes com RD proliferativa, comparado com os pacientes com RD não proliferativa. Sasongko e col. também demonstraram a relação dos níveis de PCR em pacientes com as formas mais severas da retinopatia diabética (VTDR) e essa associação era maior nos pacientes com IMC $\geq$ 30 kg/m^2

(52). A obesidade é fortemente associada com níveis crônicos subagudos de inflamação, estimulando a produção de citocinas inflamatórias, incluído PCR e TNF-α (140) entretanto, no nosso estudo, não encontramos diferença nos níveis de IMC entre casos e controles. A PCR é uma proteína inflamatória inespecífica e seu nível sérico pode estar relacionado com com resistência a insulina e controle glicêmico ruim em pacientes com DM1 (141). Níveis séricos elevados de PCR também são relacionados com a presença de DM1 mesmo na ausência de complicações micro ou macrovasculares, sugerindo um estado inflamatório crônico (50,142). Como nossos pacientes com RD proliferativa também apresentavam níveis elevados de hemoglobina glicada, talvez isso possa justificar o fato de nossos pacientes que RD proliferativa, também apresentarem níveis séricos mais elevados de PCR.

Os nossos resultados não mostraram associação entre os níveis de IL-6 e a RD proliferativa. A IL-6 é uma citocina pró-inflamatória que regula em parte, a produção de PCR pelo fígado, além de poder promover a expressão do VEGF em situação de hipóxia, estando assim indiretamente relacionada com o aumento da permeabilidade vascular e neovascularização, na retinopatia diabética (49,143). Alguns estudos demonstraram relação dos níveis de IL-6 com RD (56,144). Uma metanálise incluindo 31 estudos, com pacientes com diabetes tipo 1 e tipo 2, em que alguns dosavam IL-6 no soro e outros no vítreo, demonstrou que o nível de IL-6 era maior nos pacientes com retinopatia diabética do que nos pacientes sem RD. Além disso, os níveis de IL-6 eram mais altos no grupo de pacientes com RD proliferativa do que no grupo de RD não proliferativa (33). Ainda neste estudo de metanálise, os dois estudos que envolviam apenas pacientes com DM1, sendo a população de ambos os estudos composta por crianças, não encontraram diferença estatisticamente significativa entre os casos (pacientes com RD) e os controles (pacientes sem RD) (55,145). El Asrar e col., em 1992 demonstraram que o nível de IL-6 estava positivamente associado com a severidade da RD, quando dosado no vítreo, mas não quando dosado no soro dos pacientes (146). No estudo de Ozturk e col. os níveis de IL-6 no soro de pacientes com DM tipo 2 não apresentaram diferença significativa entre os grupos com e sem RD (147). Alguns estudos tem ainda demonstrado níveis elevados de IL-6 no soro de pacientes com DM1, independente da presença de complicações do diabetes, relacionado principalmente, com a inflamação crônica do DM1 que é resultado de um aumento na produção de marcadores inflamatórios, como a IL-6 (29,148). Outra evidência da relação da IL-6 com o DM1 está na sua capacidade de promover a diferenciação de linfócitos B e ativação de célulat T *killer*, podendo levar a dano permanente nas células beta pancreáticas (149).

No nosso estudo, os resultados mostraram que não houve associação entre os níveis séricos de TNF-α e a RD proliferativa. O papel do TNF-α na fisiopatologia da RD está

