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RETINOPATIA E MACULOPATIA DIABÉTICAS: PATOGÊNESE E FATORES DE RISCO

Tese apresentada ao Curso de Pós-graduação da Faculdade de Ciências Médicas da Universidade de Campinas para obtenção do Título de Doutor em Clínica Médica, área de concentração Clínica Médica.

Orientador: Prof Dr. José Butori Lopes de Faria

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Data:

HORIZONTE

Ó MAR anterior a nós, teus medos
Tinham coral e praias e arvoredos
Desvendadas a noite e a cerração,
As tormentas passadas e o mysterio,
Abria em flor o Longe, e o Sul siderio
Splendia sobre as naus da iniciação.

Linha severa da longínqua costa -
Quando a nau se approxima ergue-se a encosta
Em árvores onde o Longe nada tinha;
Mais perto, abre-se a terra em sons e cores:
E, no desembarcar, há aves, flores,
Onde era só, de longe a abstracta linha.

O sonho é ver as fórmas invisíveis
Da distância imprecisa, e, com sensiveis
Movimentos da esprança e da vontade,
Buscar na linha fria do horizonte
A arvore, a praia, a flor, a ave, a fonte -
Os beijos merecidos da Verdade.

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PREFÁCIO

A retinopatia diabética, acompanhada ou não do edema macular, é a principal complicação microvascular ocular que ocorre em pacientes com *diabetes mellitus* (DM) tipos 1 e 2 sendo importante causa de cegueira. A razão pela qual alguns pacientes diabéticos desenvolvem as formas severas da retinopatia não está totalmente esclarecida. É possível que a identificação de fatores associados às formas graves de retinopatia diabética (RD) possa determinar a adoção de condutas clínicas que visem o retardo ou mesmo a prevenção dessa complicação do DM.

Num primeiro estudo, investigamos fatores oculares e sistêmicos associados ao edema macular difuso e focal em pacientes diabéticos tipos 1 e 2 acompanhados em um serviço de referência em doenças do vítreo e retina. Neste trabalho observamos que entre outros fatores, a presença da hipertensão arterial aumentava em 3;2 vezes o risco de edema macular difuso em pacientes diabéticos (95% intervalo de confiança [IC]: 1,5 a 6,9).

Com o intuito de melhor entender a interação entre hipertensão e formas graves de retinopatia diabética, investigamos se pacientes com *diabetes mellitus* tipo 1 e RD proliferativa (RDP) apresentavam aumento do contra-transporte de sódio e lítio em hemáceas (CT Na^+/Li^+), um marcador de hipertensão arterial essencial. Observamos que pacientes com *diabetes mellitus* tipo 1 e RDP apresentam aumento do CT Na^+/Li^+ quando comparados àqueles sem RDP, sugerindo que a predisposição à hipertensão arterial torne tais pacientes mais propensos a desenvolver formas mais graves de RD.

Os eventos iniciais da retinopatia diabética presumem-se ocorrer na rede capilar da retina, envolvendo principalmente pericitos e células endoteliais. Entretanto, o papel da retina neural nas anormalidades funcionais visuais iniciais ainda permanece obscuro. Para investigar as possíveis alterações na retina neural nos pródomos da retinopatia diabética, realizamos trabalhos clínicos com eletrofisiologia visual, onde técnicas de potenciais visuais

evocados (PVE) foram desenvolvidas e aplicadas em estudo caso-controle com pacientes diabéticos tipo 1 sem evidências de retinopatia. Estes estudos demonstraram que pacientes diabéticos tipo 1 sem retinopatia apresentam diminuição acentuada e não seletiva dos PVE envolvendo as vias visuais, desde as camadas internas da retina (células ganglionares) atingindo os sistemas magno- e parvo- celulares no nervo óptico e em centros corticais visuais. O conhecimento da importância de outras células, além dos pericitos e células endoteliais, na patogênese da retinopatia diabética poderá contribuir para adoção de novas alternativas de tratamento.

RETINOPATIA E MACULOPATIA DIABÉTICAS

Epidemiologia

A retinopatia diabética (RD) é a principal complicação microvascular ocular que ocorre em pacientes com *diabetes mellitus* (DM) tipos 1 e 2 sendo importante causa de cegueira em pessoas entre 20 a 74 anos de idade nos Estados Unidos (Patz 1991) e na Europa ocidental (Ghafour 1983).

As complicações oculares na retinopatia diabética (RD) incluem as formas não-proliferativa e proliferativa, e a maculopatia que pode estar presente em ambas. A principal causa de diminuição moderada da acuidade visual é o envolvimento macular (Patz 1973; McMell 1977; Sigelman 1980; Ferris 1984; Ferris 1987) sendo a proliferativa a forma mais severa da RD com grande risco de perda visual devido a hemorragia vítrea ou descolamento de retina tracional.

Aproximadamente 60% dos pacientes diabéticos tipo 1 (insulino- dependentes) apresentam a forma proliferativa, com pico de incidência a partir da segunda década e declínio na terceira década de duração do DM (Klein (*a*) 1984). Este padrão não pode ser explicado exclusivamente pelo controle metabólico, e sugere que um subgrupo de pacientes são mais susceptíveis às complicacões retinianas mais graves do DM. Entre os pacientes com DM tipo 2 em uso de insulina ocorre um comportamento semelhante ao observado em pacientes com DM tipo 1 (Klein (*b*) 1984).

No Brasil, dados regionais de Londrina-PR mostram que 29% dos pacientes com DM tipos 1 e 2 atendidos no Sistema Único de Saúde apresentam algum grau de retinopatia diabética (Casella 1994). Outro estudo transversal realizado na região de Franco da Rocha-SP revelou que as formas mais graves da retinopatia estão relacionadas com o tempo de duração da doença, tendo seu pico de incidência na segunda década de duração do DM com 20% e 26% de prevalência das formas pré-proliferativa e proliferativa, respectivamente

(Steck 1993). Provavelmente, esta prevalência foi mais baixa do que a descrita anteriormente na literatura devido à duração máxima dos DM tipos 1 e 2 observada entre os pacientes estudados ser de 15 anos.

Similarmente, a prevalência e incidência do edema macular diabético aumentam com a duração da doença (Aiello 1981; Klein *(c)* 1984; Klein 1989) em pacientes com DM tipos 1 e 2. Em trabalhos do grupo de Wisconsin para estudos sobre retinopatia diabética, foram reportados aumento da prevalência de edema macular de 0% em pacientes com DM tipo 1 com menos de 5 anos de doença para 29% em pacientes com mais de 20 anos de duração do DM (Klein *(c)* 1984). Em pacientes com DM tipo 2 em uso de insulina, as prevalências foram de 3% e 28% em pacientes com menos de 5 anos e com mais de 20 anos de duração da doença, respectivamente. Em relação a gravidade da RD, em pacientes com retinopatia diabética não-proliferativa leve, a prevalência de edema macular foi de 2-6%, e em pacientes com retinopatia diabética proliferativa, prevalências mais elevadas foram observadas, variando entre 20 e 60% (Klein (c) 1984).

Os dados brasileiros, a partir de estudos de Casella e colaboradores (Casella 1994), revelam que a ocorrência da maculopatia em pacientes com *diabetes mellitus* tipos 1 e 2 em um Hospital Universitário foi de 36%, 61% e 87% nas formas não-proliferativa, pré-proliferativa e proliferativa, respectivamente. Tais dados superam as estatísticas mundiais. Uma possível explicação para estes dados é quanto à diferença metodológica na avaliação macular.

Em resumo, o grande impacto dessa complicação na população diabética se deve tanto à sua alta frequência quanto ao potencial comprometimento visual irreversível desses pacientes.

Retinopatia diabética e os fatores de risco

Vários estudos têm descrito fatores associados com a retinopatia diabética (RD), incluindo a duração do DM (Chase 1989, Klein 1995), o controle glicêmico avaliado pelos níveis de hemoglobina glicosilada (Chase 1989, Klein 1988, Klein 1995); pressão arterial sistólica (Bodansky 1982) e diastólica (Chase 1990), idade e sexo do paciente (Bodansky 1982), tabagismo (Klein 1983) e elevados níveis de colesterol séricos (Miccoli 1987, Chew 1996).

Interessantemente, são relativamente escassos os estudos sobre os fatores associados ao edema macular diabético (Sebag 1984, Nasrallah 1988, Dodson 1991), principalmente distinguindo as formas focal e difusa (ETDRS 1995).

A hipertensão arterial tem sido cada vez mais reconhecida como o fator de risco independente mais importante para o desenvolvimento das complicações micro e macrovasculares (Klein 1983; Krolewski 1988; Chase 1990; Hsueh 1992). Entretanto, se o efeito da hipertensão na vasculatura retiniana é reflexo dos efeitos hemodinâmicos ou se representa uma relação mais complexa com a genética da hipertensão, ainda não foi adequadamente estudado. Estudos experimentais (Gin 1996) para se avaliar o efeito da hipertensão arterial no desenvolvimento das complicações microvasculares do DM demonstraram que o controle pressórico diminui os efeitos deletéricos do DM na microvasculatura retiniana e renal em ratos espontaneamente hipertensos (SHR). Ensaios clínicos prospectivos, como o Estudo Europeu de Complicações do Diabetes, confirmaram que além do controle glicêmico o bom controle pressórico é de grande importância no retardo e mesmo prevenção das complicações microvasculares em patientes diabéticos tipo 1 (Forrest 1997).

Retinopatia diabética e fatores familiares

Várias linhas de evidências têm sugerido a existência de susceptibilidade ao desenvolvimento das formas graves da retinopatia diabética. Apesar do controle glicêmico ser o fator necessário no desenvolvimento das complicações retinianas diabéticas em pacientes diabéticos tipos 1 e 2 (DCCT 1993, DCCT 1995, UKPDS 1998, Baldeweg 1999, Laakso 1999), um subgrupo de pacientes parece ser particularmente susceptível às complicações vasculares do DM, incluindo retinopatia (Barnett 1981). Investigando a concordância de retinopatia proliferativa em gêmeos idênticos, Leslie e Pyre (Leslie 1982) revelaram que em 31 pares de gêmeos, 21 apresentam semelhante gravidade de retinopatia diabética. Posteriormente, Dorman e colaboradores (Dorman 1991) demonstraram que pares de irmãos com DM apresentavam concordância para a presença da retinopatia diabética proliferativa.

A procura de marcadores de predisposição genética a retinopatia proliferativa se dirigiu primeiramente aos antígenos de histocompatibilidade (HLA). Porém, os resultados desses estudos foram inconsistentes, alguns reportando associação entre B8 e retinopatia proliferativa (De Moerloose 1978, Standl 1980), outros autores com B15 (Moller 1978, Deckert 1979, Barbosa 1980) e outros ainda não demonstraram qualquer associação entre o sistema HLA e retinopatia diabética (Becker 1977, Johnston 1982, Bodansky 1982). Dados mais recentes sugerem que mecanismos imunológicos determinados geneticamente possam contribuir para o desenvolvimento da retinopatia diabética proliferativa (Cruickshanks 1992).

Mais recentemente, o Diabetes Control Complications Trial (DCCT), grande estudo clínico multicêntrico, randomizado e prospectivo em pacientes com DM tipo 1, com o objetivo de investigar os efeitos do tratamento glicêmico intensivo nas complicações microvasculares do DM em particular a retinopatia, identificou que o aumento na frequência do RDP em pacientes diabéticos tipo 1 ocorria dentro de determinadas famílias (DCCT 1997). Esta observação gerou a hipótese de que fatores familiares, provavelmente genéticos,

estejam relacionados às formas severas da retinopatia diabética. Entretanto este último estudo não sugere os mecanismos pelos quais fatores familiares tornariam indivíduos diabéticos susceptíveis a formas mais graves da RD.

Uma vez que, além do controle glicêmico, a hipertensão arterial é o fator mais importante na determinação das formas graves da RD, alguns pesquisadores têm investigado se alterações em genes associados à hipertensão arterial estão presentes em pacientes com RDP. Recentemente, Rabensteiner e colaboradores (Rabensteiner 1999) identificaram associação entre o polimorfismo do gene da enzima conversora de angiotensina (ACE) e a presença da retinopatia diabética proliferativa em pacientes diabéticos tipo 1. Essa última observação sugere que um mecanismo comum poderia estar envolvido no desenvolvimento da hipertensão e retinopatia proliferativa em pacientes com *diabetes mellitus* tipo 1.

Contra-transporte de Na^+/Li^+

Fisiologia

O significado da atividade do contra-transporte de sódio-lítio em hemáceas (CT Na^+/Li^+) na fisiologia celular ainda não é claro, mas este transporte de membrana celular apresenta similaridades com o transporte de membrana de Na^+/H^+, sistema presente em praticamente todas as células vivas eucarióticas (Mahanesmith 1985, Zerbini 1998). Este sistema está envolvido em funções celulares básicas como o controle do pH e volume celulares, resposta a genes mitóticos, a fatores de crescimento e hormônios, e ainda na reabsorção de sódio pelos túbulos renais (Mahanesmith 1985, Escobales 1986). Estudos populacionais em Salt Lake City e Rochester sugerem que até 80% da variação interindividual do CT Na^+/Li^+ está sob o controle genético (Dadone 1984, Boerwinkle 1986).

Entretanto, o CT Na^+/Li^+ é influenciado por fatores ambientais como índice de massa corpórea, glicemia, colesterolemia e consumo de álcool (Cirillo 1999).

Contratransporte de sódio e lítio em hemáceas e aspectos clínicos

Em 1980, Canessa e colaboradores demonstraram que os valores do CT Na^+/Li^+ em hemáceas de pacientes hipertensos se apresentavam elevados quando comparados àqueles observados em indivíduos normais (Canessa 1980). Posteriormente, outros estudos sugeriram que o CT Na^+/Li^+ poderia representar uma predisposição genética a hipertensão arterial (Morgan 1986, Carr 1989, Yap 1989, Walker 1990, Turner 1996, Laurenzi 1997, Strazzullo 1998).

A forte natureza hereditária do CT Na^+/Li^+ foi confirmada por estudos que demonstraram aumento neste contra-transporte em filhos normotensos de pais hipertensos, e ausência de alteração do CT Na^+/Li^+ após a redução da pressão arterial com drogas anti-hipertesivas (Trevisan 1983, Beuckelmann 1985, Beuckelmann 1986, Monciotti 1997, Chiarelli 1999). Além disso, forte correlação dos valores do CT Na^+/Li^+ foi observada em gêmeos idênticos discordantes para a presença de *diabetes mellitus* tipo 1 (Dubrey 1991).

Vários estudos (Krolewski 1988, Mangili 1988, Lopes de Faria 1992) mas não todos (Jensen 1990, Elving 1991), demonstraram que pacientes diabéticos tipo 1 com nefropatia apresentam aumento da atividade do CT Na^+/Li^+. Esta importante informação foi recentemente confirmada em estudos prospectivos. Os autores desses estudos concluíram que o risco de doença renal em pacientes com *diabetes mellitus* tipo 1 poderia estar associada com a predisposição familiar, provavelmente genética, à hipertensão arterial (Krolewski 1988, Mangili 1998, Lopes de Faria 1992, Monciotti 1997, Chiarelli 1999). Estudos familiares com esses pacientes contribuiram com novas informações na relação entre CT Na^+/Li^+, hipertensão arterial e nefropatia diabética. Walker e colaboradores (Walker 1990)

demonstraram que CT Na^+/Li^+ em pais não-diabéticos de pacientes diabéticos tipo 1 com nefropatia é significativamente mais elevado do que em pais não-diabéticos de pacientes sem nefropatia.

Estudos recentes com cultura de fibroblastos de pele de pacientes diabéticos tipo 1 com nefropatia demonstram que estes pacientes apresentam aumento significativo do *antiport* Na^+/H^+ quando comparados aos pacientes diabéticos sem lesão renal (Davies 1992, Trevisan 1992). Além disso, tem sido demonstrado que o aumento na atividade do *antiport* Na^+/H^+ está associado à elevação na proliferação celular (Trevisan 1992) e à alteração do metabolismo do colágeno (Trevisan 1997, Jin 1998). Uma vez que estas células foram mantidas em cultura por várias passagens, os autores postularam que as correlações entre atividade do *antiport* Na^+/H^+, a proliferação celular e a expressão do RNAm do colágeno tipo I possam ser geneticamente regulados.

