UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

DOUTORADO

AGRUPAMENTO DE COMPONENTES DA SÍNDROME METABÓLICA COMO FATOR DE RISCO PARA COMPLICAÇÕES CRÔNICAS EM PACIENTES COM DIABETE MELITO TIPO 2 E ASSOCIAÇÃO COM POLIMORFISMOS GENÉTICOS.

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Porto Alegre, Julho de 2002.

AGRADECIMENTOS

Ao Prof. Jorge Gross, agradeço pela brilhante orientação sem a qual este projeto não teria alcançado o seu objetivo. A sua inteligência e conhecimento científico, imensuráveis, acrescidos de uma capacidade irretocável de orientação foram qualidades fundamentais para a conclusão deste trabalho.

Ao Dr. Luís Henrique Canani pioneiro, entre nós, no estudo da genética do diabete melito, que foi incansável e imprescindível na assessoria estatística e pelas importantes sugestões.

À Profa. Ana Luiza Maia que sempre me incentivou e que muito colaborou para a qualidade do laboratório de Biologia Molecular do Serviço de Endocrinologia que nos permite realizar as análises genéticas.

À colega e amiga Profa. Sandra Silveiro que sempre me incentivou na organização deste banco de dados de pacientes com diabete melito tipo 2. E um agradecimento especial pelas suas correções gramaticais, fundamentais para a finalização deste trabalho.

À Dra. Maria Cristina Boelter que realizou, de modo exemplar, grande parte dos exames de fundoscopia destes pacientes.

Aos acadêmicos de medicina Cristiana Weber, Desirê Bortolanza e Rafael Shaeffel, cuja participação na coleta dos dados, acompanhamento dos pacientes e organização do banco de dados foram fundamentais.

Às biólogas Clarissa Capp e Paula Eichler pelo excelente trabalho realizado de análise dos polimorfismos genéticos e auxílio na organização do banco de DNA dos pacientes com diabete melito tipo 2.

Aos Serviços de Patologia Clínica, Medicina Nuclear e Cardiologia do Hospital de Clínicas de Porto Alegre, pela realização dos exames subsidiários deste projeto.

Aos meus pais, irmãs, irmão e avó que sempre me apoiaram em tudo e me ajudaram com palavras de incentivo e entusiasmo.

Ao meu marido, José Ricardo e as minhas filhas Júlia e Rafaela, por facilitar de várias formas a execução deste trabalho, principalmente entendido a minha ausência e propiciado momentos maravilhosos que facilitaram a chegada nesta etapa da minha vida.

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INTRODUÇÃO

O diabete melito tipo 2 (DM2) é uma doença metabólica de etiologia múltipla caracterizada pela presença de hiperglicemia crônica, resultante de defeitos da secreção de insulina (defeito na função da célula β), na ação da insulina (resistência insulínica) ou ambos (1). O DM2 representa no momento de seu diagnóstico, o estágio final de um processo crônico e progressivo de alterações patológicas ocasionadas pela resistência insulínica e defeito na função da célula β (2). Os efeitos a longo-prazo do DM2 incluem o desenvolvimento de complicações crônicas específicas: retinopatia com risco de cegueira, nefropatia que pode levar à insuficiência renal e neuropatia com risco de úlceras, amputações, articulação de Charcot, e distúrbios graves da função autonômica. Estes pacientes também apresentam uma progressão acelerada do processo aterosclerótico que favorece o desenvolvimento de eventos vasculares nas artérias coronarianas, cerebrais e dos membros inferiores. Pacientes diabéticos apresentam um risco dois a quatro vezes (3, 4, 5) maior de cardiopatia isquêmica, a qual constitui-se na principal causa de morte nestes pacientes (6).

Uma das questões mais importantes e não totalmente respondida no DM é a patogênese das complicações crônicas micro-macrovasculares e neuropáticas. Evidências clínicas e epidemiológicas demonstram que estas complicações podem preceder o diagnóstico da hiperglicemia. Cerca de 20% dos pacientes com DM2 apresentam retinopatia diabética ao diagnóstico, o que sugere a presença de um estado hiperglicêmico não reconhecido por pelo menos 6 a 7 anos antes (7). No estudo UKPDS cerca de 50% dos pacientes apresentava alguma complicação microvascular ao diagnóstico (8). Diferentemente do que parece acontecer com os eventos microvasculares, em relação às complicações macroangiopáticas (ateroesclerose de grandes vasos), existem evidências de que estas não sejam,

necessariamente, uma complicação do DM, mas que estas duas entidades – DM2 e doença cardiovascular – apresentem antecedentes ambientais e genéticos comuns para o seu desenvolvimento (9). A síndrome metabólica (SM) associada à resistência insulínica pode representar este fator em comum para o desenvolvimento do DM2 e doença cardiovascular (10).

De uma maneira geral, as complicações crônicas do DM2 estão associadas à presença de hiperglicemia por um tempo prolongado (duração do DM). Um estudo clássico observacional realizado por Pirart (11) demonstrou claramente esta ligação quando acompanhou cerca de 4.400 pacientes diabéticos do tipo 1 (DM1) e DM2 por 25 anos e mostrou que conforme a duração do DM aumentava, a prevalência de retinopatia, nefropatia e neuropatia eram mais altas naqueles com um pobre controle da glicemia e mais baixa naqueles com um bom controle. Os resultados de estudos que avaliaram de modo prospectivo o controle intensivo da glicemia (12, 13) e o desenvolvimento de complicações crônicas, reforçaram a presença da hiperglicemia como fator importante na patogênese destas complicações. Uma variedade de anormalidades bioquímicas, incluindo incorporação enzimática e não-enzimática da glicose em proteínas solúveis, bem como as alterações no metabolismo dos polióis e mioinositol, constituem a ligação entre hiperglicemia com / ou sem deficiência de insulina e o desenvolvimento de complicações crônicas do DM (14).

Além destas anormalidades bioquímicas, distúrbios hemodinâmicos e de coagulação sangüínea, podem contribuir para o desenvolvimento das complicações crônicas. Estas anormalidades incluem fluxo e pressão arterial capilar aumentados, viscosidade sangüínea aumentada e hipersensibilidade das plaquetas aos agentes agregantes (15). Outros fatores como dislipidemia, níveis elevados de pressão arterial e tabagismo favorecem o

desenvolvimento das complicações crônicas do DM, principalmente a doença coronariana (16).

Evidências clínicas e epidemiológicas e de estudos de agregação familiar sugerem fortemente que fatores genéticos também estão envolvidos no aparecimento das complicações crônicas do DM (17-20).

Em pacientes diabéticos seguidos por 25 a 30 anos (21), a microangiopatia aumentou progressivamente, alcançando picos de prevalência de 80%. Os 20% restantes de pacientes que não desenvolveram esta complicação, não diferiram em relação ao controle metabólico ou duração do DM, mas diferiram em relação à predisposição genética às complicações. Possivelmente, ocorreu a falta de um componente genético para o desenvolvimento das complicações ou existe a presença de um fator que confere proteção contra a evolução de doença microangiopática e /ou neuropática. Na avaliação do desenvolvimento de micro-macroalbuminúria, foi observado que pacientes que não apresentaram esta complicação nos primeiros 10 a 15 anos após o início do DM parecem ser protegidos desta complicação (22). Isto sugere que, além dos fatores de risco tradicionais para o desenvolvimento de nefropatia diabética, tais como hiperglicemia, hipertensão arterial sistêmica (HAS), dislipidemia e tabagismo, existe também um componente genético que predispõe ao seu desenvolvimento.

Conforme descrito por Matthaei et al (23), o DM2 representa apenas a ponta de um “iceberg”, cuja base seria o tempo de exposição a distúrbios metabólicos com efeito, a longo prazo no sistema vascular e demais órgãos e nos tecidos correlacionados. O desenvolvimento de micro e/ou macroangiopatia pode preceder em mais ou menos 10 anos a alteração da homeostase da glicose, como descrito previamente (24). Estes achados confirmam a relação entre a etiologia da hiperglicemia e a sua associação com fatores de risco cardiovasculares. Conseqüentemente, todos os esforços são necessários para que se possa reconhecer estes

pacientes susceptíveis a estas alterações metabólicas, muitas vezes silenciosas, evitando que eles atinjam a ponta do “iceberg”. Atualmente tem sido aceita a idéia de que outro ou outros fatores, além dos três descritos anteriormente contribuem para o desenvolvimento não apenas do DM2, mas também de suas complicações crônicas. Em estudo realizado na Escandinávia (25), foi demonstrado, que apesar da hiperglicemia crônica constituir um sinalizador importante do risco das complicações micro- e macroangiopáticas em pacientes com DM2, este risco é modificado pela presença dos componentes da SM.

Freqüentemente, um indivíduo com tolerância alterada à glicose (DM2 ou tolerância diminuída à glicose – TDG) apresentará, pelo menos um ou mais componentes que levam ao risco aumentado de doença cardiovascular. Este grupamento de fatores de risco cardiovascular tem recebido, a partir de sua primeira descrição em 1923 (26) como síndrome X (26-28), várias denominações: síndrome plurimetabólica (29, 30, 31), síndrome de resistência insulínica (32-36), quarteto letal (37) ou mais recentemente, SM (38).

A denominação de SM, proposta pela Organização Mundial da Saúde (OMS) em 1999 (38), deixa de lado a terminologia “resistência insulínica”, provavelmente por não estar ainda comprovado que a resistência insulínica causaria todos os componentes da síndrome. De acordo com a OMS define-se como SM a ocorrência de resistência insulínica ou tolerância diminuída à glicose (TDG/DM2) e pelo menos dois dos seguintes: HAS, obesidade/obesidade visceral, dislipidemia e microalbuminúria (figura 1).

Outros fatores também têm sido descritos como possíveis componentes da SM, apesar de não serem considerados indispensáveis no diagnóstico (38). Entre estes se destacam: hiperuricemia, distúrbios de coagulação, elevação do inibidor-1 do ativador do plasminogênio (PAI-1), aumento de partículas pequenas de colesterol de baixa densidade (LDL), entre outros.