relacionado com seus potenciais angiogênicos e pró-inflamatórios, induzindo a leucostasia (adesão dos leucócitos na parede endotelial dos vasos retinianos), levando a uma aumento da permeabilidade vascular (15,36). Devido a importância do papel do TNF- α na fisiopatologia da RD, já foi sugerido como tratamento do edema macular diabético o uso de medicamento anti TNF- α. Sfikakis e col. demonstraram que o uso sistêmico, com infusão venosa do inibidor de TNF- α infliximab, gerou uma melhora na acuidade visual e na espessura do edema macular em 6 olhos de 5 pacientes com edema macular diabético refratário ao tratamento com fotocoagulação a laser (150). Devido aos potenciais efeitos colaterais pelo uso sistêmico do infliximab, aventou-se a possibilidade de uso intra-vítreo, porém estudo envolvendo 39 olhos com edema macular diabético refratário, não apresentou benefício clínico (151). Evidências científicas sugerem uma relação de níveis elevados de TNF-α com a retinopatia diabética, tendo sido sugerido inclusive como marcador de RD, segundo recente artigo de metanálise (39). Gustavsson e col. compararam níveis séricos de marcadores inflamatórios, incluindo TNF-α , IL-6, IL-1 e PCR em pacientes com DM1 com RD proliferativa e não proliferativa. O TNF-α foi o único marcador inflamatório relacionado com a RD proliferativa (37). Entretanto, a população desse estudo não foi pareada por duração nem por gênero, ao contrário do nosso estudo. Roy e col. demonstraram também que TNF-α, juntamente com outros fatores inflamatórios, foi um preditor significativo de RD, em pacientes com DM1 (57). Contudo, o estudo de Cheung e col., que investigou os marcadores inflamatórios e fatores angiogênicos no humor aquoso de pacientes diabéticos com e sem RD, não encontraram associação com TNF-α, apenas com IL-6 e VEGF que apresentaram níveis mais altos em pacientes com RD (144). Loukovaara et al. também não encontraram diferença no nível de TNF-α no vítreo de pacientes com RD proliferativa comparado com pacientes com RD não proliferativa (152).

Assim como o TNF-α, os níveis séricos de VEGF no nosso estudo também não se associaram com a RD proliferativa. O papel do VEGF na fisiopatologia da RD está relacionado principalmente com a neovascularização, característica da forma proliferativa da retinopatia, e também com seu potencial inflamatório, promovendo aumento da permeabilidade vascular (29), porém, as investigações científicas sobre a relação os níveis de VEGF no soro, no plasma e no vítreo, são conflitantes. Um estudo recente de metanálise mostrou que o VEGF no soro e não no plasma, estava relacionada a presença e severidade da RD e atribuíram essa diferença devido o uso de anticoagulantes no plasma evitando a formação de coágulos sanguíneos, reduzindo assim, o número de plaquetas que são uma rica fonte de VEGF (41). Schlingemann e col. fizeram um estudo em que comparam o nível de VEGF no plasma de pacientes com DM1

com o de pacientes sem diabetes. Eles colheram amostra de sangue de cada paciente em 2 tubos com diferentes reagentes para obtenção do plasma. Observaram que não houve diferença do resultado dos níveis de VEGF entre os pacientes com e sem DM1 no frasco que continha um reagente que reduzia mais a ativação plaquetária (prostaglandina E1 e teofilina). Nos tubos que continham citrato, o plasma de pacientes com DM1 apresentou níveis maiores de VEGF , o que foi atribuído a possível maior liberação desse fator devido ativação plaquetária, nos indivíduos com controle glicêmico ruim (153). Mysliwec e col. encontraram níveis elevados de VEGF, assim como de TNF-alfa e IL-6 no soro de crianças com DM1 e RD em comparação ao grupo sem RD (55). Ahuja e col. sugerem inclusive que o nível sérico de VEGF poderia sem utilizado como biomarcador de gravidade da RD (154). Baharivand e col. compararam os níveis de VEGF no soro e no vítreo de pacientes com RD proliferativa e pacientes com RD não proliferativa e encontraram que os níveis de VEGF tanto no soro e no vítreo eram maiores nos pacientes com RD proliferativa (155). Entretanto, trata-se de um estudo com um número reduzido de pacientes, sem pareamento por duração da doença. Outros estudos também tem revelado resultados significativos de dosagem de VEGF intra-vítreo, sugerindo uma ação localizada do VEGF intraocular, estando fortemente relacionado com a severidade da RD (156–159). Contudo, assim como nosso estudo, os resultados de Crosby-Naomi et col. também não encontraram associação entre níveis séricos de VEGF e RD proliferativa (160).

