LUCILENE REZENDE ANASTÁCIO

ASPECTOS CLÍNICOS E COMPORTAMENTO DOS MARCADORES BIOQUÍMICOS DA SÍNDROME METABÓLICA NO PÓS-OPERATÓRIO TARDIO DO TRANSPLANTE HEPÁTICO

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto da Faculdade de Medicina da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutora em Ciências Aplicadas à Saúde do Adulto.

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“Todo caminho da gente é resvaloso.
Mas também, cair não prejudica demais.
A gente levanta, a gente sobe, a gente volta!...
O correr da vida embrulha tudo, a vida é assim:
Esquenta e esfria, aperta e daí afrouxa,
Sossega e depois desinquieta.
O que ela quer da gente é cora[illegible]
Ser capaz de ficar alegre e mais alegre no meio da ale
E ainda mais alegre no meio da triste.

João Guimarães Rosa

Resumo

Introdução: A síndrome metabólica (SM) tem sido amplamente descrita em pacientes nos primeiros anos após o transplante hepático (TxH), mas há poucos estudos sobre essa condição em pacientes submetidos ao TxH em longo prazo. Também, embora haja grande quantidade de dados sobre o papel das adipocinas, marcadores inflamatórios e de resistência à insulina na SM, até o momento, esses dados são desconhecidos em pacientes submetidos ao transplante de fígado. **Objetivos:** Descrever longitudinalmente a prevalência da SM, respectivos componentes e fatores associados e, em um subgrupo, estudar o comportamento das concentrações séricas de adipocinas, marcadores inflamatórios e de resistência à insulina em pacientes submetidos ao TxH em longo prazo. **Métodos:** Trata-se de estudo longitudinal no qual 117 pacientes após o TxH foram avaliados em dois tempos distintos (2008 e 2012) quanto à presença de SM (utilizando os critérios do *National Cholesterol Education Program Adult Treatment Panel* III revisado – NCEP ATP III, *International Diabetes Federation* - IDF e *Harmonizing the Metabolic Syndrome* - HMS) e respectivos componentes, a composição corporal e a ingestão alimentar também foram analisadas. Dados demográficos, socioeconômicos e clínicos foram coletados. Em 34 pacientes, foram realizadas transversalmente as dosagens de adiponectina, resistina, fator de necrose tumoral alfa (TNF-a), proteína quimiotática de monócitos 1 (MCP-1), interleucina 6 (IL-6), ácidos graxos livres (AGL) e proteína C reativa (PCR). As dosagens de insulina e glicemia de jejum foram realizadas no intuito de se obter o cálculo do índice HOMA-IR (*Homeostatic Model Assessment*). **Resultados:** A SM foi diagnosticada em 43,1% (IDF/HMS) e 34,3% (NCEP) dos pacientes com tempo de seguimento pós-TxH mediano de 3 anos (0 a 13 anos) e, após tempo de seguimento mediano de 7 anos (3 a 17 anos), essa foi de 53,3% (p=0,12) e 44,8% (p=0,03), respectivamente. A prevalência de hiperglicemia elevou-se de 34,2% para 48,6% (p<0,01). O perímetro da cintura médio que foi de 93,3±14,3cm evoluiu para 99,4±14,9cm (p<0,01) e o percentual de gordura corporal médio, de 30,3±8,9% para 31,8±10,3% (p=0,03). A prevalência de obesidade abdominal pelo critério do IDF/HMS foi de 66,7% para 72,0% (p=0,09). Os fatores associados à SM foram: maior idade (OR: 1,05; IC: 1,02-1,11; p=0,02), história familiar de diabetes (OR: 3,38; IC: 1,19-9,61; p=0,02), maior índice de massa corporal (IMC) anterior à doença hepática (OR: 1,39; IC: 1,19-1,63; p<0,01) e maior percentual de gordura corporal (OR: 1,09; IC: 1,03-1,14). Os componentes da SM (obesidade abdominal, pressão arterial e hiperglicemia) associaram-se à maior idade (p<0,05). A obesidade abdominal esteve associada ao maior Índice de Massa Corporal (IMC) (p<0,01), à maior quantidade de gordura corporal (p=0,01) e à falta de exercícios físicos (p=0,01). O HDL reduzido esteve associado ao maior IMC (p<0,01), à maior ingestão de gorduras (p=0,03) e de carboidratos (p=0,03). A hipertrigliceridemia foi associada ao uso atual de corticoesteróides (p=0,04), história familiar de diabetes mellitus (p=0,04), maior IMC (p<0,01) e maior ingestão de gorduras (p<0,01). Os pacientes com SM apresentaram valores superiores de adiponectina (6,7±4,5 μg/mL *versus* 3,2±1,2 μg/mL, p<0,01). Maiores concentrações séricas de AGL (0,8±0,3 mEq/L *versus* 0,5±0,3 mEq/L, p<0,05) e altos valores de HOMA-IR (4,9±3,8 *versus* 1,6±0,8) também foram observados entre os pacientes submetidos ao TxH com SM. Maior relação cintura-quadril e HDL reduzido foram preditores independentes das concentrações de adiponectina (p<0,05). Menores quantidades de resistina foram observadas em pacientes com pressão arterial elevada e superiores, naqueles com obesidade abdominal (p<0,05). Os valores de HOMA-IR foram independente preditos por obesidade e SM (p<0,05). Variáveis independentes associadas ao TNF-a, MCP-1, IL-6 e AGL não foram identificadas. **Conclusão:** No pós-operatório tardio de TxH, foi observada SM em mais da metade dos pacientes. Houve aumento da prevalência de SM ao longo dos anos, bem como aumento do perímetro da cintura e da glicemia. SM e respectivos componentes estiveram associados a fatores potencialmente modificáveis, como maior IMC e

gordura corporal, falta de exercícios físicos, utilização de corticosteroides e maior ingestão de carboidratos e gorduras. A SM e respectivos componentes estiveram relacionados com as concentrações de AGL, bem como o HOMA-IR aumentados. Adiponectina, resistina e demais marcadores inflamatórios avaliados (TNF-a, IL-6, MCP-1 e PCR) não retratam a SM nesta amostra de pacientes submetidos ao TxH.

Descritores: transplante hepático, síndrome metabólica, obesidade, diabetes mellitus, agentes imunossupressores, adipocinas, resistência à insulina

Abstract

Background: Metabolic syndrome (MS) has been widely reported among liver graft recipients on the early years after liver transplantation (LTx), however, there are few studies of this condition in the long-term. Furthermore and despite the amount of data on the role of adipokines under this condition, there is scarce information after LTx. **Objetivos:** To longitudinally describe the prevalence of MS, its components and associated factors in long-term LTx survivors. In a subgroup of individuals, we have studied the pathophysiological mechanisms by means of serum adipokines, inflammatory and insulin resistance markers. **Methods:** This was a longitudinal study in which 117 patients were evaluated in two distinct times (2008 e 2012) for the presence of MS (National Cholesterol Education Program Adult Treatment Panel III revised – NCEP ATP III, International Diabetes Federation - IDF e Harmonizing the Metabolic Syndrome – HMS criteria) and its components, as well as body composition parameters and dietary intake. Demographic, socioeconomic and clinical data were also collected. In 34 patients, serum dosages of adiponectin, resistin, Tumor Necrose Factor (TNF-a), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), (MCP-1), free fatty acids (FFA), C-protein reactive (CPR), fasting insulin and blood glucose for HOMA-IR (Homeostatic Model Assessment) calculation were transversally assessed. Potential associated factors with MS and its components were assessed using multivariate logistic regression. Potential associated factors to adipokines, inflammatory and insulin resistance markers were studied using multiple linear regression. **Results:** MS was diagnosed in 43.1% (IDF/HMS) and 34.3% (NCEP) in patients who underwent LTx with median follow-up of 3 years (range 0-13 years) and these numbers were 53.3% ($p=0.12$) and 44.8% ($p=0.03$), respectively, when patients had median of 7 years of follow-up (range 3-17 years) ($p=0.12$ e $p<0.01$, respectively). The prevalence of hyperglycemia increased from 34.2% to 48.6% ($p<0.01$). The waist circumference rose from 93.3±14.3cm to 99.4±14.9cm ($p<0.01$) and body fat percentage from 30.3±8.9% to 31.8±10.3% ($p=0.03$). Abdominal obesity prevalence by the IDF/HMS criteria increased from 66.7% to 72.0% ($p=0.09$). Associated factors to MS were: older age (OR: 1.05; CI: 1.02-1.11; $p=0.02$), familiar history of diabetes (OR: 3.38; CI: 1.19-9.61; $p=0.02$), greater body mass index (BMI) prior to liver disease (OR: 1.39; CI: 1.19-1.63; $p<0.01$) and body fat percentage (OR: 1.09; CI: 1.03-1.14). MS components (abdominal obesity, arterial hypertension, hyperglycemia) were associated to older age ($p<0.05$). Abdominal obesity was associated to greater body fat amount ($p=0.01$) and lack of exercise ($p=0.01$). Low HDL was associated to increased BMI ($p<0.01$), greater fat ($p=0.03$) and carbohydrate intake ($p=0.03$). High triglycerides were associated to current corticosteroid use ($p=0.04$), family history of diabetess ($p=0.04$), greater BMI ($p<0.01$) and increased fat intake ($p<0.01$). Higher adiponectin concentrations (6.7±4.5 μg/mL *versus* 3.2±1.2 μg/mL. $p<0.01$) and FFA concentrations (0.8±0.3 mEq/L *versus* 0.5±0.3 mEq/L. $p<0.05$) and greater HOMA-IR values (4.9±3.8 *versus* 1.6±0.8) were observed in LTx receptors diagnosed with MS. Increased waist-hip ratio and low HDL were independent of adiponectin concentrations ($p<0.05$). Lower resistin concentrations were seen in patients with high blood pressure and greater, in those with abdominal obesity ($p<0.05$). HOMA-IR values were independently predicted by obesity and MS ($p<0.05$). Independent variables associated to TNF-a, MCP-1, IL-6 and FFA were not identified. **Conclusion:** MS was observed in more than half of the liver graft recipients on the long-term. There was an increase in the prevalence of MS over the years, as well as for the waist circumference and blood glucose. MS and its components were associated to potentially modifiable factors, as greater BMI and body fat, lack of exercises, current use of corticosteroids and increased intake of fat and carbohydrates. MS and its components were associated with FFA, as well as with HOMA-IR, increasing both of them. Adiponectin, resistin and other

inflammatory markers (TNF, IL-6, MCP-1 and CRP) did not portray the MS in this samp patients who underwent LTx.

Key-words: liver transplantation, metabolic syndrome, obesity, diabetes mellitus, immunosuppressive agents, adipokines, insulin resistance

Lista de abreviaturas e siglas

AGL – Ácidos Graxos Livres

cm – Centímetros

CI – Confidence interval

CRP – C-reactive protein

dL – Decilitros

FFA – Free Faty Acids

HDL – High Density Lipoprotein

HMS – *Harmonizing the Metabolic Syndrome*

HOMA - *Homeostatic Model Assessment*

IC – Intervalo de confiança

IDF – *International Diabetes Federation*

IL-2 – Interleukin 2

IL-6 – Interleucina 6

LDL – Lipoproteína de baixa densidade

LTx – Liver transplantation

MCP-1 – Proteína quimiotática de monócitos-1

MS – Metabolic syndrome

NCEP ATP III – *National Cholesterol Education Program Adult Treatment Panel* III

NFAT - Nuclear factor of activated T cells

mg – Miligramas

mmHg – Milímetros de mercúrio

OR – Odds Ratio

PCR – Proteína C reativa

SM – Síndrome metabólica

TxH – Transplante hepático

TNF-a – Tumor Necrose Factor alfa / Fator de necrose tumoral alfa

SUMÁRIO

1. CONSIDERAÇÕES INICIAIS

1.1. Introdução

O transplante de fígado (TxH) constitui-se, muitas vezes, na opção terapêutica de escolha para os pacientes com diagnóstico de falência hepática aguda e crônica, permitindo-lhes melhora dos índices de qualidade de vida e aumento da sobrevida (Braun et al. 2009). É fato também que nas duas últimas décadas, tem sido observado aumento das taxas de sobrevida após o TxH. Adam e Hoti (2009) relatam 85% de sobrevida após cinco anos e Duffy et al. (2010), 56% após 20 anos. Isso ocorreu principalmente devido aos avanços das técnicas cirúrgicas e da terapia imunossupressora, assim como dos cuidados antes, durante e após o ato cirúrgico (Neuberger, 2009).

No entanto, o aumento da sobrevida dos pacientes submetidos ao TxH foi também acompanhado por aumento da prevalência de doenças crônicas e, em taxas superiores, quando comparadas às da população em geral (Laish et al. 2011; Simo et al. 2011). Neste contexto, figuram a obesidade (Anastácio et al. 2012) e a síndrome metabólica (SM) e respectivos componentes (Anastácio et al. 2011). Estas afecções expõem os pacientes submetidos ao TxH a riscos maiores de desenvolver doenças cardiovasculares (Laryea et al. 2008), câncer (Mathur et al. 2013), esteatose (Sprinzl et al. 2013), esteatohepatite não alcoólica (El Atrache et al. 2012) e fibrose do enxerto (Hanouneh et al. 2008). Ademais, geram impacto negativo na sobrevida destes pacientes em longo prazo (Watt et al., 2010).

O conhecimento sobre a SM e as repercussões após o TxH ainda é incipiente. Embora o impacto negativo sobre a saúde dos pacientes submetidos ao TxH venha sendo documentado, a maioria dos estudos refere-se a dados de seguimentos de pacientes com menos de um ano do TxH ou com tempo de acompanhamento pós-transplante de três a cinco anos (Bianchi et al. 2008; Laish et al. 2010; Kallwitz et al. 2013; Lunati et al. 2013). Ainda que o pós-operatório tardio do TxH seja considerado do sexto mês do ato cirúrgico em diante, o aumento da sobrevida destes pacientes torna importante estudos com maior tempo de seguimento. Além disso, até o momento, apenas se especula sobre o comportamento das adipocinas e dos marcadores bioquímicos da resistência à insulina e de inflamação em pacientes submetidos ao transplante hepático, assim como a relação com a SM e respectivos componentes. Informações sobre a prevalência da SM no pós-operatório tardio e os mecanismos fisiopatológicos, bem como a avaliação de possíveis

fatores de risco poderão fomentar estratégias de intervenção, de tal forma a prevenir e tratar esse distúrbio em grupos específicos, bem como promover o melhor entendimento nessa população.

1.2. Revisão da Literatura

O TxH é o segundo tipo de transplante de órgão sólido mais realizado no Brasil, depois do transplante renal. No ano de 2013, foram realizados 1.723 transplantes de fígado por 60 equipes atuantes no país (ABTO, 2014). Em Minas Gerais, a Equipe de Transplantes do Instituto Alfa de Gastroenterologia do Hospital das Clínicas/UFMG iniciou seus trabalhos em 1994 e, nesses 20 anos, realizou mais de 800 transplantes.