Contratransporte de sódio e lítio em hemáceas e a retinopatia diabética proliferativa

Apenas um estudo investigou uma possível associação entre o CT Na^+Li^+ em hemáceas e a presença de retinopatia diabética (RD) (Krolewski 1988). Estes autores não observaram associação entre o aumento do CT Na^+Li^+ e a presença da retinopatia diabética. Entretanto uma possível associação entre o aumento do CT Na^+Li^+ e a presença de retinopatia proliferativa não foi investigada. Os achados deste estudo não foram surpresa uma vez que, diferentemente da retinopatia proliferativa que acomete um subgrupo de indivíduos diabéticos, a RD acometerá praticamente todos os pacientes diabéticos com longa duração da doença.

Diabetic Macular Edema: Risk Factors and Concomitants

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Abstract

Purpose: To investigate the systemic and ocular factors associated with diffuse macular edema in patients with diabetic retinopathy (DR) and compare with patients with focal or no macular edema.

Methods: From 160 consecutive patients with DR we obtained medical and ocular histories, blood pressure and visual acuity. Macular edema was determined by biomicroscopy, stereoscopic fundus photography and fluorescein angiography, and the vitreoretinal relationship by preset lens biomicroscopy.

Results: Among adult-onset diabetes mellitus (DM) patients, 55% had diffuse, 23.5% had focal and 21.5% had no macular edema (P=0.01). The risk of developing diffuse macular edema was 3.2 times greater in patients with high blood pressure (HBP) (95% confidence interval (CI), 1.5 to 6.9). Patients with cardiovascular disease (CVD) had a higher prevalence of diffuse (58.0%) than focal (26.0%) or no maculopathy (16.0%) (P=0.01). The odds for development diffuse macular edema was 3.4 times greater in patients with vitreomacular adhesion (95% CI, 1.15 to 13.30) than in those with complete posterior vitreoretinal attachment or vitreoretinal separation. The prevalence of diffuse maculopathy was higher in patients with preproliferative (PPDR) and proliferative (PDR) diabetic retinopathy (58.0% and 67.0%, respectively) than focal (19.0% and 17.0%) or no macular edema (19.0% and 16.0%) (P=0.04 and P=0.0001, respectively). The risk of diffuse macular edema was 6.2 (95% CI, 1.83 to 21.04) and 7.7 times greater (95% CI, 3.12 to 19.12) in patients with PPDR and PDR, respectively.

Conclusions: In this study, adult-onset DM, HBP, CVD, vitreomacular adhesion and advanced retinopathy were associated with increased risk of development of diffuse diabetic macular edema.

Introduction

Diabetic retinopathy (DR), the most important ocular complication in patients with diabetes mellitus (DM),[1] is the leading cause of new cases of blindness in patients under 60 years of age in the United States and in individuals between the ages of 30 and 64 in the United Kingdom,[2] and accounts for approximately 12%-14% of new cases of blindness resulting from all causes.[3] The ocular complications of DR include nonproliferative and proliferative DR and macular edema, which can be present in both types of DR. Macular involvement is the major cause of visual loss in patients with DR.[3] In epidemiological studies in the United States, the prevalence of patients with proliferative DR is approximately 700,000 cases and 500,000 for macular edema. These prevalences represent 65,000 new cases of macular edema annually.[3] Individual factors reported to be associated with DR include the duration of diabetes,[4,5] glucose control assessed by glycohemoglobin values[4-6] diastolic[7] and systolic blood pressure, age, sex,[8] smoking[9] and elevated cholesterol levels.[10,11] Few studies have investigated the factors associated with diabetic macular edema[5,12-14] and mainly they omit the distintion between focal and diffuse forms.

Diabetic maculopathy can result from ischemia, retinal edema, or a combination of both. Diabetic maculopathy may be either focal and diffuse. The diffuse form usually presents areas of capillary nonperfusion[15] (ischemia), with or without cystic changes, while the focal form is characterized by focal leakage from specific capillary lesions (microaneurysms), often associated with a ring exudate. Most studies to date have failed to distinguish between these two types of diabetic maculopathy, even though such a distinction is important for treatment and subsequent visual prognosis.

The Early Treatment Diabetic Retinopathy Study (ETDRS) demonstrated the benefit of laser photocoagulation in clinically significant macular edema.[16,17] In contrast,

diffuse macular edema in which extensive leakage from the posterior retinal capillary bed is observed, is more difficult to manage and the use of laser photocoagulation is not as efficient as in focal edema.

The purpose of this study was to investigate the ocular and systemic factors that are prevalent in patients with diffuse diabetic macular edema compared to individuals with focal or no macular edema, and to determine the risk factors for developing diffuse macular edema.

Patients and Methods

A cross-sectional study of a 160 patients with DR consecutively examined at the Schepens Retina Associates was performed. The ocular, family, and medical histories (cardiovascular disease, high blood pressure, respiratory, gastrointestinal and genitourinary disorders) as well as information on the use of systemic and ocular medications, were collected. The blood pressure was measured to the nearest 2 mmHg using a standard sphygmomanometer after the patient had been rested in a sitting position for five minutes. The refractive error was measured, and the best-corrected visual acuity for distance was determined using a modified ETDRS chart protocol.[18] Intraocular pressures were measured by applanation tonometry. Slit-lamp examination was applied to determine anterior chamber depth and the presence of iris neovascularization. Biomicroscopy of the posterior pole was performed with a flat contact lens (+90-, +78-, or +60-diopter lens) to determine the presence and nature of macular edema. Indirect fundus ophthalmoscopy and intravenous fluorescein angiography were also performed. Stereoscopic color fundus photographs of seven standard fields were taken. The vitreoretinal relationship was examined using a preset lens with slit-lamp, which allows observation of the dynamics of the vitreous condition with high magnification of the macular area[19]. This technique was performed by the

same examiner (in order to abolish the inter-observer variation) and helps differentiate a premacular liquefied lacuna from the detached premacular cortical vitreous. The vitreous study was not performed in every case, since it is not an ordinary test in the evaluation of the DM cases. When the visual acuity was worse than 20/400, one of the following levels was recorded: 20/800 (counting fingers [CF] 3'), 20/1600 (CF 1'), 20/3200 (hand motion), light perception, and no light perception (NLP).

Macular edema was considered to be present if there was retinal thickening within 500 μm of the center of the fovea, or hard exudates at or within 500 μm of the center of the fovea associated with adjacent retinal thickening, or a zone of retinal thickening one disc area, or larger, in size located one disc diameter (1,500 μm) or less from the center of the fovea or presence of photocoagulation scars in the macular area (compatible with previous development of macular edema). Macular edema was defined as *focal* if there were focal areas of retinal thickening and intraretinal leakage (Fig 1) in the macular area, which were often associated with a hard exudate ring, and *diffuse* if the retinal thickening involved the entire macular area with intraretinal leakage from dilated capillaries and microaneurysms, with or without cystoid macular edema (Fig 2). Patients with NLP visual acuity were not included in the analysis. Similarly, patients with other ocular diseases such as cataract, glaucoma, corneal opacities, amblyopia that could affect visual acuity were excluded. Also, eyes with macular degeneration, preretinal fibrosis, central vein occlusion or macular ischemia, (determined by capillary non perfusion of more than two quadrants of the macular area on fluorescein angiography), were not included into the study.

DR was classified as nonproliferative (NPDR) (presence of microaneurysms and intraretinal hemorrhages greater than ETDRS standard photograph 2A[20], hard exudates and or venous looping, soft exudates, venous beading and intraretinal abnormalities questionable present, and foveal avascular zone abnormalities), preproliferative (PPDR) (presence of microaneurysms and intraretinal hemorrhages in

all four quadrants, venous beading in 2 or more quadrants, intraretinal microvascular abnormalities greater than the ETDRS standard photograph 8A[20] at least in one quadrant, or proliferative (PDR) (presence of neovascularization on the disc or elsewhere on the retina, or preretinal or vitreous hemorrhage, and fibrous proliferation).

For the purpose of data analysis, the eye with the more severe pathology was used in patients with bilateral, clinically significant macular edema.[21] All diabetic patients with DR were classified according to the presence of diffuse, focal, or absent diabetic macular edema at the time of examination.

Definitions

The following definitions were used in this study:

Age at diagnosis of DM indicates the age at the time of the initial diagnosis of DM recorded by a physician.

The treatment of DM indicates diet only, insulin, or oral hypoglycemic drugs.

Duration of DM indicates the time period between diagnosis and the time of examination.

Early-onset DM indicates the onset of DM earlier than 35 years of age.

Adult-onset DM indicates the onset of DM after 35 years of age.

Cardiovascular disease was determined by a history of cardiovascular disease if there was a physician-verified history of angina, heart attack, or stroke, or if the patient was taking cardiovascular medications.

HBP indicates systolic or diastolic measurement above or equal to 160 and 90 mmHg, respectively, or if the patient was taking antihypertensive medications.

Vitreoretinal relationship was classified into complete posterior vitreous attachment to retina, vitreomacular adhesion only (adherence of posterior vitreous on

posterior pole and macular area) or posterior vitreous separation (separation between the posterior vitreous and the retina).

Statistical analysis

Analysis of variance (ANOVA) was used for descriptive variables. The Chi-square test was used to analyze the association among the three patient groups and other variables separately. A multiple regression model [22] was used to evaluate the association of systemic and ocular factors with the prevalence of diffuse diabetic macular edema. P values less than 0.05 were considered to be statistically significant.

Results

Of the 160 patients with DR, 66 patients (41%) were early-onset DM and 94 (59%) were adult-onset DM. Forty-eight patients (30%) had no macular edema, 32 (20%) had focal macular edema, and 80 (50%) had diffuse macular edema. Among the three groups, there was no significant difference in sex (P=0.67), age (P=0.80) and duration of DM (P=0.87) (Table 1). In 94 adult-onset DM patients, the frequency of diffuse macular edema (55.5%) was significantly higher than focal (23.0%) or no macular edema (21.0%) at the time of examination (P=0.01). By contrast, patients with early-onset DM did not show difference in the prevalence of diffuse and focal or no macular edema (42.5%, 15.0% and 42.5%, respectively). The odds ratio for diffuse diabetic macular edema was 1.6 times greater in adult-onset DM compared with early-onset DM (95% CI, 0.89 to 3.64). In the adult-onset DM group, there was no difference in the frequency of patients on a diet, oral hypoglycemic drug, or insulin treatment who presented diffuse, focal or no macular edema (P=0.91) (Table 1).

The prevalence of high blood pressure was 71.0% (38/53) among patients with diffuse macular edema, 9.0% (5/53) among those with focal, and 20.0% (10/53) among those with no macular edema (p=0.03). The odds for diffuse macular edema was 3.2 times greater in HBP patients than in those without HBP (95% CI, 1.5 to 6.9). Thirty-one of 44 (70.0%) patients with a systolic blood pressure higher than 160 mmHg had diffuse maculopathy, six (14.0%) had focal maculopathy, and seven (16.0%) had no maculopathy (p=0.007) (Table 2). The odds ratio for diffuse macular edema at increments of 10 mmHg over 160 mmHg adjusted for age was 1.23 (95% CI, 1.0 to 1.03), *i.e.*, the relative risk of diffuse macular edema in patients with a high systolic blood pressure was 23% greater at each 10 mmHg increment exceeding 160 mmHg. There was no significant difference in the diastolic blood pressure levels among the three groups (P=0.28). However, there was a tendency for the patients with a diastolic blood pressure level over 91 mmHg to present severe diabetic maculopathy: thus, 16 out of 27 such patients (60.0%) had diffuse macular edema, whereas six patients (22.0%) had focal edema, and five (18.0%) had no macular edema. Cardiovascular disease was more prevalent in patients with diffuse diabetic macular edema (58.0%) than in patients with focal (26.0%) and no macular edema (16.0%) (P=0.01). The risk for developing diffuse macular edema was 53% higher in patients with CVD in comparison with those with history of CVD (odds ratio: 1.53; 95% CI, 0.77 to 1.11)

There was an association between the severity of DR and maculopathy. In patients with PPDR, 16 out of 28 patients (58.0%) had diffuse macular edema, six (21.0%) had focal edema, and six (21.0%) had no macular edema (P=0.04) (Table 3). Among patients with PDR, 50 out of 75 (67.0%) patients had diffuse edema, 13 (17.0%) had focal edema, and 12 (16.0%) had no macular edema (P=0.0001). The risk for diffuse macular edema was 6.2 times greater in patients with PPDR (95% CI, 1.83 to 21.04) and 7.7 times in patients with PDR (95% CI, 3.12 to 19.12) greater when

compared with patients having NPDR. As expected, a visual acuity worse than 20/200 was more frequent among patients with diffuse macular edema (Fig 1).

The vitreoretinal interface of 74 patients in whom a vitreous study was performed at examination is shown in Table 4. Of 61 patients with vitreoretinal adhesion, 47 (77%) presented complete posterior vitreous adhesion and 14 (23%) patients presented vitreomacular adhesion (on the posterior pole and macular area only). Thirteen DM patients (13/74) showed total vitreoretinal separation. Among patients with vitreomacular adhesion (N=14), 1 (7%) patient had no macular edema, 2 (14%) had focal and 11 (79%) patients diffuse macular edema. Diffuse diabetic macular edema was more prevalent in patients with vitreomacular adhesion (11 out of 14) than in those with complete posterior vitreous attachment (25 out of 47) or vitreoretinal separation (6 out of 13); p=0.02, Chi-square test. The odds for developing diffuse macular edema in patients with vitreomacular adhesion only was 3.4 times greater (95% CI, 1.15 to 13.30) compared with those with complete posterior vitreous attachment or vitreoretinal separation.

Discussion

The ability of laser therapy to stabilize or restore the visual acuity in diffuse macular edema is not as good as in focal macular edema, and may result in central vision loss. For this reason, identification of the risk factors for diffuse macular edema may allow better management of this complication of DR. The goal of the present study was to determine the ocular and systemic factors that may be associated with an increased risk of diffuse macular edema in DR patients compared with patients having focal or no macular edema. Most of the studies failure in distinguish diffuse from focal diabetic macular edema. The importance of classify the diabetic macular edema into focal and diffuse lies on the different pathologic processes involved in both. Diffuse

macular edema reflects a complex deterioration involving a combination of diffuse capillary "drop-out"[15] and inner blood-retinal barrier and retinal pigmented epithelium degeneration[23], and focal edema represents localized intraretinal microaneurysms at the macular area. Additionally, there is no comprobatory information that the focal and diffuse diabetic macular edema are different stages of the same entity.

The importance of the vitreous gel in some macular disorders has ultimatelly been studied.[13, 24-27] In our study, diabetic patients with posterior vitreous adhesion, mainly those with vitreoretinal adherence on posterior pole and macula were more likely to present diffuse macular edema. It was reported[27] higher prevalence of complete posterior vitreous attachment in comparison with vitreomacular adherence only in patients with diffuse diabetic macular edema. These findings differ from ours since the previous investigation considered new cases of diabetic macular edema during a short period follow up. The destabilization of the biochemical and anatomic vitreous structure in diabetic eyes due to nonenzymatic glycation of the collagen fibers[28,29] may induce traction over the macula, and therefore contribute to the persistence of the diffuse macular edema in DM[14].

Patients with PPDR and PDR were found to have an increased risk of developing diffuse macular edema. As expected, a visual acuity worse than 20/200 in the studied eye was observed in 54% of the patients with diffuse macular edema. These findings confirm a previous report which showed a higher incidence of poor visual acuity in patients with diffuse macular edema and severe DR.[30] The visual impairment in patients with no diabetic macular edema at the time of examination was attributed to optic neuropathy, retinal traction lines in the macular area resulting from fibrovascular proliferation at the optic disc or along the temporal vascular arcades or epiretinal membranes, and changes in the retinal pigment epithelium of the macula subsequent to the resolution of previous macular edema.