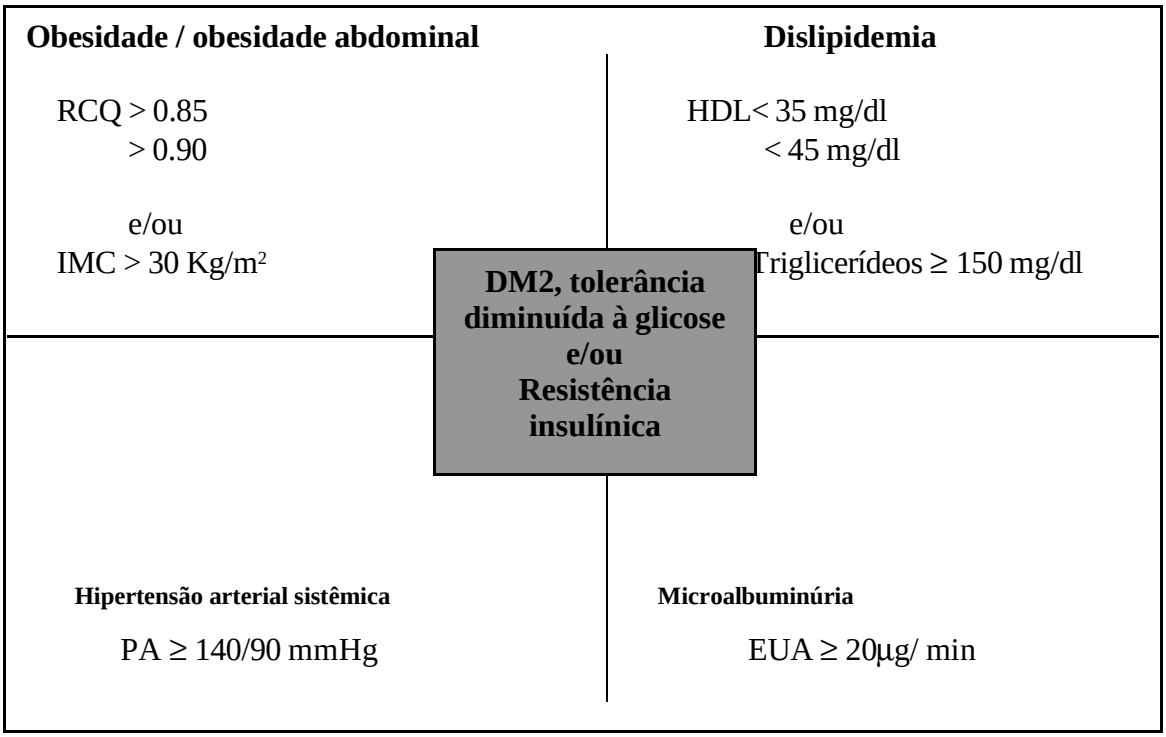

Figura 1. Critérios diagnósticos para os componentes da síndrome metabólica – OMS, 1999 (38). RCQ: razão cintura quadril; IMC: índice de massa corporal; HDL: colesterol de alta densidade; PA: pressão arterial; EUA: excreção urinária de albumina

1. Definição da resistência insulínica e alterações da homeostase glicêmica

A resistência insulínica parece ser um elemento fundamental para o desenvolvimento da SM (27, 29, 31).

A resistência insulínica é definida como uma diminuição do efeito biológico da insulina nas células sensíveis em diversos níveis: pré-receptor (insulina anormal), receptor (diminuição do número ou afinidade do receptor), transportador da glicose (diminuição das moléculas GLUT-4), ou pós-receptor (transdução e fosforilação anormal do sinal) (39, 40). A resistência insulínica reflete um defeito da ação da insulina predominantemente no músculo esquelético e fígado. O tecido adiposo é um terceiro sítio metabólico de ação insulínica que vem recebendo atenção nos últimos anos. O mecanismo da resistência insulínica no tecido adiposo está relacionado à produção local de algumas citocinas (fator de necrose tumoral e interleucina 6) ou hormônios pelo tecido adiposo (resistina). Além disto, a resistência insulínica ao nível do tecido adiposo favorece o desenvolvimento de resistência em outros tecidos. A mobilização excessiva de ácidos graxos decorrentes no menor efeito da insulina no tecido adiposo (aumento da lipólise), ocasiona um aumento da oferta de ácidos graxos livres (AGL) ao músculo esquelético e é acompanhada de um aumento da oxidação de ácidos graxos, que por sua vez inibe enzimas chaves da via glicolítica e do ciclo de Krebs. Esta relação é conhecida como o ciclo de Randle ou ciclo glicose-ácidos graxos (41). Além disso, a maior oferta de AGL ao fígado estimula a gliconeogênese hepática, através de mecanismos relacionados a aumento da oxidação dos ácidos graxos na célula hepática. Os efeitos da maior oferta de AGL aos tecidos muscular e hepático determinam hiperglicemia, respectivamente, através da diminuição do consumo periférico de glicose e aumento da produção hepática. (42, 43).

O método padrão para estabelecer a presença deste distúrbio metabólico é o – *clamp* hiperinsulinêmico –euglicêmico. Por esta técnica, os níveis de insulina estão elevados (usualmente 8-10 vezes acima dos níveis basais), enquanto os níveis de glicose são mantidos em euglicemia (80-100 mg/dl) através da variação na taxa infundida de glicose. Em pacientes com resistência insulínica, a quantidade da glicose necessária para manter estáveis os níveis glicêmicos para os mesmos níveis de insulina sérica é menor do que em indivíduos normais (44). Este método é laborioso, caro e complexo e pode ser conduzido apenas em um número limitado de pacientes. Foram desenvolvidos métodos alternativos que medem a presença da resistência insulínica de uma forma mais simples e prática, porém menos acurada que o *clamp*. Entre estes se citam: insulina de jejum e relação insulina / glicose (45), teste de tolerância à glicose intravenosa (FSIVGTT) (46), índice de quantificação da sensibilidade insulínica (QUICKI) (47), infusão constante de baixas concentrações de glicose (CIGMA) (48). Um método simples e utilizado freqüentemente em estudos epidemiológicos é medida do índice de homeostase da resistência insulínica ($HOMA_{IR}$), que utiliza na sua fórmula a insulina plasmática e glicose plasmática em jejum (49). Independente do método utilizado, é fundamental que sejam determinados valores de referência da sensibilidade insulínica para cada população, pois há diferenças acentuadas entre os diversos grupos étnicos (50).

A resistência insulínica está presente na maioria dos pacientes com TDG ou DM2 (51-53). É observada também em cerca de 25% de indivíduos não obesos e com tolerância normal ao estímulo da glicose oral (54). Esta observação fortalece a importância da capacidade da célula β em compensar a resistência insulínica, exercendo, portanto importante função de manutenção da homeostase da glicose.

Os fatores que ligam a resistência insulínica e dislipidemia aterogênica, HAS, um estado alterado de coagulação e alteração da homeostase glicêmica são bastante complexos e

mediados por diferentes vias metabólicas. A insulina pode apresentar um mecanismo direto para aterogenicidade em decorrência de sua capacidade de estimular a síntese lipídica no tecido arterial e a proliferação das células musculares lisas arteriais (55). A capacidade da insulina de estimular a vasodilatação do endotélio encontra-se acentuadamente comprometida em indivíduos obesos resistentes à insulina (56).

O diagnóstico das diferentes categorias de hiperglicemia está bem definido e foi recentemente revisado pela OMS (38). As categorias são as seguintes: glicemia de jejum alterada: glicose plasmática de jejum ≥ 110 mg/dl e < 126 mg/dl, tolerância oral à glicose diminuída: glicose plasmática 2 horas após 75 g de glicose oral ≥ 140 mg/dl e < 200 mg/d, até DM2: glicose plasmática em jejum ≥ 126 mg/dl e/ou 2 horas após 75 gramas de glicose oral ≥ 200 mg/dl. Todas estas situações caracterizam alterações na homeostase da glicose com subseqüente hiperglicemia e são considerados no diagnóstico da SM.

2. Componentes da síndrome metabólica

2.1. Hipertensão arterial sistêmica e síndrome metabólica

A resistência insulínica tem sido descrita em pacientes com HAS (33, 57, 58, 59). Pacientes com DM2 e HAS apresentam um defeito mais intenso da homeostase glicêmica do que indivíduos com DM2 normotensos, achado que não está relacionado com o IMC (60).

Foi observada uma relação direta entre a concentração de insulina plasmática e a pressão arterial e foram propostos diversos mecanismos para especificar a HAS induzida pela resistência insulínica. Primeiro, por estimulação do sistema nervoso simpático, em que o aumento da concentração da insulina plasmática está associada com o aumento significativo da concentração plasmática de catecolaminas, independente de qualquer alteração nos níveis plasmáticos de glicose (61, 62). O rim é o outro provável sítio no qual a resistência insulínica

e a hiperinsulinemia agem elevando os níveis pressóricos (31). Existem evidências de que a insulina age em animais (63) e em seres humanos (64) promovendo a reabsorção tubular de sódio, assim como ao nível de túbulo proximal aumentando a reabsorção de água (65), com conseqüente, sobrecarga de volume. O aumento da resposta à angiotensina é também ocasionado pela hiperinsulinemia, o que leva ao aumento dos níveis pressóricos (66).

2.2. Dislipidemia: hipertrigliceridemia e redução de HDL colesterol

A presença de níveis elevados de triglicerídeos plasmáticos (≥ 150 mg/dl) e/ou reduzidos de HDL colesterol (< 35 mg/dl em homens e < 45 mg/dl em mulheres) define a dislipidemia típica da SM (38). A hipertrigliceridemia ocorre, comumente, em conjunto com outros componentes da síndrome (67).

As bases aterogênicas da SM são atribuídas ao resultado de múltiplos fatores de risco que, combinados, produzem um aumento no risco de doença coronariana (67). Hipertrigliceridemia e a redução dos níveis plasmáticos de HDL colesterol são fatores independentemente aterogênicos. Diversos investigadores observaram que a hipertrigliceridemia está fortemente associada à resistência insulínica (68-70). Como referido anteriormente, a resistência insulínica ao nível do tecido adiposo favorece um aumento da mobilização de ácidos graxos. A maior oferta de AGL ao fígado estimula a síntese hepática de triglicerídios e, portanto há um aumento da produção hepática de lipoproteínas (VLDL) ricas em triglicerídios. O catabolismo destas partículas está diminuído devido à redução da atividade da lipase lipoprotéica nos pacientes com DM2, o que contribui para um maior aumento dos níveis de triglicerídios séricos.O menor metabolismo destas partículas faz com que ocorra menos transferência de fosfolipídios e apolipoproteínas para as partículas de HDL (71). Alem disto, o maior tempo de circulação das partículas de VLDL ricas em triglicerídios

permite que ocorra um maior transferência de triglicerídios pela ação da proteína de transferência de ésteres de colesterol para as partículas de HDL e um aumento da passagem de ésteres de colesterol do HDL para o VLDL. Desta maneira as partículas de HDL ficarão menores e mais ricas em triglicerídios o que determina um aumento do seu catabolismo e redução dos níveis séricos (72, 73).

As partículas pequenas e densas de LDL são consideradas também neste espectro de dislipidemia aterogênica (74). Estas partículas pequenas de LDL colesterol são inclusive mais aterogênicas do que as partículas normais de LDL (74, 75). Todavia, apesar da forte associação destas partículas pequenas de LDL com infarto agudo do miocárdio e doença arterial coronariana (74, 75), elas não representam, estatisticamente, um fator de risco independente para doença cardiovascular em estudos prospectivos. Portanto, a consideração destas partículas dentro da definição de SM, como fator de risco para doença cardiovascular, não é tão forte como para o aumento e redução dos níveis plasmáticos, respectivamente, de triglicerídeos e HDL colesterol.