A relevência do nosso estudo baseia-se principalmente no pioneirismo em realizar um estudo multicêntrico sobre pacientes com diabetes tipo 1 em um país com uma população miscigenda e com dimensões continentais, incluindo um número expressivo de pacientes. De acordo com os nossos resultados o percentual de pacientes referenciáveis foi de 18%, evidenciando a importância de um programa de investigação diagnóstica em larga escala que permita selecionar os pacientes que deveriam ser encaminhados para o oftalmologista, o que facilitaria o fluxo de avaliação desses pacientes. Os nossos resultados mostraram ainda que a presença da retinopatia diabética pode ser considerada um indicador de risco para doença cardiovascular. Dessa forma, o diagnóstico da retinopatia diabética pode sugerir quais pacientes com diabetes tipo 1 deveriam ser investigados para doença cardiovascular. Devido a alta taxa de mortalidade mundialmente atribuída a doença cardiovascular, todo e qualquer esforço em identificar e prevenir esta doença, é importante. O exame de fundo de olho é não invasivo e de baixa complexidade, podendo auxiliar na identificação desses pacientes.

Nosso estudo apresenta algumas limitações: o fato do desenho do estudo ser transversal, não permite o estabelecimento da relação de causa-efeito baseada nos resultados; o diagnóstico de hipertensão arterial foi baseado segundo informação dos pacientes, o que pode ter

subestimado a prevalência. Entretanto, existe evidência científica de que há concordância entre a informação dada pelo paciente e dados obtidos de prontuário sobre hipertensão (aproximadamente 82% de sensibilidade e 92,2% de especificidade) (43). O diagnóstico do diabetes tipo 1 foi clínico, realizado por médico endocrinologista, porém não foi utilizado como critério diagnóstico avaliação de anticorpo anti-ilhotas nem peptídeo-c. Além disso, a avaliação da retinopatia diabética foi feita por oftalmoscopia indireta, apesar da retinografia de sete campos ser considerada o método padrão pelo ETDRS. Porém estudos revelam que existe concordância entre os dois métodos (44), além da fundoscopia ser frequentemente utilizada em estudos epidemiológicos, com boa concordância entre observadores (109,110). Os nossos dados de doença cardiovascular foram baseados em informações de prontuário e não foi realizado investigação clínica em todos os pacientes, de acordo com recomendação da ADA de não investigar DCV em pacientes assintomáticos e sim avaliação anual de fatores de risco cardiovasculares. Apesar disso, nossa prevalência de doença cardiovascular foi semelhante à de outros estudos envolvendo pacientes com diabetes tipo 1.

CONCLUSÃO

Os objetivos principais da presente tese eram apresentar a prevalência da RD e seus fatores de risco em estudo multicêntrico no Brasil, em pacientes com DM1. Encontramos uma prevalência da RD, em concordância com a estimativa mundial. Os fatores de risco que apresentaram uma associação mais bem definida com a RD foram o tempo de duração do diabetes, o controle glicêmico, a hipertensão arterial e os níveis de ácido úrico sérico. A retinopatia diabética se mostrou como importante indicador de risco para a presença da doença cardiovascular, sugerindo que novos estudos são necessários para esclarecer a real função da retinopatia como preditora dessa fatal comorbidade.

Dentre os fatores inflamatórios pesquisados, encontramos uma associação positiva da RD proliferativa apenas com os níveis séricos da PCR. Entretanto, devido a característica inespecífica da PCR e as evidências científicas do papel do VEGF, TNF- α, e de IL-6 na fisiopatologia da RD, acreditamos que novos estudos, incluindo a dosagem intra-vítreo, seriam necessários para melhor avaliação da relação dessa citocinas com a RD proliferativa.

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ANEXO - Aprovação do Comitê de Ética

HOSPITAL UNIVERSITÁRIO PEDRO ERNESTO/ UERJ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Relação da retinopatia diabética com mediadores inflamatórios no Diabetes Tipo 1 em estudo multicêntrico brasileiro.

Pesquisador: LAURA GOMES NUNES DE MELO

Área Temática:

Versão: 1

CAAE: 52641215.9.1001.5259

Instituição Proponente: Hospital Universitário Pedro Ernesto/UERJ

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.401.712

Apresentação do Projeto:

O estudo será realizado a partir do estudo multicêntrico : Estratégias de rastreamento e diagnóstico da retinopatia e neuropatia diabética e
identificação de biomarcadores de complicações crônicas em pacientes com Diabetes Mellitus tipo 1 associado a análise econômica do tratamento,
realizado em 2011-2013, em 13 centros de 4 regiões do Brasil (Sul, Sudeste, Norte/Nordeste,e Centro-Oeste) que incluiu 1760 pacientes.Estudo de
corte transversal, realizado em pacientes com Diabetes mellitus tipo 1 (DM1) acompanhados regularmente em cada centro.