Ainda que a sobrevida destes pacientes tenha aumentado ao longo dos anos (Duffy et al. 2010), a prevalência de doenças cardiovasculares (Simo et al. 2011; Rubín et al. 2013) também aumentou e se tornou importante causa de morbidade e mortalidade neste grupo de pacientes. Aumento da prevalência de fatores de risco associados às doenças cardiovasculares também tem sido descrita, especialmente o excesso de peso (Nair et al. 2002; Ribeiro et al. 2012). A taxa de obesidade pode atingir 40% dessa população já no primeiro ano pós-transplante (Stegall et al. 1995) e, três anos após o ato cirúrgico, cerca de 70% dos pacientes apresenta sobrepeso ou obesidade (Richards et al. 2005). Embora não se tenha demonstrado a relação entre ingestão alimentar excessiva e o ganho de peso pós-transplante (Muñoz et al. 1991; Richardson et al. 2001; Krasnoff et al. 2006), sabe-se que pacientes submetidos ao transplante hepático redescobrem o apetite e o prazer de antigos hábitos alimentares após meses de restrições (Heyman et al. 2006). Além disso, sentem-se melhor e conseguem se alimentar em quantidades apreciáveis. Ao mesmo tempo anseiam por recuperar o peso perdido durante meses de espera pelo transplante (McCashland, 2001; Reuben, 2001), uma vez que 75% deles encontram-se desnutridos nesse período (Ferreira et al. 2010). Por outro lado, muitos pacientes não retornam ao trabalho após a realização do TxH (Saab et al. 2007) e mesmo aqueles que têm maior tempo de sobrevida possuem níveis de atividade física diária significantemente inferiores aos da população geral (Duffy et al. 2010). Além desses fatores, pacientes submetidos ao transplante utilizam drogas imunossupressoras, dentre as quais, tacrolimus, ciclosporina e corticosteroides, também implicadas na gênese do ganho de peso excessivo e/ou das desordens metabólicas pós-transplante (Mcpartland e Pomposelli, 2007; Mells e Neuberger, 2007).

Embora o ganho de peso excessivo possa não afetar as estatísticas de sobrevida dos pacientes submetidos ao transplante hepático em curto prazo, é bastante plausível afirmar que esteja envolvido no desenvolvimento de diabetes mellitus, dislipidemias, hipertensão arterial e SM, "per si" (Watt, 2010).

Os componentes da SM são muito prevalentes em pacientes submetidos ao transplante hepático. A prevalência de diabetes mellitus já foi descrita em até 61% deles (Laryea et al. 2007); de dislipidemias, em até 69% (Gisbert et al. 1997) e a de hipertensão arterial, em até 75% dos pacientes submetidos ao TxH (Rubín et al. 2013). Recentemente, tem sido postulado que tais distúrbios podem não surgir de forma isolada nesses pacientes, mas sim em conjunto, sob a forma de SM e acometer entre 44,5% e 63,5% dessa população (Laryea et al., 2007, Hanouneh et al., 2008; Bianchi et al., 2009; Kallwitz et al. 2013), sendo essa apontada como fator de risco para doenças cardiovasculares (Laryea et al., 2007; Laish et al., 2010), esteatose (Sprinzl et al. 2013) e fibrose hepática (Hanouneh et al., 2008).

De acordo com a *Harmonizing the Metabolic Syndrome* (Alberti et al. 2009), a SM é diagnosticada quando há presença de pelo menos três, dos seguintes critérios: obesidade abdominal (perímetro da cintura ≥ 80 cm para mulheres e ≥ 90 cm para homens sul-americanos), pressão arterial sistólica ≥ 130 mmHg e/ou pressão arterial diastólica ≥ 85 mmHg e/ou uso de medicamentos anti-hipertensivos, glicemia de jejum ≥ 100 mg/dL e/ou uso de hipoglicemiantes orais, triglicérides ≥ 150 mg/dL), lipoproteína de alta densidade (HDL) < 50 mg/dL para mulheres e < 40 mg/dL para homens).

Trata-se de desordem metabólica complexa e multifatorial, cujos maiores fatores de risco são a resistência à insulina e a obesidade (Grundy et al. 2005). A resistência insulínica pode ser secundária à obesidade, mas fatores genéticos também podem estar presentes (Grundy, 2006). A resistência à insulina pode ser medida por meio do HOMA-IR (*Homeostatic Model Assessment*) a partir de glicemia e da insulinemia. De modo sucinto, o tecido adiposo e células do sistema imunológico secretam uma série de substâncias envolvidas na gênese da resistência à insulina, da inflamação, da disfunção endotelial e da aterosclerose, dentre esses, o fator de necrose tumoral alfa (TNF-a), a interleucina 6 (IL-6), a proteína quimiotática de monócitos 1 (MCP-1) e a resistina (Trayhurn; Wood, 2004; Fonseca-Alaniz et al. 2006). Todos encontrados, em concentrações séricas maiores em indivíduos obesos quando comparados à população geral (Kern et al. 1995; Maury e Brichard, 2010). Por outro lado, o tecido adiposo também secreta

adiponectina (o que também acontece no músculo esquelético e cardíaco e, em células endoteliais). A adiponectina possui efeito anti-inflamatório e anti-aterogênico (Fonseca-Alaniz et al. 2006). Entretanto, está reduzida em indivíduos da população geral com resistência à insulina, hiperglicemia (Arita et al. 1999) e SM (Stenholm et al. 2010).

Ao se considerar todo o tecido adiposo corporal, o visceral é o maior responsável pela gênese da SM. A obesidade visceral apresenta comportamento metabólico que a difere do tecido adiposo subcutâneo periférico ou glúteo-femoral. O tecido adiposo abdominal está mais sujeito à lipólise devido ao maior número de receptores de glicocorticóides e maior sensibilidade à ação das catecolaminas (Montague e O'rahilly, 2000). O resultado desta lipólise é a maior secreção de ácidos graxos livres para a corrente sanguínea, principalmente para a circulação portal, na qual são liberados em grande quantidade, corroborando para o aumento da resistência tecidual à ação insulínica, tanto no tecido hepático quanto periférico (Gagliardi e Wittert, 2007). A resistência tecidual à ação insulínica tem como consequência a hiperinsulinemia, a qual promove ativação do sistema nervoso simpático e estimula a reabsorção de sódio, mecanismo associado à elevação da pressão arterial (Carneiro et al. 2003). Além disso, o aporte aumentado de ácidos graxos no fígado contribui para o aumento do estoque de gordura hepática e síntese aumentada de lipoproteína de muito baixa densidade (VLDL), gerando aumento de triglicérides na corrente sanguínea (Vanni et al. 2010). O aumento de triglicérides circulantes modifica a composição e o metabolismo do HDL, reduzindo o seu conteúdo de colesterol e aumentando o de triglicérides, o que promove aumento do seu *clearance* na circulação (Eckel et al. 2005).

O estudo da SM pós TxH é recente. Ainda que possa acometer mais da metade dessa população (Laish et al. 2011; Kallwitz et al. 2013) e que seja fator de risco para doenças cardiovasculares (Laryea et al. 2007) e fibrose (Hanouneh et al. 2008), pode-se afirmar que os fatores preditivos ainda são mal elucidados. Em relação às adipocinas, não foram encontrado registros de estudos que avaliaram o comportamento nessa população de pacientes.

Dentre os fatores preditivos da SM descritos em populações norte-americana e europeia submetidas a TxH, constam a maior idade, a indicação para o transplante por cirrose etanólica, a cirrose criptogênica e por vírus da hepatite C, a presença de esteatohepatite não alcoólica antes do transplante, o diagnóstico de diabetes mellitus, o maior índice de massa corporal (IMC) pré-transplante, as mudanças no IMC pós-transplante e a maior ingestão de calorias e de gorduras saturadas (Laryea et al. 2007; Bianchi et al. 2009; Laish et al. 2011; Lunati et al. 2013).

No Brasil, a média geral de prevalência da SM por meio de uma série de vários estudos sistematizados em uma revisão foi de 29,6% (Vidigal et al. 2013). Este valor é bem inferior às taxas de prevalência da síndrome em enfermos submetidos a transplante hepático, conforme constatado por nós (Anastácio et al. 2011). Nesse estudo, a prevalência de SM foi descrita em 50,0% da amostra e essa foi associada à maior idade, à história de sobrepeso anterior à doença hepática e à indicação ao transplante por cirrose etanólica. Além disso, fatores ambientais potencialmente modificáveis também foram apontados como preditivos para o desenvolvimento desta afecção, como o auto-relato de sedentarismo pós-transplante hepático (como causa para o ganho de peso excessivo) e a ingestão reduzida de cálcio, fibras, potássio e ácido fólico. Por último, o menor tempo pós-transplante também foi fator preditivo para a SM nesses pacientes (Anastácio et al. 2011). Neste contexto, baseado em outras séries de casos, sabe-se que o menor tempo de TxH esteve relacionado ao maior ganho de peso relativo (Richards et al., 2005) e à maior prevalência de diabetes (Xu et al. 2008). Condições presumidamente associadas à maior carga de terapia imunossupressora (Navasa et al., 1996) utilizadas nesse período e apontadas como responsáveis pelos distúrbios metabólicos pós-transplante (Mcpartland e Pomposelli, 2007; Mells e Neuberger, 2007). Contudo, diversos trabalhos não conseguiram demonstrar associação entre o uso dessas drogas e a presença de SM de forma multivariada entre este grupo de pacientes (Laryea et al. 2007; Hanouneh et al. 2008; Bianchi et al., 2009; Anastácio et al. 2011; Laish et al. 2011).

Dessa forma, o seguimento em longo prazo de pacientes submetidos ao TxH faz-se importante, pois, embora tenha sido demonstrado que o menor tempo desde a operação possa afetar as taxas de SM, sabe-se que, com o passar dos anos, a prevalência de doenças crônicas tende a aumentar, inclusive na população submetida ao transplante hepático (Simo et al. 2011). Além disto, o estudo de marcadores inflamatórios e de resistância à insulina que retratam a SM também é relevante, uma vez que, até o momento, esses dados são desconhecidos em pacientes submetidos ao TxH.

1.3. Referências Bibliográficas

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2. OBJETIVOS

2.1. Objetivo Geral

Avaliar de modo longitudinal a prevalência da SM e os mecanismos fisiopatológicos em pacientes submetidos ao transplante hepático no Hospital das Clínicas da UFMG.

2.2. Objetivos Específicos

- ✓ Avaliar, em dois tempos distintos, a prevalência dos respectivos componentes da SM (obesidade abdominal, hiperglicemia, elevação da pressão arterial, elevação da concentração de triglicérides e diminuição da concentração de HDL);

- ✓ Identificar fatores associados à prevalência da SM e respectivos componentes;

- ✓ Estudar o comportamento das adipocinas adiponectina e resistina, dos marcadores inflamatórios e de resistência à insulina (TNF-a, MCP-1, IL-6, PCR e índice HOMA-IR) na SM e respectivos componentes;

- ✓ Observar as modificações ocorridas, com o decorrer dos anos, na composição corporal e ingestão alimentar dos indivíduos submetidos ao transplante hepático.

3. MÉTODOS

3.1. Desenho do estudo

Trata-se de estudo coorte no qual o diagnóstico da SM, respectivos componentes e possíveis fatores associados foram avaliados em dois tempos distintos (2008 e 2011/2012) em pacientes submetidos ao transplante hepático acompanhados no ambulatório do Grupo de Transplantes do Instituto Alfa de Gastroenterologia do HC/UFMG. Em um subgrupo de doentes, foram realizadas dosagens de adipocinas, marcadores séricos de inflamação e resistência à insulina, relacionados com o desenvolvimento da SM, entre esses resistina, adiponectina, fator de necrose tumoral alfa (TNF-a), proteína quimiotática de monócitos-1 (MCP-1) proteína C reativa (PCR), ácidos graxos livres, insulina e glicose. Esses últimos dois com o objetivo de se obter o cálculo do índice HOMA-IR.

Na linha de base do trabalho (2008), foram incluídos os pacientes maiores de 18 anos de idade submetidos ao transplante [1] e que estavam em acompanhamento médico regular no ambulatório de Transplante Hepático do Instituto Alfa de Gastroenterologia. Nesta ocasião, foram excluídos pacientes que tiveram diagnóstico de doença hepática com ascite, neoplasia, pacientes em terapia dialítica, gestantes e aqueles que se recusaram a participar do trabalho. Estes mesmos critérios foram adotados para a exclusão de pacientes na reavaliação de 2011/2012. Em 2008, dos 165 pacientes submetidos ao TxH avaliados, em 148 foi possível prover o diagnóstico da SM. Em 2012, 117 pacientes foram avaliados, dos quais, o diagnóstico de SM foi possível em 98 (Figura 1) e em 34, realizou-se a coleta de amostras de sangue. As 13 exclusões ocorridas na segunda fase da pesquisa foram devidas a recusas em participar do trabalho (n=5), neoplasias (n=5), diagnóstico de doença hepática com ascite (n=2) e terapia dialítica (n=1).

No primeiro e segundo momentos, os pacientes foram entrevistados no dia da consulta médica com objetivo de se obter dados demográficos, socioeconômicos, de estilo de vida, clínicos, antropométricos (Apêndice A) e dietéticos (Apêndice B). Também houve coleta de dados a partir de prontuários médicos. A coleta de amostras de sangue para dosagens de adipocinas e marcadores séricos de resistência à insulina e inflamação ocorreu no ano de 2013. Para tal, foram enviadas cartas (Apêndice C) convidando os mesmos a comparecer ao hospital para tal. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais

[1] Anastácio, L.R.; Ferreira, L.G.; Ribeiro, H.S.; Liboredo, J.C.; Lima, A.S.; Correia, M.I.T.D. Metabolic syndrome after liver transplantation: prevalence and predictive factors. ***Nutrition*** 2011; 27(9): 931-37.

(protocolo ETIC 44 /08, Anexo 1) e os pacientes que aceitaram participar do trabalho assinaram o Termo de Consentimento Livre e Esclarecido (Apêndice D).

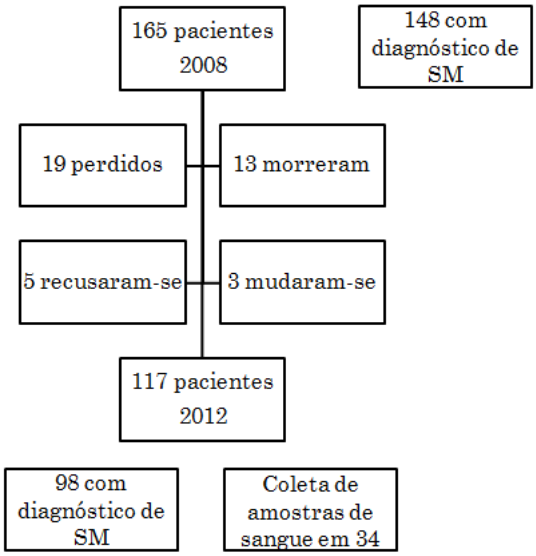

Figura 1 - Universo amostral, exclusões, recusas, perdas e número final de participantes do trabalho em 2008 e 2012

3.2 Definição da síndrome metabólica

O diagnóstico de SM foi estabelecido com base em três critérios – *National Cholesterol Education Program Adult Panel Treatment* III (NCEP ATP III) [2] revisado, *International Diabetes Federation* (IDF) [3] e *Harmonizing the Metabolic Syndrome* (HMS) [4]. A SM pelo NCEP ATP III revisado e HMS foi diagnosticada na vigência de pelo menos três dos cinco critérios descritos abaixo. No caso do IDF, o paciente precisava ter, obrigatoriamente, obesidade abdominal e pelo menos dois dos quatro critérios restantes. Os componentes da SM por estas classificações são: obesidade abdominal (IDF e HMS: perímetro da cintura ≥ 80 cm para mulheres e ≥ 90 cm para homens sul-americanos; NCEP ATP III revisado: perímetro da cintura ≥ 88 cm para mulheres e ≥ 102 cm para homens); pressão arterial sistólica ≥ 130 mmHg e/ou pressão arterial diastólica ≥ 85 mmHg e/ou uso de medicamentos anti-hipertensivos, glicemia de

[2] Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. ***Circulation***. 2004 27;109(3):433-8.