2.3. Adiposidade visceral e obesidade

A obesidade geral e em particular a obesidade central ou visceral, está associada com um aumento do risco de doença cardiovascular (76, 77). A obesidade visceral é caracterizada pelo aumento da razão cintura/quadril (RCQ > 0,90 em homens e > 0,85 em mulheres) ou apenas pelo aumento da circunferência da cintura (> 102 cm em homens e > 88 cm em mulheres). As recomendações para considerar a RCQ, assim como apenas a medida da circunferência do abdomen estão, igualmente, relacionadas com os fatores de risco para doença coronariana como HAS, hiperglicemia ou dislipidemia (78), assim como estão

associadas com o aumento da probabilidade de ter um ou mais fatores de risco cardiovascular (79).

A distribuição regional da gordura corporal, a qual não pode ser determinada pelo IMC somente, é mais deletéria do que a gordura corporal total (80-83). Adicionalmente, tem sido demonstrado que a associação entre gordura visceral e doença cardiovascular ocorre em indivíduos com IMC < 25 kg/m^2 (84).

O conhecimento atual de obesidade ampliou-se mais recentemente com as descobertas de diversas proteínas produzidas pelo tecido adiposo, permitindo que este seja considerado um órgão endócrino (85). Entretanto os mecanismos que ligam a adiposidade e doença cardiovascular são complexos e precisam ser melhores compreendidos. Duas formas de ligação entre estes eventos são as alterações da hemóstase e fibrinólise (86) e o desencadeamento de um quadro inflamatório crônico subclínico (87).

2.4. Microalbuminúria e síndrome metabólica

A inclusão de microalbuminúria (excreção urinária de albumina em urina de 24 horas ≥ 20 μg/min) como parte da SM é questionada, pois alguns estudos não demonstraram associação com resistência insulínica (88, 89). Por outro lado, a presença de microalbuminúria foi acrescida na definição atual de SM da OMS (38) devido a forte associação demonstrada com hiperglicemia e hiperinsulinemia (60, 90). A microalbuminúria é um fator de risco de morte por doença cardiovascular (91, 92) e progressão para nefropatia clínica (93-95).

A microalbuminúria é considerada um marcador importante de disfunção vascular. Os pacientes com DM2 e microalbuminúria têm níveis elevados de fator de von Willebrand (FWV) como um sinal de disfunção endotelial (96). Em adição, a microalbuminúria está

associada a um aumento do risco de doença cardiovascular somente em pacientes com concentração plasmática elevada de FWV (96). Este risco também é modificado pela presença de níveis reduzidos de HDL colesterol e hipertensão (60).

O entendimento da participação da resistência insulínica na patogênese da microalbuminúria é tão importante quanto o fato desta constituir um forte indicador de doença cardiovascular avançada e mortalidade cardiovascular.

3. Síndrome metabólica: prevalência e riscos associados

Estudos epidemiológicos demonstram que esta síndrome ocorre comumente em diferentes grupos étnicos incluídos: europeus, afro-americanos, mexicanos-americanos, índios asiáticos e chineses, aborígenes australianos, polinésios e micronésios (50, 97).

Os estudos iniciais da prevalência da SM diferiram muito nos seus resultados devido aos diversos critérios utilizados e porque selecionaram subgrupos específicos da população (98). Em estudo de base populacional, a SM, definida como a presença concomitante de dislipidemia e de resistência insulínica (estabelecida como tolerância anormal à glicose ou insulina plasmática de jejum ≥ 13 UI/l) estava presente em 17 % dos homens não-diabéticos e em 8 % das mulheres não-diabéticas (99). Em outro estudo populacional (100) – *Atherosclerosis Risk in Commumities (ARIC)* -, a combinação de HAS e dislipidemia foi observada em 10 % dos indivíduos.

A prevalência da SM e de seus componentes é fortemente dependente da definição das diferentes situações que compõem a síndrome. Em estudo realizado na Suécia e Finlândia, Isomaa et al (92), utilizando como definição recomendações da OMS (38), encontraram a presença de SM em ~10 % dos indivíduos com tolerância normal à glicose, em ~50% dos indivíduos com TDG e em ~80% dos pacientes com DM2.

A própria definição de SM – agrupamento de fatores que conferem risco aumentado de doença cardiovascular - demonstra a importância do diagnóstico preciso e tratamento intensivo destes pacientes.

Apesar de datarem dos idos de 1900 (26) os primeiros relatos de uma possível associação patológica entre doenças como HAS e obesidade, foi apenas a partir dos últimos anos que estas e outras situações clínicas foram unificadas (38), permitindo então, que se possa realmente identificar os pacientes de menor ou maior risco. A presença da SM está associada com um risco aumentado de morbi-mortalidade cardiovascular (Razão de chances = 3 para morbidade e 1,8 para mortalidade) (92). A prevalência de doença coronariana, infarto do miocárdio e acidente vascular cerebral foi aproximadamente três vezes maior em indivíduos com a SM. O risco de morbidade por doença coronariana associada ao agrupamento de fatores de risco (componentes da SM) é maior do que o risco de cada componente individualmente (92).

4. Aspectos etiológicos da síndrome metabólica

A definição de SM representa um progresso no entendimento da sua fisiopatologia e de como os seus diferentes componentes interagem aumentando o risco de doença cardiovascular. Todavia, a sua etiopatogênese ainda permanece obscura. As diferentes anormalidades associadas na SM – resistência e/ou alterações da tolerância à glicose e obesidade/ obesidade abdominal, HAS, dislipidemia e microalbuminúria apresentam evidências de hereditariedade (101). No entanto, a herança da SM, propriamente dita, não está totalmente comprovada. Os agrupamentos destes achados envolvem componentes genéticos e ambientais, mas não existe um modelo único explicando as suas razões.

4.1.Fatores ambientais

Entre as condições ambientais que favorecem o aparecimento da SM está a falta de atividade física (sedentarismo). Do ponto de vista de saúde pública, a falta de atividade física e a obesidade são os principais fatores para o desenvolvimento da SM na população dos Estados Unidos (67). Em duas recentes revisões (102, 103), o aumento da prática de atividade física, juntamente com a redução da ingestão calórica, representa a base do tratamento da resistência insulínica. As evidências indicam que a tolerância à glicose e a sensibilidade insulínica, o perfil lipídico, a pressão arterial, e a atividade fibrinolítica melhoram com a prática regular de exercícios em indivíduos com resistência insulínica (104). Os exercícios não necessitam ser intensos, considerando que longas caminhadas (105) e corridas (106) que produziram nenhuma ou mínima alteração na $VO_{2\ max,}$ mostram significante melhora na resistência insulínica, perfil lipídico e pressão arterial.

Foi demonstrado que a redução da atividade física (baixa $VO_{2\ max}$) está associada com adiposidade intra-abdominal em recém-nascidos de pacientes com DM2 (107) e está associada com defeito na sensibilidade insulínica (108). É amplamente comprovado (109) o efeito favorável do exercício sobre a resistência à insulina através do estímulo dos transportadores intracelulares (GLUT4) para captação de glicose. Este efeito é realizado através da ativação da 5’ - AMP- quinase e ocorre normalmente mesmo em indivíduos com resistência insulínica, portanto representando um importante fator terapêutico para o controle da glicemia em indivíduos com DM2 (110). Além de melhorar o metabolismo glicídico, reduzindo os níveis de glico-hemoglobina de pacientes com DM2, a prática regular de exercício traz benefícios a outros componentes da SM, melhorando os níveis pressóricos e o perfil lipídico do plasma (elevação do HDL-colesterol).

Outros fatores ambientais que influem no grau de resistência insulínica são a composição da dieta, a idade e alguns hormônios, particularmente glicocorticóides (111) e andrógenos (112). Dietas com altos níveis de carboidrato reproduzem alguns dos achados da SM (113). No estudo CARDIA (114) - *Coronary Artery Development in Young Adults* - os autores observaram que a ingestão de leite e seus derivados reduzia em cerca de 50% o risco de desenvolver a SM nos pacientes com sobrepeso.

4.2. Fatores neonatais e genótipo de "privação" ou de "economia"

A observação de que a má nutrição intra-uterina ocasiona um baixo peso ao nascer e aumenta o risco de desenvolvimento de SM na vida adulta deu origem a uma outra hipótese para a etiologia da SM denominada de fenótipo de privação (economia) (115). O risco do fenótipo de privação para a SM está particularmente aumentado em indivíduos com história familiar de HAS, tanto paterna (116) como materna, sugerindo um gene de "privação". Estas observações foram recentemente comprovadas em um estudo que descreveu que polimorfismo no substrato-1 do receptor da insulina está associado ao baixo peso ao nascer (117).

A hipótese da SM ou de alguns dos seus componentes, como p.ex. a obesidade, representarem uma adaptação genética às condições ambientais a que o indivíduo é exposto – alcançando um novo estado de homeostase foi descrito como hipótese do gene de "privação" ou genótipo de "privação" (118). Esta hipótese é baseada no fato de que indivíduos expostos a situações ambientais precárias, com reduzido suprimento alimentar, iriam melhorar a sua sobrevida se pudessem maximizar a sua capacidade de armazenar energia; sendo assim, os indivíduos sobreviventes, por seleção natural, seriam aqueles capazes de reduzir o gasto energético em situações de privação ou jejum prolongado. Todavia, o eficiente

armazenamento de energia leva ao acúmulo de gordura e ganho de peso. Portanto, genes que afetam o peso corporal e a distribuição de gordura podem predispor à SM e conseqüentemente ao DM2.

4.3.Fatores genéticos

As doenças metabólicas humanas raramente podem ser explicadas como simples fenótipos mendelianos. Mais comumente, envolvem múltiplos e complexos fatores etiológicos como a influência de numerosos efetores incluindo os fatores sociais, fisiológicos, metabólicos e moleculares. O entendimento das bases genéticas destes fatores requer uma definição fenotípica cuidadosa, amostras representativas de indivíduos, estudo de agrupamentos familiares, estudo de genes candidatos e estudo do genoma humano com marcadores microsatélites.

A procura de uma explicação genética para a etiologia da SM e de seus componentes tem conquistado grande espaço na pesquisa médica nos últimos anos. Existem evidências substanciais de que o DM2 (componente da SM) é uma doença hereditária, conforme demonstrado em estudos com gêmeos, estudos de agrupamentos familiares de DM2 e estudos epidemiológicos em alguns grupos étnicos (1, 50).

As observações de que a resistência insulínica está agrupada em famílias e que 45% dos parentes de primeiro grau de pacientes com DM2 apresentam resistência insulínica quando comparados com 20 % de indivíduos sem história familiar de DM2 (1, 119, 120), favorecem a participação genética na etiopatogênese da SM. As interações dos efeitos do genótipo e ambiente foram analisadas em gêmeos monozigóticos, através de manipulação no balanço energético. Os autores observaram que embora existem diferenças individuais na resposta ao balanço energético negativo e positivo, a resposta, é muito semelhante nos pares

de gêmeos (121, 122). Esta similaridade intrapares é também observada, em relação à gordura visceral (123).