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo principal deste projeto, é analisar a relação de biomarcadores inflamatórios, principalmente a proteína C reativa, com a presença de
retinopatia diabética, e seus diferentes níveis.

Avaliação dos Riscos e Benefícios:

Riscos:

Uma vez que os pacientes envolvidos no estudo foram submetidos a exames laboratoriais, de

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sangue e urina, além de inquerito com questionário
padrão, não foi identificado nenhum risco iminente.
Benefícios:
Os pacientes envolvidos diretamente no estudo, realizaram exames laboratoriais, e investigação clínica rigorosa, permitindo detectar complicações
ou descontrole da doença, possibilitando um melhor acompanhamento clínico.

Comentários e Considerações sobre a Pesquisa:
A análise estatística será realizada através dos programas SPSS (versão 22.0) e Epi Info (versão 7.1.5). Para comparação entre grupos, utilizaremos
análise de variância (ANOVA) seguida do teste Tukey – Krammer e os testes de Mann Whitney ou Kruskal-Wallis (com correção de Bonferroni nas
comparações múltiplas). Para análise de variáveis categóricas utilizaremos o teste do Qui-Quadrado com correção de Yates e o teste exato de
Fisher. Para correlação entre variáveis numéricas utilizamos a correlação de Pearson e correlação de Spearman (rs) e regressão múltipla em
"stepwise". Os dados serão apresentados como média ± desvio padrão (DP) ou mediana (mínimo /máximo). Consideraremos como significante um
valor de p bicaudal < 0,05. Quando indicado será apresentado o limite de confiança (95% CL).

Considerações sobre os Termos de apresentação obrigatória:
Todos os documentos de apresentação obrigatória foram enviados a este Comitê, estando dentro das boas práticas e apresentando todas dados necessários para apreciação ética.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:
O trabalho pode ser realizado da fora como está apresentado. Diante do exposto e à luz da Resolução CNS nº466/2012, o projeto pode ser enquadrado na categoria – APROVADO. Para ter acesso ao PARECER CONSUBSTANCIADO: Clicar na "LUPA" (DETALHAR) - Ir em "DOCUMENTOS DO PROJETO DE PESQUISA ", clicar na opção da ramificação (pequeno triangulo no entrocamento do organograma) de pastas chamada – "Apreciação", e depois na Pasta chamada "Pareceres", o Parecer estará nesse local.

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente, o CEP recomenda ao Pesquisador: 1. Comunicar toda e qualquer alteração do projeto e termo de consentimento livre e esclarecido. Nestas circunstâncias a inclusão de pacientes deve ser temporariamente interrompida até a resposta do Comitê, após análise das mudanças propostas. 2. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 anos para possível auditoria dos órgãos competentes. 3. O Comitê de Ética solicita a V. Sª., que encaminhe relatórios parciais e anuais referentes ao andamento da pesquisa ao término da pesquisa encaminhe a esta comissão um sumário dos resultados do projeto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_641925.pdf	18/12/2015 00:20:59		Aceito
Projeto Detalhado / Brochura Investigador	RELACAO_RD_MARCADORES_INFLAMATORIOS.pdf	18/12/2015 00:19:13	LAURA GOMES NUNES DE MELO	Aceito
Declaração de Instituição e Infraestrutura	Vinculo_do_Pesquisador_UERJ.jpeg	18/12/2015 00:18:38	LAURA GOMES NUNES DE MELO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_CONSENTIMENTO.pdf	18/12/2015 00:16:34	LAURA GOMES NUNES DE MELO	Aceito
Cronograma	CRONOGRAMA.jpeg	18/12/2015 00:14:43	LAURA GOMES NUNES DE MELO	Aceito
Folha de Rosto	FOLHA_DE_ROSTO_PDF.pdf	18/12/2015 00:14:12	LAURA GOMES NUNES DE MELO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 03 de Fevereiro de 2016

Assinado por:
WILLE OIGMAN
(Coordenador)

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