[3] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. ***Diabet Med***. 2006; 23(5):469-80.

[4] Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interi statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. ***Circulation***. 20 2009;120(16):1640-1645.

jejum ≥ 100 mg/dL e/ou uso de hipoglicemiantes orais; triglicérides ≥ 150 mg/dL ou HDL < 50 mg/dL para mulheres e < 40 mg/dL para homens.

3.3 Investigação de fatores preditivos para a síndrome metabólica

Fatores preditivos relacionados à presença de SM foram investigados com base em variáveis levantadas em 2008 e coletadas em 2011/2012. São esses variáveis demográficas, socioeconômicas, de estilo de vida, clínicas, antropométricas e dietéticas. Os componentes da síndrome (obesidade abdominal, distúrbios do metabolismo glicêmico, pressão arterial e desordens lipídicas) bem como os valores das respectivas medidas (do perímetro da cintura, da glicemia de jejum, da pressão arterial, dos triglicérides e do HDL) não foram incluídos no modelo estatístico para determinação dos fatores preditivos da síndrome.

Os dados demográficos e socioeconômicos coletados foram idade, sexo, estado marital, execução de atividade profissional remunerada, escolaridade e renda per capita. Dados referentes ao estilo de vida foram os seguintes: horas de sono por noite, tabagismo atual e ex-tabagismo e nível de atividade física diária efetuada (auto-relato). Em relação ao último, os pacientes foram questionados sobre as atividades diárias e as respostas foram transformadas em MET (*Metabolic Equivalent Energy*) correspondentes [5]. As atividades diárias em MET foram multiplicadas pelo respectivo tempo gasto e os resultados, somados e divididos por 24 horas. Esse valor foi categorizado de acordo com o nível de atividade realizada conforme os pontos de corte estipulados pela *World Health Organization* de 1995 (caso o resultado do MET/24 horas seja < 1,3, o indivíduo é considerado sedentário; se o resultado for de 1,3 a 1,5, pouco ativo; entre 1,5 a 1,8, ativo e > 1,9, muito ativo) [6].

Os dados clínicos coletados foram os seguintes: tempo em meses desde o transplante, indicação para transplante, dados dos doadores (sexo, idade e índice de massa corporal – IMC), utilização atual de corticoesteróides, de tacrolimus ou de ciclosporina, presença de hipertensão arterial; glicemia ≥ 100 mg/dL ou diabetes mellitus pré-transplante. História familiar de hipertensão arterial, diabetes mellitus, excesso de peso e doença cardiovascular também foram questionados. A glicemia de jejum, o colesterol total, o LDL (lipoproteína de baixa densidade), os triglicérides

[5] Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. ***Med Sci Sports Exerc***. 2000 Sep;32(9 Suppl):S498-504.

[6] World Health Organization W. ***Physical status. the use and interpretation of anthropometry***. Genebra1995.

e o HDL séricos foram obtidos a partir dos exames de rotina realizados pelo pacientes em data próxima à da coleta de dados. A pressão arterial do paciente foi obtida pela equipe de enfermagem do ambulatório, no dia da consulta. O colesterol total foi estratificado como "elevado" quando os valores ultrapassaram 240mg/dL e o LDL, quando acima de 160mg/dL [7].

Os dados antropométricos coletados foram perímetro da cintura (medida dois dedos acima da cicatriz umbilical), perímetro do quadril (medido na região de maior perímetro), razão cintura-quadril, peso, estatura, IMC e respectiva classificação (baixo peso: IMC<18,5kg/m²; eutrofia: IMC entre 18,5-24,9kg/m²; sobrepeso: IMC entre 25,0-29, 9kg/m²; obesidade: IMC>30,09kg/m²) [8] – antes da disfunção hepática e no dia da avaliação. O peso habitual antes da disfunção hepática foi obtido a partir do relato do paciente. Dados relativos à composição corporal dos pacientes: percentual de gordura corporal, percentual de água corporal, ângulo de fase, água intra e extracelular, foram obtidos por meio da avaliação de bioimpedanciometria (Systems® Quantum RJL, Clinton Twp, MI, USA)

Os pacientes foram ainda questionados quanto à dieta habitual (método História Dietética) e também foram solicitados a preencher o Registro Alimentar Três Dias não Consecutivos (anexo 2), sendo um referente ao final de semana. Quando não foi possível ter acesso ao registro alimentar do paciente, os dados referentes à dieta habitual foram utilizados. O consumo alimentar foi avaliado considerando-se calorias, carboidratos, frutose, proteínas, gorduras totais, gorduras saturadas, gorduras monoinsaturadas, gorduras poliinsaturadas, colesterol, fibra total, vitamina A, vitamina C, vitamina D, vitamina E, tiamina, riboflavina, niacina, ácido pantotênico, vitamina B_6, ácido fólico, vitamina B_{12}, cálcio, ferro, magnésio, sódio, potássio e zinco, com o auxílio do software Excel (Microsoft Corp, Redmond, WA) e da tabela de composição de alimentos de Philippi et al. [9].

[7] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP). ***JAMA*** 2001: 285; 2486–97.

[8] World Health Organization (WHO). ***Obesity: preventing and managing the global epidemic.*** Genebra: World Health Organization1998.

[9] Philippi ST. ***Tabela de composição de alimentos: suporte para decisão nutricional.*** ANVISA, editor. Brasília: FINATEC/NUT-UnB; 2001.

3.4 Investigação de marcadores inflamatórios e de resistência à insulina

A análise de adipocinas, marcadores de inflamação e resistência à insulina foi realizada por meio de dosagens em amostra sanguínea. A coleta foi realizada no período da manhã, após jejum de oito horas. O sangue foi centrifugado para a obtenção do soro, o qual foi armazenado em freezer a -80 ^{o}C até o momento das dosagens. Essas incluíram adiponectina, resistina, TNF-a, MCP-1, PCR, ácidos graxos livres (AGL) e insulina para a realização do índice HOMA-IR (calculado a partir da fórmula: insulina de jejum (μU/mL) × glicose de jejum (mg/dL)/405. A resistência à insulina foi diagnosticada quando os valores de HOMA-IR ultrapassaram 2,7 [10].

A análise dos AGL foi realizada utilizando-se o kit da WAKO (Pure Chemical Industries, Japan). O perfil inflamatório foi determinado pela dosagem, no soro, de adiponectina (Linco Research, St Charles, MI, USA), resistina e TNF-a (Millipore Corp., Bedford, Massachusetts, USA), IL-6 e MCP-1 (BD PharMingen or Endogen, Woburn, MA), utilizando-se a técnica de ELISA. A dosagem de PCR foi realizada pelo teste reativo VITROS® (Johnson & Johnson, EUA), o qual tem sensibilidade funcional de 5 mg/dL e linearidade entre 5 e 90 mg/dL. Por tal, os pacientes foram estratificados em aqueles com PCR < 5mg/dL e pacientes com valores de PCR > 5 mg/dL. As dosagens foram realizadas no Laboratório Imunofarmacologia do Instituto de Ciências Biológicas e no Laboratório do Hospital das Clínicas da UFMG. Foram adotadas as instruções do fabricante para a realização dos ensaios.

3.5 Amostra e análise estatística

O cálculo amostral para o diagnóstico de SM determinou que seriam necessários no mínimo 96 pacientes dos 148 com diagnóstico avaliados em 2008 (considerando-se erro amostral de 5%, intervalo de confiança de 90% e 50% de prevalência de SM). As análises estatísticas foram realizadas utilizando-se o programa SPSS para Windows versão 17.0 (SPSS Inc., Chicago, IL). As variáveis numéricas foram avaliadas quanto à normalidade (teste de Kolomogorov-Smirnov), para a seleção da apresentação dos dados (se em média e desvio-padrão ou valor mínimo, máximo e mediano). As variáveis categóricas foram apresentadas sob a forma de percentuais. Os

[10] Geloneze B, Vasques AC, Stabe CF, et al. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). ***Arq Bras Endocrinol Metabol.*** 2009;53(2):281-287.

pacientes foram comparados pelas suas características, em 2008 e 2012, usando o teste de

McNemar e t pareado ou Wilcoxon (de acordo com a distribuição dos dados). O teste t de Student ou Mann-Whitney (de acordo com a distribuição dos dados) e qui-quadrado (ou teste exato de Fisher quando apropriado) foram utilizados na análise univariada para determinar os fatores associados com SM e respectivos componentes. As variáveis com p <0,1 na análise univariada foram incluídas nas análises de regressão logística e linear múltipla realizadas. O ajuste do modelo de regressão logística foi verificada pelo teste de Hosmer-Lemeshow (p> 0,05). O nível de significância foi fixado em 5%.

4. ARTIGOS

4.1 Artigo 1

Prospective evaluation of metabolic syndrome and its components among long-term liver recipients

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Abbreviations:

BMI – Body Mass Index

HDL – High Density Lipoprotein

IDF – International Diabetes Federation

LDL – Low Density Lipoprotein

MS – Metabolic syndrome

MET – Metabolic Equivalent Energy

NCEP - National Cholesterol Education Program

US – United States

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Abstract

Background: Survival after liver transplantation (LTx) has increased. Metabolic syndrome (MS) is widely reported in patients in the early years after LTx; few studies have researched this condition in relatively long-term liver recipients. Aims: To describe, prospectively, the prevalence of MS, its components and its associated factors in relatively long-term liver recipients. **Methods:** A total of 117 patients were evaluated in 2008 (median of 3 years after LTx, range 0-13 years) and in 2012 (median of 7 years after LTx, range 3-17 years) for the presence of MS (using modified NCEP Adult Treatment Panel III and IDF criteria); its components; and its associated factors, including demographic, socioeconomic, lifestyle, clinical, body composition (measured using bioelectric impedance) and dietetic factors. **Results**: MS increased over the years (IDF, 43.1% to 53.3%, p=0.12; and NCEP, 34.3% to 44.8%, p=0.03). Blood glucose increased over the years (98.8±24.7 mg/dL to 109.2±33.3 mg/dL, p<0.01), which resulted in an increased prevalence of hyperglycemia (34.2% to 48.6%, p<0.01). Waist circumference (93.3±14.3 cm to 99.4±14.9 cm, p<0.01) and body fat (30.3±8.9% to 31.8±10.3%, p=0.03) also increased. The MS associated factors were age (OR 1.05, CI 1.02-1.11), family history of diabetes (OR 3.38, CI 1.19-9.61), body mass index (BMI) prior to liver disease (OR 1.39, CI 1.19-1.63) and body fat (OR 1.09, CI 1.03-1.14). The MS components were associated with greater age, family history of diabetes, current and previous BMI, body fat, current corticosteroid use, lack of exercise and greater carbohydrate and fat intakes. **Conclusion:** MS prevalence increased over the years after LTx because of the increase**s** in waist circumference and blood glucose. MS and its components are associated with modifiable factors, such as greater BMI, body fat and carbohydrate and fat intake.

Keywords: liver transplantation, metabolic syndrome, obesity, diabetes mellitus, immunosuppressive agents

Introduction

Liver transplantation is often the only solution for fulminant and chronic hepatic failure, allowing increased survival and quality of life [1]. In the last two decades, survival rates after liver transplantation increased to 85% after 5 years [2] and 56% after 20 years [3], mainly because of advances in surgical techniques, immune management and care before, during and after surgery [4].

However, the increased survival of patients undergoing liver transplantation has been accompanied by an increase in the prevalence of chronic diseases, which is usually higher than that found in the general population [5-6]. For instance, these patients often experience excessive weight gain, obesity [7] and metabolic syndrome (MS) and its components [8]. These morbidities expose patients to increased risks of developing cardiovascular disease, kidney disease and nonalcoholic steatohepatitis of the graft, which have important morbidity and mortality consequences in these patients in the long term [9].

The study of MS after liver transplantation is still new. Although the disorder can affect up to half of these patients [6] and is a risk factor for fatty liver disease [10], cardiovascular disease [6] and fibrosis [11], most studies have not focused on MS in patients many years after LTx [6, 12-14]. As the shorter duration of follow-up after transplantation may be related to the higher prevalence of metabolic syndrome [8], studies of longer-term liver recipients are required to better describe this condition after liver transplantation. Furthermore, the assessment of associated factors, including demographic, socioeconomic, lifestyle, clinical, anthropometric (waist circumference and body composition, as analyzed by bioelectrical impedance) and dietetic data, among liver recipients has not been widespread. The aim of this study was to prospectively evaluate MS and its components and associated factors among longer-term liver recipients.

Methods

This prospective study of the presence of MS and its components and associated factors after liver transplantation was conducted between 2008 and 2012. We previously assessed the prevalence of MS and its associated factors among 148 adult liver transplant recipients in 2008 [8] a median of 3 years after LTx (range 0-13 years). In 2012, a median of 7 years after LTX (range

3-17 years), 117 of these patients were reassessed. For this evaluation, we considered a minimum sample size of 108 patients (standard error of 5% confidence interval of 95%).

Outpatients followed at Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Universidade Federal de Minas Gerais, Brazil, were once again invited to participate in this study. Patients who presented with recurrence of liver disease with ascites or any sign of decompensation, women who were pregnant during the study period, patients diagnosed with cancer after the first evaluation, patients diagnosed with renal disease stages 3, 4 and 5 [15] and those who refused to participate were excluded from the study. The study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (ETIC protocol 44/08).

The MS diagnosis was made based on the modified National Cholesterol Education Program NCEP Adult Treatment Panel III [16] and the International Diabetes Federation criteria (IDF) [17]. Using the former criteria [16], patients presented with MS if they had at least three of the following disorders: abdominal obesity (waist circumference ≥ 88 cm for women and ≥ 102 cm for men), high blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg and/or the use of antihypertensive medications), high fasting glucose (≥ 100 mg/dL and/or oral hypoglycemic agents), hypertriglyceridemia (triglycerides ≥ 150 mg/dL), reduced high-density lipoprotein (HDL) (< 50 mg/dL for women and < 40 mg/dL for men). According to the IDF criteria, MS was present when patients had abdominal obesity (waist circumference ≥ 80 cm for women and ≥ 90 cm for men in South America) and at least two of the following disorders: high blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg and/or the use of antihypertensive medications), high fasting glucose (≥ 100 mg/dL and/or oral hypoglycemic agents), hypertriglyceridemia (triglycerides ≥ 150 mg/dL) and reduced HDL (< 50 mg/dL for women and < 40 mg/dL for men).

Patients were interviewed during routine medical appointments to obtain demographic, socioeconomic, lifestyle, clinical, anthropometric and dietary information, which was analyzed to determine predictive factors for the presence of MS and its components. Information was retrospectively collected from medical records and from the 2008 survey of the same population [8]. The demographic and socioeconomic data consisted of age, gender, marital status, implementation of paid professional activity, schooling and per capita income. Lifestyle data related to the number of hours of sleep per night (self-reported), current and past history of smoking, physical exercise performed and daily physical activity level. Patients were categorized

as sedentary, very little activity, active or very active according to the World Health Organization criteria [18] and the number of metabolic equivalents (METs) spent on their daily activities [19].