Aproximadamente, 40% da variação da gordura corporal, especialmente da gordura abdominal, é atribuída a fatores genéticos (124). Vários genes candidatos e marcadores genéticos estão associados à obesidade, gordura corporal e sua distribuição em humanos. O mapa genético humano para obesidade (125), baseado em estudos em seres humanos e animais, inclui diversos genes ou locis potencialmente envolvidos na etiologia da obesidade.

Os demais achados da SM: HAS (126), hipertrigliceridemia e reduzidos níveis de HDL colesterol (127, 128), microalbuminúria (129) parecem estar também sob algum controle genético.

Baseado na teoria do genótipo de "privação", genes que afetam o peso, a distribuição de gordura, a taxa de lipólise e o metabolismo da glicose, podem predispor à SM e seus componentes. Genes relacionados com o aparecimento de resistência insulínica podem estar associadas ao desenvolvimento do DM2 e suas complicações crônicas. Na tabela 1 estão apresentadas as principais alterações (mutações/ polimorfismos) estudadas até o momento, e que estão associadas à resistência insulínica e/ou a algum componente da SM.

Tabela1. Alterações genéticas descritas em associação com alteração da sensibilidade insulínica ou com componente da síndrome metabólica.

Gene	Polimorfismo	Efeito/ fenótipo	Referência
IRS-1	Gly972Arg	Impede a sinalização da insulina estimulada	130, 131
IRS-2	Gly1057Arg	Alteração da ação insulínica	132
ACE	Inserção/Delesão	Associação com SM	133
PPARγ	3p25 Pro12Ala	Diminui a atividade transcricional e aumenta a sensibilidade insulínica	134, 135 136, 137

LEPR	1p31	Obesidade abdominal e aumento de gordura corporal	138, 139
ENPP1	K121Q	Inibição da atividade do receptor tirosina quinase/ resistência insulínica	140, 141
FABP2	Ala54Thr	Aumenta a absorção de AGL Aumenta a oxidação de gordura Diminui a ação insulínica	142, 143, 144
APOD	3q26.2-qter	Aumento do IMC	145
UCP1	4q28-q31	Aumento de gordura corporal	146

5. Tratamento da síndrome metabólica e perspectivas futuras

O tratamento da SM ou de seus componentes de forma isolada é extremamente importante para determinar redução de morbi - mortalidade cardiovascular e o desenvolvimento de DM2. Seria uma tentativa de diminuir a freqüência de indivíduos que, atualmente, atingem o topo do “iceberg” descrito por Mathaaei et al (23). A resistência insulínica é comum, porém freqüentemente não é detectada até uma fase tardia da vida, quando aparece o comprometimento da homeostase da glicose, principalmente o DM e suas complicações crônicas, além de HAS, dislipidemia ou obesidade.

Está bem demonstrado que mudanças no estilo de vida, incluindo exercício e hábitos alimentares, podem reduzir o risco de progressão para o DM2 em pacientes com tolerância alterada à glicose (147-150). De um modo geral, os dados de estudos clínicos e de observação demonstram a importância da redução de peso sustentada e dos comportamentos relacionados com a obtenção de um benefício duradouro em termos de tolerância oral à glicose.

Tuomilehto et al (149) mostraram que através de intervenções no estilo de vida (atividade física e comportamento alimentar), é possível prevenir o DM 2 (3% ao ano evoluiu

para o DM no grupo de intervenção vs. 6% no grupo controle) em indivíduos de alto risco. Em agosto de 2001, o *"National Institute of Diabetes and Digestive and Kidney Disease"* finalizou outro grande estudo clínico randomizado de prevenção de diabete – *"Diabetes Prevention Program"* (DPP) (66). Neste estudo, ambas as intervenções, modificação intensiva do estilo de vida e a medicação (metformina) foram efetivas. Os participantes alocados para a intervenção intensiva no estilo de vida reduziram em 58 % seu risco de desenvolvimento de DM2, enquanto os que utilizaram metformina reduziram o aparecimento de DM2 em 31%. Em todos estes estudos, a atividade física é de intensidade moderada como p.ex. caminhadas de 30 minutos ao dia e modificações na dieta são modestas, tais como redução na ingestão de gordura e aumento na quantidade de fibras da dieta.

No entanto, apenas estas medidas não parecem ser suficientes para evitar inteiramente o surgimento da SM e de seus componentes. O manejo farmacológico da resistência insulínica é outro ponto forte de prevenção ou progressão da SM. Na atualidade, apesar de nenhum tratamento estar ainda aprovado para uso específico na resistência insulínica há diversos estudos em andamento para avaliar o efeito de alguns fármacos que influenciam a resistência insulínica no aparecimento de DM e eventos cardiovasculares. Entre estes se citam: as biguanidas (metformina) (151), os inibidores da α-glicosidase (acarbose, voglibose, miglitol) (152) e as tiazolidinedionas (TZD) (rosiglitazona, pioglitazona) (153). Outros medicamentos que não são usados como anti-hiperglicêmicos também afetam a resistência insulínica. Os inibidores da enzima conversora da angiotensina são agentes anti-hipertensivos que inibem a conversão da angiotensina I para angiotensina II e apresentam uma pequena redução da resistência insulínica, provavelmente relacionada ao efeito dilatador sobre vasos que fornecem suprimento ao fígado e ao músculo esquelético (154).

6. Conclusão

Apesar do progresso no entendimento da etiopatogênese da SM e o reconhecimento de sua associação com morbi-mortalidade cardiovascular, é fundamental que se compreenda melhor a sua possível participação no desenvolvimento das complicações crônicas do DM2. Conforme já descrito anteriormente, estes achados precedem muitas vezes o diagnóstico de hiperglicemia Portanto, se pudermos identificar uma relação de risco entre SM e o desenvolvimento das complicações micro e macrovasculares do DM2, poderemos intensificar as medidas de intervenção terapêutica neste grupo de indivíduos, reduzindo não apenas o surgimento do DM2, mas também de suas complicações as quais estão relacionadas com altos índices de morbi-mortalidade nestes indivíduos. Com este objetivo, estudou-se a prevalência de SM em um grupo de pacientes com DM2 e, a relação do agrupamento progressivo dos seus componentes com o desenvolvimento de complicações crônicas. Além disso, para melhor entender a participação dos fatores genéticos na etiologia da SM, analisamos três polimorfismos genéticos anteriormente relacionados à resistência insulínica

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ARTIGO I - Aggregation of features of the metabolic syndrome increases the proportion of diabetes complications in patients with type 2 diabetes mellitus *

* Este artigo foi submetido à publicação no Diabetes Care em maio de 2002 e será apresentado de forma oral no 38º Encontro Anual da "European Association for the Study of Diabetes", em setembro de 2002.

Aggregation of features of the metabolic syndrome increases the proportion of diabetes complications in patients with type 2 diabetes mellitus

Running title: Metabolic syndrome and diabetes complications

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word count: 3513 words

number of tables: 1

number of figures: 2

ABSTRACT

Objective: To investigate whether the aggregation of metabolic syndrome (MS) features was associated with increased prevalence of chronic diabetes complications.

Research Design and Methods: A case-control study was conducted with 345 type 2 diabetic (DM2) patients. Retinopathy was diagnosed by direct funduscopy; coronary artery disease (CAD) by the World Health Organization (WHO) cardiovascular questionnaire and/or ECG and/or myocardial scintigraphy; distal sensory neuropathy (DSN), by symptoms and vibratory and tactile sensation; peripheral vascular disease (PVD) by the WHO cardiovascular questionnaire and palpation of foot pulses; and micro- or macroalbuminuria by 24 hour urinary albumin excretion rate (UAER). MS was diagnosed in the presence of at least two of the following: hypertension, dyslipidemia, obesity, and UAER ≥ 20 μg/min.

Results: Patients with two or more MS features (n=277; 80%) presented higher prevalence of CAD (52% vs. 35%, OR: 2.0, 95%CI: 1.12-3.63), PVD (43% vs. 17.8, OR: 3.5, 95% CI: 1.46-8.83), stroke (10.1% vs. 1.9%, OR 5.83, 95% CI:0.8-119), retinopathy (53% vs. 26%, OR 3.2, 95% CI:1.68-6.14), DSN (51 vs. 28%, OR 2.6, 95% CI:1.23-5.8), and macroalbuminuria (38.2% vs. 27.1% OR 1.67, 95% CI:0.9-3.02)]. The higher the number of MS features (none or one, two, three) the higher the proportion of diabetes complications: CAD: 35%, 44%, 52% and 60%; PVD: 21%, 24%, 52% and 60%; stroke: 1.9%, 6.0%, 10.3% and 17.0%; retinopathy: 26%, 39%, 61% and 67%; DSN: 28%, 35%, 57% and 66%; macroalbuminuria: 27.1%, 32.4% and 45.1%, $P<0.05$.

Conclusion: The MS and summation of its components were significantly associated with macro- and microvascular complications in DM2 patients. Multintervention strategies directed to the components of the syndrome may be beneficial.

INTRODUCTION

About 30 to 45% of patients with type 2 diabetes mellitus (DM2) present microvascular complications [1]. In addition, macrovascular disease, which affects 20% of DM2 patients, is the main cause of death in these patients [1]. Poor glicemic control and duration of diabetes are well known risk factors for both micro- and and macrovascular problems [2]. Hypertension and dyslipidemia are also important factors especially for macroangiopathy [3]. These factors and central obesity often cluster and are related to insulin resistance [4]. The recognition of this cluster was made initially by Reaven [5] and has been recently denominated by the World Health Organization (WHO) [6] as metabolic syndrome (MS). The MS is defined by the presence of two or more of the following – hypertension, obesity, dyslipidemia, and microalbuminuria – in patients with DM2 or impaired glucose tolerance [6].

Each component of the MS is in itself a risk factor for cardiovascular disease, and the combination of several components results in greater risk [7]. The presence of the MS has also been recently associated with microvascular complications in patients with DM2 [8]. Therefore, it is plausible to assume that the number of MS elements could be associated with an increased risk for chronic macro- and microvascular diabetes complications. To test this hypothesis, a case-control study was conducted in DM2 patients grouped according to the number of MS features presented by them.

RESEARCH DESIGN AND METHODS

Patients

A case-control study was conducted with DM2 patients (WHO criteria [6]) attending the outpatient endocrinology and nephrology clinics at Hospital de Clínicas de Porto Alegre, state of Rio Grande do Sul, Brazil. Four hundred patients were initially enrolled, but 55 patients did not complete the clinical and/or laboratorial evaluation. From the 345 patients who were actually studied, 195 came from other protocols conducted in the Endocrinology Division [9, 10]; 50 patients were recruited at the Nephrology Division; and 100 were recruited consecutively among the patients receiving care at the diabetes clinic.

According to the last demographic census [11], 86% of the state population are classified (based on self-reporting) as white, 8.4% as mulatto, 4.0% as black and 0.9% as native, yellow or not defined. Our patient population reflects this ethnic distribution.