Hypertension is a major factor that contributes to the development of vascular complications in DM, including atherosclerosis, nephropathy and retinopathy[31]. Our results showed that diabetic patients with HBP have a higher risk of developing diffuse macular edema. It is usually associated with a diffusely dilated retinal capillary bed and smaller vessels. Diabetic patients with CVD and HBP are more susceptible to vascular occlusive disease, and hence to retinal ischemia and edema. The effect of the DM on the macrovasculature is the result of an acceleration of atherosclerosis and increased thrombosis, affecting cerebral, coronary and peripheral vessels mainly. Many factors are involved in the etiology of these macrovasculopathy, such as hyperlipidemia, hypertension and hyperinsulinemia which has been proposed to induce atherogenesis. The present study showed the odds ratio for diffuse macular edema in comparison with focal edema among patients with hypertension (OR=3.2) and among those with systolic blood pressure over 160 mmHg (OR=1.23). These are important indicatives of the involvement of the systemic hypertension on the development of diffuse macular edema and advanced retinopathy in diabetic patients.

It is well established that hyperglycemia is the most identifiable risk factor for microvascular disease in diabetes. The results of Diabetes Complications and Control Trial (DCCT) have proved that, in IDDM, hyperglycemia is a major contributor to microvascular disease, i.e., retinopathy, nephropathy and neuropathy, and the intensive glycaemic control can also reduce cardiovascular disease. In the present cross-sectional study, the HbA_{1c} levels differed considerably in the methodology used for their determination, thus making impossible to compare them.

In summary, our results demonstrate that adult-onset DM, HBP, cardiovascular disease, vitreomacular adhesion and severe DR were associated with increased risk of development diffuse macular edema in diabetic patients in comparison with focal macular edema. The adequate treatment of cardiovascular disease and good control of

blood pressure should be included in the management of diffuse macular edema in patients with DR.

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Legend of Figure

Figure1. Visual acuity (VA) in each type of diabetic macular edema. The eye with the worst visual acuity was used in the analysis.

Table 1. Demographic characteristics of the 160 diabetic patients studied.

Variable	*Category*	*Macular Edema*			
		None	*Focal*	*Diffuse*	*P*
Sex	Male	21	21	39	0.67†
	Female	27	11	41	
Age (yrs) (Mean±SD)		51±16	60±13	61±15	0.80†
Duration of DM (yrs) (Mean±SD)		21.3±11	24±9	22.6±12	0.87†
Age at onset of DM	≤35 years	28 (42.5%)	10 (15.0%)	28 (42.5%)	
	>35 years	20 (21.0%)	22 (23.5%)	52 (55.5%)	0.01*
Therapy for adult-onset DM after 35 years of age					
Diet only		0	0	1 (1.0%)	
Oral hypoglycemic drugs		19 (46.0%)	18 (45.0%)	27 (34.0%)	
Insulin		22 (54.0%)	22 (55.0%)	51 (65.0%)	0.91

DM : diabetes mellitus; † : Analysis of variance test; *: statistically significant

Table 2. Clinical features of the 160 patients studied.

Variable	*Category*	*Range*	*Macular Edema*			
		(mmHg)	*None*	*Focal*	*Diffuse*	*P*
			n (%)	*n (%)*	*n (%)*	
High BP	Absent		34 (32)	26 (24)	47 (44)	
	Present		10 (20)	5 (9)	38 (71)	0.03*
Cardiovascular disease						
	Absent		40 (38)	16 (15)	49 (47)	
	Present		9 (16)	14 (26)	32 (58)	0.01*
Systolic BP		<140	24 (43)	13 (23)	19 (34)	
		140-159	12 (29)	9 (22)	20 (49)	
		≥160	7 (16.0)	6 (14)	31 (70)	0.007*
Diastolic BP		<90	43 (32)	29 (22)	61 (46)	
		≥91	5 (18)	5 (22)	16 (60)	0.28

BP: Blood presure; *: statistically significant; Chi-square test.

Table 3. Relationship between the type of macular edema and the severity of diabetic retinopathy.

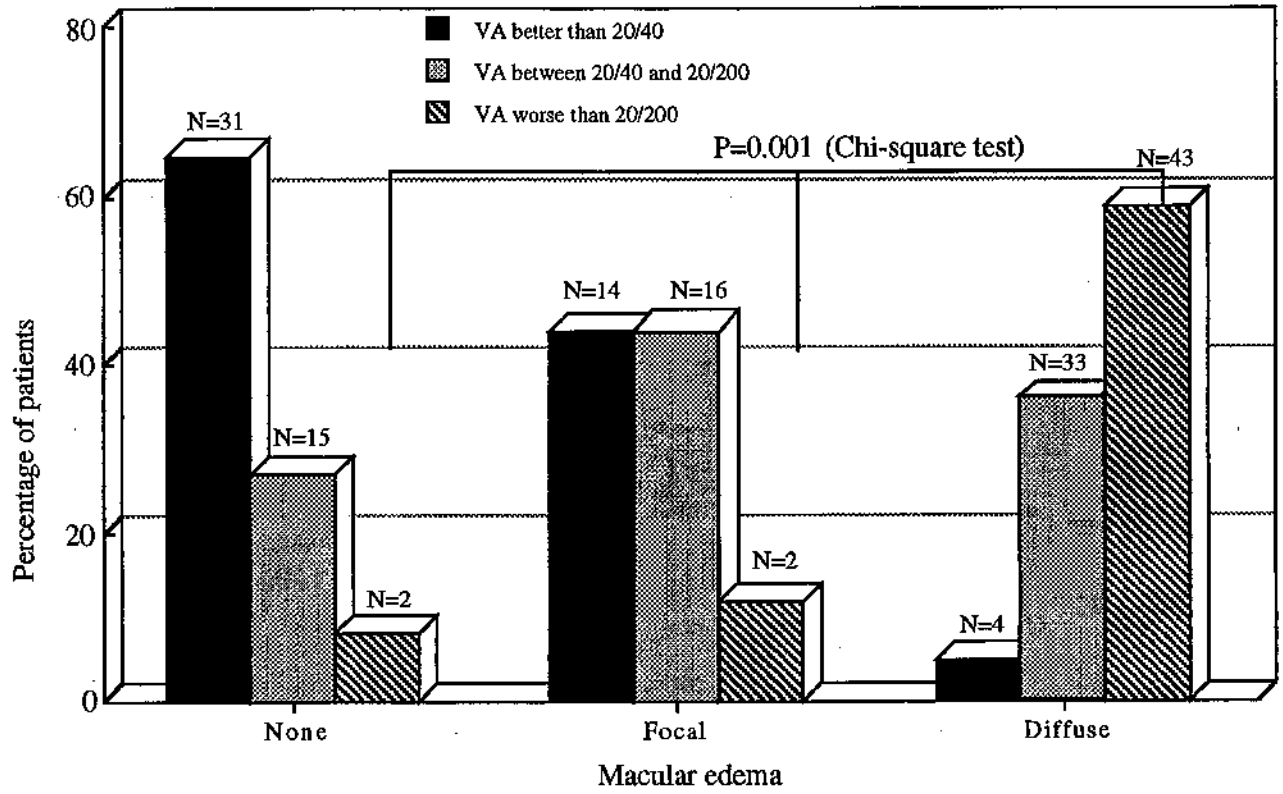
	Macular edema			
Degree of DR	*None* *n (%)*	*Focal* *n (%)*	*Diffuse* *n (%)*	*P*
Very early DR	16 (100)	0	0	
NPDR	15 (36.0)	15 (36.0)	11 (28.0)	
PPDR	6 (21.0)	6 (21.0)	16 (58.0)	0.04*
PDR	12 (16.0)	13 (17.0)	50 (67.0)	
0.0001*				

DR = diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PPDR = preproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; *: statistically significant

Table 4. Vitreoretinal interface and the severity of diabetic macular edema in 74 studied patients.

Vitreoretinal interface	*Macular edema*			
	None	*Focal*	*Diffuse*	*Total*
	n (%)	*n (%)*	*n (%)*	*n*
Complete posterior vitreous attachment	15 (32%)	7 (15%)	25 (47%)	47
Vitreomacular adhesion only	1 (7%)	2 (14%)	11 (79%)*	14
Posterior vitreoretinal separation	1 (8%)	6 (46%)	6 (6%)	13
				74

*P=0.02 (Chi-square test) for comparison between patients with complete posterior vitreous attachment and posterior vitreous separation.


VA better than 20/40
VA between 20/40 and 20/200
VA worse than 20/200
P=0.001 (Chi-square test)
Percentage of patients
80
60
40
20
0
N=31
N=15
N=2
N=14
N=16
N=2
N=4
N=33
N=43
None
Focal
Diffuse
Macular edema

Erythrocyte sodium-lithium countertransport and proliferative diabetic retinopathy

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ABSTRACT

Purpose: To investigate whether elevated erythrocyte Na^+/Li^+ countertransport (Na^+/Li^+ CT) activity is present in patients with proliferative diabetic retinopathy (PDR).

Methods: The rate of Na^+/Li^+ CT activity assayed in 21 patients with type 1 *diabetes mellitus* (DM) presenting PDR was compared with 10 patients with non-proliferative retinopathy (NPDR) and with 11 patients with normal fundi. Twelve normal volunteers with no family history of hypertension were used as a control group. The albumin excretion rate was determined by nephelometry and the glomerular filtration rate was measured by the plasma clearance of eidetic acid labeled with chromium-51.

Results: Patients with PDR showed higher diastolic blood pressure levels (mean ± SD) compared to those with NPDR or normal fundi (95±13 vs 90±09 and 82±19 mmHg, p=0.02, respectively). The albumin excretion rate was higher [geometric mean (range)] and the glomerular filtration rate was lower (mean ±SD) in patients with PDR than in those with NPDR or normal fundi [333 (2-5140) vs 32 (5.9-2200) and 6 (1.5-306) µg/min, p=0.01, and 63±33 vs 99±37 and 93±43 mL/min p=0.02, respectively]. The mean Na^+/Li^+ CT in patients with PDR was significantly higher than in patients with NPDR or normal fundi and control group (0.46±0.20 vs 0.32±0.12, 0.32±11 and 0.21±0.07 mmol/L RBC/h, respectively, p=0.0001). In a multiple logistic regression analysis, with PDR as the dependent variable, Na^+/Li^+ CT (odds ratio (OR): 4.7, confidence interval (CI): 1.2-17.6, p=0.02), diastolic blood pressure (OR: 3.4, CI: 1.3-9.6, p=0.018) and glomerular filtration rate (OR: 5.1, CI: 1.6-17.7, p=0.007) were the only variables which were maintained in the equation, indicating that they were the main determinants of PDR.

Conclusion: Patients with type 1 DM and proliferative retinopathy have elevated erythrocyte Na^+/Li^+ CT.

INTRODUCTION

Sodium-lithium countertransport activity (Na^+/Li^+ CT) in red blood cells has been found to be abnormal in subjects with essential hypertension[1]. The role of Na^+/Li^+ CT in cell physiology is still not clear, although this transport bears similarities to the physiological sodium-hydrogen exchanger [2]. The Na^+/H^+ exchanger is a membrane transporter that regulates intracellular pH, cellular volume and growth and bicarbonate reabsorption by the proximal tubules in the kidney[2]. In patients with type 1 *diabetes mellitus* (DM), cross-sectional studies have demonstrated that Na^+/Li^+ CT is associated with micro-macroalbuminuria [3-5]. This finding was recently confirmed by a prospective study[6]. Since this abnormality of cell membrane transport is probably the best reproducible cellular cation transport abnormality phenotype associated with essential hypertension in caucasian individuals[1], it has been suggested that a genetic predisposition to essential hypertension is an important factor in the susceptibility to diabetic renal disease[3-5]. To our knowledge, no study have investigated if elevated Na^+/Li^+ CT is present in patients with PDR.

The aim of the present study was to compare the rate of erythrocyte Na^+/Li^+ CT activity in patients with type 1 DM presenting PDR to that observed in patients with NPDR or normal fundi, and to explore the interactions between this abnormality in cellular transport and other risk factors for PDR.

METHODS

Patients

We estimated that to detect a difference of 0.20± 0.18 mmol/L RBC/h, that has been reported in the literature for caucasian diabetic patients with and without microvascular

complications[3], a sample size of 17 patients in the studied group will have a 90% power to detect that difference with a two-tailed significance level of 0.05.

From January 1997 to July 1998, consecutive patients with type 1 DM attending the diabetic outpatient clinic at the University Hospital of the State University of Campinas were asked to participate in the study. The criteria for inclusion in the study were patients with type 1 DM, defined as age at diagnosis less than 35 years, a history of sudden onset of severe hyperglycemic symptoms, marked weight loss, spontaneous sustained ketosis or ketonuria, an age ranging from 18 to 45 years old, caucasian, with DM for at least 10 years (to allow enough time for developing of diabetic retinal disease) and free of any endocrine, hepatic, metabolic, or cardiac disease, and non-diabetic renal disease. The exclusion criteria were pregnancy, use of contraceptives or estrogen. Such criteria were deemed appropriate to exclude patients with factors which may influence the determination of Na^+/Li^+ CT[7]. Patients with high myopia, chorioretinitis scars, posterior uveitis and glaucoma were also excluded, as were those who had undergone a previous ocular surgery, since such events may influence the development of diabetic retinopathy[8]. Of the potential 80 patients for the study attended at the outpatient clinic, 38 (48 %) did not meet the inclusion and exclusion criteria. The remaining 42 patients gave their informed consent prior to participating in the study. Twelve normal volunteers with no family history of hypertension were used as a control group. The study was carried out in conformity with the tenants of the Declaration of Helsinki, which was approved by the Ethics Committee of the University Hospital. All patients were treated with insulin and 17 (41%) who were taking antihypertensive drugs were asked to discontinue them for 48 h before the protocol.

Clinical and ophthalmological measurements. The patients attended the outpatient clinic after an overnight fast. Blood pressure was measured twice, in the supine position, by a single observer (JMLF), to the nearest 2 mmHg, using a standard cuff, five minutes apart after the subject had been resting for at least five minutes. Mean systolic and diastolic (fifth

Korotkoff's sound) blood pressures were obtained by averaging the two measurements. A blood venous sample was taken for the measurements of red blood cell (RBC) Na^+/Li^+ CT activity and biochemical analysis. A complete ophthalmologic examination was then performed, including indirect fundus ophthalmoscopy with stereoscopic color fundus photographs of seven standard fields of both eyes. The stereoscopic color photos were examined by a single observer (JMLF) unaware of the patient's Na^+/Li^+ CT status. The level of DR was classified following the ETDRS retinopathy severity classification[9]. The eye with the most severe level of DR in each subject was considered for analysis[10]. Eyes presenting level 1, or levels from 2 to 5, or levels from 6 to 8 were grouped into *normal fundi* or *non-proliferative diabetic retinopathy* (NPDR) or *proliferative diabetic retinopathy* (PDR), respectively.

Methods

Red blood cell Na^+/Li^+ CT activity was measured according to the original method described by Canessa et al[1], as previously reported by our laboratory[11]. In our laboratory, the normal range for normotensive subjects with negative familial history of hypertension (n=12) was 0.21±0.07 mmol/L RBC/h , ranging from 0.12 to 0.38 mmol/L RBC/h[11]. The intraindividual and interassay variations were approximately 9.2%, a value similar to that reported previously.

Other measurements. Three consecutive timed overnight urine samples per patient were collected for albumin measurement. Albumin concentrations were determined by nephelometry. The median value of the three specimens was used for classifying the patients into three categories: normoalbuminuric (AER≤20 μg/min), microalbuminuric (20 < AER ≤ 200 μg/min) and macroalbuminuric (AER > 200 μg/min). GFR was measured by the plasma

clearance of eidetic acid labeled with chromium-51 (^{51}CR-EDTA). For comparison between patients, GFR was corrected for body surface area (1.73 m^2). The percentage of the A_{1c} component of glycosylated hemoglobin was assessed by high performance liquid chromatography. Normal values in our laboratory were 5.6±1.3%. Serum creatinine, total cholesterol and triglycerides were measured by an automated method. In our laboratory, the normal values for serum cholesterol and triglycerides were up to 5.20 and 2.30 mmol/L, respectively.