The assessed clinical data were related to time since the transplant; indication for the transplant; donor data (gender, age and body mass index [BMI]); current use of corticosteroids; use of tacrolimus or cyclosporine; presence of hypertension, glucose intolerance (fasting glucose ≥ 100 mg/dL) or diabetes mellitus before transplant; and family history of hypertension, diabetes mellitus, overweight and cardiovascular diseases. Fasting glucose, total cholesterol, low-density lipoprotein (LDL), triglycerides and serum HDL were obtained from the routine tests performed in the outpatient clinic. Each patient's blood pressure was assessed on the interview day. The prevalence of arterial hypertension and of diabetes mellitus were estimated based on the use of medications to treat these conditions, as described in the medical records. High total cholesterol and high LDL were classified based on cutoffs of 200 mg/dL and 130 mg/dL, respectively [20].

The anthropometric data consisted of waist circumference (measured two fingers above the umbilicus), weight, height, BMI and its classification [21]. The patients also described their usual weight before experiencing liver dysfunction. Body composition data (i.e., fat mass, lean mass, total body water and phase angle) were obtained using bioelectrical impedance (RJL Systems® Quantum, Clinton Township, MI, USA).

Patients were also asked about their diets and were asked to complete a non-consecutive three-day food diary (including one weekend day). All patients were also asked about their diet histories, and for those who did not deliver the diary, this method was used to estimate food consumption. The food consumption evaluation considered calories, carbohydrates, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, total fiber, vitamin A, vitamin C, vitamin D, vitamin E, thiamin, riboflavin, niacin, vitamin B6, folic acid, vitamin B12, calcium, iron, magnesium, potassium, sodium and zinc; these data were tracked using Excel software (Microsoft Corp., Redmond, WA, USA) and the Philippi et al. [22] table food composition.

Statistical analyses were performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as percentages. Numerical variables were assessed for normality (Kolmogorov-Smirnov test) and **are** presented as the mean and standard

deviation or median, minimum and maximum. Patients were compared in terms of their characteristics in 2008 and 2012 using the McNemar and paired T test or Wilcoxon test. Student's t-test or the Mann-Whitney U-test (according to the distribution of the data) and the chi-squared test (or Fisher's exact test where appropriate) were used in the univariate analysis to assess the associated factors for MS and its components. The variables with $p<0.1$ in the presence of the syndrome in the univariate analysis were included in the multiple logistic regression analysis. Model adjustment was checked by the Hosmer-Lemeshow test ($p>0.05$). The level of significance was set at 5%.

Results

The characteristics of the 117 patients (average age, 53.3±13.0 years, 59.0% male) are listed in table 1. In 2008, the median follow-up time was 3 years (range 0-13 years), and in 2012, the median was 7 years (range 3-17 years). Of the 165 patients assessed in 2008, 13 died, eight were excluded, five refused to participate in the study, three changed their monitoring center and 19 were lost during data collection. The most commons indications for liver transplantation were hepatitis C virus cirrhosis (n=36), alcoholic cirrhosis (n=31), cryptogenic cirrhosis (n=14), auto-immune hepatitis cirrhosis (n=13) and hepatocellular carcinoma with cirrhosis (n=9). Other indications accounted for 31 cases. The patients' BMI before liver disease averaged 24.4±4.6 kg/m^2.

Tables 2 and 3 compare the patients evaluated in 2008 and revaluated in 2012, respectively, in terms of their anthropometric, body composition, clinical and biochemical data and MS and its components. Waist circumference, body fat and fasting blood glucose significantly increased in four years ($p<0.05$), while lean mass and total body water decreased ($p<0.05$). The prevalence of metabolic syndrome significantly increased in patients undergoing liver transplantation, according to the glucose criteria used to diagnose this condition ($p<0.05$).

Table 1. General characteristics of patients undergoing liver transplantation evaluated in 2012

Characteristics (n=117)	Average ± standard deviation / total n Median (minimum-maximum) / total n n (%) / total n
Demographic/socioeconomic	
Age	53.3±13.0 / 117
Male	69 (59.0) / 117
Married	84 (71.8) / 114
Schooling (years)	11.0 (0.0-16.0) / 115
Retired/unemployed/ house wife	61 (52.3) / 114
Income per capita (US$)/year	10,800 (1,344-90,000) / 114
Lifestyle	
Sleep per night (hours)	7.6±1.3 / 114
Current smoking	10 (8.5) / 114
Past smoking	41 (35.4) / 114
Physical exercise activity	62 (55.9) / 114
Physical activity level (15)	
Sedentary	40 (35.1) / 114
Very little active	56 (49.1) / 114
Active	15 (13.2) / 114
Very active	3 (2.6) / 114
Clinical	
Time since transplantation (years)	7.0 (3.0-17.0) / 117
Immunosuppressive treatment	
Tacrolimus	103 (88.0) / 117
Cyclosporine	14 (12.0) / 117
Corticosteroids + calcineurin inhibitors	19 (16.2) / 117
Family history	
Diabetes mellitus	55 (47.0) / 115
Arterial hypertension	83 (70.9) / 115
Overweight	66 (56.4) / 115
Cardiovascular disease	66 (56.4) / 115
Donor's characteristics	
Age	31±13.1 / 95
Male sex	71 (66.1) / 95
Weight	69.0±11.8 / 95
Body mass index (kg/m²)	23.0±4.8 / 95
Overweight by body mass index	3 (3.0) / 95
Obesity by body mass index	25 (25.5) / 95

Table 2. Anthropometric, body composition, clinical and biochemical parameters of patients undergoing liver transplantation evaluated in 2008 and 2012

Parameters	Average ± standard deviation / total n		
(n=117)	2008	2012	p
Time since liver transplantation	3 years (0-13)	7.0 years (3-17)	
Anthropometric/body composition			
Weight (kg)	71.9±16.0 / 117	73.2±17.0 / 117	0.05
Body mass index (kg/m²)	26.5±5.4 / 117	26.7±4.9 / 117	0.60
Waist circumference (cm)	93.3±14.3 / 117	99.4±14.9 / 117	<0.01*
Waist-hip ratio	0.91±0.09 / 117	0,94±0.09 / 117	0.05
Body fat (%)	30.3±8.9 / 115	31.8±10.3 / 114	0.03*
Body fat (kg)	22.2±9.3 / 115	23.9±10.7 / 114	<0.01*
Lean mass (%)	69.7±8.9 / 115	67.4±9.9 / 114	<0.01*
Lean mass (kg)	49.7±10.9 / 115	49.0±11.5 / 114	0.22
Total body water (%)	52.0±6.9 / 115	50.5±6.8 / 114	<0.01*
Total body water (L)	37.1±8.1 / 115	36.8±8.2 / 114	0.37
Phase angle	6.0±1.1 / 115	6.4±2.4 / 114	0.11
Clinical and biochemical			
Systolic blood pressure (mmHg)	122.1±17.8 / 117	121.9±23.1 / 117	0.95
Diastolic blood pressure (mmHg)	78.6±12.0 / 117	80.0±12.1 / 117	0.31
Fasting blood glucose (mg/dL)	98.8±24.7 / 117	109.2±33.3 / 117	<0.01*
Total cholesterol (mg/dL)	164.1±41.1 / 101	166.9±36.3 / 98	0.47
LDL (mg/dL)	89.8±32.7 / 101	93.1±31.2 / 98	0.39
HDL (mg/dL)	46.4±16.3 / 101	46.0±12.5 / 98	0.70
Triglycerides (mg/dL)	138.2±84.6 / 102	136.9±75.1 / 99	0.63

Paired t test; * $p < 0.05$

Of all the patients, 98 were assessed for all MS criteria in 2008 and 2012. Considering the IDF criteria, 6.1% (n=6) of patients who were diagnosed with MS in 2008 no longer had the syndrome in 2012; 79.6% (n=78) of patients who were diagnosed with MS (n=39) or without MS (n=39) in 2008 also had those conditions in 2012; and 14.3% (n=14) of the patients who were not diagnosed with MS in 2008 had this condition in 2012. Using the NCEP criteria, 5.1% (n=5) of patients who were diagnosed with MS in 2008 no longer had the syndrome; 78.6% (n=77) of the patients who were diagnosed with (n=30) or without MS (n=46) in 2008 also had that condition in 2012; and 16.3% (n=16) of patients who were not diagnosed with MS in 2008 retained that condition in 2012.

Table 3. Metabolic syndrome and its components in patients undergoing liver transplantation evaluated in 2008 and 2012

Parameters	Prevalence (n; %) / / total n		
(n=117)	2008	2012	p
Time since liver transplantation	3 years (0-13)	7.0 years (3-17)	
Metabolic syndrome			
IDF classification	47 (43.1) / 108	56 (53.3) / 108	0.12
NCEP revised classification	37 (34.3) /107	47 (44.8) / 107	0.03*
Anthropometric/ body composition			
Underweight by BMI	4 (3.4) / 117	2 (1.7) / 117	0.50
Normal weight by BMI	44 (37.6) / 117	48 (40.7) / 117	0.63
Overweight by BMI	44 (37.6) / 117	40 (33.9) / 117	0.56
Obesity by BMI	25 (21.4) / 117	28 (23.7) / 117	0.58
Abdominal obesity by IDF	78 (66.7) / 117	85 (72.0) / 117	0.09
Abdominal obesity by NCEP	45 (38.5) / 117	51 (43.2) / 117	0.24
Clinical and biochemical			
Blood pressure criteria	62 (52.5) / 117	64 (54.2) / 117	0.69
Arterial hypertension	34 (28.8) / 117	41 (34.7) / 117	0.21
Glucose criteria	40 (34.2) / 117	56 (48.6) / 117	<0.01*
Diabetes mellitus	21 (17.9) / 117	29 (24.6) / 117	0.08
High total cholesterol	16 (16.3) / 101	18 (17.8) / 98	0.82
High LDL	8 (8.2) / 101	15 (14.9) / 98	0.21
Low HDL	52 (51.5) / 101	45 (44.6) / 98	0.17
High triglycerides	30 (29.7) / 102	34 (33.3) / 99	0.99

McNemar test; * p <0.05

Patient intake of calories, macronutrients and micronutrients at the 2008 and 2012 evaluations are shown in table 4. The patients significantly increased their calorie and macronutrient intakes ($p<0.01$). The consumption of fiber, vitamin E and vitamin C also increased ($p<0.05$), while the consumption of thiamin, riboflavin, iron, sodium and potassium decreased ($p<0.05$).

Table 4. Nutrient intake of patients undergoing liver transplantation evaluated in 2008 and 2012

Nutrients	Average ± standard deviation		
	2008 (n=116)	2012 (n=114)	p
Time since liver transplantation	3 years (0-13)	7.0 years (3-17)	
Calories (kcal)	1,920.9±633.1	2,016.7±666.1	<0.01*
Carbohydrates (g)	258.5±78.8	260.3±92.2	<0.01*
Carbohydrates (%)	53.3±6.9	51.3±7.0	0.46

Proteins (g)	72.6±26.9	74.2±29.6	<0.01*
Proteins (%)	14.8±4.4	14.5±3.5	<0.01*
Fat (g)	68.4±28.5	74.6±30.0	<0.01*
Fat (%)	30.8±5.6	32.7±6.1	0.90
Polyunsaturated fat (g)	18.4±8.9	20.7±10.1	<0.01*
Polyunsaturated fat (%)	8.1±2.7	8.9±3.2	0.02
Monounsaturated fat (g)	18.1±8.8	20.7±9.5	<0.01*
Monounsaturated fat (%)	8.0±2.1	8.8±2.6	0.85
Saturated fat (g)	20.2±10.0	21.0±10.1	0.04*
Saturated fat (%)	8.9±2.6	9.0±2.6	0.47
Cholesterol (mg)	204.0 (39.0-614.0)	190.0 (50.0-1,038.0)	0.98
Fiber (g)	16.4±7.2	17.5±7.2	<0.01*
Vitamin A (RE)	657.0 (111.0-3,905.0)	534.0 (55.0-4,300.0)	0.33
Vitamin D (mcg)	2.0 (0.0-8.0)	1.0 (0.0-10.0)	0.87
Vitamin E (mg)	20.0 (5.0-47.0)	22.5 (7.0-75.0)	<0.01*
Vitamin C (mg)	66.0 (7.0-595.0)	94.0 (3.0-1,092.0)	0.02*
Thiamin (mg)	1.22±0.65	1.04±0.73	0.02*
Riboflavin (mg)	1.10±0.78	0.87±0.68	<0.01*
Niacin (mg)	17.0 (1.0-40.0)	17.0 (5.0-49.0)	0.22
Vitamin B_6 (mg)	1.19±1.01	1.15±0.77	0.09
Vitamin B_{12} (mg)	4.5±5.0	3.3±4.7	0.59
Folic acid (mcg)	186.2±74.3	167.9±75.9	0.25
Iron (mg)	13.0 (4.0-37.0)	11.0 (5.0-60.0)	<0.01*
Calcium (mg)	582.0 (183.0-1,577.0)	562.0 (148.0-3,193.0)	0.20
Sodium (mg)	2,206.8±823.2	2,190.6±965.4	<0.01*
Magnesium (mg)	233.8±111.9	201.6±118.2	0.42
Potassium (mg)	2,255.1±769.6	2,157.1±816.4	0.02*
Phosphorus	707.4±262.8	751.5±309.4	0.06

Paired t test or Wilcoxon test; * p <0.05

Associated factors of MS and its components are described in table 5. Greater age was the most common independent predictor of MS by the abdominal obesity, glucose and blood pressure criteria. BMI and body composition data also correlated with MS and its components, including body mass index prior to liver disease, which was an independent predictor of MS diagnosis (by the IDF criteria) and reduced HDL. Nutrient intake also impacted MS components, particularly fat intake, which was considered an independent predictor for low HDL and high triglycerides.

Table 5. Predictors of metabolic syndrome and its components in patients undergoing liver transplantation evaluated in 2012

Condition (% of correct prediction; Hosmer-Lemeshow p value)	Associated factors	Odds ratio	Confidence interval 95%	p
Metabolic syndrome by IDF (72.8%; p=0.43)	Age (years)	1.05	1.02-1.11	0.02
	Familial history of diabetes mellitus	3.38	1.19-9.61	0.02
	BMI prior to liver transplantation (kg/m²)	1.39	1.19-1.63	<0.01
Metabolic syndrome by NCEP (73.5%; p=0.24)	Age (years)	1.05	1.02-1.09	<0.01
	Body fat (%)	1.09	1.03-1.14	<0.01
Abdominal obesity by IDF (86.8%; p=0.91)	Age (years)	1.11	1.04-1.19	<0.01
	Body fat (kg)	1.19	1.04-1.36	0.01
	Current BMI (kg/m²)	1.71	1.23-2.39	<0.01
Abdominal obesity by NCEP (94.5%; p=0.99)	Total body water (%)	0.58	0.43-0.77	<0.01
	Age (years)	1.09	1.02-1.18	0.02
	Current body mass index (kg/m²)	1.99	1.34-2.97	<0.01
	Lack of physical exercise	14.49	1.87-111.11	0.01
Blood pressure criteria (63.5; p=0.11)	Age (years)	1.04	1.01-1.07	0.02
Glucose criteria (59.0%; p=0.57)	Age (years)	1.06	1.02-1.09	<0.01
Reduced HDL (72.9%; p=0.77)	Body mass index prior to liver transplantation (kg/m²)	1.18	1.06-1.32	<0.01
	Fat intake (g)	1.09	1.01-1.18	0.03
	Carbohydrate intake (g)	1.03	1.01-1.06	0.03
	Calories intake (kcal)	0.99	0.99-1.00	0.04
Hypertriglyceridemia (78.7%; p=0.13)	Fat intake (g)	1.04	1.02-1.06	<0.01
	Current body mass index (kg/m²)	1.20	1.07-1.35	<0.01
	Familial history of diabetes mellitus	3.11	1.05-9.17	0.04
	Current corticosteroid use	4.05	1.05-15.71	0.04

BMI – body mass index; multiple logistic regression analysis

Discussion

We chose to follow patients who underwent liver transplantation and who were evaluated in 2008 [8] because of the limitations at that time, particularly regarding the earlier study's cross-sectional nature. Additionally, few previous studies have focused on patients many years after

LTx, instead following up patients for a median of 3 to 5 years post-transplant [6, 13-14]. In 2008, shorter time since transplantation was associated with the presence of metabolic syndrome in our sample, and this made us reflect on the possible transitory nature of this condition, suggesting the need to follow patients and evaluate them for longer periods after transplantation. Interestingly, many more patients developed the syndrome than went into remission.