A standard questionnaire was used to collect information about age, age at diabetes diagnosis and drug treatment. All patients underwent a complete physical examination and laboratory tests. They were weighed in light outdoor clothes without shoes, and height was recorded. BMI was calculated as weight (kg) divided by height (m^2). Waist and hip circumference were measured and waist to hip ratio (WHR) was calculated. Blood pressure was measured twice in the sitting position after a 10 minute rest with a mercury sphygmomanometer (Korotkoff phases I and V). Hypertension was considered to be present when blood pressure was ≥ 140/90 mm Hg, or if the patient was taking antihypertensive drugs. The presence of retinopathy was assessed by an ophthalmologist and graded as: 1) no signs of diabetic retinopathy; 2) non-proliferative retinopathy (microaneurysms, hemorrhages, hard exudates); or 3) proliferative retinopathy (newly formed blood vessels and/or fibrous tissue into the vitreous cavity). The diagnosis of distal symmetric neuropathy

(DSN) was based on abnormal results on Achilles tendon reflexes, vibration or sensory perception by a 10 g Semmes-Weinstein monofilament at the hallux on each foot. Presence of intermittent claudication, assessed by the WHO questionnaire for cardiovascular disease [12] and/or absence of posterior tibial pulse upon clinical examination indicated the presence of peripheral vascular disease (PVD).

The presence of cerebrovascular disease was established by history of stroke and/or presence of compatible findings (sequelae). The diagnosis of coronary artery disease (CAD) was based on the presence of angina or possible infarct according to the WHO questionnaire for cardiovascular disease [12], and/or on the presence of resting ECG abnormalities [Minnesota Code: Q and QS patterns (1.1-2, 1.3); S-T junction (J) and segment depression (4.1-4); T-wave items (5.1-3) and complete left bundle branch block (7.1)] [13], and/or on the presence of perfusion abnormalities (fixed or variable) upon myocardial scintigraphy at rest and after dipyridamole administration.

Patients with two or more of the components listed below were considered to present the MS [6]: hypertension (blood pressure ≥ 140/90 mm Hg and/or on antihypertensive treatment); dyslipidemia (plasma triglycerides ≥ 150 mg/dl and/or HDL-cholesterol < 35 mg/dl in men or < 39 mg/dl in women); obesity (BMI > 30 kg/m^2 and/or WHR > 0.90 in males or > 0.85 in females); micro- or macroalbuminuria [urinary albumin excretion rate (UAER) ≥ 20 μg/min]. The patients diagnosed with MS were defined as cases and grouped according to the number of MS components they presented (two, three or four components; Group 2; Group 3; Group 4, respectively).

For the analysis of the association between the MS and abnormal UAER, the presence of microalbuminuria was not considered. Therefore, for this specific analysis, a maximum of three associated MS features was taken into consideration. Patients with DM2

presenting only one or none of the features of the MS were classified as controls (Group 1). The protocol was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre and all patients signed an informed consent form.

Methods

UAER was measured in 24-h timed sterile urine samples by immunoturbidimetry (Microalb, Ames-Bayer, Tarrytown, NY, intra- and inter-assay coefficients of variation: 4.5% and 11.0%, respectively). The presence of microalbuminuria (UAER 20-200 μg/min) or macroalbuminuria (UAER > 200 μg/min) was confirmed by at least two measurements made 3 to 6 months apart. Glucose levels were determined by a glucose oxidase method; creatinine by the Jaffé reaction; HbA_{1c} by an ion-exchange HPLC procedure (Merck-Hitachi L-9100 Glycated hemoglobin Analyser; reference range: 2.7-4.3%), and triglycerides and cholesterol levels by enzymatic methods. LDL-cholesterol was calculated using the Friedewald equation. Myocardial scintigraphy was performed at rest and after dipyridamole stress, as previously described [13]. Cardioactive medications were suspended 7 days before the scintigraphy.

Statistical Analysis

Analysis of the data was carried out in two steps. First, the patients were grouped into two categories: MS cases (two or more features of MS) or controls (none or only one feature of MS). Second, the data were analyzed according to the number of associated MS features (none or one feature and two, three or four features). Continuous data were expressed as means ± SD or number of cases and percent of individuals affected. Chi-square, or Student's t-test, or one-way analysis of variance (ANOVA) were used to compare cases and controls in terms of clinical and laboratory characteristics. Variables non-Gaussian distribution where log-transformed. The trends associated with the proportion of complications observed in patients grouped according the number of features of the MS were analyzed by chi-square for

linear trends. A P value (two sided) < 0.05 was considered to be significant. Multiple logistic regression analyses (backward stepwise Wald method) were performed with the chronic complications as the dependent variable. Statistically significant variables in the univariate analysis and known risk factors for development of chronic complications (HbA_{1c} and total cholesterol) were included in the model. All calculations were performed using the SPSS statistical package.

RESULTS

Among the 345 patients studied, 277 (80%) presented two or more features of the MS. There were no differences between the groups with or without the MS in terms of mean age (60 ± 9.7 years *vs*. 59 ± 9.4 years, P=0.21) and proportion of males (50.5% vs. 49.5%, P=0.77).

Twenty six percent of the patients were classified as non-white. The prevalence of the MS was similar in the white and non-white groups (79% vs. 84%, P=0.16). Patients with MS had longer duration of diabetes (15 ± 9 vs. 12 ± 6 years, P=0.01) and were more often treated with insulin (46% vs. 26%, P=0.02). HbA_{1c} values were similar in the two groups (6.46% ± 1.84 vs. 6.74% ± 1.81, P=0.27). As expected based on the definition of the MS, the prevalence of hypertension (83.4% vs. 16.2%, P<0.01) and the blood pressure levels were higher (146/83 mmHg vs. 124/78 mmHg, P<0.01) in the group with the MS. The MS group also had higher WHR (0.94 ± 0.09 vs. 0.91 ± 0.09, P=0.02), higher BMI (28.6 ± 5.1 kg/m^2 vs. 25.6 ± 3.5 kg/m^2, P<0.01) and higher triglyceride levels (202 ± 117 mg/dl vs. 111 ± 53 mg/dl, P<0.01). HDLc levels were lower in the MS group (42 ± 12.3 mg/dl vs. 48 ± 8.7 mg/dl, P<0.01). Total cholesterol and LDLc were not considered for the diagnosis of MS, however they were higher in patients with the MS (total cholesterol 222 ± 43 mg/dl vs. 207 ± 43 mg/dl, P=0.02; LDLc 145 ± 43 mg/dl vs. 131 ± 33 mg/dl, P=0.01).

The frequency of chronic complications in all patients was: CAD in 51%, PVD in 39%, stroke in 8.5%, retinopathy 48%, DSN in 47% and micro- or macroalbuminuria in 46%. Patients with 2 or more MS features presented a higher prevalence of CAD [52% vs. 35%, odds ratio (OR) 2.0, 95%CI: 1.12-3.63, P=0.01], PVD (43% vs. 17.8, OR 3.5, 95%CI: 1.46-8.83, P<0.01), stroke (10.1% vs. 1.9%, OR 5.83, 95%CI: 0.8-119, P=0.04), retinopathy (53% vs. 26%, OR 3.2, 95%CI: 1.68-6.14, P<0.01), and DSN (51 vs. 28%, OR 2.6, 95%CI: 1.23-5.8, P<0.01). Micro- or macroalbuminuria were not different in the two groups when UAER > 20 μg/min was excluded from the definition of MS (OR 1.4, 95%CI: 0.87-2.32, P=0.14). The frequency of complications increased with the duration of diabetes in both groups. However, the increase was more marked among patients with the MS (figure 1). For CAD, PVD, DR and DSN the difference became significant after 10 to 15 years of diabetes duration (p<0.05).

When the patients were grouped according the number of MS features (none or one, two, three or four), there was a significant linear increase in the proportion of complications associated with the number of MS features (figure 2): (CAD – 35%, 44%, 52% and 60%; PVD – 21%, 24%, 52% and 60%; stroke – 1.9%, 6.0%, 10.3%, 17.4%; retinopathy – 26%, 39%, 61% and 67%; DSN – 28%, 35%, 57% and 66%; P for trend <0.05).

The prevalence of proliferative retinopathy showed the same pattern of association (9.4%, 19.2, 38.1% and 50%, respectively; P for trend < 0.001). The frequency of micro- and macroalbuminuria increased with the aggregation of MS features, although the statistical significance was borderline (40%, 45% and 54%, P=0.06). Macroalbuminuria alone was significantly associated with the clustering of MS elements: 27.1%, 32.4% and 45.1%, P=0.02.

The clinical and laboratorial characteristics of DM2 patients according to the clustering of MS features are described in table 1. The group with three MS features had longer duration of diabetes than the control group (P=0.02). Blood pressure levels (systolic and diastolic) and BMI were higher in the groups with the MS compared to controls (P<0.01), however both features were similar within all MS groups (P>0.05). The WHR was higher in Groups 3 and 4 in comparison to Groups 2 and 1 (controls). Total cholesterol and HbA_{1c} were similar in all groups. HDLc was lower in Group 4 than in the other groups (P<0.01). LDLc levels were higher in Group 4 than in controls (P=0.04). As expected by the definition of the MS, triglyceride levels and UAER were lower in the control group compared to the groups with the MS (P<0.01). However, a significant increment was observed in the mean triglyceride and UAER values with the increase in the number of MS features (P for trend <0.01).

Logistic regression models were performed having chronic diabetes complications as the dependent variable. Presence of the MS, duration of diabetes, HbA_{1c} and total cholesterol levels were entered as independent variables. After control of these variables, the MS was associated with CAD (OR 1.83, 95% CI 1.01-3.32, P=0.05), PVD (OR 2.95, 95% CI 1.26-6.89, P=0.01), retinopathy (OR 3.20, 95% CI 1.65-6.24, P<0.01) and DSN (OR 2.07, 95% CI 1.02-4.33, P=0.05). Micro- and macroalbuminuria presented a borderline significant association (OR 1.71, 95% CI 0.99-2.93, P=0.05). However, when patients with macroalbuminuria only were analyzed, we observed a significant association with presence of the MS (OR: 2.5, 95% CI: 1.24-5.0, P=0.01). Stroke, when controlled for diabetes duration, HbA1c and total cholesterol levels, was not associated with the MS (OR 4.75, 95% CI: 0.6-36).

Hypertension is a well-defined risk factor for developing retinopathy, and it is part of the definition of the MS. In a logistic model without the MS, the presence of hypertension had a lower OR for retinopathy (OR 2.0 95% CI 1.19-3.5) than the MS.

CONCLUSION

The MS occurred in 80% of this sample of DM2 patients, and was associated with increased prevalence of macro- and microvascular complications even when metabolic control and duration of diabetes were taken into account. Moreover, the aggregation of features of the MS was associated with a linear increase in the proportion of complications.