Statistical analysis. Clinical data and laboratory determinations were compared for significance using *Analysis of Variance* (ANOVA), *Fisher* and *Schefe F* tests from a commercial software package (SPSS® for Power Macintosh). Because of the interrelationship of several variables, the univariate logistic analysis was followed by multiple logistic regression analysis, with PDR as the dependent variable. The results were expressed as the mean ± standard deviation (SD), unless otherwise stated. The null hypothesis was rejected below the conventional (two-tailed) 0.05 level.

RESULTS

Among the studied group, 21 patients had proliferative DR (PDR), 10 patients presented non-proliferative DR (NPDR) and 11 patients had normal fundi. The demographic and laboratory characteristics of the patients are summarized in table 1. The three groups of patients had similar distributions for sex, age, body mass index (BMI), duration of DM and systolic blood pressure. However, patients with PDR had diastolic BP significantly higher than those with NPDR and normal fundi (95±13 vs 90±09 and 82±19 mmHg, p=0.02, respectively) and were more likely to be on antihypertensive therapy (p=0.001) (table 1). The metabolic control, evaluated by HbA_{1c} levels, was similar in all groups of studied

patients (table 1). Albumin excretion rate (AER) [geometric mean (range)] was higher and GFR (mean ± SD) was lower in patients with PDR than in those with NPDR and normal fundi (333 (2-5140) vs 32 (5.9-2200) and 6(1.5-306) μg/min, p=0.01, and 63±33 vs 99±37 and 93±43 mL/min p=0.02, respectively). The frequency of patients with micro or macroalbuminuria was higher in patients with PDR (80%) than those with NPDR (40%) and normal fundi (18%) PDR, p=0.001. In addition, the mean level of the total cholesterol was significantly higher in patients with PDR than in the other groups (6.53±1.80 vs 5.10±0.80 and 4.8±1.2 mmol/L, p=0.008).

Figure 1 shows the individual values of the erythrocyte Na^+/Li^+ CT activity. The mean value for Na^+/Li^+ CT activity in patients presenting PDR was significantly higher than that observed in patients with NPDR, normal fundi or control group (0.46±0.20 vs 0.32±0.12, 0.32±11 and 0.21±0.07 mmol/L RBC/h, respectively, p=0.0001). This difference in Na^+/Li^+ CT activity remains significant even when the micro and macroalbuminuric patients were excluded from the calculation [0.55±0.29 vs 0.32±0.09 and 0.34±0.12 mmol/L RBC/h, $p<0.05$ for patients with PDR (n=4), NPDR (n=6) and normal fundi (n=9), respectively], suggesting that difference in mean Na^+/Li^+ CT activity cannot be accounted solely by this group of patients. The presence of laser therapy had no effect on Na^+/Li^+ CT since the activity of this cation transport was similar in patients with PDR that had laser therapy (n=8) and those without laser therapy (n=13) (0.48±0.21 vs 0.42±0.20 mmol/L RBC/h, p=0.60). The Na^+/Li^+ CT activity was above the upper limit of normal (>0.38 mmol/L RBC/h) in 12 out of 21 patients with PDR (58%) and in 2 out of 10 patients with with NPDR (20%) and in 2 out of 11 patients with normal fundi (18%) (p=0.03).

To determine the relationship between PDR with other variables (serum creatinine, albumin excretion rate, GFR, cholesterol, blood pressure and Na^+/Li^+ CT activity) in the univariate analyses, binary logistic regression models were used while controlling for

multiple potential cofounders. Subsequently, a multiple logistic regression analysis was carried out, with PDR as the dependent variable. In this model, Na^+/Li^+ CT (odds ratio (OR): 4.7, confidence interval (CI): 1.2-17.6, p=0.02), diastolic blood pressure (OR: 3.4, CI: 1.3-9.6, p=0.018) and glomerular filtration rate (OR: 5.1, CI: 1.6-17.7, p=0.007) were the variables which were maintained in the equation, indicating that they were the main determinants of the presence of PDR (table 2).

DISCUSSION

We have demonstrated, for the first time, that patients with type 1 DM and proliferative retinopathy have elevated erythrocyte Na^+/Li^+ CT activity. The more severe retinopathy in these patients with increased Na^+/Li^+ CT activity cannot be attributed either to the duration of diabetes nor to the level of metabolic control as indexed by glycated hemoglobin values since these parameters did not differ between the three group of patients. This observation is important since it may provide further insights into the pathogenesis and susceptibility to this complication of *diabetes mellitus*.

At present, the nature of the association between proliferative diabetic retinopathy and elevated Na^+/Li^+ CT is unclear. We have observed that patients with type 1 DM, proliferative diabetic retinopathy and elevated Na^+/Li^+ CT also have a higher frequency of diabetic nephropathy (micro/macroalbuminuria). Although this finding represents a cofounding factor, this is not surprising since in these patients a close association between proliferative diabetic retinopathy and micro and macroalbuminuria, abnormalities associated with elevated Na^+/Li^+ CT [3-5], has been established by large epidemiological studies[12]. However, we believe that the increased Na^+/Li^+ CT observed in our patients cannot be

accounted solely by the presence of nephropathy. In agreement with this hypothesis, the mean Na^+/Li^+ CT rates remained higher in patients with proliferative diabetic retinopathy even when patients with micro or macroalbuminuria were eliminated from the analysis, though the number of patients in the group with PDR was quite small.

In summary, patients with type 1 DM presenting PDR have a higher mean erythrocyte Na^+/Li^+ CT activity than patients without PDR. Further studies are needed with a larger number of patients to determine the validity of this preliminary observation.

ACKNOWLEDGMENTS

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LEGEND OF FIGURE

Figure 1: Rates of red blood cell sodium-lithium countertransport (Na^+/Li^+ CT) in 21 patients with type 1 *diabetes mellitus* with proliferative diabetic retinopathy (PDR), 10 patients with non-proliferative diabetic retinopathy (NPDR), 11 patients with normal fundus and in 12 normal volunteers without family history of essential hypertension used as a control group.

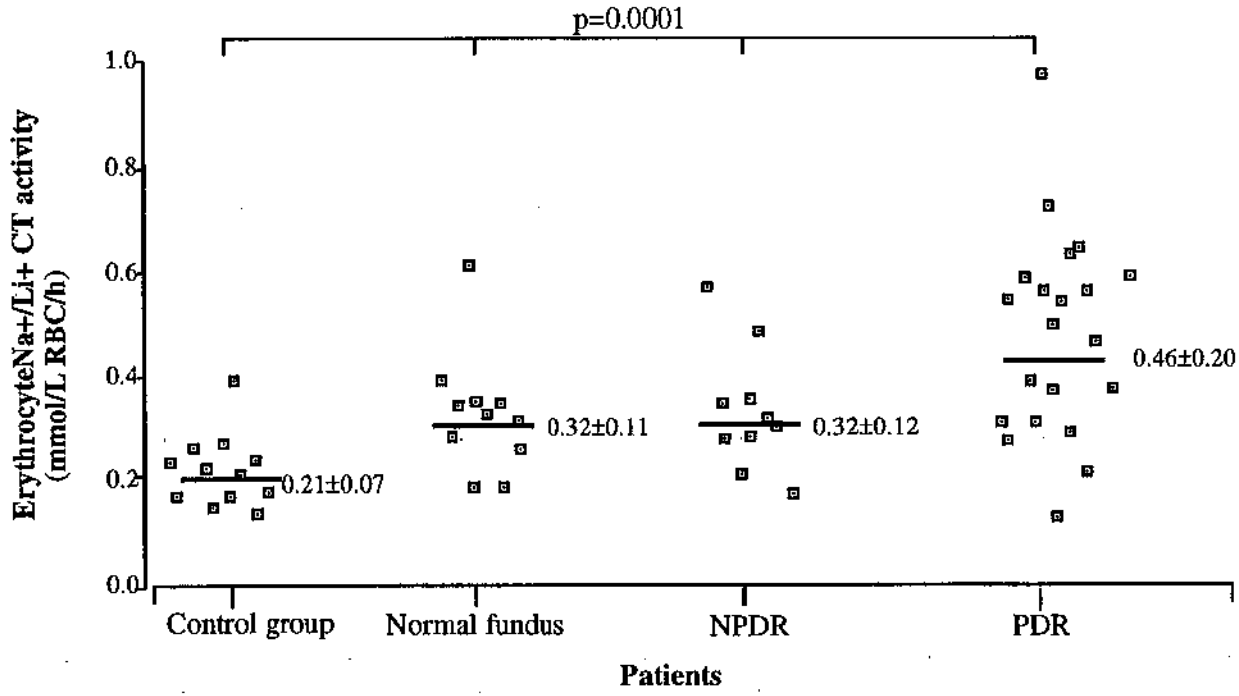
p=0.0001
1.0
0.8
0.6
0.4
0.2
0.0
ErythrocyteNa+/Li+ CT activity
(mmol/L RBC/h)
0.21±0.07
0.32±0.11
0.32±0.12
0.46±0.20
Control group
Normal fundus
NPDR
PDR
Patients

Table 1. Clinical and laboratory characteristics of the studied patients.

Variables	with PDR	with NPDR	normal fundus	p
Sex (M:F)	11:10	7:3	6:5	0.75
Age (yrs)	33±9	37±7	37±8	0.28
BMI (kg/m^2)	23.9±4.2	24.7±3.5	25.2±3.7	0.63
Duration of DM (yrs)	18±6	18±5	15±4	0.29
Antihypertensive therapy (P:N)	11:10	5:5	1:10	0.001*
Systolic BP (mmHg)	141±34	141±18	127±19	0.33
Diastolic BP (mmHg)	95±13	90±09	82±19	0.02*
Glycated hemoglobin A_{1c} (%)	9.3±2	8.1±2.5	8.4±2.7	0.32
Serum creatinine (µmol/L)	124±79	74±12	70±10	0.02†
Cholesterolemia (mmol/L)	6.53±1.80	5.10±0.80	4.8±1.20	0.008†
Triglyceridemia (mmol/L)	4.8±4.8	2.5±1.3	5.4±6.5	0.36
AER (µg/min)‡	333(2-5140)	32(5.9-2200)	6(1.5-306)	0.01†
Normo/micro/macroalbuminuria (n)	4/1/16	6/2/2	9/1/1	0.001
GFR (mL/min)	63±33	99±37	93±43	0.02†

PDR: proliferative diabetic retinopathy; NPDR: non-proliferative diabetic retinopathy; M: male, F: female, P:positive and N: negative for antihypertensive therapy; BMI:body mass index= [weight (kg)/height ^{2} (m^2)], BP: blood pressure; AER: albumin excretion rate; GFR: glomerular filtration rate; *: comparison between PDR and normal fundus; †: comparison between PDR and normal fundus and PDR and NPDR; ‡: geometric mean (range).

Table 2. Variables that appeared as main determinants of proliferative diabetic retinopathy in the multiple logistic regression model.

Variables	*Odds ratio*	*95% CI*
Na^+/Li^+ CT	4.7	1.2-17.6
Diastolic BP	3.4	1.3 - 9.6
GFR	5.1	1.6 - 17.7

APÊNDICE

Introdução à eletrofisiologia da visão

As funções visuais podem ser estimadas por método subjetivo ou objetivo. Estes testes são psicofísicos (sensitividade ao contraste, visão de cores, função de recuperação macular) ou eletrofisiológicos (eletrorretinografia e potencial visual evocado). A mácula, que possui a maior densidade de fotoreceptores (cones), é responsável pelas funções visuais mais significativas como acuidade visual, visão de cores e sensitividade ao contraste. Assim, estes testes demonstram uma boa correlação com a função macular.

O teste de sensitividade ao contraste é um método psicofísico de avaliação visual que provê informações sobre a discriminação de contraste. Tem sido demonstrado que a medida da sensitividade ao contraste pode dar informações que não são acessíveis pelas medidas-padrão de acuidade visual. Esta dissociação entre a acuidade visual e a sensitividade ao contraste sugere que ambas representem funções visuais diferentes.

O conceito de função de sensitividade ao contraste (FSC) surgiu com Campbell e Robson (Campbell 1968). A FSC é importante porque ela revela a habilidade visual do indivíduo em ambiente normal de baixo e médio contraste onde os objetos estão. A FSC teve um importante papel no diagnóstico e entendimento de processos patogênicos em doenças com alterações do sistema visual, como a esclerose múltipla (Regan *(a)* 1977), ambliopia (Hess 1977), doenças de retina (Wolkstein 1980), galucoma (Regan 1984) e o *diabetes melitus* (Sokol 1985). O denominador comum nestas diferentes doenças é a dissociação entre a medida de acuidade visual e a sensitividade ao contraste. A FSC pode ser avaliada através de métodos psicofísicos ou eletrofisiológicos. Estes últimos constituem-se dos potenciais visuais evocados (PVE). O conceito de potencial visual evocado de estímulo padrão (PVER) foi introduzido por Spekreijse há mais de 30 anos (Spekreijse 1966), com a vantagem de ser um método não invasivo, objetivo, e portanto exequível na população pediátrica, não-verbal ou em pacientes não cooperativos. Desde o original trabalho de Spekreijse, muitos estudos têm demonstrado a reprodutibilidade dos potenciais visuais evocados a partir de estímulos padrão (PVER) (Wright 1987).

Nas vias ópticas, as respostas visuais são processadas numa série de múltiplos canais, sendo cada canal sensível a uma determinada frequência espacial (Enroth-Cugell 1966, Campbell 1968). Cada canal pode ser representado por um único grupo de neurônios, sendo que as atividades dos subgrupos de células ganglionares respondem a determinados estímulos visuais. A partir desses subgrupos de células ganglionares, é gerado no nervo óptico e em áreas corticais visuais os sistemas magno e parvo celulares para a detecção de estímulos de baixa e alta frequências espaciais, respectivamente (Merigan 1991).

Um novo método para registrar os PVER, o *sweep* PVER (SPVER) foi introduzido por Regan (Regan *(b)* 1977). Esta técnica utiliza uma série de estímulos de diferentes frequências espaciais apresentada sequencialmente. Similarmente, com uso de um algoritmo com diferentes níveis de contrastes apresentado sequencialmente, a avaliação da sensitividade ao contraste é possível de modo rápido e objetivo, como ocorre com o contraste SPVER (Norcia 1989, Lopes de Faria 1998).

Classicamente, presume-se que as alterações iniciais da RD ocorram nos pequenos vasos da retina, com a perda de pericitos, espessamento da membrana basal e subsequente desenvolvimento de capilares acelulares. Apesar das alterações visuais funcionais em pacientes com *diabetes mellitus* serem desconhecidas, evidências sugerem que anomalias neurosensoriais precedam, ou mesmo contribuam para o desenvolvimento da RD e da neuropatia óptica diabéticas. As alterações funcionais na retina neural causadas pelo DM podem levar a diminuição da visão em ambientes com baixa luminosidade, mesmo com acuidade e campo visuais normais. Esta complicação pode estar relacionada com a diminuição da detecção do contraste em frequências espaciais baixas (Hyvrinen 1983). Assim, em pacientes com DM, a FSC revela alterações subclínicas da função macular melhor que a avaliação de acuidade visual e pode ser, portanto, útil na detecção dos efeitos precoces da RD nas camadas neurais da retina e vias ópticas (Yamazaki 1982; DiLeo 1992). Além da alteração da sensitividade ao contraste, outras funções visuais estão alteradas em pacientes diabéticos com acuidade visual normal e sem retinopatia, como a visão de cores (Kinnear 1972), eletrorretinografia (Bresnick 1984), e estudos de potenciais visuais evocados (PVE) (Puvanendran 1983; Pozzessere 1988; Algan 1989; Moreo 1995). Assim, testes psicofísicos e eletrofisiológicos da visão podem ser úteis no diagnóstico de disfunções macular e neural das vias ópticas em pacientes diabéticos sem retinopatia.

Estudos anátomo-patológicos vêm documentando desmielinização e degeneração do quiasma óptico em pacientes diabéticos, sugerindo comprometimento de sistema nervoso central em casos avançados de retinopatia diabética (Reske-Nielsen 1965). Mais recentemente, Kamijo (Kamijo 1993) demonstrou que, similarmente à neuropatia periférica, existe atrofia axonal e disjunção axo-glial na neuropatia óptica diabética humana.