The prevalence of MS in liver transplant recipients is greater than that found in the general population [6]. The current prevalence of MS in the present population is 44.8% (using the revised NCEP criteria) or 53.3% (using the IDF classification). Among adults in the United States of a similar age to the patients of our study, the prevalence of MS using the revised NCEP criteria is 34.3%, and it is 38.5% using the IDF classification [23]. Among Brazilian adults of a similar age, the prevalence of the syndrome using the NCEP criteria is 29.8% [24]. There is no single agreed-upon definition of metabolic syndrome, so we used two diagnostic methods (IDF and revised NCEP criteria). Of the various available diagnostic criteria, both of the IDF as the revised NCEP criteria have components that are easily obtained, but IDF classification was, in some studies, the best in predicting acute coronary syndrome [25] and cardiovascular disease [26]. However, NCEP proved to be more associated with uncontrolled hypertension and cardiovascular disease than IDF in the studies of Cortez-Dias [27] and Choi et al. [28], respectively. The IDF definition of metabolic syndrome seems to be more appropriate for transplant patients. The IDF definition considers abdominal obesity a mandatory condition for the presence of the metabolic syndrome, but it seems to be more appropriate to consider the other components of metabolic syndrome, based on this findings. However, most work on metabolic syndrome in patients undergoing liver transplantation has used the NCEP definition, making it difficult to use another criterion for comparison.

The emergence of new cases of MS in patients undergoing liver transplantation also appears to occur in a shorter period of time when compared to the general population. After four years, 16.3% of patients who did not have MS in 2008 were diagnosed with this condition in 2012. In a prospective study in the general population, the prevalence of MS increased from 27.0% to 32.2% in men and from 38.6% to 45.0% in women over three years [29]. This increase represented a 5.2% new syndrome diagnosis in men and in 6.4% in women.

The increased incidence of MS could have been related to the increase in waist circumference (from 93.3±14.3 cm to 99.4±14.9 cm; $p<0.01$) and the increase in fasting blood glucose (from 98.8 mg/dL to 109.2 mg/dL, $p<0.01$), or to the number of patients who met the glucose-related criteria for MS diagnosis (from 34.2% to 48.6%, $p<0.01$) and the number of diabetics (from 17.9% to 24.6%, $p=0.08$). New-onset diabetes mellitus is a condition widely described after transplant [30], and it is mainly attributed to immunosuppressive therapy with prolonged use of corticosteroids and tacrolimus [31], although these associations were not evident in the present study. The maintenance dose of tacrolimus is approximately 15 ng/mL in the first two months post-transplant and decreases to 10 ng/mL at the end of the first year post-operation. Thereafter, the maintenance dose of tacrolimus was approximately 5 ng/mL. The maintenance dose of cyclosporine is approximately 300 ng/mL in the first and second months post-transplant and approximately 150 ng/mL in the first postoperative year. After the first year, the cyclosporine dose is 50 ng/mL. Prednisone is tapered over 3 to 4 months after LTx, except for those patients with autoimmune diseases. Nineteen patients (16.2%) in the current study were taking prednisone, as 10 had autoimmune hepatitis, four had primary sclerosing cholangitis, one had primary biliary cirrhosis, and one had autoimmune cholangiopathy. Our protocol specifies that prednisone be kept at a dose of 5 mg ad infinitum in patients who are transplanted because of autoimmune diseases. The remaining three patients also received tapered prednisone for treatment of previous graft rejection.

Weight, percent body fat and kilograms of body fat also significantly increased during the four years. The overall general population also gained weight over the four years. The natural history of body weight gain in overweight people is approximately 0.25 kg per year [32], which is less than the weight gain found in this study (average 1.3 kg in four years and 0.325 kg per year, which means a 34% higher weight gain). However, liver transplantation recipients experience a much larger increase in waist circumference than that found in the general population. While waist circumference increased on average 6.1 cm in four years according to the National Health and Nutrition Examination Survey, waist circumference increased only 3.0 cm among adult men and 3.2 cm among adult women in approximately ten years [33].

Lack of exercise was considered a predictor for abdominal obesity in the present study, and physical inactivity most likely contributed to the increase in waist circumference of these patients. Long-term liver recipients were mostly sedentary or slightly active (84.2%), and most of them did not engage in paid professional activity (52.6% of them were retired, unemployed or

housewives). This result is similar to other studies in which transplant survivors had lower physical scores than the general population of the same age [3]. Although the higher consumption of calories and macronutrients was not independently associated with abdominal obesity, reassessment of patients revealed that they increased their food consumption over the years, hence the weight gain. Calorie consumption rose an average of approximately 100 kcal in four years, and this as well as low physical activity most likely contributed to their weight gain and their high prevalence of overweight and obesity.

Another factor associated with abdominal obesity (by the NCEP criteria) was the low amount of total body water. Patients with less body water most likely have less lean body mass and, consequently, they expend less energy, which also contributes to an increased weight gain. This observation may be indicative of sarcopenic obesity, described in the general older population [34], and this supposedly also occurs in patients undergoing liver transplantation [35]. Liver recipients lose lean mass while they are cirrhotic [36], and they continue to have low levels of physical activity thereafter, which certainly contributes to the reduction of lean mass and body water observed over the years, as in the present work.

Greater age was predictive for MS, abdominal obesity, blood glucose and blood pressure. The prevalence of metabolic syndrome increases with time, both in the general population [23] and in patients undergoing liver transplantation [6], perhaps as a result of inadequate lifestyle for longer and/or the effect of age on many metabolic disturbances [6]. Family history of diabetes mellitus was considered an independent factor associated with MS (using the IDF criteria), as was high triglycerides. However, this relationship was not associated with the glucose criteria for MS in this study, which was surprising because a family history of diabetes mellitus can reflect a genetic predisposition to diabetes mellitus and has been cited as a risk factor for new-onset diabetes mellitus after transplantation in many studies, increasing the risk by as much as sevenfold [30].

We choose to not add to the model of MS components to identify the associated factors of the syndrome that were not directly connected to it, although these additional components are part of the diagnosis of MS and are certainly associated with it. Although waist circumference was not included in the models, several anthropometric features remained after the multiple logistic regression analysis of the associated factors of MS and its components, primarily body mass

index (prior to liver disease and current), body fat (percentage and kilograms) and total body water (percentage).

Higher carbohydrate and fat intake were associated with dyslipidemia in the present study. In the model of low HDL, caloric intake remained most likely to maintain the significance of carbohydrate and fat consumption because when this variable was removed, the others lost their significance. Total fat intake was associated with MS prevalence in a Brazilian-Japanese descendant population [37]. High carbohydrate and fat diets are causes of high triglycerides [20]. Corticosteroid use is also recognized as a cause of increased triglycerides [20], as found in the present study.

The cause of liver disease was not independently associated with metabolic syndrome or its components in this study. Some indications for transplantation were previously found to be related to metabolic syndrome and/or its components, such as ethanolic cirrhosis [8, 13, 38], hepatitis C virus cirrhosis [6, 13] and cryptogenic cirrhosis [6, 13]. Some patients undergoing transplantation for cryptogenic cirrhosis may have nonalcoholic steatohepatitis (NASH) as an etiologic factor. However, as cirrhosis progresses, the components of steatosis and inflammation disappear in liver biopsies, making the diagnosis of NASH difficult [6].

In conclusion, the prevalence of MS increased over time after LTx because of increases in waist circumference and blood glucose. MS and its components are associated with potentially modifiable factors, such as greater body mass index, body fat and increased carbohydrate and fat intake.

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4.2 Artigo 2

Adipokines, inflammatory and insulin resistance parameters: can they be good markers of metabolic syndrome after liver transplantation?

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Abstract

Background: Metabolic syndrome (MS) and obesity are widely prevalent among liver transplant (LTx) recipients. Although there is lot of data on the role of adipokines in these diseases, studies after LTx are scarce. **Aim:** To investigate the concentrations of adipokines, inflammatory and insulin resistance markers among liver recipients according to MS and its components. **Methods:** This was a cross-sectional study in which serum samples from 34 patients (55.9% male; average age 54.9±13.9 years; average time of 7.7±2.9 years after LTx) were evaluated for analysis of adiponectin, resistin, tumor necrosis factor-alpha (TNF-a), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), C-protein reactive (CPR), HOMA-IR and free fatty acids (FFA) in 2012/2013. The dosages were uni and multivariate analyzed considering metabolic syndrome (using the Harmonizing the MS criteria) and its components. **Results:** Half of the patients evaluated had MS (n=17). Higher concentration of adiponectin was observed among liver recipients that had MS (6.7±4.5 μg/mL versus 3.2±1.2 μg/mL; $p<0.01$). Low HDL and high waist hip ratio were considered independent predictors of adiponectin concentrations. Lower amounts of resistin were observed in those patients with high blood pressure (4.5±1.6 ng/mL versus 6.2±2.3 ng/mL; $p<0.01$) and higher, in those with abdominal obesity (5.5±4.2 ng/mL versus 4.3±1.6 ng/mL). Increased FFA (0,8±0,3 mEq/L *versus* 0,5±0,3 mEq/L, $p<0,05$) and HOMA-IR (4,9±3,8 *versus* 1,6±0,8) were observed in patients with MS. Independent risk factors were not identified for TNF-a, MCP-1, IL-6 and FFA. **Conclusions:** MS and its components are related to increased FFA concentration and HOMA-IR. Adiponectin, resistin and inflammatory markers, such as TNF, IL-6, MCP-1 and CRP, were not good markers of metabolic syndrome in this sample of patients who underwent liver transplantation.

Key-words: liver transplantation, metabolic syndrome, obesity, adipokines, insulin resistance

Introduction

Obesity and metabolic syndrome (MS) are highly prevalent after liver transplantation (LTx) [1-2] and have been considered risk factors for developing cardiovascular disease [3], cancer [4], steatosis [5], nonalcoholic steatohepatitis [6], graft fibrosis [7], and negatively impact on long term patient survival [8].

Adipose tissue is the largest endocrine organ. A range of bioactive polypeptides and proteins are secreted by adipocytes and collectively named as adipokines [9]. These adipokines play a role locally, peripherally and centrally in many physiological processes including energy balance and food intake, insulin action, lipid metabolism, angiogenesis, homeostasis and regulation of blood pressure [10-11]. Among the various secreted adipokines, adiponectin stands out as an abundant adipokine from adipose tissue, which acts as an anti-inflammatory and anti-obesity hormone. This molecule improves insulin sensitivity and has also anti-atherogenic properties [12-13]. While adiponectin acts improving the metabolism, other molecules like resistin, tumor necrosis factor-alpha (TNF-a), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), C-protein reactive (CPR) and free fatty acids (FFA) are associated to inflammation and insulin resistance [14-15].

Many studies assessing the general population have demonstrated that adiponectin concentrations are low [16-17] and resistin [18], TNF-a [19], IL-6[19], MCP-1 [20] CPR [19, 21] and FFA[22] levels are high among patients with MS or its components, such as obesity or diabetes. These markers have already been studied in patients undergoing solid organ transplantation[23-24], especially in kidney recipients[25-28]. However, to date and to our knowledge, no record of this type of study was found in hepatic transplant patients. The aim of the current study was to investigate if concentrations of adipokines, inflammatory and insulin resistance are good markers of MS and its components, among patients who underwent LTx.

Methods

This is a cross-sectional study in which serum samples from patients who underwent liver transplantation were assessed for adiponectin, resistin, TNF-a, MCP-1, IL-6, CPR, FFA, insulin and fasting blood glucose (the latter to calculate the Homeostatic Model Assessment - HOMA-IR). The prevalence of MS, its features and obesity were also assessed in order to predict concentrations of adipokines, inflammatory and insulin resistance. This was a convenience sample, in which the number of individuals with (n=17) and without metabolic syndrome (n=17) was evaluated in the same quantity. MS prevalence in this set of patients is in agreement with other studies that showed it to affect between 44.5 to 63.5% of liver graft recipients. [29-30]

This study was carried out between 2012 and 2013. Outpatients followed at Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Universidade Federal de Minas Gerais, Brazil, were invited to participate in this study. Patients who presented with recurrence of liver disease, those with ascites or any sign of non-compensated liver disease, women who were pregnant during the study period, patients diagnosed with cancer, those diagnosed with renal disease stages 3, 4 and 5 and those who refused to participate were excluded. The study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (ETIC protocol 44/08).

The MS diagnosis was based on the Harmonizing the Metabolic Syndrome criteria [31]. Patients presented with MS if they had at least three of the following disorders: abdominal obesity (waist circumference ≥ 80 cm for women and ≥ 90 cm for men, in South America), high blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg and/or in use of antihypertensive medications), high fasting glucose (≥ 100 mg/dL and/or oral hypoglycemic agents), hypertriglyceridemia (triglycerides ≥ 150 mg/dL) and reduced high density lipoprotein (HDL) (<50 mg/dL for women and <40 mg/dL for men). The prevalence of arterial hypertension and of diabetes mellitus was estimated based on the use of medications to treat these conditions, as described in the medical records.

Patients were interviewed during routine medical appointments to obtain demographic, clinical and anthropometric information. The demographic data consisted of age and sex. The assessed clinical data were related to time since the transplant, indication for the transplant, use of tacrolimus or cyclosporine. Fasting glucose, triglycerides and serum HDL were obtained from

the routine tests performed in the outpatient clinic. Each patient's blood pressure was assessed on the interview day.

The anthropometric data consisted of waist circumference (measured two fingers above the umbilicus), hip circumference, waist/hip ratio, weight, height, body mass index (BMI) and its classification (overweight: BMI ≥ 25 kg/m²; obesity: BMI ≥30 kg/m²) [32]. Body composition data (i.e., fat mass, lean mass, total body water and phase angle) were obtained using bioelectrical impedance (RJL Systems® Quantum, Clinton Township, MI, USA).

Blood sample collection in order to analyze adipokines, inflammatory and insulin resistance markers was performed in the morning after an overnight fasting of eight hours. The blood was centrifuged to obtain the serum, which was stored in a freezer at -80°C. Adiponectin (Linco Research, St Charles, MI, USA), resistin and TNF-a (Millipore Corp., Bedford, Massachusetts, USA), MCP-1 and IL-6 (BD PharMingen or Endogen, Woburn, MA), and FFA (WAKO; Pure Chemical Industries, Japan) were assessed by ELISA kits and according to the manufacturer's instructions. When CRP values were less than 5 mg/dL, the reactive test Vitros® (Johnson & Johnson, EUA) was used, which has a functional sensitivity of 5 mg/L and a linearity between 5 and 90 mg/L. Results were divided into those with low (CPR<5.0 mg/dL) and high (CPR>5.0 mg/dL) concentrations of CPR. HOMA-IR was calculated by the formula: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL)/405. Insulin resistance was classified using the HOMA-IR cut-off of 2.7[33] and, for this analysis, we excluded those diabetic patients in use of insulin.