As far as we know, up to this point only two other studies [7, 14] have followed WHO criteria to assess the prevalence of the MS. Interestingly, that population-based study in Sweden and Finland and the Chinese case-control study found the same proportion of the MS in DM2 patients [7, 14]. In studies using other criteria, the prevalence of the MS varied according to the population studied [15-18]. According to the HOMA index and other methods, the prevalence of insulin resistance has a large variability in patients with DM2 of different ethnic origins [19]. The population of our state is composed mainly of white people of European descent, and people of African and Latin-American ancestry are less represented. However, even in white people some genetic heterogeneity is expected. In fact, about 1% of the persons identified as white in our population are heterozygous for hemoglobin S [20]. The results of our study and those of the Scandinavian and Chinese studies [7, 14], following WHO criteria, suggest that the prevalence of the MS in DM2 patients is independent of ethnic factors.

The fact that there was no significant increase in the prevalence of complications among patients with shorter diabetes duration (<10-15 years) suggests that the presence of

the MS before the diagnosis of diabetes does not play a major role in the development of these complications in the pre-diabetes state. On the other hand, the presence of the MS markedly increased the development of macro- and microvascular complications 10 to 15 years after the known onset of hyperglycemia.

The risk of CVD and PVD was increased by a factor of 2 to 3 in the presence of the MS. This may be due to an accelerated atherosclerotic process resulting from the clustering of cardiovascular risk factors [7].

In the logistic model, stroke was not associated with the MS. However, this could be reflecting a type 2 error, since very few patients had this complication, and almost all of them presented the MS (96%). Only one did not have a diagnosis of MS. This would explain the wide confidence interval (0.6-36) observed. In fact, we observed a linear increase in the proportion of macroangiopathies (CAD, PVD, stroke) with the increase in the number of MS features. These findings are in agreement with the observation that diabetic patients are more susceptible to the development of cardiovascular events when there is an aggregation of cardiovascular risk factors [21]. The atherogenic basis of the MS is believed to be result of multiple risk factors that combine to produce a large increase in risk for coronary disease and general atherosclerosis. It is possible that the MS may have other features besides those analyzed in the present study (obesity, hypertriglyciredemia, low HDL cholesterol, hypertension), such as abnormalities of fibrinolysis and coagulation and endothelial dysfunction. We have previously reported that dyslipidemic DM2 patients presented higher levels of endothelin-1 as compared to dyslipidemic non-diabetic individuals, and that this was significantly associated (adjusted r^2 = 0.42) with serum triglyceride levels, age, insulin sensitivity index and albuminuria levels [22].

Retinopathy was more prevalent in patients with the MS and increased linearly according the number of features of the syndrome. Proliferative retinopathy followed the same pattern. Other authors have also observed that proliferative retinopathy, but not retinopathy in general, was more common in patients with the MS [8]. In the logistic analysis with retinopathy as the dependent variable, we observed that the MS remained significantly associated, with an OR 3.2. Considering that hypertension is also a strong risk factor for retinopathy [23] and it is one of the criteria to establish the diagnosis of MS, hypertension was included in the logistic analysis instead of MS. The observed OR was 2.0. Therefore, the association of MS and retinopathy seems to be stronger than the association between hypertension alone and retinopathy.

In the present study we did not observe an increase in the proportion of patients with micro and macroalbuminuria in the MS group. However, the increased prevalence of micro- and macroalbuminuria reached a borderline statistical significance when the cluster of metabolic abnormalities was analyzed. The same pattern was observed in the multivariate model. This association became more evident when macroalbuminuria was analyzed in isolation. In fact, isolated microalbuminuria was neither associated with the MS nor increased with the aggregation of MS components (data not shown). The association of microalbuminuria with the MS in DM2 patients is controversial. Studies addressing this question in different populations and using different methods to define the MS have failed to demonstrate an association between insulin resistance, hyperinsulinemia, and/or dyslipidemia and the presence of microalbuminuria [24-26]. Also, microalbuminuria in non-diabetic patients with hypertension is not considered to be a determinant of insulin resistance [27]. On the other hand, in Finland, microalbuminuria was described to be associated with insulin resistance [28] and MS features in patients with DM2 [8].

DSN was also more frequent in patients with MS and increased progressively with the clustering of metabolic abnormalities. Very few studies have analyzed the association between peripheral neuropathy and MS features. Peripheral neuropathy consistent with bilaterally symmetric distal axonal loss was reported in a small number of patients with some MS components: obesity, hypertension, borderline plasma glucose levels, and hyperinsulinemia [29]. Isoma et al. observed a weak association of distal neuropathy with MS, but only HbA_{1c} remained statistically significant in a multivariate model [8]. It is possible that features of the MS contributed to impaired microcirculation of the peripheral nerves, causing impaired nerve function.

One potential limitation of the present study regarding the prevalence of MS-is that it was conducted with a sample of patients attending an outpatient clinic at a tertiary center offering specialized care in chronic diabetes complications. However, the proportion of chronic complications observed in these patients was similar that reported in population-based studies [1], although the frequency of CAD was higher. This may be due to the broad criterion used to define the presence of CAD (diagnosis according to one out of three different tests, the WHO questionnaire, ECG, and scintigraphy).

In conclusion, the MS in general, and also the clustering of its components, were associated with the presence of macrovascular, microvascular and distal neuropathy in patients with DM2. The MS is very frequent in diabetic patients, and these patients would probably benefit from an intensive multifactorial intervention treatment.

Acknowledgments: This study was partially supported by grants from the Programa de Apoio a Núcleos de Excelência and Hospital de Clínicas de Porto Alegre. LAC was recipient

of scholarships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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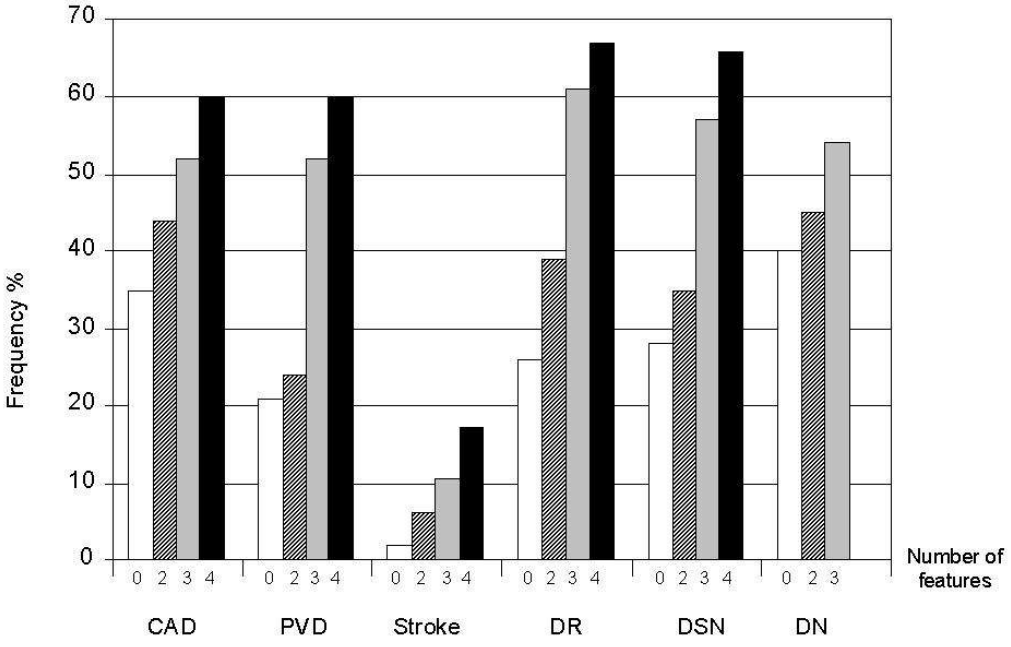

Figure 1. Distribution of diabetic chronic complications according to the number of metabolic syndrome features associated. CAD: coronary heart disease; PVD: peripheral vascular disease; DR: diabetic retinopathy; DSN; distal sensory neuropathy; DN: diabetic nephropathy. * P for trend <0.05

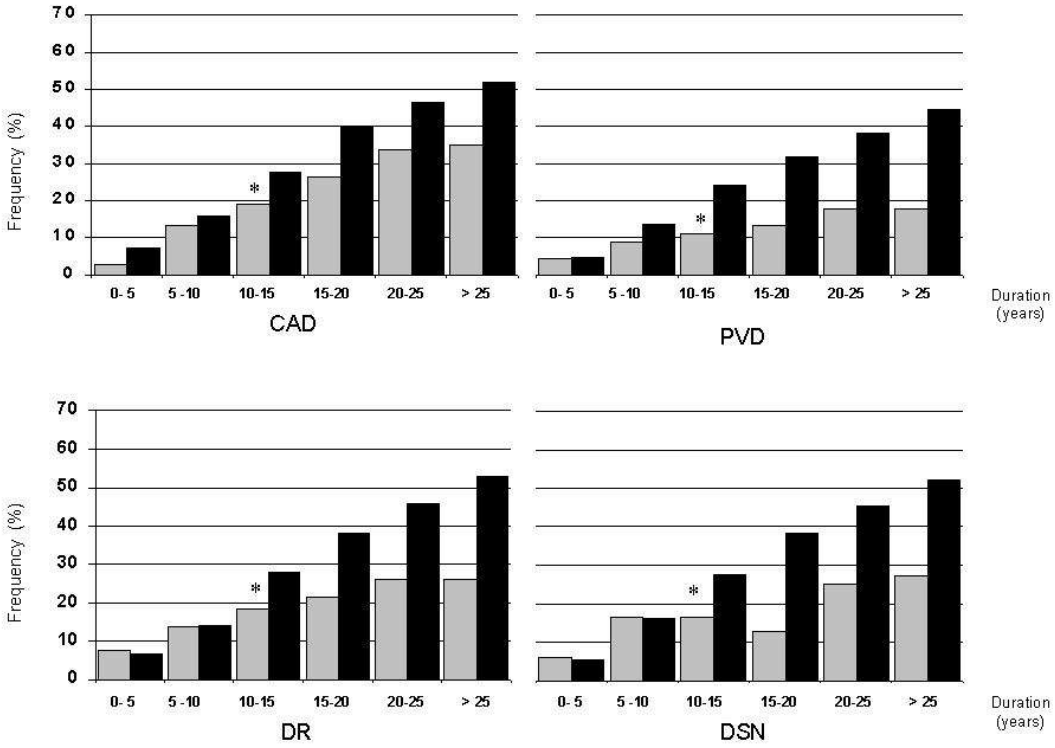

Figure 2. Prevalence of diabetic chronic complications according to diabetes duration and the presence or absence of the metabolic syndrome (MS). Gray bars: MS absent. Black bars: MS present. CAD: coronary heart disease; PVD: peripheral vascular disease; DR: diabetic retinopathy; DSN; distal sensory neuropathy. * P<0.05

Table 1. Clinical and laboratorial characteristics of type 2 patients according to aggregation of components of the metabolic syndrome