Adicionalmente, com o objetivo de se avaliar as camadas neurais da retina diabética, Barber e colaboradores demonstraram que em ratos espontaneamente hipertensos (SHR) diabéticos ocorre aumento do número de células em apoptose na retina neural em comparação com o grupo controle normal (Barber 1998). Este achados indicam que a neurodegeneração pode ser um importante fenômeno na fisiopatogênese da retinopatia diabética.

Objective Measurement of Contrast Sensitivity Function Using Contrast Sweep Visual Evoked Responses

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Abstract

Aim/background: The contrast sensitivity function (CSF) measurement provides information that is not accessible by standard visual acuity measurements. In order to measure CSF objectively in the clinical practice, we used the contrast sweep pattern reversal visual evoked responses (CSVER) technique.

Methods. We measured the contrast thresholds in five spatial frequencies in 10 normal subjects. The contrast sweep PVER (CSVER) were recorded with sinusoidally modulated vertical gratings at 10 contrast levels, 96, 64, 48, 32, 16, 8, 4, 2, 1, and 0.5%, presented in five spatial frequencies, 0.5, 1.0, 2.0, 4.0, and 8.0 cycles per degree. Each of 10 contrast levels was displayed for 2 seconds at the spatial frequencies. The CSVER amplitudes at the second harmonic were calculated by discrete Fourier transform. The results were compared with those obtained using a psychophysical method.

Results. Findings showed an inverted-U shaped CSF peaking at 2.0 cycles per degree with a contrast sensitivity of 34.5 (contrast, 2.9%). The electrophysiologically assessed CS was 0.62 to 0.79 log unit lower than the sensitivity measured using the psychophysical method. However, the overall shapes were highly correlated.

Conclusion. With CSVER, one can objectively measure CSF and it may be useful in patients in whom the psychophysical method is limited.

Introduction

The contrast sensitivity function (CSF) is a basic measurement of human spatial vision that provides a clinical evaluation of visual function over a wide range of spatial frequencies. CSF measurement is important because it reflects the subject's visual ability in his or her low-contrast living environment in which there are numerous objects. The CSF concept, developed by Campbell and Robson,[1] plays an important role in psychophysical[1] and electrophysiological[2] studies of the visual system. In addition, CSF measurements have contributed to the diagnosis and understanding of visual disorders in diseases such as multiple sclerosis, amblyopia, and diabetes mellitus.[3-7]

The CSF measurement provides information that is not accessible by standard visual acuity measurements typically obtained with high-contrast optotypes.[6-9] The clinical application of CSF in ophthalmology has been delayed because of the complexity of the method that limited it to use with alert and cooperative subjects. Recently, some studies have demonstrated much simpler and less time-consuming psychophysical CSF methodologies.[7,10] However, when assessing vision in a pediatric population or in nonverbal or mentally deficient patients in whom these tests may not be effective, an "objective" test for measuring CSF is desirable. The pattern reversal visual evoked response (PVER),[11] which primarily reflects macular function [12-16] is useful for CSF measurement.[17-20] Although the results of electrophysiological measurements of CSF roughly parallel the psychophysical measurements, the methodology using the standard PVER for CSF measurement is time-consuming, the CSF derived from VER is less sensitive than the psychophysical test [18,21] in addition to the irregularities of the VER signals[22], making the test impractical for clinical use.

The spatial frequency sweep PVER (SPVER), which was introduced by Regan[23] and later by Tyler et al.,[24] allows visual sensory threshold evaluation within a short time.

Although the spatial frequency sweep PVER analyze the visual function in the spatial domain, this algorithm can be used to analyze the visual function at the contrast domain by sweeping the stimulus at different contrast levels. In the present study, instead of sweeping spatial frequencies, a large number of contrast stimuli were displayed consecutively without interruption for 20 seconds at each spatial frequency. We thus obtained the contrast threshold at each spatial frequency within a very short time. This methodology is referred to here as contrast sweep VER (CSVER).

In this study, we measured the contrast threshold of normal adults derived from VER (CSVER), analyzed the effect of contrast ratio on the amplitude-response at different spatial frequencies, and compared the findings with the subjective (conventional) CSF.

Materials and Methods

Patients

Ten ophthalmologically normal volunteers (6 men, 4 women; age range, 25 to 48 years) participated in this study. All subjects had a corrected visual acuity of 20/20 or better at the time of the recording. Each subject underwent monocular electrophysiological and psychophysical testing of a randomly selected eye with an undilated pupil in a dark room. Before the measurements were recorded, the procedures were fully explained to each subject, and informed consent was obtained in all cases. This study was carried out in conformity with the tenets of the Declaration of Helsinki.

Recording CSVER

The stimulus pattern was displayed on 19-inch, high-resolution television monitor (P7A24K-931, Pixelink) with a spatial resolution of 960 horizontal raster lines. The overall

stimulus field was 25 cm x 25 cm. The stimulus field size subtended a visual angle of 14° x 14° at the testing distance of 100 cm. The mean luminance was maintained at 50 cd/m^2. The CSVER was recorded using standard electroencephalogram cup occipital electrodes positioned at Oz/Pz (the electrodes were placed anterior to the inion, Oz at 10% of the inion-nasion measurement and Pz at 30% of inion-nasion measurement), amplified with a 0.5 to100 Hz bandwidth isolated differential amplifier (Model Enfant 4010, Neuroscientific Corp., Farmingdale, NY), digitized at 180 Hz, and harmonically synchronized (phase-coherent) to the stimulus presentation. The digitized samples were divided into analysis records (epochs) of 180 points each (i.e., 1 second). Each record was analyzed using discrete Fourier transform (DFT), which values were vector-averaged and converted into polar form to arrive at the value of magnitude and phase for the second harmonic frequencies (12 Hz) of the mean value of the Fourier coefficients. Sinusoidally modulated vertical gratings of 10 different contrast levels, ranging from the highest contrast level (96%) to the lowest (0.5%) were swept at five spatial frequencies: 0.5, 1.0, 2.0, 4.0, and 8.0 cycles per degree (cpd). The contrast levels measured were 96, 64, 48, 32, 16, 8, 4, 2, 1, and 0.5%. The pattern reversal rate was fixed at 12 reversals per second (6 Hz). Each of 10 contrast levels was displayed for 2 seconds, for a total recording time of 20 seconds at each of the five spatial frequencies. The mean contrast threshold and the 95% confidence interval (CI) were calculated using the manufacturer's software. The results were displayed on a video monitor of a personal computer immediately.

Determining Contrast Threshold Electrophysiologically

The contrast threshold was determined as follows [25]: after finishing the recordings, two points were manually selected for analysis, one at the highest contrast level that records the first peak of PVER amplitude and the other at the lowest contrast level which produces

the minimum but separate from the noise level on the descending curve of the CSVER amplitude-contrast function. Derived from an extensive empirical data set, any evoked response is at least three times greater than the average noise level and the phase of response is steady (within 20 degrees) or slightly leading the stimulus (no more than 90 degrees of phase shift between points).[26] The best-fit regression line (within 95% confidence interval, because the CSVER function is not always a simple linear function of log contrast) was then drawn by the Enfant system between these two points including all datapoints between them. The contrast threshold was determined on the intersection of the linear regression line with zero microvolts. The determination of the contrast threshold at each spatial frequency generally takes 30 to 60 seconds.

Measuring Psychophysical CSF

The gratings used were sinusoidally modulated and generated under computer control on a cathode-ray display. Luminance of 50 cd/m^2 was constant during the test. Using a 12 x 12 cm screen, a 6.8° stimulus field size was obtained when viewed at a distance of 100 cm from the screen and a 5° stimulus field size at a distance of 300 cm. Sinusoidally modulated gratings of 31 contrast levels, ranging from 98% to 0.2% were displayed in five spatial frequencies (0.5, 1.0, 2.0, 4.0, 8.0 cpd) in gratings oriented at 90∞ (vertical), 45∞ (slanted to right) and 135∞ (slanted to left) which levels were presented depending on the subject's response, by indicating the orientations using a 3-alternative, forced-choice algorithm (i.e., staircase procedure:[27] each correct response decreased the contrast level 0.1 log unit and each incorrect response increased the contrast level by 0.2 log unit), under a pseudorandom order. Threshold was computed as the contrast required for correct choices at a probability of 0.84%.

Statistical Analysis

The intersubject homogeneity of CSVER amplitude under different contrast conditions was tested via single-factor repeated measures of analysis of variance (ANOVA). When the test was highly significant, the Scheffe's test of multiple comparison was applied. A P value < 0.05 was considered statistically significant.

Results

Figure 1A-D shows the CSVER amplitudes at 0.5, 2.0, 4.0, and 8.0 cpd with 10 stimulus conditions from the same normal subject. The PVER amplitude-contrast function showed the mean value of the PVER amplitude with 95% CI, calculated with the DFT program. The contrast thresholds obtained were 0.96,1.67, 1.59, and 5.01% at 0.5, 2.0, 4.0, and 8.0 cpd, respectively. The largest CSVER amplitude was recorded at 4.0 cpd, peaking at 48% of contrast (5.78 μv of PVER amplitude).

Figure 2 shows the PVER amplitude (μv) contrast stimulus level (%) function at 0.5, 1.0, 2.0, 4.0, and 8.0 cpd. The amplitude was the mean of the values of 10 normal subjects. There was a general tendency for the CSVER amplitude to increase as the contrast increased. At 0.5 cpd, the CSVER amplitude increased continuously. However, at 1.0 to 8.0 cpd, the CSVER amplitude increased to the 64% contrast level, at which point the amplitude responses then decreased with a further increase of stimulus contrast. As a result, a high band pass filter curve was obtained.

The CSVER amplitudes (mean ± SD) of 10 different contrast stimuli (0.5 to 96%) at five spatial frequencies from 10 normal subjects are shown in Figure 3A-E. At 0.5 cpd, the overall F values from the repeated ANOVA measures to test the homogeneity of responses

were highly significant at contrasts up to 32% (F=4.08, *P*=0.0001). Statistically, at contrast levels higher than 32%, the CSVER amplitude contrast stimuli function showed no difference. Similar findings were observed at 1.0, 2.0, 4.0, and 8.0 cpd up to 32, 32, 32, and 48% of contrast, respectively (F=5.47, *P*=0.001; F=6.59, *P*=0.001; F=4.63, *P*=0.0001; F=3.86, *P*=0.0002, respectively). At those levels of contrast, we observed the saturation phenomena as a feature of CSVER.

Figure 4 shows the mean CSVER amplitudes of five spatial frequencies for the 10 contrast levels. A very distinct high pass filter function was observed at contrasts of 16 to 96%, peaking at 4.0 cpd with 64% contrast. At lower contrast levels (0.5 to 8%), this response pattern was not observed: at 8% and 4% of contrast, the peak was observed at 2.0 cpd; at 2%, 4.0 cpd; and at 1% and 0.5%, a low pass filter curve was seen, peaking at 1.0 cpd. From 16 to 96%, the PVER amplitudes were higher than in PVER derived from contrasts of 8% to 0.5% (approximately 0.5 μv of amplitude voltage), in which the flattened shape is evident.

The mean electrophysiologic (CSVER) values compared with the psychophysical sensitivities are showed at Figure 5. The curves have essentially the same band pass filter shape with the electrophysiologic CSF lower by 0.65, 0.68, 0.79, 0.76, and 0.62 log unit at 0.5, 1.0, 2.0, 4.0, and 8.0 cpd, respectively. Both peaked at 2.0 cpd with the electrophysiologic contrast threshold at 2.9% of contrast (CS, 34.5) and the psychophysical contrast threshold at 0.45% of contrast (CS, 222). The mean and the intersubject variations were 1.26 ± 0.175 at 0.5 cpd, 1.522 ± 0.247 at 1.0 cpd, 1.532 ± 0.218 at 2.0 cpd, 1.482 ± 0.255 at 4.0 cpd and 1.157 ± 0.202 at 8.0 cpd in the electrophysiologic CSF, and 1.91 ± 0.082 at 0.5 cpd, 2.2 ± 0.072 at 1.0 cpd, 2.321 ± 0.107 at 2.0 cpd, 2.242 ± 0.202 at 4.0 cpd, and 1.78 ± 0.286 at 8.0 cpd in the psychophysical method.

The statistical correlation between the individual values of both contrast thresholds (psychophysical and electrophysiological) is showed as a scattergram (Figure 6) at 0.5, 1.0,

2.0, 4.0, and 8.0 c.p.d. of ten normal subjects. The solid line represents the linear regression between both of them. The correlation coefficient for CSVER vs. psychophysics was approximately 0.89 indicating that the contrast threshold derived from the VER provides good prediction of conventional (psychophysical) threshold under similar conditions ($p=0.0001$).

Discussion

The importance of the contrast sensitivity (CS) evaluation lies in its ability to detect visual function abnormalities in patients who, despite a good Snellen acuity, complain of visual disturbances. These patients often complain of "misty" vision. Isolated CS losses are present in certain diseases, such as optic nerve disease, and often the loss is more prominent and disturbing to the patient than a decrease in visual acuity. Clinical applications of CSF are found in review articles by Sekuler,[28] and DeValois and DeValois.[29]

Campbell and Robson[1] interpreted their CSF results (psychophysically) as the existence of "multiple or separate channels" within the visual system, which are selectively sensitive to a narrow spatial frequency and the resultant CSF was an inverted U-shape curve, peaking at approximately 5.0 cpd. The concept of separate channels for different spatial frequencies is in a sense a restatement of the fact that the retina is not uniform. Channels may be the expression of single classes of neuron function. Activities of ganglion cell subpopulations of the visual pathway can be isolated by different spatial frequencies. There has been much speculation concerning the source of the central/peripheral field origin[11] to cortical manifestation of the magnocellular and parvocellular pathways,[21,30-32] to their representing separate motion and pattern discrimination mechanisms. [33] Only the fovea is specialized for high visual acuity and must, therefore, process all information involving high

spatial frequencies. In the retinal periphery, only lower frequency channels are represented.[7] The clinical value of this test is that it allows rapid assessment of peripheral (low spatial frequencies) and central retinal function (high spatial frequencies).

In the present study, we estimated the CSF values derived from the steady-state visual evoked potentials (VEPs) and psychophysically under similar conditions. In electrophysiologic recordings, we observed the saturation phenomena as one feature of the contrast VER. It may be influenced by several conditions such as temporal and spatial frequencies, the mean retinal luminance, the type of pattern, and the size of the pattern elements.[34] The contrast VER recordings showed the saturation phenomena at 32% or 48% of contrast at the spatial frequencies tested; these data are supported by previous studies.[12,34] The CSVER recorded in this present investigation showed a nearly linear function, which allowed the extrapolation to zero voltage and the rapid measurement of the contrast threshold, confirming the results of other studies.[11,20,35] By plotting both psychophysical and electrophysiological CSF against five different spatial frequencies under similar conditions, we observed inverted U-shaped functions, almost parallel, with the latter 0.62 to 0.79 log units lower at all spatial frequencies. Previous studies also demonstrated a good correlation between the CSF derived from steady-state VEP and the psychophysical method [18,20,30, 36-41] and systematically the electrophysiologic CSF was less sensitive compared with the former. The methodology of our study does not permit to clarify mechanisms of responses of both visual pathways. As well known, the psychophysical threshold is a perception in which only a few neurons need to be activated to obtain the threshold, whereas a greater number of them need to be activated to produce evident electrical potentials in occipital cortex during the electrophysiological test. Additionally, the stimulus field is a very important factor: the size of stimulus field and number of elements (number of check sizes in cases when the checkerboard pattern is used and number of grating when gratings are used as visual stimulus). It seems that there is certain correlation between the number of elements

and magnitude of responses especially in the case of PVER. [42] In our study, it would have been ideal if could be possible to use similar stimulus field size in both psychophysical and electrophysiological tests, but due to the limitation especially on the side of PVER, it is recommended to use relatively large stimulus field size in order to record good response in the area of low spatial frequency conditions. Those differences between the experiments, especially the stimulus field size, grating orientation, and temporal modulation, are factors that cannot be dismissed. This study could be compared with the Allen et al's paper [20] where they tested different electrode positions for the measurement of contrast thresholds derived from sweep VER and compared with the psychophysical method. Using similar technique (frame rate of 7.5 Hz, the contrast levels increased from 0.5 to 40% during the trial every each 0.5 seconds, mean luminance of 80 cd/m^2), the PVER amplitude obtained were lesser (up to 1.0 μv) compared with our results. In addition, in the present study, the CSF derived from the electrophysiologic method had slightly higher intersubject variations than the psychophysical method, confirming those of Cannon,[18] who used checkerboard patterns, and Allen,[16] who showed that the sweep-VEP standard deviations were not significantly greater than the psychophysical standard deviations. Accordingly, objective CSF from a normal population agrees with the psychophysical CSF.