Statistical analyses were performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as percentages. Numerical variables were assessed for normality (Kolmogorov-Smirnov test) and were presented as the average and standard deviation or median, minimum and maximum. Patients were compared in terms of their serum dosages using the Student t Test or the Mann-Whitney test and the Pearson or Spearman correlation. The chi-squared test or Fisher's exact test when appropriate were also used in the univariate analysis to assess the associated factors for insulin resistance by HOMA-IR and CPR classifications. Variables that had $p<0.1$ in the univariate analysis were included in the multiple linear regression analysis which was undertaken in a stepwise, backward method. P values < 0.05 were considered to be statistically significant.

Results

Thirty four patients were assessed (55.9% male; average age 54.9±13.9 years). These patients had 7.7±2.9 years since LTx was performed (range 3 years to 15 years). The leading cause for liver transplantation was C virus hepatitis cirrhosis (41.1%; n=14). Half of the patients had MS (n=17), and the majority had abdominal obesity (76.5%; n=26), hyperglycemia (52.9%; n=18) and high blood pressure (55.9%; n=19). Patients had 2.6±1.6 number of components of MS with diabetes mellitus present in 29.4% (n=10, two using insulin) and hypertension, in 44.1% (n=15).

Adiponectin level was 5.0±3.7 μg/mL and resistin, 5.2±2.1 ng/mL. Median TNF-a value was 35.3 pg/mL, range 16.6 to 495.5 pg/mL, and median IL-6 value was 14.3 pg/mL, range 2.0 to 305.2 pg/mL. MCP-1 was 292.6±231.6 pg/mL and HOMA-IR 3.3±3.2. Concentrations of FFA were 0.7±0.3 mEq/L. Serum concentrations of adiponectin, HOMA-IR and FFA were statistically affected by the presence of MS (figure 1). General characteristics, MS and its components and concentrations of the serum markers are depicted in tables 1 and 2.

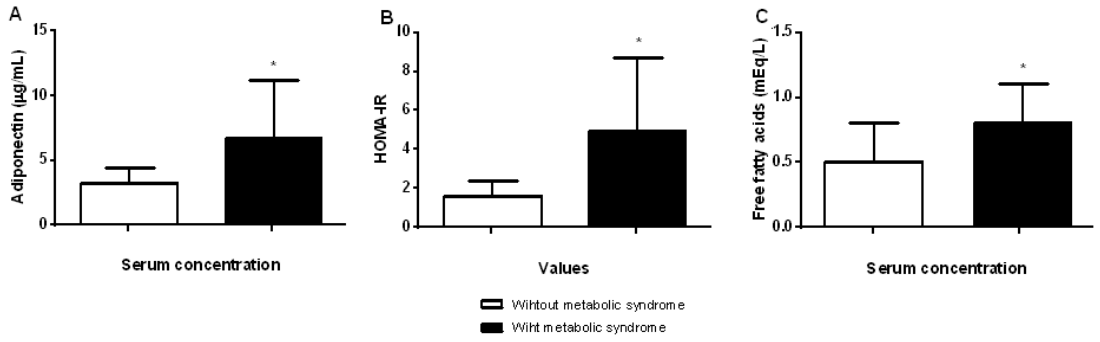

Figure 1. Serum concentration of adiponectin (A), values of HOMA-IR (B) and serum concentration of free fatty acids (C) among liver-transplant recipients with or without metabolic syndrome. T-student Test. * $p<0.05$.

It was observed increased concentration of adiponectin among liver recipients that had low HDL concentrations (8.0±4.3 μg/mL versus 3.2±1.9 μg/mL; $p<0.01$) and MS (6.7±4.5 μg/mL versus 3.2±1.2 μg/mL; $p<0.01$), those who were overweight (6.5±4.2 μg/mL versus 3.6±2.6 μg/mL; $p<0.05$) and those with abdominal obesity (5.7±3.9 μg/mL versus 2.6±0.6 μg/mL; $p<0.01$). Adiponectin was also positively correlated to waist hip ratio (correlation coefficient of 0.63; $p<0.01$); number of components of MS (correlation coefficient of 0.56; $p<0.01$), triglycerides (correlation coefficient of 0.53; $p<0.01$) and waist circumference (correlation coefficient of 0.35;

p<0.05). Adiponectin concentrations were also inversely correlated to HDL concentrations (correlation coefficient of -0.52; p<0.01).

Table 1. Average and standard deviation or median, minimum and maximum of adipokines, inflammatory and insulin resistance markers of patients who underwent liver transplantation according to their characteristics, obesity, metabolic syndrome and its components

Characteristic	General n (%)	Adiponectin (µg/mL)	Resistin (ng/mL)	TNF-a (pg/mL)	MCP-1 (pg/mL)	IL-6 (pg/mL)	HOMA IR	FFA (mEq/L)
Sex								
Male	19 (55.9)	5.6±3.6	5.1±1.8	26.3 (16.6-488.2)	261.3±226.1	19.9 (2.0-244.3)	3.5±3.8	0.6±0.3
Female	15 (44.1)	4.2±3.8	5.3±2.3	57.4 (16.9-459.9)	317.4±239.1	13.3 (5.9-305.2)	2.9±2.3	0.7±0.3
Immunossupressor								
Tacrolimus	23 (85.3)	4.9±3.8	5.3±2.1	49.8 (16.6-495.9)*	285.9±230.2	13.3 (4.6-305.2)	3.3±3.2	0.7±0.3
Ciclosporin	11 (14.7)	5.3±2.9	4.8±2.4	19.2 (16.9-28.3)	331.7±263.3	19.9 (2.0-52.1)	3.1±3.2	0.5±0.3
Prednisone								
Yes	8 (23.5)	4.9±3.3	4.3±1.2	27.3 (16.6-495.9)	318.6±249.4	13.4 (5.9-78.1)	3.9±2.6	0.9±0.5
No	26 (76.5)	5.0±5.1	5.6±2.2	46.9 (24.3-175.0)	208.3±142.1	15.5 (2.0-305.2)	3.0±3.4	0.6±0.3
Indication for LTx								
HCV cirrhosis	14 (44.1)	3.7±2.5	5.4±1.7	50.3 (16.7-495.9)	263.8±227.8	15.5 (4.6-305.2)	3.7±4.1	0.7±0.3
Alcoholic cirrhosis	6 (17.7)	6.7±4.4	6.1±3.1	23.8 (16.6-488.2)	281.4±276.9	31.2 (4.7-244.3)	3.7±3.4	0.8±0.3
Cryptogenic cirrhosis	5 (14.7)	4.3±2.1	5.1±2.7	20.5 (18.5-110.3)	221.5±119.7	10.4 (2.0-261.9)	1.2±0.7	0.7±0.6
Auto-immune cirrhosis	4 (11.8)	2.2±0.8	4.3±1.3	113.5 (49.8-175.1)	371.3±254.5	18.4 (6.0-78.1)	2.9±2.4	0.4±0.2
HCC with cirrhosis	4 (11.8)	4.9±4.4	3.9±1.3	79.0 (26.3-175.1)	374.4±255.1	16.6 (6.1-71.7)	1.2±0.2	0.6±0.3
Others	8 (23.5)	6.9±4.9	4.4±3.1	25.7 (18.9-170.2)	277.6±253.6	9.5 (5.9-81.1)	3.4±2.8	0.6±0.3
Overweight (by BMI)								
Yes	16 (47.1)	6.5±4.2*	5.8±2.4	42.3 (16.6-488.2)	263.4±243.3	31.8 (5.9-244.3)	3.5±2.7	0.6±0.3
No	18 (58.9)	3.6±2.6	4.8±1.7	29.3 (18.5-495.9)	318.6±224.5	10.4 (2.0-305.2)	3.1±3.6	0.7±0.4
Obesity (by BMI)								
Yes	7 (20.6)	5.2±3.6	5.0±1.1	54.9 (26.3-169.6)	272.9±195.9	10.3 (6.0-78.1)	6.0±5.2*	0.8±0.4
No	27 (79.4)	4.9±3.8	5.3±2.3	25.2 (16.6-495.9)	297.7±244.9	14.9 (2.0-305.2)	2.7±2.3	0.6±0.3
Metabolic syndrome								
Yes	17 (50.0)	6.7±4.5**	5.0±1.1	40.5 (16.6-170.2)	342.6±251.8	10.4 (4.6-244.3)	4.9±3.8**	0.8±0.3*
No	17 (50.0)	3.2±1.2	5.3±2.3	40.0 (16.7-495.9)	260.9±208.5	18.2 (2.0-305.2)	1.6±0.8	0.5±0.3
Abdominal obesity								
Yes	26 (76.5)	5.7±3.9**	5.5±2.2**	46.9 (16.6-488.2)	262.1±229.3	14.3 (4.6-244.3)	3.9±3.3	0.7±0.3
No	8 (23.5)	2.6±0.6	4.3±1.6	21.5 (18.5-495.9)	391.9±224.6	15.5 (2.0-305.2)	1.1±0.6	0.6±0.3
Hyperglycemia								
Yes	18 (52.9)	5.8±4.3	5.4±2.3	25.8 (16.6-170.2)	226.6±203.4	12.9 (4.7-71.7)	4.5±4.1*	0.7±0.3
No	14 (41.2)	3.9±2.6	5.1±1.9	50.8 (18.2-495.9)	366.9±245.0	14.9 (2.0-305.2)	2.1±1.1	0.6±0.4
High blood pressure								
Yes	19 (55.9)	5.7±4.0	4.5±1.6**	28.3 (18.5-170.2)	303.1±226.3	10.4 (2.0-261.9)	4.3±3.7**	0.7±0.4
No	15 (44.1)	3.9±3.1	6.2±2.3	49.8 (16.6-495.9)	279.3±245.5	16.2 (7.7-305.2)	1.7±0.8	0.6±0.3
High triglycerides								
Yes	15 (44.1)	6.3±4.0	4.9±1.6	42.3 (19.2-488.2)	243.2±192.4	14.9 (4.6-244.3)	5.1±3.6**	0.8±0.3*
No	19 (55.9)	4.1±3.3	5.3±2.3	28.3 (16.6-495.9)	342.7±251.7	13.3 (2.0-305.2)	1.4±0.7	0.6±0.3
Low HDL								
Yes	12 (35.3)	8.0±4.3**	4.9±2.3	24.9 (16.6-170.2)	298.2±198.6	14.9 (4.7-261.9)	5.2±4.7**	0.6±0.2
No	22 (64.7)	3.2±1.9	5.2±1.9	49.8 (16.7-495.9)	301.8±251.7	13.6 (2.0-305.2)	2.4±1.7	0.7±0.4

Legend: LTx: liver transplant patients, HCV: Hepatitis C Virus, BMI: Body Mass Index, HDL: High Density Lipoprotein, TNF-alpha: Tumor Necrosis Factor-alpha, MCP-1: Monocyte chemoattractant protein-1, IL-6: Interleukin 6; FFA: Free Fatty Acids. Student t test or Mann-Whitney test; *p<0.05; **p<0.01.

Decreased concentrations of resistin were observed in those patients with high blood pressure (4.5±1.6 ng/mL versus 6.2±2.3 ng/mL; p<0.01) and increased, in those with abdominal obesity (5.5±4.2 ng/mL versus 4.3±1.6 ng/mL). An inverse correlation between resistin concentrations and time since transplant was observed (correlation coefficient of -0.36; p<0.05).

The inflammatory markers (TNF-alpha, MCP-1, IL-6 and CPR) were poorly associated with MS and its components. Only body fat percentage was directly correlated to TNF-alpha (R: 0.37; $p<0.05$) and diastolic blood pressure was inversely correlated to MCP-1 (R: -0.48; $p<0.01$).

Table 2. General characteristics and correlation coefficient (R) between metabolic syndrome, its components and adipokines, inflammatory and insulin resistance markers of patients who underwent liver transplantation.

Characteristic	General	Adiponectin	Resistin	TNF-a	MCP-1	IL-6	HOMA IR	FFA
Age (years)	54.9±13.9	0.15	0.25	-0.05	-0.17	-0.18	0.09	-0.21
Time since transplantation (years)	7.7±2.7	0.03	-0.36*	-0.23	0.01	0.23	-0.01	0.14
Body Mass Index (kg/m²)	27.3±4.3	0.26	0.19	0.18	-0.12	0.03	0.53**	0.24
Waist (cm)	97.3±4.3	.035*	0.15	0.19	-0.24	-0.06	0.67*	0.24
Body fat (%)	32.1±10.7	0.08	0.05	0.37*	-0.29	0.04	0.29	0.28
Waist-hip ratio	0.94±0.09	0.63**	0.18	0.05	-0.14	-0.02	0.71**	0.07
Component numbers of MS	2.6±1.6	0.56**	0.10	-0.06	-0.22	-0.16	0.67**	0.28
Fasting glucose (mg/dL)	98.6 (64-248)	0.32	0.14	0.08	-0.24	-0.10	0.37	0.17
Systolic blood pressure (mmHg)	118.6±26.6	0.02	-0.05	0.06	-0.48**	-0.15	0.32	0.45**
Diastolic blood pressure (mmHg)	80.3±12.9	0.01	-0.11	0.01	-0.29	-0.23	0.37	0.35*
Triglycerides (mg/dL)	143.4±75.5	0.53**	0.03	0.09	-0.15	-0.18	0.52**	0.29
High density lipoprotein (mg/dL)	47.8±15.5	-0.52**	-0.08	0.08	0.13	0.08	0.31	0.02

Legend: TNF-alpha: Tumor Necrosis Factor-alpha, MCP-1: Monocyte chemoattractant protein-1, IL-6: Interleukin 6, CPR: Protein C Reactive. Pearson or Spearman correlation test; *p<0.05; **p<0.01.

Higher values of HOMA-IR were observed in those patients who were obese, had MS and its components (hyperglycemia, high blood pressure, high triglycerides and low HDL). BMI, waist, waist-hip ratio, number of components of MS and triglycerides were also correlated to HOMA-IR values. FFA concentrations were higher in patients with MS, those with elevated triglycerides and they were correlated to systolic and diastolic blood pressure.

Half of patients had increased CPR values (n=17) and 35.3% (n=12) had insulin resistance by HOMA-IR. Increased CPR was not related with MS, its features or obesity. Insulin resistance and diabetes were more common in obese patients, those with high triglycerides and abdominal obesity. Patients who had MS and high blood pressure also presented with insulin resistance (table 3).

Independent variables associated with adiponectin, resistin and HOMA-IR values are listed in table 4. Of all variables associated to adiponectin, only waist-hip ratio and low HDL concentrations were able to predict it by multiple linear regression. Resistin was positively correlated to abdominal obesity and negative correlated to high blood pressure. HOMA-IR values were predicted by abdominal obesity and MS. Independent variables were not identified

for TNF-a, MCP-1, IL-6 and FFA since multiple linear regression models could not be carried out with their inclusion.