	Controls	Metabolic Syndrome			
	Group 1	Group 2	Group 3	Group 4	P*
	N=68	N=109	N=109	N=59	
Age (years)	59 ± 9	59 ± 10	60 ± 10	63 ± 9	0.14
DM duration (years)	11.5 ± 6	13.4 ± 9	15.6 ± 9	14.9 ± 8	0.02
Systolic blood pressure (mm Hg)	124 ± 14	141 ± 22	148 ± 22	150 ± 25	<0.01
Diastolic blood pressure (mm Hg)	78 ± 7	85 ± 12	86 ± 14	87 ± 11	<0.01
Waist-to-hip ratio	0.91 ± 0.09	0.91 ± 0.10	0.94 ± 0.09	0.98 ± 0.06	< 0.01
Body mass index (kg/m^2)	25.6 ± 3.5	28.4 ± 5.6	28.3 ± 4.9	29.6 ± 4.6	<0.01
HbA_{1c} (%)	6.75 ± 1.81	6.36 ± 1.78	6.56 ± 1.94	6.44 ± 1.77	0.62
Creatinine	0.94 ± 0.95	1.49 ± 2.25	1.69 ± 2.06	2.29 ± 2.70	<0.01
Total cholesterol (mg/dl)	207 ± 43	219 ± 43	225 ± 42	224 ± 46	0.08
HDLc (mg/dl)	48 ± 8.7	46 ± 12.3	42 ± 12.0	37 ± 11.2	<0.01
LDLc (mg/dl)	131 ± 33	140 ± 41	145 ± 39	153 ± 52	0.04
Triglycerides (mg/dl)†	111 ± 53	164 ± 94	203 ± 103	257 ± 146	<0.01
Albuminuria (μg/min)†	42.2 ± 198	182.7 ± 456	261.2 ± 473	733.8 ± 1244	<0.01

Data are expressed as number of cases (%) or mean values ± SD. *One-way analysis of variance (ANOVA). †Significance test performed on log-transformed data.

ARTIGO II - Angiotensin Converting Enzyme insertion/deletion, Fatty Acid-Binding Protein 2 A54T and Ecto-Nucleotide Pyrophosphate/Phosphodiesterase 1 K121Q polymorphisms are not associated with metabolic syndrome in white Brazilian patients with type 2 diabetes mellitus.**

****** Este artigo será submetido à publicação em periódico internacional

Angiotensin Converting Enzyme insertion/deletion, Fatty Acid-Binding Protein 2 A54T and Ecto-Nucleotide Pyrophosphate/Phosphodiesterase 1 K121Q polymorphisms are not associated with metabolic syndrome in white Brazilian patients with type 2 diabetes mellitus.

Running title: Metabolic syndrome and genetic polymorphisms

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word count: 2529

number of tables:2

ABSTRACT

Objective: To analyse whether the metabolic syndrome (MS) is associated with DNA polymorphisms in three genes previously reported to be related to insulin resistance: the angiotensin converting enzyme gene (*ACE*) I/D, the fatty acid-binding protein 2 (*FABP2*) A54T and the ecto-nucleotide pyrophosphate/phosphodiesterase 1 (*ENPP1*) K121Q.

Research design and methods: A case control study was conducted in 253 white Brazilian patients with type 2 diabetes mellitus (DM2). Patients underwent a clinical and laboratorial investigation and the DNA polymorphisms were genotyped using PCR and restriction enzyme protocols. MS was defined [World Health Organization criteria (WHO)], by the presence of 2 or more of the following features: hypertension, dyslipidemia, obesity and the presence of microalbuminuria.

Results: Approximately 80% (199) of white patients with DM2 presented the MS according WHO definition. The proportion of *ACE* DD genotype was 30% (n = 75), ID 51% (n = 129) and II 19% (n = 49). For the *FABP2* the frequency of AA genotype was 57% (n=145), AT 40% (n = 100) and TT 3% (n = 8), and for *ENPP1* KK was present in 60% (n = 152), KQ in 37% (n = 93) and QQ in 3 % (n=8) The *ACE* risk allele for developing MS is the D allele. For *ENPP1* is the presence of Q and for *FABP2* is the presence of T allele. The proportion of carriers of the risk allele of these three genes was similar among patients with or without the features of the MS (*ACE* 81% vs. 77%, P=0.55; *ENPP1* 37% vs. 50%, P=0.08 and *FABP2* 43% vs. 43%, P=0.99).

Conclusion: The variants of *ENPP*1, *FABP2* and *ACE* genes are not associated with the MS in Brazilian patients with DM2. These findings indicate that these genes probably do not play a major role in the development of the MS in these patients.

INTRODUCTION

The metabolic syndrome (MS) is an important risk factor for cardiovascular (1) and microvascular complications (2) in type 2 diabetes mellitus (DM2). Recently, World Health Oraganization (WHO) recommended an operational definition of MS characterized by variable coexistence of hyperglycemia and/or insulin resistance, dyslipidemia, obesity and hypertension (3). According to this definition, MS is observed in 80% of type 2 diabetic patients (1) and 22-24% of the general population (4). The presence of MS is influenced by both environmental and genetic background, the latter being mostly unknown (5, 6, 7). Insulin resistance – a key pathogenic element of MS - clusters in families (8). About 45% of first-degree relatives of patients with DM2 are insulin resistant as compared to 20% of individuals without a family history of diabetes (7, 8, 9,). The other components of MS are influenced by genetic factors (10, 11, 12). Indeed, we have previously demonstrated that type 2 diabetic patients with diabetic nephropathy presented an increased familial history of hypertension and cardiovascular disease (13). MS is a complex disease and probably has a polygenic background. Although, the familial inheritance of isolated metabolic abnormalities associated with the MS are well established, the heritability of the syndrome itself has not been reported.

One approach to study the genetic component of the MS is through the analysis of polymorphisms in putative candidate genes. Candidate genes are chosen based on the present knowledge of the components that define the syndrome. So any gene that is associated with obesity or abdominal obesity, dyslipidemia, hypertension and DM2 or insulin resistance may be considered to contribute to the development of the MS. Recently, it was demonstrated that the angiotensin-converting enzyme (*ACE*) I/D polymorphism was associated with MS in Chinese patients with DM2 (14). Ecto-nucleotide pyrophosphate/phosphodiesterase (*ENPP1*)

and fatty acid binding protein 2 *(FABP2)* polymorphisms have been associated with insulin resistance in diabetic and non-diabetic subjects (15, 16). However, these associations are not present in all populations (17, 18, 19), and the role of these genes in predisposing diseases might be dependent of the ethnic background.

Therefore we analysed the association of polymorphisms of *ACE I/D*, *FABP2* and *ENPP1* genes with MS in a Brazilian sample of white type 2 diabetic patients.

RESEARCH METHODS AND DESIGN

Patients

A nested case-control study with white type 2 diabetic patients was conducted. Two hundred fifty three white patients with DM2 who participate in a cross-sectional study for detection and study of diabetic chronic complications were identified and included in the case-control study. The diabetic chronic complication study group is a composed of three centers located in the southernmost part of Brazil. In this region of the country the majority of the population has an European heritage and 86% report themselves as white (20).

DM2 was diagnosed according to WHO criteria (3). The patients answered a standard questionnaire including age, age of diabetes diagnosis, and drug treatment. They underwent a complete physical examination and laboratory tests. They were weighed in light outdoor clothes without shoes, and height was recorded. Body mass index (BMI) was calculated as the weight (kg)/height (m^2) ratio. Waist and hip circumference were measure and waist to hip ratio (WHR) was calculated. Blood pressure was measured twice in the sitting position after a 10-minute rest with a mercury sphygmomanometer (phases I and V of the Korotkoff). Hypertension was considered to be present when blood pressure was ≥ 140/90 mm Hg, or if the patient was taking antihypertensive drugs. The MS was defined according to the WHO criteria (3). Diabetic patients with two or more of the components listed below were considered to present MS (cases): hypertension (blood pressure ≥ 140/90 mm Hg and/or antihypertensive treatment); dyslipidemia (plasma triglycerides ≥ 150 mg/dl and/or HDL-cholesterol (< 35 mg/dl in men; < 39 mg/dl in women); obesity [BMI > 30 kg/m^2 and/or WHR > 0.90 in males or > 0.85 in females]; micro- macroalbuminuria (albumin excretion rate ≥ 20 μg/min).

The protocol was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre and all patients signed an informed consent.

Methods

Urinary albumin excretion rate (UAER) was measured in 24-h timed sterile urine samples by immunoturbidimetry (Microalb, Ames-Bayer, Tarrytown, NY, intra- and inter-assay coefficients of variation: 4.5% and 11.0%, respectively). The presence of microalbuminuria (UAER 20-200 μg/min) or macroalbuminuria (UAER > 200 μg/min) was confirmed by at least two measurements 3 to 6 months apart Glucose levels were determined by a glucose oxidase method; creatinine by the Jaffé reaction; HbA_{1c} by an ion-exchange HPLC procedure (Merck-Hitachi L-9100 Glycated hemoglobin Analyser; reference range: 2.7-4.3%), and fructosamine by a colorimetric method (normal range: 1.87-2.87 mmol/L); and triglycerides and cholesterol levels by enzymatic methods. LDL-cholesterol was calculated using the Friedewald equation.

DNA was isolated from peripheral lymphocytes under standard procedures (21). Subjects were genotyped for previously described polymorphisms in *ENPP1* (K121Q) and *FABP2* (A54T) genes by polymerase chain reaction (PCR) – restriction fragment length polymorphism (PCR-RFLP) based method. Genomic DNA was amplified by PCR with a specific pair of primers for each fragment (*FABP2* FORWARD 5'- ACA GGT GTT AAT ATA GTG AAA AG-3 AND REVERSE 5'- TAC CCT GAG TTC AGT TCC GTC-3'; *ENPP1* FORWARD 5'- CTG TGT TCA CTT TGG ACA TGT TG –3' and reverse 5'- GAC GTT GGA AGA TAC CAG GGT TG- 3') and the amplicons were digested using specific restriction enzymes (*FABP2* A54T - *HHA*I and *ENPP1* K121Q - *AVA*II) (15, 16). The fragments were then separated by electrophoresis on agarose gel. Following the enzymatic digestion, the *FABP2* wild allele produces a PCR of 180 bp and the variant allele a fragment of 99 and 81 bp. The *ENPP1* had a PCR fragment size of 238 bp for K variant and two fragments of 148 and 90 bp for the Q variant.

The *ACE* I/D polymorphism was genotyped using the following set of primers: forward 5'- CTG GAG CCA CTC CCA TCC TTT CT – 3' and reverse 5'-GAT GTG GCC ATC ACA TTC GTC AGA T- 3'. The genotypes for the insertion allele were confirmed using a nested PCR with an internal primer as previously described (FYM 5'-ATC ACG AGG TCA GGA GAT CGA GAC-3' (22, 23). The final PCR product was analysed in an agarose gel. The deletion allele produced one fragment of 274 bp and the insertion, two fragments of 376 bp (FYM) and 561 bp (24).