In summary, we presented a objective technique to measure CSF, which correlates well with the psychophysical method. The intersubject difference was reliable, the evoked responses were reproducible and the test can be rapidly performed (20 seconds at each spatial frequency). Further studies are needed to evaluate the use of this technique with uncooperative patients. Because of the relationship between the contrast sensitivity and visual performance, the easy assessment of the CSF determined from the VEP may be a valuable tool for clinical work, infant vision evaluation, and visual research.

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Legends

FIGURE 1. A-D. Actual contrast sweep visual evoked response (CSVER) from one normal female subject in four spatial frequencies. In each figure, the mean VER amplitude-contrast function with 95% confidence interval (CI) was plotted at each of the 10 different contrasts. The dashed lines indicate the best-fit linear regression with 95% CI in each spatial frequency. The contrast threshold was determined by extrapolating to 0 μV on X-axis.

FIGURE 2. Mean of visual evoked response (CSVER) amplitude-contrast level function at five spatial frequencies from 10 normal volunteers. cpd, cycles per degree; PVER, pattern visual evoked response.

FIGURE 3. A-E. Mean ± SD of visual evoked response (CSVER) amplitudes plotted against 10 contrast levels in five different spatial frequencies (0.5, 1.0, 2.0, 4.0 and 8.0 cycles per degree [cpd]). The arrows at the X-axis indicate the individual contrast thresholds (mean interception of 0 μV on X-axis) obtained from the best-fit regression.

FIGURE 4. Mean of visual evoked response (CSVER) amplitude-spatial frequency function at 10 contrast levels from 10 normal volunteers. PVER, pattern reversal visual evoked response; cpd, cycles per degree.

FIGURE 5. Comparison of the mean electrophysiologic (CSVER) and psychophysical contrast sensitivity values at five different spatial frequencies; cpd, cycles per degree.

FIGURE 6. Scattergram showing the correlation between the all individual psychophysical and electrophysiological CSF of ten normal subjects at 0.5, 1.0, 2.0, 4.0 and 8.0 c.p.d., under similar conditions.

Figure 1

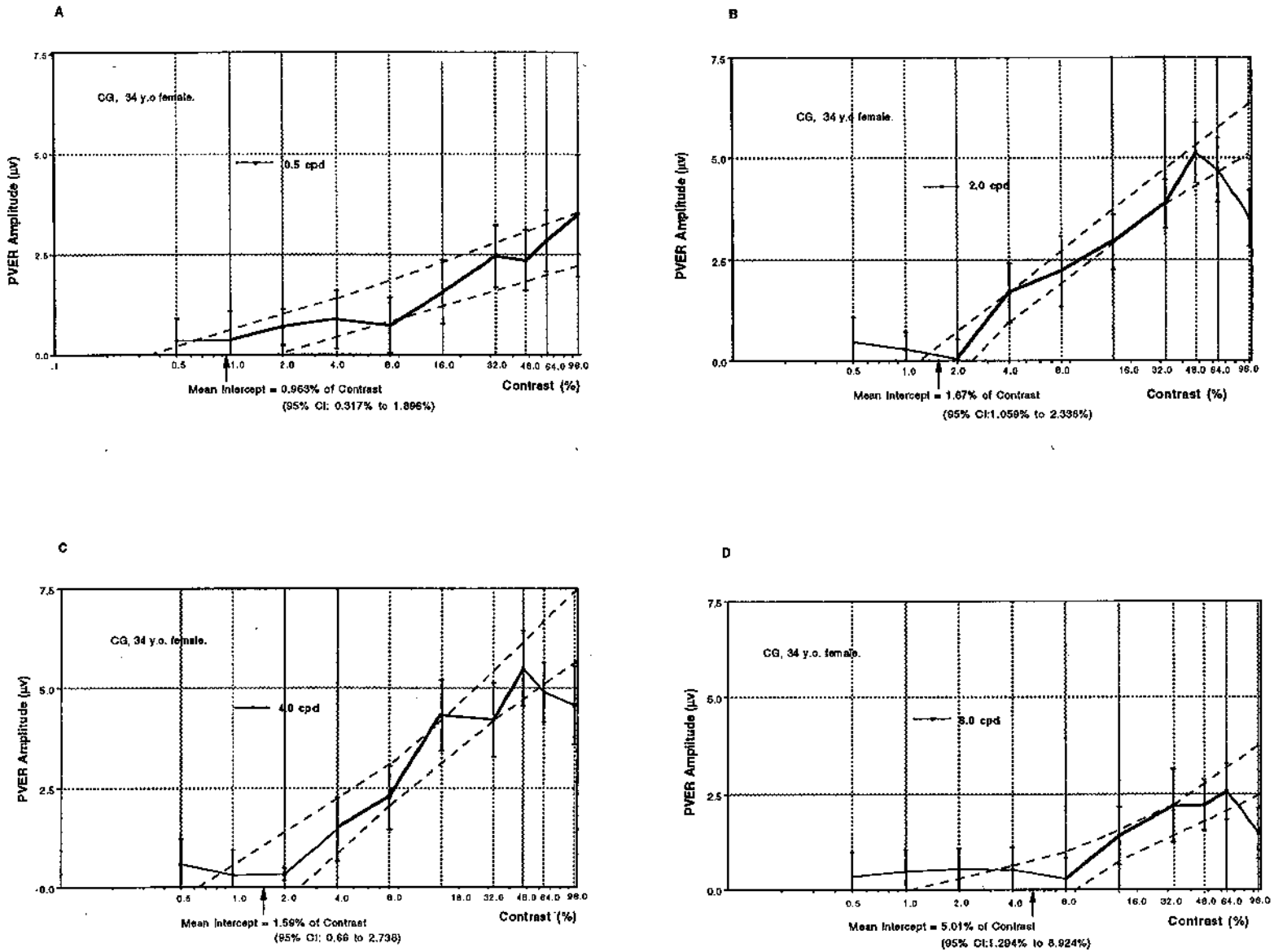

A
CG, 34 y.o female.
0.5 cpd
PVER Amplitude (µv)
Contrast (%)
Mean Intercept = 0.963% of Contrast
(95% CI: 0.317% to 1.896%)
B
CG, 34 y.o female.
2.0 cpd
PVER Amplitude (µv)
Contrast (%)
Mean Intercept = 1.67% of Contrast
(95% CI:1.059% to 2.336%)
C
CG, 34 y.o. female.
4.0 cpd
PVER Amplitude (µv)
Contrast (%)
Mean Intercept = 1.59% of Contrast
(95% CI: 0.66 to 2.738)
D
CG, 34 y.o. female.
8.0 cpd
PVER Amplitude (µv)
Contrast (%)
Mean Intercept = 5.01% of Contrast
(95% CI:1.294% to 8.924%)

Figure 2

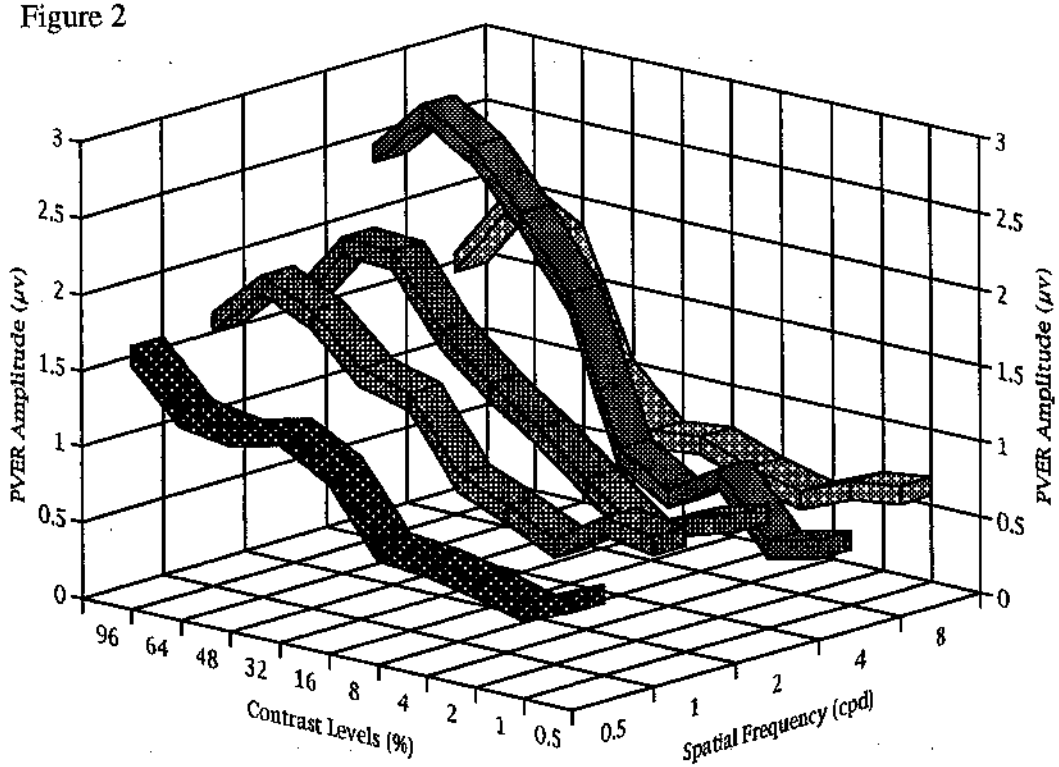

PVER Amplitude (μv)
3
2.5
2
1.5
1
0.5
0
96
64
48
32
16
8
4
2
1
0.5
Contrast Levels (%)
0.5
1
2
4
8
Spatial Frequency (cpd)
PVER Amplitude (μv)

Figure 3

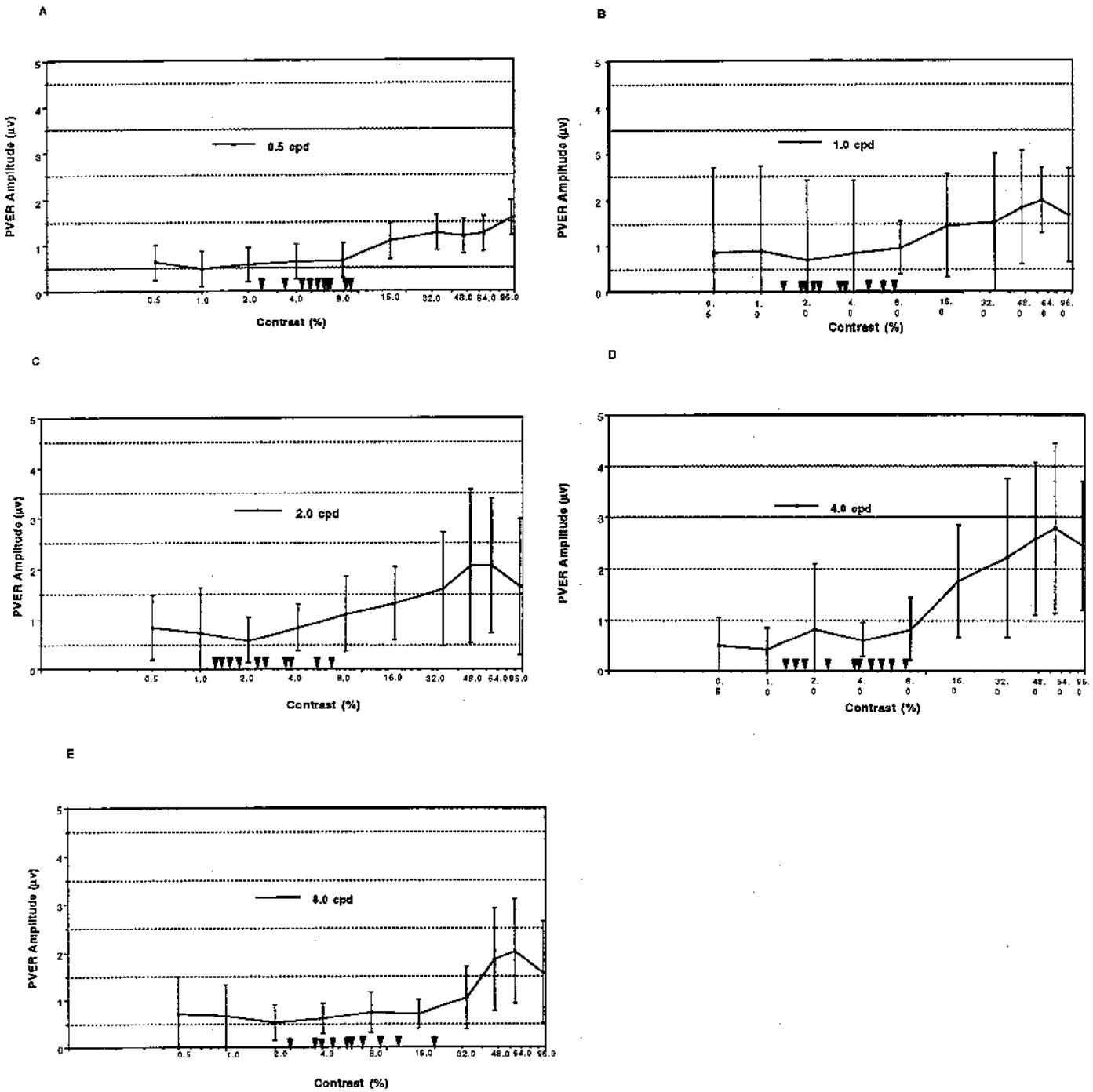

A
PVER Amplitude (µv)
0.5 cpd
Contrast (%)
B
PVER Amplitude (µv)
1.0 cpd
Contrast (%)
C
PVER Amplitude (µv)
2.0 cpd
Contrast (%)
D
PVER Amplitude (µv)
4.0 cpd
Contrast (%)
E
PVER Amplitude (µv)
8.0 cpd
Contrast (%)

Figure 4

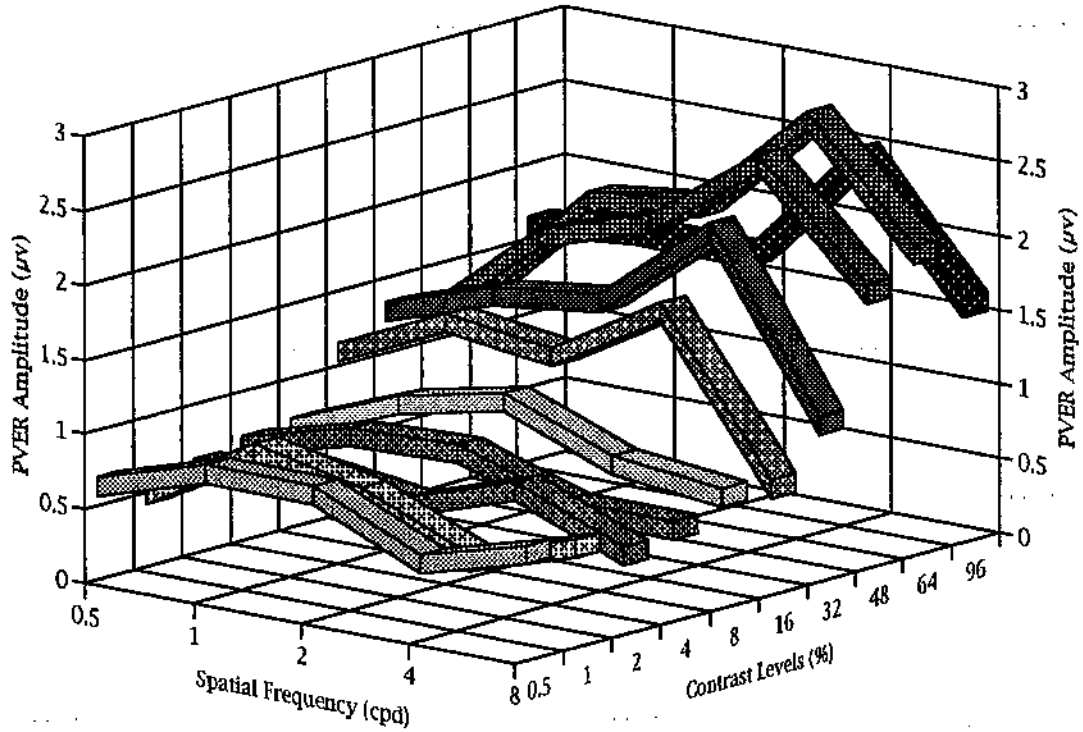

PVER Amplitude (μv)
3
2.5
2
1.5
1
0.5
0
0.5
1
2
4
8
Spatial Frequency (cpd)
0.5
1
2
4
8
16
32
48
64
96
Contrast Levels (%)
PVER Amplitude (μv)