Table 3. Metabolic syndrome and HOMA-IR associated features in patients who underwent liver transplantation

Variables associated to insulin resistance by HOMA-IR/Diabetes mellitus	**% (n)**	**OR**	**CI 95%**
High triglycerides			
Yes	64.3% (9)	21.6**	2.1-218.6
No	7.7% (1)		
Metabolic syndrome			
Yes	64.3% (9)	21.6**	2.1-218.6
No	7.7% (1)		
High blood pressure			
Yes	52.9% (9)	11.3*	1.2-108.4
No	9.1% (1)		
Obesity (BMI>30 kg/m²)			
Yes	80.0% (4)	11.3*	1.1-122.5
No	26.1% (6)		
Abdominal obesity			
Yes	47.6% (10)	1.9*	1.3-2.9
No	0.0% (0)		

Legend: BMI: Body Mass Index; OR: Odds Ratio; CI: Confidence Interval

Qui-Square or Fisher's Exact Test; * p<0.05; ** p<0.01

Table 4. Independent variables associated to adiponectin, resistin and HOMA-IR values in patients who underwent liver transplantation

Variables associated to Adiponectin	β	CI 95%
Waist-hip ratio	25.8	11.4 - 40.3**
High HDL	3.4	1.3 – 5.5**
Waist circumference	-0.9	-0.18 – 0.01
Constant	-11.9	-21.3 – (-) 2.5*
Variables associated to Resistin	β	CI 95%
Abdominal obesity	1.7	0.1 – 3.3*
High blood pressure	-2.0	-3.3 – (-) 0.63 **
Constant	5.1	3.6 – 6.5*
Variables associated to HOMA-IR	β	CI 95%
Obesity (BMI>30 kg/m²)	0.5	0.1 – 0.9*
Metabolic syndrome	0.5	0.2 – 0.8**

Legend: BMI: Body Mass Index; OR: Odds Ratio; CI: Confidence Interval

Multiple logistic regression; * p<0.05; ** p<0.01

Discussion

MS and its features were associated to increased concentrations of adiponectin, FFA and HOMA-IR in patients who underwent liver transplantation after 7.7±2.9 years. Higher adiponectin concentrations were observed in those liver recipients with MS (6.7±4.5 μg/mL versus 3.2±1.2 μg/mL) contrary to what we expected. Adiponectin has anti-inflammatory, anti-atherogenic, anti-oxidant and insulin-sensitizing properties[14]. Therefore, having low adiponectin concentrations is expected to play a pathogenic role in the development of the MS [16]. In the general population[16-17, 34-35] and in other groups of patients who underwent transplantation[24-26, 28], adiponectin concentrations were lower among patients with MS or its features. In a study with 271 kidney transplant patients of similar age (52.3±12.6 years old) and follow-up since transplantation (9.0±5.9 years), those diagnosed with MS had lower values of adiponectin (21.8±13.9 μg/mL) compared to patients without MS (28.7±18.7 μg/mL) [26]. In another study with 94 kidney transplant patients (40.0±11.0 years old and median of 3.9 years of follow-up, range 1 month–8.8 years), patients with MS also had lower concentrations of adiponectin (11.9±5.1 μg/mL versus 17.7±8.5 μg/mL) [25]. In the general population, a study with 312 elderly Korean individuals without diabetes demonstrated that subjects with MS also had lower concentrations of adiponectin (13.9 μg/mL versus 17.0 μg/mL) [36]. Bahia and colleagues evaluated adiponectin concentrations among 19 Brazilian obese subjects with MS (average age of 40.8 ± 9.4 years) old and they also had lower values than the controls (9.5 μg/mL versus 19.5 μg/mL) [34]. Also, adiponectin concentrations were correlated with HOMA-IR index in this study (data not shown; R: 0.59; $p<0.01$) and this may explain the link between insulin resistance and this adipokines in this population. It may well be that these patients were adiponectin resistant and this may impede the insulin-sensitizing and anti-obesity effects. Nonetheless, comparisons of measured values of adiponectin in our sample with those from others studies are not appropriated, since adiponectin values are variable and could be affected by methods, kits, renal and cardiac function, smoking status, dietary factors and physical exercise [37]. Also, reference values of adiponectin have not been proposed to date, which complicates any reviews on the classification of adiponectin concentrations in these patients. However, a study conducted with 225 male patients that evaluated the association between adiponectin and coronary heart disease, categorized plasma adiponectin concentrations according to the first quartile of distribution (< 4 μg/mL) as “hypoadiponectinemia” [38]. Although it was not the purpose of this study, most (58.8%, n=20) of the patients assessed had “hypoadiponectinemia” . Thus, more studies are needed to verify the reason for these findings.

HOMA-IR (4.9±3.8 versus 1.6±0.8) and FFA (0.8±0.3 versus 05.±0.3) were also significantly increased among liver recipients with MS, which was expected, since the MS is a consequense of insulin resistance. Plasma FFA concentrations are chronically elevated in human obesity because of the blunted capacity of insulin to inhibit lipolysis, as well as a concomitant excessive consumption of dietary lipids. It is also known that the excess of fatty acids induces hepatic insulin resistance and provides substrate for lipoprotein synthesis and neutral lipid storage in hepatocytes [14]. HOMA-IR values in the present study (3.3±3.2) were very similar to the findings of Bianchi and colleagues[29] (3.1±2.3) who assessed patients after LTx. In that study, 41.4% of patients had insulin resistance, a similar prevalence to the 35.3% described in our patients. HOMA-IR was also independently predicted by obesity (BMI>30.0 kg/m²), more than by the other anthropometric measures (waist circumference, waist-hip ratio, body fat percentage) and the used classification (overweight and abdominal obesity). It must be highlighted that, although BMI does not discriminate between body compartments (as fat mass and fat-free mass), obese people certainly have higher measures of waist circumference, far superior to measures of 80 cm for women and 90 cm for men, proposed as cut-off points by the MS criteria adopted [31]. Although distribution of body fat is more important than its amount, the low cut-off points suggested in this classification [31] may have influenced our results. Considering this aspect, it should also be emphasized, the impact of waist-hip ratio as a predictor for adiponectin concentrations. This measure was more important than waist circumference and abdominal obesity, body fat percentage, BMI as well as overweight and obesity in predicting adiponectin concentrations. Increased waist-hip ratio values demonstrate greater and disproportionate distribution of abdominal fat. Intra-abdominal fat, more than subcutaneous fat (for example, that of the hip region), is more metabolically active due to increased responsiveness to cathecholamines and lower sensibility to suppression of lipolysis mediated by insulin [39]. Metabolic products (FFA, inflammatory molecules, angiotensinogen, and cortisol) of intra-abdominal fat are released into the portal vein, which provides direct delivery to the liver, causing dyslipidemia and insulin resistance [14]. In the present study, waist-hip ratio was a better predictor of adiponectin values when compared to the waist measurement alone. If abdominal obesity has a higher impact on cardiovascular disease, then, the best assessment should be the one that better predicts these affections, however there is little consensus on such aspect. In a meta-analysis of 15 articles and 258,114 participants, although both waist circumference and waist hip-ratio were associated with cardiovascular disease, the last one was more strongly associated with the events [40]. Another meta-analysis of 82,864 participants from nine cohort

studies[41] showed that waist-hip ratio and waist circumference were equivalents considering the capacity of better predicting cardiovascular disease when comparing to BMI. .

Abdominal fat accumulation was related to increased values of resistin, in the current study. Such high concentrations have been associated with obesity, visceral fat, insulin resistance, type 2 diabetes, inflammation, metabolic syndrome and its components [18], but not in all studies [42]. Surprisingly, in this study, lower concentrations of resistin were observed in those LTx recipients with high blood pressure (4.5±1.6 ng/mL versus 6.2±2.3 ng/mL). Therefore, more investigations are needed to explain this finding.

In the present study, none of the assessed inflammation markers (TNF-a, MCP-1, IL-6 and CPR) were independently associated with metabolic syndrome and its components. Circulatory concentrations of TNF-a, IL-6, MCP-1 and CPR are usually elevated in obese subjects and play a role in insulin resistance as well as are related to the inflammatory process that occurs in cardiovascular disease [14]. However, these cytokines are not specific from adipose tissue and maybe associated to other factors other than MS and its features such as the immune system response to the transplanted organ as well as the use of the imnuossupressive drugs. The latter may interfere with the production of inflammatory markers. As tacrolimus and cyclosporine inhibit the activation of nuclear factor of activated T cells (NFAT), preventing the transcription of interleukin 2 (IL-2), among other cytokines, which affect the immune response and the production of other cytokines, as TNF-a [43]. Prednisone also affects these markers and, although we found no differences in those patients in use of this medication, it is known that prednisone reduces transcription of TNF-a, IL-6, MCP-1 [44-46]. Also, following LTx, hepatic innervation is transected; thus, liver allografts are completely isolated from neural control of their hosts. Insulin resistance, postprandial hyperglycemia and changes in ingestion behavior are supposed to be the major side effects of absent liver innervations [47]. As we do not know if patients with a denervated liver have different production of adipokines, inflammatory and insulin resistance markers when compared to matched controls, this is a potential limitation of our study. Also, the small sample of patients may have affected our results and the statistical analyses. However, we should highlight that to our knowledge this is the first study assessing these markers in this population. Although MS after LTx has been increasingly studied, information on the pathophysiology of this disorder is still very incipient.

In conclusion, MS and its features were associated with increased concentrations of FFA and HOMA-IR. Adiponectin, resistin and inflammatory markers (TNF, IL-6, MCP-1 and CRP) did not portray the pathophysiology of the metabolic syndrome in this sample of patients.

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5. CONSIDERAÇÕES FINAIS

5.1. Discussão do Método

Neste estudo, adotamos três classificações de SM (*National Cholesterol Education Program Adult Treatment Panel* III revisado – NCEP ATP III, *International Diabetes Federation* - IDF e *Harmonizing the Metabolic Syndrome* - HMS). As classificações da NCEP ATP III e IDF já haviam sido utilizadas na linha de base do estudo, em 2008. Como o trabalho proveniente desta avaliação já havia sido publicado, optamos por manter os mesmos critérios daquela época para a avaliação prospectiva dos pacientes, descrita no artigo 1. Para o artigo 2, optamos pelo critério da HMS por ser se tratar de classificação mais recente para SM, na qual os critérios do NCEP ATP III revisado e da IDF foram unificados. A principal diferença entre as classificações do NCEP ATP III revisado e IDF diz respeito à medida de obesidade central, sendo este um componente obrigatório na definição da IDF, menor do que na definição do NCEP ATP III revisado e específico conforme a etnia. Na classificação de SM do HMS, o critério de obesidade abdominal não é condicionante da SM, mas os pontos de corte são os mesmos propostos pelo IDF. Vale ressaltar que, no presente estudo, a mesma população classificada com SM pelo IDF também foi pelo HMS, pois todos os pacientes com a síndrome por essas classificações possuíam também obesidade abdominal por este critério.

Na avaliação da ingestão alimentar, embora o aumento ou decréscimo da ingestão da maioria dos nutrientes tenha sido estatisticamente constatado, deve-se ressaltar que a média de ingestão de alguns nutrientes sofreu pequena variação nas duas avaliações (como, por exemplo, a ingestão média de proteínas e carboidratos). Ainda, destaca-se que os dados sobre a ingestão de sódio não são fidedignos, uma vez que os pacientes não souberam quantificar a utilização de sal e temperos no preparo dos alimentos. Outras limitações relativas à avaliação da ingestão alimentar devem ser consideradas. Embora a adoção do Registro Alimentar de Três Dias poderia oferecer-nos melhores dados da ingestão alimentar, apenas 60 pacientes (51,3%) enviaram-nos os registros de volta. Para os pacientes em que o registro não estava disponível, utilizamos a História Dietética. Outros métodos de inquérito alimentar, como o Questionário de Frequência Alimentar e Recordatório Alimentar de 24 horas não foram possíveis de serem aplicados. Os pacientes já submetidos ao transplante hepático são geralmente os primeiros a chegar ao ambulatório e ficam receosos em realizar qualquer atividade que possa prejudicar a ordem de seu atendimento

médico. Por isso, o tempo disponível para a coleta de dados era muito restrito, o que tornava necessária a adoção de método de inquérito alimentar menos delongado. Ainda, muitos dos pacientes submetidos ao transplante avaliados moram em outras cidades ou estados, se deslocam grandes distâncias no dia anterior à consulta ou ficam hospedados em hotéis ou em casas de parentes. Ademais, muitos pacientes realizam coleta de sangue para exames no dia anterior à consulta. Todos estes fatores afetariam a ingestão alimentar nas 24 horas antecedentes à coleta de dados. Outra limitação da avaliação dietética refere-se à tabela de composição de alimentos adotada (Philippi et al., 2001) e concordamos que a utilização da Tabela Brasileira de Composição de Alimentos (TACO) desenvolvida com análise de alimentos cultivados em solo brasileiro seria mais apropriada. No entanto, a avaliação dietética na linha de base desta pesquisa, em 2008, levou em conta a utilização da tabela de Philippi et al. (2001), tornando inviável a utilização de outra base de dados de composição de alimentos. Embora imbuída de limitações, a avaliação dietética é importante e os dados dietéticos obtidos no presente estudo talvez possam ser mais bem explorados em outro trabalho, comparando-se, por exemplo, a ingestão alimentar dos pacientes submetidos ao transplante hepático avaliados com as Ingestões Dietéticas de Referência e verificando a associação do consumo de nutrientes com outras variáveis, como as antropométricas, de composição corporal e os marcadores bioquímicos dosados.

Limitações relativas ao tamanho amostral pequeno do artigo 2 e à ausência de controles não submetidos ao transplante hepático para a comparação dos valores das adipocinas, marcadores inflamatórios e de resistência à insulina dosados devem ser destacadas. No desenho inicial do artigo 2, optamos por realizar amostra de conveniência com prevalência da SM similar à descrita em outros trabalhos para que nossos pacientes sem SM fossem considerados controles dos demais. No entanto, nossos resultados fizeram-nos refletir sobre a possibilidade da terapia imunossupressora e da perda da inervação hepática poderem afetar os marcadores dosados na população avaliada. Dessa forma, estudos sobre os mecanismos fisiopatológicos da SM em pacientes submetidos ao TxH com amostras maiores e que tenham comparação com controles sadios são necessários.

Embora as limitações do trabalho mereçam evidência, este foi o primeiro estudo, até o momento, sobre a associação das adipocinas, marcadores inflamatórios e de resistência à insulina com a SM e respectivos componentes, em amostra de população submetida ao transplante hepático. A SM é um distúrbio cada vez mais descrito em pacientes submetidos ao transplante hepático e

dado o impacto sobre a morbi-mortalidade desses pacientes, trabalhos como este são importantes para o entendimento desse distúrbio.

5.2. Conclusões

Neste estudo, avaliou-se de modo prospectivo a prevalência da SM e respectivos componentes em pacientes no pós-operatório tardio de transplante hepático. Os mecanismos fisiopatológicos da síndrome também foram investigados nestes pacientes, por meio de análises de adipocinas e marcadores inflamatórios e de resistência à insulina.

No pós-operatório tardio de TxH, a SM foi observada em mais da metade dos pacientes e houve aumento da prevalência dessa morbidade com o passar dos anos. A SM e respectivos componentes afetaram as concentrações séricas de AGL, bem como o HOMA-IR, havendo aumento de ambos. Adiponectina, resistina e marcadores inflamatórios (TNF-a, IL-6, MCP-1 e CRP) não caracterizaram a fisiopatologia da SM nesta amostra de pacientes.

Dos componentes da SM, o perímetro da cintura e glicemia aumentaram significativamente da primeira para a segunda avaliação. Em virtude desse último aumento, houve incremento da prevalência do critério “hiperglicemia” ao longo dos anos.