All the reactions were run in final 25 μL using 50 ng of genomic DNA, containing 20 mM Tris HCl, (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit of Taq polymerase and 1 μM of specific primer. The thermal-cycler parameters for each PCR was based in previously published conditions (15, 16, 22).

Statistical Analysis

The patients were grouped in two categories: MS cases (two or more features of MS) or controls (none or only one feature of MS). Continuous data were expressed as mean ± SD or number of cases and percent of affected individuals. Chi-square or Student's t-test or one-way analysis of variance (ANOVA) were used to compare clinical and laboratory characteristics among the different genotypes. Variables with non-normal distribution where log-transformed. A P value (two sided) < 0.05 was considered to be significant.

RESULTS

Approximately 80% (199) of white patients with DM2 presented the MS according WHO (3) definition. There were no difference in the mean age (61 ± 9.3 years vs. 59 ± 9.1 years, P=0.94) and the proportion of males (54% vs. 46%, P=0.44) Patients with MS had longer duration of diabetes (15 ± 9 vs. 11 ± 6 years, P= 0.02) and were more often treated

with insulin (46% vs. 26%, P=0.02). HbA1c was similar between the two groups (6.46% ± 1.84 vs. 6.74% ± 1.81, P=0.27).

In table 1 are presented the frequency of alleles and genotypes for each polymorphism. The proportion of *ACE* DD genotype was 30% (n = 75), ID 51% (n = 129) and II 19% (n = 49). For the *FABP2* the frequency of AA genotype was 57% (n=145), AT 40% (n = 100) and TT 3% (n = 8), and for *ENPP1* KK was present in 60% (n = 152), KQ in 37% (n = 93) and QQ in 3 % (n=8). All polymorphisms were in Hardy-Weinberg equilibrium.

The *ACE* risk allele for developing MS is the D allele. For *ENPP1* is the presence of Q and for *FABP2* is the presence of T allele. The proportions of carriers of the risk allele of these three genes were similar among patients with or without the features of the MS (*ACE* 81% vs. 77%, P=0.55; *ENPP1* 37% vs. 50%, P=0.08 and *FABP2* 43% vs. 43%, P=0.24) (table 2). The carriers for the risk allele were similar to the non-carriers regarding gender distribution, mean age, duration of diabetes and BMI (table 2). The WHR did not differ between the carriers and non-carriers for the *FABP2* (T allele) and *ENPP1* (Q allele) risk alleles. The *ACE* D carriers had a lower WHR than the non-carriers (0.92 vs. 0.96, P=0.02). The metabolic control indices and lipid profile did not differ significantly in groups with or without risk allele for all three genes (table 1).

CONCLUSION

In this sample of white type 2 diabetic patients the proportion of patients with MS was ~ 80% and we did not observe any association of *ENPP1* K121Q, *FABP2* A54T, and *ACE* I/D polymorphisms with the MS.

Ethnical variability has been described for the *FABP2* A54T and *ACE* I/D polymorphisms. In this study, only white patients were included. Nevertheless, some degree

of ethnical admixture in Brazilian people self-reported as white can be expected. (25). However, the allele frequencies of the *ACE* and *FABP2* polymorphisms analysed were similar to the frequencies described in other studies with Caucasian subjects (19, 26). These findings suggest that, for these two genes, our sample of Brazilian white patients is comparable to other Caucasian populations.

ENPP1 is a specific inhibitor of insulin signalling and over expression of the gene is directly correlated with the degree of insulin resistance (27). A nucleotide change in a start codon in exon 4 substitutes a glutamine for a lysine (K121Q) and is associate with insulin resistance in non-obese, non-diabetic individuals (15) and diabetic subjects (28). However, there were no association between MS and *ENPP1* K121Q polymorphism in these Brazilian white DM2 patients. In two others studies in different Caucasians populations (15, 17) the authors found discrepancy in the results: in Sicilian population (15) human *ENPP1*-gene was associated with DM2 and insulin resistance and in Danish population (17) it was not. These findings might be due to differences in genetic background. One hypothesis is that the K121Q polymorphism may need to interact with other genetic mutations to end up in a phenotype of MS and such interactions may be more frequent in some populations.

The *FABP2* gene encodes an intestinal fatty acid-binding protein that is expressed only in the columnar intestinal epithelial cells of the small intestinal villous (29). Defects in the *FABP2* gene could affect the binding capacity of the *FABP2* protein, increasing fatty acid absorption and leading to enhanced fatty acid oxidation. These events would lead to impairment in insulin action (29). Actually, the polymorphism Ala→Thr in codon 54 of the *FABP2* has been shown to be associated with insulin resistance in non-diabetic Pima Indians (16) and in aborigines Canadians (30). Our results suggest that the polymorphism in the codon 54 of the *FABP2* gene is not a major contributing factor to MS in white DM2 patients.

Furthermore, Japanese and Finnish studies also did not observe an association of the A54T polymorphism with insulin sensitivity or obesity (18, 19). The frequency of the T-caring allele differs among the populations. It varies from 0.14 in aborigines Canadians up to 0.41 in Japanese. (30, 18). This may explain the discrepant findings among the studies.

It has been proposed that the presence of allele D of *ACE* gene is associated with diabetes, hypertension, coronary heart disease, elevated plasma triglycerides and total cholesterol (31-34). In addition, the renin-angiotensin system may be involved in the regulation of adipose tissue physiology, probably participating in the process of adipogenic differentiation and in the regulation of body weight (35). Finally, angiotensin II has been found to be a modulator of insulin sensitivity in diabetic and non-diabetic subjects. Therefore, *ACE* would be a good candidate gene for a MS. Recently, in Chinese patients with DM2 it was observed an association of MS with the presence of allele D (14). Although, we had noticed a similar prevalence of MS in our patients with DM2, the association with D allele was not observed. It is well known that *ACE* I/D polymorphism has an ethnical distribution and in the Asiatic the D allele is less frequent than in Caucasian (26). In fact, in our sample the proportion of type 2 diabetic patients with allele D (80%) was higher than in the Chinese patients (44.3%). The observation that the prevalence of MS in both studies was very similar (75% vs. 80%), despite of the significant difference in the alleles frequency, suggest that the presence of D allele did not influence the development of MS.

In conclusion, the variants of *ENPP1*, *FABP2* and *ACE* genes are not associated with the MS in Brazilian patients with DM2. These findings indicate these genes probably do not play a major role in the development of the MS in these patients.

Acknowledgments: This study was partially supported by grants from the Programa de Apoio a Núcleos de Excelência and Hospital de Clínicas de Porto Alegre. LAC was recipient of scholarships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Table 1. The genotypes distribution for *ACE* I/D, *FABP2* A54T and *ENPP1* K121Q polymorphisms and allele frequencies in patients with DM2

	Genotype		n	Allele	
		(%)		%	
ACE	**DD**	**ID**	**II**	**D**	**I**
	75 (30)	129 (51)	49 (19)	55.1	44.9
***FABP*2**	**AA**	**AT**	**TT**	**A**	**T**
	145 (57)	100 (40)	8 (3)	77.1	22.9
***ENPP*1**	**KK**	**KQ**	**QQ**	**K**	**Q**
	152 (60)	93 (37)	8 (3)	78.5	21.5

Table 2 – Clinical and laboratorial characteristics of patients with DM2 grouped according the presence of D, T and Q alleles of the *ACE*, *FABP*2, and *ENPP1* genes.

	ACE		*FABP*2		*ENPP1*	
	II	**ID/DD**	**AA**	**AT/TT**	**KK**	**KQ/QQ**
Metabolic syndrome (% yes)	76	79	79	79	82	73
Age (years)	60 ± 9	61 ± 9	60 ± 9	61 ± 10	61 ± 10	60 ± 9
Gender (% males)	59	52	50	57	52	55
DM duration (years)	16.2 ± 10.1	14.0 ± 9.0	14.0 ± 9.0	14.5 ± 10.0	14.4 ± 9.0	14.4 ± 9.0
Body mass index (kg/m^2)	28.1 ± 3.9	27.5 ± 4.4	27.2 ± 4.2	28.2 ± 4.3	27.4 ± 3.8	27.8 ± 4.3
Waist-to-hip ratio	0.96 ± 0.07*	0.92 ± 0.08	0.93 ± 0.08	0.94 ± 0.08	0.93 ± 0.09	0.93 ± 0.09
Fasting plasma glucose (mg/dl)	170.8 ± 77	167.0 ± 65	171.4 ± 72	162.0 ± 61	165.0 ± 60	169.0 ± 73
HbA_{1c} (%)	6.68 ± 1.66	6.48 ± 1.72	6.70 ± 1.77	6.28 ± 1.59	6.32 ± 1.69	6.70 ± 1.63
Total cholesterol (mg/dl)	212 ± 40	220 ± 42	220 ± 42	217 ± 40	218 ± 40	220 ± 44
HDLc (mg/dl)	45 ± 13	42 ± 10	43 ± 11	43 ±12	42 ± 11.2	44 ± 11.3
LDLc (mg/dl)	139 ± 36	142 ± 44	143 ± 43	140 ± 41	141 ± 43	142 ± 44
Triglycerides (mg/dl)†	188 ± 114	187 ± 112	181 ± 94	197 ± 134	187 ± 116	185 ± 107

Data are expressed as number of cases (%) or mean values ± SD

† Significance test performed on log-transformed data.

* II vs. ID/DD, P=0.027

COMENTÁRIOS FINAIS E PERSPECTIVAS FUTURAS

No primeiro estudo, observou-se uma elevada (80%) prevalência de síndrome metabólica (SM) em pacientes com diabete melito tipo 2 (DM2), empregando a definição da Organização Mundial da Saúde de 1999. No mesmo grupo de indivíduos, quando se analisou o agrupamento progressivo dos componentes da SM, observou-se um aumento linear da prevalência das complicações crônicas do Diabete Melito – doença cardiovascular (cardiopatia isquêmica, doença vascular periférica e acidente vascular periférico), neuropatia periférica, retinopatia e nefropatia. Outro achado de relevância é de que este aumento da prevalência das complicações, apesar de associado à SM, ocorre significativamente a partir de 10-15 anos de exposição ao DM2. Com isto, pode-se concluir que a hiperglicemia permanece como um fator fundamental para o desenvolvimento das complicações micro-macroangiopáticas do DM2.

O segundo artigo analisou a associação de três polimorfismos (*ACE* I/D, *ENPP1* – K121Q e *FABP2* – A54T) de genes candidatos para resistência insulínica e/ou SM. Apesar de não ter sido observada nenhuma associação destes polimorfismos com SM, acredita-se que esta é uma área de grande interesse para melhor entendimento do desenvolvimento da SM. Este aspecto será melhor respondido com a ampliação deste projeto para o estudo de agregação familiar dos pacientes com DM 2 com e sem SM, estudo de diferentes grupos étnicos, além do estudo de outros genes candidatos e /ou de outros polimorfismos destes mesmos genes.