Figure 5

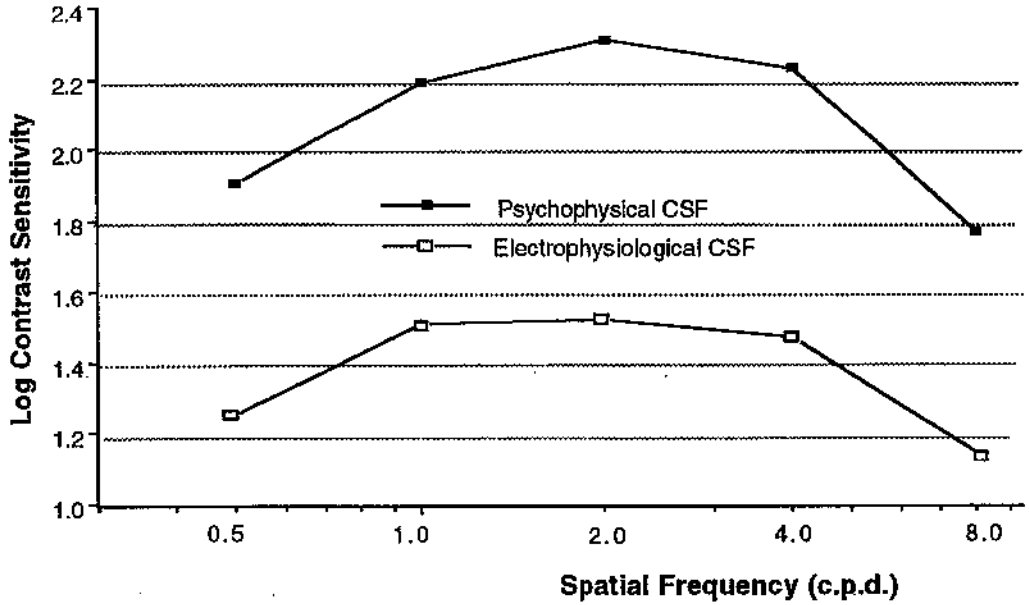
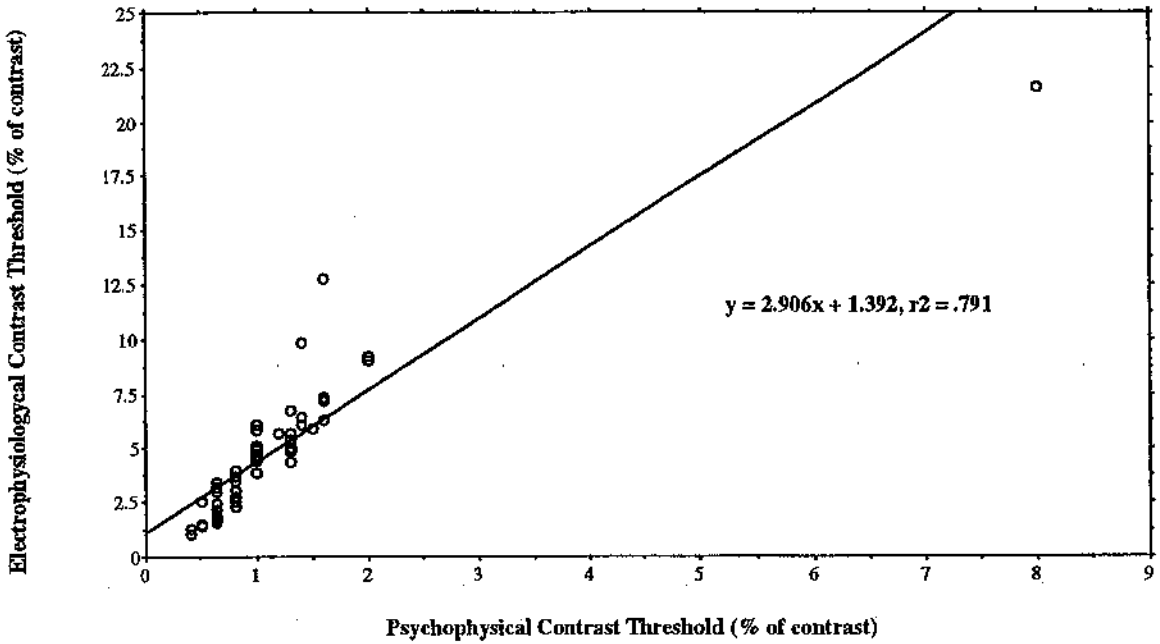

2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Log Contrast Sensitivity
Psychophysical CSF
Electrophysiological CSF
0.5
1.0
2.0
4.0
8.0
Spatial Frequency (c.p.d.)

Figure 6


Electrophysiologycal Contrast Threshold (% of contrast)
Psychophysical Contrast Threshold (% of contrast)
y = 2.906x + 1.392, r2 = .791
25
22.5
20
17.5
15
12.5
10
7.5
5
2.5
0
0
1
2
3
4
5
6
7
8
9

Neurovisual abnormalities preceding the retinopathy in patients with long-term type 1 *diabetes mellitus*

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Short title: Visual evoked potentials in type 1 DM patients

Key-words: diabetic retinopathy, electrophysiology, visual evoked potentials, contrast sensitivity.

Proprietary interest: N

Section code: EL

3 figures and 2 tables

Word count: 2670

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Abstract

Purpose: To detect the functional abnormalities preceding the retinopathy in patients with type 1 *diabetes mellitus*.

Methods: The visual evoked responses were recorded with sinusoidally modulated vertical gratings at 10 spatial frequencies presented sequentially on a high-resolution monitor in patients with type 1 *diabetes mellitus* and in normal volunteers. Similarly, the contrast visual evoked responses of 10 contrast levels were recorded at five spatial frequencies. Both amplitudes at the second harmonic were calculated by discrete Fourier transform. The visual acuity and contrast thresholds were determined objectively.

Results: There was dissociation between the Snellen and the estimated visual evoked response acuity measurements in patients with diabetes. The saturation phenomena were observed at lower levels of contrast stimuli when compared with normal individuals at 1.0, 2.0, 4.0 and 8.0 cycles per degree ($p=0.0001$). The values of the area under the curve of the visual evoked response amplitude-contrast level function at five spatial frequencies were smaller in patients with diabetes than in the control group ($p<0.05$) at all spatial frequency tested.

Conclusion: A significant and nonselective neuronal visual loss involving the visual pathway, from the inner retinal layers to high cortical levels, precedes the ophthalmoscopicaly detectable retinopathy in patients with type 1 *diabetes mellitus* .

Introduction

The initial changes in diabetic retinopathy (DR) are presumed to occur in the small vessels of the retina, including thickening of basal membrane, intramural pericyte loss and development of acellular capillaries (1,2). However, it is still unclear whether functional changes are present in the neural retina before the onset of the above mentioned abnormalities in the pathogenesis of the DR.

Changes in the retina caused by diabetes may lead to visual impairment in dim light, even with good visual acuity and visual field. This feature may be related to a decreased contrast sensitivity at low spatial frequencies (3). Contrast sensitivity function (CSF) can be assessed through psychophysical tests or by pattern visual evoked responses (PVER) (4,5). PVER are now widely used to evaluate the visual pathway and macular function (6-8). It has been described that electrophysiological evaluation of CSF using the sweep PVER technique allows a rapid and objective assessment of the visual sensory threshold even in a clinical setting (9,10).

Previous studies demonstrated that in patients with *diabetes mellitus* and good visual acuity there is a dissociation between the CSF and Snellen acuity (11-13). These studies have suggested that when the Snellen acuity of patients with diabetes and retinal disease is slightly affected, the contrast sensitivity is strongly altered. However, not all investigators have found a functional impairment before the appearance of vasculopathy in DM (14).

Recently, evidences of the involvement of the neuroglial elements of the retina in the pathogenesis of the DR were demonstrated (15,16), indicating that the vascular cells are not the only cells affected by the *diabetes mellitus* in the retina.

To gain further insights into the neurovisual abnormalities before the onset of retinopathy, we applied electrophysiological methods to evaluate the visual pathway, from the inner retinal layers to high cortical levels, in patients with long term type 1 *diabetes mellitus* without detectable retinopathy. The sweep VEP technique was performed because it allows to acquire rapid, objective and reproducible potentials from the tested patients using different spatial frequencies and contrast levels. The standard psychophysical contrast

sensitivity was not included into the analysis since both measurements are highly correlated (r=0.91) (17).

Methods

Patients

A case control study was conducted among the out-patients of the Diabetic Unit at Massachusetts General Hospital, Boston, MA, USA. All tests were performed at Schepens Retina Associates, Boston, MA, by one of the authors (JMLF) and the analysis for the regression line were done by the co-investigator (OK) unaware of the clinical features of the tested subject (blindly fashion). The criteria for inclusion in the study were patients with type 1 *diabetes mellitus*, age between 18 and 45 years old, no systemic disease other than DM, a Snellen visual acuity of 20/25 or better with best optical correction, no other ocular disease, such as very early cataract, an IOP less than 21 mmHg and clear media. Based on stereoscopic color fundus photographs, the patients were classified in level 1 (normal fundus) of the Airlie House classification modified by the Wisconsin Study Group. The exclusion criteria were the use of any medication other than insulin, presence of a systemic disease other than *diabetes mellitus*, uncontrolled glaucoma, opaque media or advanced retinopathy. Ten ophthalmologically normal age-similar volunteers with a corrected visual acuity of 20/25 or better at the time of the recordings served as the control group (10). For each subject, the age at onset of disease, duration of disease and glycosylated hemoglobin levels (HbA1c) as assessed by HPLC within three months of the examination date were determined. The recordings were performed monocularly using randomized eyes with natural pupil in a dark room. Before any measurements were obtained, the procedures were fully explained to each subject, and an informed consent was obtained in all cases. The study was carried out in conformity with the tenants of the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Massachusetts General Hospital and the Schepens Eye Research Institute, Boston, MA, USA.

Recording of sweep VER (SPVER)

The system for recording has been described elsewhere (18,19). The stimulus pattern was displayed on a 9-inch, high-resolution television monitor (P7A24K-931, Pixelink) with a spatial resolution of 960 horizontal raster lines. The overall stimulus field was 25 cm x 25 cm, subtending a visual angle of 14° x 14° at a testing distance of 100 cm. The mean luminance was maintained at 50 cd/m2. The SPVER were recorded using standard electroencephalogram cup occipital electrodes positioned at Oz/Pz (the electrodes were placed anterior to the inion, with Oz and Pz at 10% and 30% of the inion-nasion measurement, respectively), amplified with a 0.5-100 Hz bandwidth isolated differential amplifier (Model Enfant 4010, Neuroscientific Corp., Farmingdale, NY), digitized at 180 Hz.

The sinusoidally modulated vertical gratings of 10 different spatial frequencies, ranging from 0.5 to 30.5 cycles per degree (CPD) were presented sequentially on the stimulus television monitor with each frequency stimulus lasting for 2 seconds. The actual values of all spatial frequencies were 0.515, 1.031, 2.063, 3.013, 4.126, 6.026, 7.589, 10.27, 15.20, and 30.40 CPD. The digitized samples were divided into analysis records (epochs) of 180 points each (i.e., 1 second).

Recording of contrast SPVER (CSVER)

Under the same parameters of the SPVER recordings, the CSVER were evoked with sinusoidally modulated vertical gratings of 10 different contrast levels, ranging from the highest contrast level (96%) to the lowest (0.5%) at five spatial frequencies (0.5, 1.0, 2.0, 4.0, and 8.0 CPD), as previously described (10). The contrast levels measured were 96, 64, 48, 32, 16, 8, 4, 2, 1, and 0.5% lasting for 2 seconds each one.

In both recordings, the reversal rate was 6 Hz. Each record was analyzed using discrete Fourier transform (DFT) at the second harmonic frequencies (12 Hz) of the mean

value of the Fourier coefficients. The recordings were monocular with the fellow eye occluded by a patch. The refractive error of the tested eye was fully corrected at the time of the recording. The results were displayed on the video monitor of a personal computer immediately after the recording.

Electrophysiological determination of the visual acuity

The visual acuity was determined as described (18). Briefly, after finishing the recordings on the graph of SPVER amplitude-spatial frequency function, two points were selected for analysis: one at the highest spatial frequency that recorded the first peak of SPVER amplitude and the other at the lowest spatial frequency which produced a minimum potential (different from the background noise level) on the descending curve of the SPVER amplitude-spatial frequency function. Based on an extensive empirical data set, any evoked response was at least three times greater than the average noise level. The best-fit regression line (within the 95% confidence interval, since the SPVER function is not always a simple linear function of log spatial frequency) was then drawn by the Enfant system between these two points and included all datapoints between them. The visual acuity was determined by the intersection of the linear regression line with zero microvolts. This procedure required 30 to 60 seconds for completion.

Electrophysiological determination of the contrast threshold

The contrast threshold was determined similarly as described for the visual acuity determination and previously described (10). Two points were selected for analysis on the graph of CPVER amplitude-contrast level function, one at the highest contrast level that represented the first peak of CSVER amplitude and the other at the lowest contrast level which produced a minimum potential on the descending curve for each spatial frequency tested separately. The best-fit regression line (within the 95% confidence interval) was then

drawn by the Enfant system. The contrast threshold was determined by the intersection of the linear regression line with zero microvolts.

Contrast thresholds in which the best fit-regression line (within the 95% CI) included positive or negative infinite values (due to large variations of amplitude or amplitude of voltage below than 0.10 μv of the evoked potentials) were considered as unreliable, and therefore were not included into the analysis. The estimated contrast sensitivity (CS) was defined as the inverse of the electrophysiological contrast threshold.

Statistical analysis

The intersubject homogeneity of the CSVER amplitudes under different contrast conditions was tested by single-factor repeated measures analysis of variance (ANOVA). When the test was significant, *Scheffe*'s test for multiple comparison was applied.

To analyze the serial measurements of the CSVER amplitude functions in patients and normal individuals, the area under the curve (AUC) (20) was calculated at each spatial frequency, followed by *Student*'s unpaired t-test. This approach was used because the successive observations on a given subject were strongly correlated. The AUC was calculated by adding the areas under the function between each pair of consecutive observations (trapezoidal rule).The correlation was calculated for each case as appropriate. The *Fisher* exact test was used to analyze discrete variables. The null hypothesis was rejected below the conventional (two-tailed) 5% level.

Results

Ten patients with type 1 *diabetes mellitus* (age range 25 to 45 years, mean age 39 years) were compared with 10 sex- and age-similar normal volunteers (age range 25 to 46 years, mean age 35 years) (table 1).

The correlations between the Snellen and the estimated SPVER acuity measurements in the normal volunteers and the patients with DM show that both measurements were highly correlated among the normal volunteers (r^2=0.526, p=0.02). By contrast, in the patients with *diabetes mellitus* the regression coefficient disclosed a discrepancy between both acuity measurements (r^2=0.077, p=0.44) (figure 1).