A SM e respectivos componentes estiveram associados a fatores potencialmente modificáveis, como maior IMC e gordura corporal, falta de exercícios físicos, utilização de corticosteroides e maior ingestão de carboidratos e gorduras. Idade e história familiar de diabetes mellitus também foram fatores associados à SM e respectivos componentes.

A SM esteve independentemente associada apenas ao índice HOMA-IR. De forma univariada, maiores valores séricos de adiponectina, AGL e índice HOMA-IR foram observados em pacientes submetidos ao TxH com SM. O componente da SM que mais impactou em adiponectina, resistina e HOMA-IR foi a obesidade, representada pela maior relação cintura-quadril, obesidade abdominal e obesidade pelo critério de IMC, respectivamente. De forma independente, maiores concentrações de adiponectina foram observados em indivíduos com HDL reduzido e, menores concentrações de resistina foram encontrados em pacientes com pressão arterial elevada. Os marcadores inflamatórios (TNF-a, MCP-1, IL-6, PCR) não se associaram à SM ou respectivos componentes. Estes dados não condizem com os achados da literatura e mais estudos são necessários para elucidar estes resultados.

Em relação às modificações ocorridas na composição corporal de forma prospectiva, foi observado aumento no percentual e na quantidade de gordura corporal e decréscimo no percentual de massa magra e água corporal. A ingestão alimentar também foi modificada ao longo do tempo. Foi observado aumento na ingestão calórica contemplando tanto carboidratos, proteínas e gorduras, bem como a ingestão de fibras, vitaminas E e C. Foi também observado decréscimo na ingestão de tiamina, riboflavina, ferro, sódio e potássio.

APÊNDICES

Apêndice A – Instrumento de coleta de dados

Aspectos clínicos e fisiopatológicos da síndrome metabólica no pós-operatório tardio do transplante hepático / Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto, Faculdade de Medicina-UFMG

Data:____/____/_____

BLOCO A: Dados demográficos e socioeconômicos

Nome:_____Prontuário n°:_____

Endereço:_____Cep:_____

Telefone:_____ Celular:_____

Profissão:_____Ocupação atual:_____

Renda mensal individual:_____ Renda mensal familiar:_____n° pessoas:_____

Escolaridade: ( ) analfabeto ( ) primeiro grau incompleto ( ) primeiro grau completo ( ) segundo grau incompleto ( ) segundo grau completo ( ) terceiro grau incompleto ( ) terceiro grau completo. Anos de estudo:_____

Estado Marital: ( ) casado/amasiado ( ) solteiro ( ) divorciado ( ) viúvo

BLOCO B: Dados sobre estilo de vida

Horário de dormir:_____Horário de acordar:_____Horas dormidas:_____

Tabagismo: ( ) não fumante ( ) ex fumante

Atividade física: horas gastas com: _____ atividades assentadas (televisão, leitura); _____ atividades assentadas (computador, trabalho); _____deslocamento a pé; _____atividades diárias (banho, alimentação); _____ atividades domésticas (varrer casa, cozinhar, lavar roupa,); _____ exercícios físicos/tipo:_____; trabalho/tipo:_____

Outras atividades/tempo despedido:_____

BLOCO C: Dados bioquímicos e clínicos

Glicemia de jejum atual:_____

TG:_____ CT:_____ LDL:_____ HDL:_____ DATA:_____

Medicamentos em uso:_____

_____

História familiar de doenças/parentesco: ( ) diabetes mellitus:_____ ( )hipertensão arterial:_____ ( ) excesso de peso:_____ doenças cardiovasculares:_____

BLOCO D: Dados antropométricos e dietéticos

Medida de perímetro da cintura (cm): _____

História do Peso Corporal (kg): Anterior à disfunção hepática:_____ Atual:_____

História habitual da dieta – descreva os horários habituais de suas refeições, os alimentos e as quantidades que a compõem:

Horário/Refeição	Alimentos/Quantidades

Apêndice B - Registro Alimentar de Três Dias não Consecutivos

Nome:_____Dias escolhidos:_____

INSTRUÇÕES PARA O PREENCHIMENTO	DATA	**1° Dia**
O preenchimento deste registro alimentar e seu posterior envio pelo correio é fundamental para que sua alimentação possa ser avaliada da melhor forma possível, em caso de qualquer dúvida, ligue para 031 8898-7637 ou 031 3351-0992. Uma vez recebido, esse registro será quantificado em calorias, macro e micronutrientes e será comparado às suas necessidades nutricionais a fim de verificar se a sua alimentação habitual atende às suas exigências individuais. Para preencher o registro, anote tudo o que foi consumido (alimentos e bebidas) no dia em questão com o maior detalhamento possível: ▪ Hora e local onde foi feita a refeição ▪ Se o alimento for industrializado, a sua marca ▪ Quantidades detalhadas (ex:1 colher de sopa cheia de Nescau®; 1 copo americano na risca de leite integral; 1 escumadeira rasa de arroz; 4 folhas de alface com 5 gotas de azeite extra-virgem) Atenciosamente, Lucilene Rezende Nutricionista	Local e Hora	Alimentos / Quantidades

DATA	**2° Dia**	DATA	**3° Dia**
Local e Hora	Alimentos / Quantidades	Local e Hora	Alimentos / Quantidades

Apêndice C - Carta de devolutiva para os pacientes e pedido de exames

Prezado (a) _____,

nos anos de 2008 e 2011/2012, você participou da pesquisa sobre "Síndrome Metabólica pós-transplante hepático". A Síndrome Metabólica é uma condição na qual as pessoas que têm excesso de gordura abdominal desenvolvem pelo menos dois dos seguintes fatores: aumento da pressão arterial, aumento da glicose; dos triglicérides e redução do HDL (lipoproteína de alta densidade) no sangue.[1]

Em nossos trabalhos, avaliamos 165 pacientes do pós-transplante em 2008 e 116 em 2011/2012 e a Síndrome Metabólica esteve presente em pelo menos metade deles.[2] Na população geral adulta (não transplantada), essa Síndrome ocorre em menor número de indivíduos – aproximadamente 30% deles,[3] o que demonstra a necessidade de mais estudos desse problema na população submetida ao transplante, bem como adoção de estratégias que visem a prevenção desse distúrbio, uma vez que o mesmo é considerado fator de risco para doenças cardiovasculares[4] e para problemas no fígado.[5]

Gostaria de agradecer sua participação em nosso trabalho e me prontificar a retirar quaisquer dúvidas sobre seus resultados na pesquisa e nossas sugestões, que estão anexados a essa carta. Conseguimos recentemente financiamento da nossa pesquisa pela Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e estamos complementando nossos resultados com dosagens de alguns marcadores bioquímicos da Síndrome Metabólica no sangue (resistina, adiponectina, interleucina 1 e 6, fator de necrose tumoral, proteína C reativa, insulina e ácidos graxos livres).

Gostaria, mais uma vez, de contar com a sua colaboração na coleta de amostra do seu sangue para realização dessas dosagens na próxima vez em que você for ao laboratório do Hospital das Clínicas para fazer os exames de rotina do Ambulatório de Transplante. O procedimento de coleta será o mesmo (inclusive o jejum de 8 horas). Para isso, você deve anexar aos seus pedidos, o pedido de exame anexado a essa carta. Assim que as dosagens estiverem concluídas, também enviaremos os seus resultados a você.

Obrigada,

Lucilene Rezende Anastácio

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Resultados de _____ no estudo sobre
"Síndrome Metabólica pós-transplante hepático"

Tabela 1. Composição corporal, pressão arterial, exames bioquímicos, diagnóstico de Síndrome Metabólica do paciente submetido ao transplante avaliado nos anos de 2008 e 2011/2012

Valores	Resultados em 2008	Resultados em 2011/12	Referência
Peso			
Índice de Massa Corporal			
Perímetro da Cintura			
Percentual de gordura corporal			
Pressão arterial			
Glicemia de jejum			
Triglicérides			
Colesterol total			
LDL colesterol			
HDL colesterol			
Síndrome Metabólica			

Comentários:

Tabela 2. Ingestão de quilocalorias, macro e micronutrientes do pacientes submetido ao transplante hepático avaliado no ano de 2011/12

Nutrientes	**Ingestão Alimentar**	**% g/kg**	**Necessidades Nutricionais**	**Nutrientes**	**Ingestão Alimentar**	**Necessidades Nutricionais**
Quilocalorias				Vit.C (mg)		
Carboidratos (g)				Vit.B_1 (mg)		
Proteínas (g)				Vit.B_2 **(mg)**		
Lipídeos (g)				Niacina (mg)		
Lip.Poliinsaturados (g)				Vit.B_6 (mg)		
Lip.Monoinsaturados (g)				Vit.B_{12} (mcg)		
Lip.Saturados (g)				Folato (mcg)		
Colesterol(mg)				Ferro (mg)		
Fibras (g)				Cálcio (mg)		
Vit.A (RE)				Sódio (mg)		
Vit.D (mcg)				Fósforo (mg)		
Vit.E (mg)						

Comentários:

Apêndice D

Termo de Consentimento Livre e Esclarecido

Eu, _____,
estou sendo convidado pelas pesquisadoras Lucilene Rezende Anastácio e Hélem de Sena Ribeiro, nutricionistas, para participar do estudo **"Reavaliação do diagnóstico de síndrome metabólica decorridos três anos, em pacientes submetidos ao transplante hepático"**, para obtenção do título de Doutora pelo Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto da Faculdade de Medicina e de Mestre pelo Programa de Pós-Graduação em Ciências dos Alimentos da Faculdade de Farmácia, Universidade Federal de Minas Gerais, respectivamente.

O estudo em questão visa conhecer a prevalência de pacientes com síndrome metabólica entre aqueles submetidos ao transplante hepático que permaneceram nessa condição desde 2008. A Síndrome Metabólica é condição na qual os portadores apresentam pelo menos três dos seguintes distúrbios: obesidade abdominal (perímetro da cintura ≥ 88 cm para mulheres e ≥ 102 cm para homens), pressão arterial elevada (pressão arterial sistólica ≥ 130 mmHg e/ou pressão arterial diastólica ≥ 85 mmHg e/ou uso de medicamentos anti-hipertensivos), glicemia de jejum elevada (≥ 100 mg/dL e/ou uso de hipoglicemiantes orais), hipertrigliceridemia (triglicérides ≥ 150 mg-dL), HDL reduzido (< 50 mg/dL para mulheres e < 40 mg/dL para homens). O conhecimento do número de pacientes que continuam portadores desse distúrbio é interessante, visto que há possibilidade do problema nessa população ter caráter transitório ou não. Para tanto, deverei estar em jejum de 12 horas para realização do próximo exame bioquímico, responder algumas perguntas sobre idade, residência, ocupação, sexo, cor da pele, escolaridade, renda, estado civil, número de filhos, hábito de fumar, hábito de consumo de bebidas alcoólicas, atividade física, ingestão de medicamentos, antecedentes pessoais e familiares de doenças e me submeter à medida da perímetro da cintura, do abdome e do quadril. Preciso também me submeter ao teste de bioimpedância elétrica, para determinação da composição corporal e ao teste de calorimetria indireta, que medirá o gasto energético de repouso. Esses testes foram realizados em sala com temperatura ambiente controlada, baixa luminosidade e sem ruídos, após repouso de 20 minutos. No primeiro, quatro eletrodos (tipo esparadrapo) foram colados na mão (dois) e no pé (dois) por onde passa uma corrente elétrica que não se percebe e não causa dor ou qualquer outra sensação e tem como objetivo avaliar a quantidade de gordura, água e músculo do corpo. No segundo, utiliza-se um aparelho chamado calorímetro ao qual estará conectada uma

máscara que foi fixada no seu rosto, durante 30 minutos, para que possa respirar somente nela, tranquilamente, enquanto está deitado. Para a realização desses testes, deverei estar em jejum de 12 horas, não ter praticado exercícios físicos e ingerido bebidas alcóolicas no dia anterior, trajar roupas leves. Fui esclarecido que os dados foram sigilosos e que poderei ter acesso às informações em qualquer momento sobre os riscos e benefícios relacionados ao estudo, inclusive que poderei tirar minhas dúvidas em qualquer momento. Fui ainda garantido sobre a confidencialidade do sigilo e privacidade dos dados. Concordo e aceito que em nenhum momento solicitarei remuneração ou recompensa financeira para participar do respectivo estudo. A minha decisão de participar ou não do estudo é inteiramente voluntária e estou esclarecido também que a decisão não afetará o meu tratamento. Além disso, poderei retirar-me do estudo a qualquer momento, para isso deverei entrar em contato com o pesquisador. Fui esclarecido que o resultado da avaliação nutricional estará à minha disposição, mediante o contato com as pesquisadoras, na próxima consulta no ambulatório Bias Fortes, desde que tenha enviado o registro alimentar de 72 horas. Estou ciente que os dados foram exclusivamente para estudo com posterior publicação dos resultados obtidos. Após respondidas todas as minhas dúvidas, assino o presente documento em duas vias.

Belo Horizonte, ____ de _____ de 20__.

Assinatura do paciente:_____

Assinatura do pesquisador:_____

Assinatura da testemunha:_____

Assinatura da testemunha: _____

Endereço do Comitê de Ética em Pesquisa da UFMG: Av. Antônio Carlos, 6627, Unidade Administrativa II - 2º andar, Campus Pampulha, Belo Horizonte, MG , CEP: 31270-901. Telefone:0XX 31 3409-4592

Doutoranda: Lucilene Rezende Anastácio (31) 8898 7637 email: lucilene.rezende@gmail.com

Orientador: Prof. Dr. Eduardo Garcia Vilela

Co-Orientadora: Profa. Dra. Maria Isabel T.D. Correia.

Mestranda: Hélem de Sena Ribeiro Telefone: (31) 8674 5337 email: helemsena@yahoo.com.br

Orientador: Profa. Dra. Maria Isabel T.D. Correia.

Anexo 2 – Aprovações do Comitê de Ética

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 44/08

Interessado(a): Profa. Maria Isabel Toulson Davisson Correia
Departamento de Cirurgia
Faculdade de Medicina - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 3 de abril de 2008, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Síndrome metabólica em pacientes submetidos a transplante hepático: prevalência e causas associadas"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
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UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 44/08

Interessado(a): Profa. Maria Isabel Toulson Davisson Correia
Departamento de Cirurgia
Faculdade de Medicina - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP analisou e aprovou, no dia 18 de outubro de 2011, a extensão do projeto de pesquisa intitulado **"Síndrome metabólica em pacientes submetidos a transplante hepático: prevalência e causas associadas".**

A aprovação é válida por um ano (14 de outubro de 2011 a 13 de outubro de 2012).

O relatório final ou parcial deverá ser encaminhado ao COEP um anos após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

Anexo 2 – Carta de aceite e prova do Artigo 1 / Revista *Liver International*

Decision Letter (LIVint-13-01047.R1)

From: editorliverinternational@gmail.com
To: isabel_correia@uol.com.br
CC: liv@oxon.blackwellpublishing.com
Subject: Liver International - Manuscript LIVint-13-01047.R1
Body: 04-Feb-2014

Re:Correiaet al "Prospective evaluation of metabolic syndrome and its components among long-term liver recipients "

Dear Dr. Correia,

We are pleased to inform you that your manuscript has been accepted for publication in Liver International.

Any additional corrections will be sent with the proof, which you will receive in due course. We appreciate your contribution to the journal and look forward to receiving future submissions from you.

Please note that articles exceeding 9 journal pages are charged 100 GBP per excess page.

Yours Sincerely

Prof. Rajiv Jalan
Editor-in-chief, Liver International

Date Sent: 04-Feb-2014