The tridimensional graph (figure 2A) shows the mean VER amplitude (μv) contrast stimulus level functions (%) at 0.5, 1.0, 2.0, 4.0, and 8.0 CPD in the studied patients. The VER amplitudes in the control group (figure 2B) showed similar shapes, but with higher amplitude. At 0.5 CPD, the overall F values from the repeated ANOVA measures for heterogeneity of the responses were highly significant at contrasts up to 32% (F=7.53, p=0.0001), indicating the saturation phenomenon as the point in the VER amplitude-contrast stimulus function in which further stimulus increment does not evoke higher amplitude of potentials. At contrast levels higher than 32%, the VER amplitude-contrast stimulus functions showed no significant difference. Similar findings were observed at 1.0, 2.0, 4.0 and 8.0 CPD up to 8, 16, 8 and 4% of contrast, respectively (F=6.54, p=0.0001; F=7.32, p=0.0001; F=7.54, p=0.0001; F=6.57, p=0.0001, respectively). There was a marked tendency towards a decreased level of saturation at 1.0, 2.0, 4.0 and 8.0 CPD in patients with type 1 *diabetes mellitus* without retinopathy compared to the normal group, in which saturation phenomena were observed at 32, 32, 32, 32 and 48% of contrast at 0.5, 1.0, 2.0, 4.0 and 8.0 CPD, respectively.

The estimated contrast sensitivity (CS) derived from the CSVER of the 10 patients with type 1 *diabetes mellitus* are depicted in table 2. Overall, patients with DM presented an abnormal CS function pattern with several unreliable responses. Among the normal volunteers, the CS values were reliable at all spatial frequencies tested, and the values of the log CS (standard deviation) observed were 1.26 (0.17), 1.52 (0.24), 1.53 (0.22), 1.48 (0.25) and 1.15 (0.2) at 0.5, 1.0, 2.0, 4.0 and 8.0 CPD, respectively, leading to a U-shaped band pass filter curve. The estimated CS at 0.5 CPD were recorded only in patients in whom the HbA1c levels were lower than 7.6% (mean level of the studied patients) but not in those patients which HbA1c levels were above 7.6% (p=0.05). At the higher spatial frequency

tested (8.0 CPD), there was no recordable CS among the patients with *diabetes mellitus*, independently of the level of HbA1c. These findings suggest an interaction between the electrophysiologycal CS at low spatial frequency and the metabolic control as indexed by HbA1c.

The serial measurements of the CSVER amplitude functions in patients and normal individuals at each spatial frequency were analyzed by the area under the curve (AUC) (figure 3). The control group had higher AUC values at all spatial frequencies with significant differences at 0.5 (p=0.01), 1.0 (p=0.02), 4.0 (p=0.04) and 8.0 (p=0.001) CPD in comparison with the patients with diabetes. In addition, the correlations between the duration of disease and the AUC values of the CSVER function at each spatial frequency was very poor, indicating that the contrast-spatial frequency loss is not related with the duration of the disease (r^2 =0.05 and p=0.5, r^2 =0.14 and p=0.3, r^2 =0.08 and p=0.4, r^2 =0.08 and p=0.4, r^2 =0.2 and p=0.2 at 0.5, 1.0, 2.0, 4.0, and 8.0 CPD, respectively). However, all of the studied patients have had *diabetes mellitus* for long duration (the mean duration of the group is 26 years, ranging from 15 to 38 years).

Discussion

We have shown that in type 1 *diabetes mellitus* patients with long duration of disease but without retinopathy the correlation between the Snellen visual acuity and the estimated SPVER acuity is very poor (r^2=0.077, p=0.02). Similar discrepancy between the two methods of estimating visual acuity has been previously reported in patients with optic nerve disorders (19). It is possible that the poor correlation between both acuity measurements observed in patients with type 1 *diabetes mellitus* is an indicative of neural dysfunction and it may precede the clinical and detectable diabetic microangiopathy in the retina.

In our investigation, the visual potentials evoked in the contrast and spatial frequency domains from patients with diabetes showed two major characteristics: lower amplitude of responses and lower level of saturation phenomena at each spatial frequency tested. Other

investigators (21,22) have shown that P100 is abnormal in patients with *diabetes mellitus* compared with normal individuals, even under strict metabolic control (23). Although, it is difficult to identify the specific neural processes derived from the visual evoked potentials, it is strongly suggestive that different neural mechanisms are involved in these abnormal responses from the patients with *diabetes mellitus*.

In conclusion, this study demonstrated that patients with type 1 *diabetes mellitus* have a significant and nonselective spatial-frequency and contrast detection losses involving systems of the inner retina spreading to the parvo and magnocellular systems at optic nerve and higher levels of the visual pathway, preceding the ophthalmoscopicaly detectable retinopathy. However, these functional neurovisual abnormalities may not preclude the ultrastructural changes at the pericyte and capillary levels described as the earliest changes in the pathogenesis of diabetic retinopathy. To the present, the pathophysiology underlying the above visual abnormalities in patients with type 1 *diabetes mellitus* is unknown, some evidences suggest that neurosensory anomalies precede or even contribute to the development of both diabetic retinal and optic nerve diseases. Knowledge the importance of cells other than endothelial cells and pericytes in the pathogenesis of diabetic retinopathy may provide novel targets for intervention and earns further studies.

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Figure Legends

Figure 1: Correlation between the Snellen and the estimated SPVER acuity measurements in normal volunteers (⧈) and in patients with type 1 *diabetes mellitus* without retinopathy (■). The solid lines represent the linear regression; r2= regression coefficient.

Figure 2A: PVER amplitude-contrast stimulus functions in 10 patients with type 1 *diabetes mellitus* at 0.5, 1.0, 2.0, 4.0 and 8.0 CPD.

Figure 2B: PVER amplitude-contrast stimulus functions in normal individuals at 0.5, 1.0, 2.0, 4.0 and 8.0 CPD.

Figure 3: The area under the curve (AUC) derived from CSVER amplitude-contrast level functions at five spatial frequencies in patients with type 1 *diabetes mellitus* (—⧈—) and in normal individuals (—◈—). *p<0.05, Student's t-test.

Table 1. Clinical features of 10 patients with type 1 diabetes mellitus studied.

Patient	*Age*	*Sex*	*Duration of DM*	*HbA1c†*	*Snellen VA*	*Estimated SPVER VA*
	(yr)		*(yr)*	*(%)*		
1	42	m	33	4.5	20/20	20/43
2	45	m	28	6.3	20/20	20/50
3	45	m	20	6.5	20/20	20/67
4	45	f	22	6.8	20/25	20/83
5	26	f	15	7.0	20/25	20/83
6	40	f	37	8.3	20/20	20/61
7	34	m	18	8.4	20/15	20/65
8	35	m	29	9.5	20/20	20/37
9	39	m	22	10.2	20/20	20/75
10	39	f	38	11.4	20/25	20/80
Mean	39	6m/4f	26.2	7.6		
(SD)	6		8	2.5		

DM: diabetes mellitus; HbA1c: Glycosilated hemoglobin; VA: visual acuity; SPVER: sweep pattern visual evoked responses; SD: standard deviation.

Figure 1

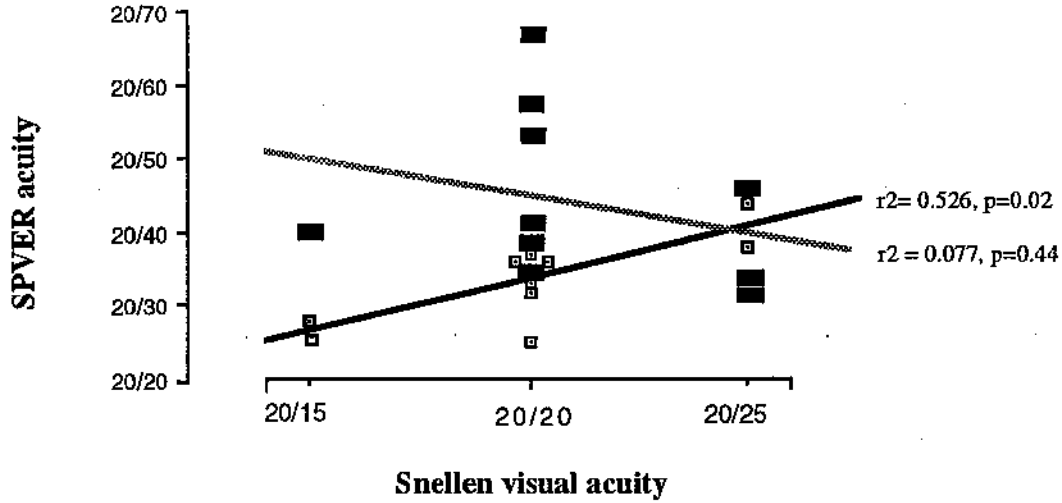

20/70
20/60
20/50
20/40
20/30
20/20
SPVER acuity
20/15
20/20
20/25
Snellen visual acuity
r2= 0.526, p=0.02
r2 = 0.077, p=0.44

Figure 2

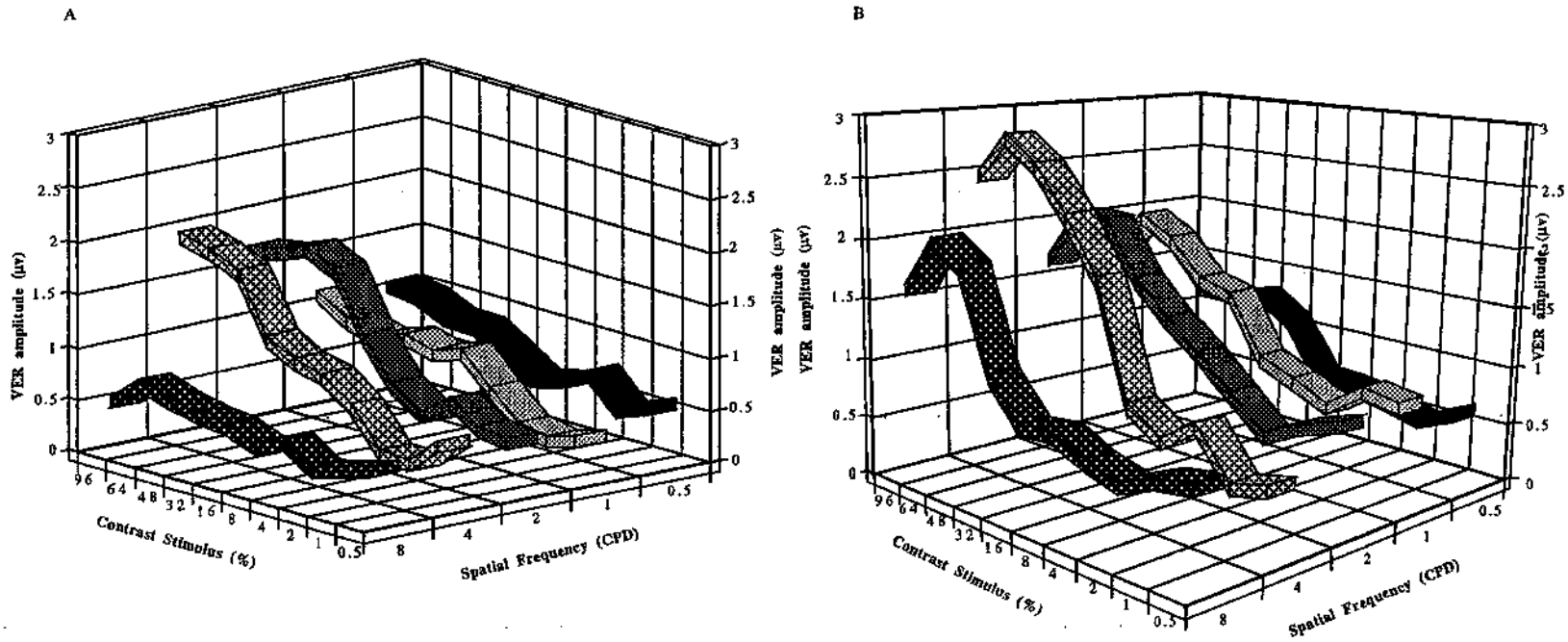
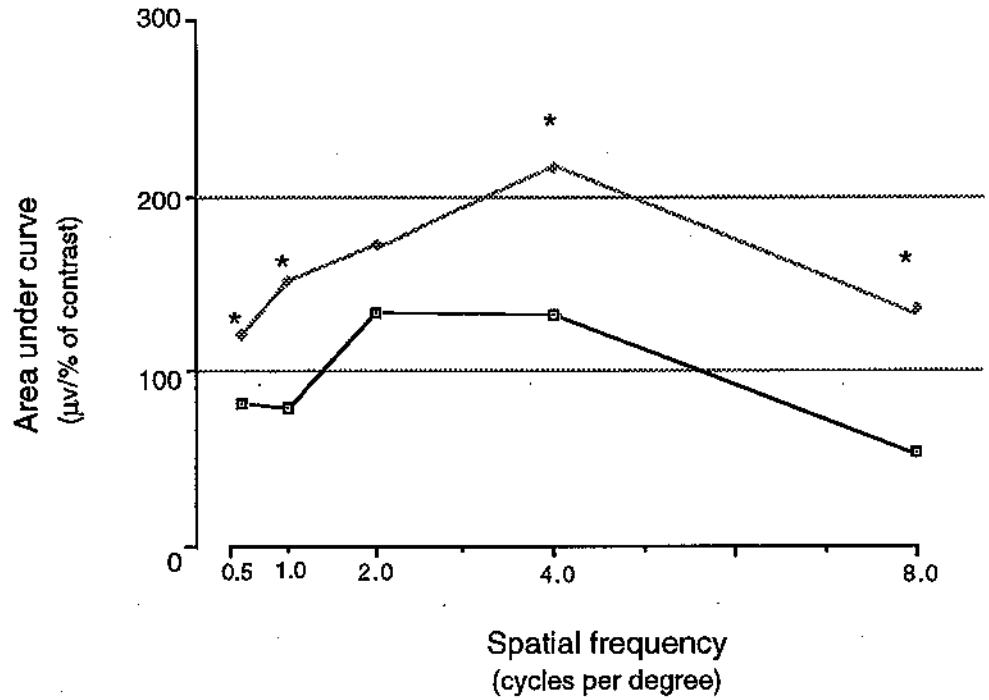

A
B
VER amplitude (μv)
Contrast Stimulus (%)
Spatial Frequency (CPD)
96
64
48
32
16
8
4
2
1
0.5
0
0.5
1
1.5
2
2.5
3
8
4
2
1
0.5

Table 2. The estimated contrast sensitivity (CS) values obtained from the CSVER of the 10 patients with type 1 diabetes mellitus.

Patients	*Estimated contrast sensitivity (log scale) Spatial Frequency (CPD)*				
	0.5	*1.0*	*2.0*	*4.0*	*8.0*
1	1.23	2.50	2.20	2.02	UR
2	1.9	UR	UR	UR	UR
3	0.54	0.50	2.10	1.92	UR
4	1.42	1.45	0.83	UR	UR
5	UR	UR	0.92	UR	UR
6	UR	UR	1.33	1.81	UR
7	UR	UR	UR	1.93	UR
8	UR	UR	1.65	0.57	UR
9	UR	UR	1.43	0.92	UR
10	UR	1.70	1.42	2.10	UR

CPD: cycles per degree; UR: unreliable response.

Figure 3


300
200
100
0
Area under curve
(μv/% of contrast)
*
*
*
*
0.5
1.0
2.0
4.0
8.0
Spatial frequency
(cycles per degree)

SUMÁRIO E CONCLUSÕES

1- A hipertensão arterial, além do DM tipo 2, presença de doença cardiovascular, adesão vitreomacular e retinopatia grave estão associadas ao edema macular difuso diabético.

2- Pacientes com retinopatia diabética proliferativa apresentam aumento do contratransporte de sódio e lítio em hemáceas, alteração associação à hipertensão arterial.

3- Alterações funcionais da neurorretina e das vias visuais podem preceder e mesmo contribuir para a patogênese das alterações anatômicas classicamente descritas na retinopatia diabética.

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