FERNANDA ESTEVES NASCIMENTO BARROS

INFLUÊNCIA DAS ADIPOCINAS E DA FERRITINA NA RESISTÊNCIA À INSULINA EM PACIENTES INFECTADOS PELO VÍRUS DA HEPATITE C

Londrina
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Londrina, como requisito parcial à obtenção de título de Mestre em Ciências da Saúde.

Orientador: Prof. Dr.Isaias Dichi.
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Londrina
2014

Catalogação elaborada pela Divisão de Processos Técnicos da Biblioteca Central da Universidade Estadual de Londrina

Dados Internacionais de Catalogação-na-Publicação (CIP)

B277i Barros, Fernanda Esteves Nascimento.
Influência das adipocinas e da ferritina na resistência à insulina em pacientes infectados pelo vírus da hepatite C / Fernanda Esteves Nascimento Barros. – Londrina, 2014.
69 f. : il.

Orientador: Isaias Dichi.
Coorientador: Andréa Name Colado Simão.
Dissertação (Mestrado em Ciências da Saúde) – Universidade Estadual de Londrina, Centro de Ciências da Saúde, Programa de Pós-Graduação em Ciências da Saúde, 2014.
Inclui bibliografia.

1. Hepatite C – Teses. 2. Resistência à insulina – Teses. 3. Tecido adiposo – Teses. 4. Ferro no organismo – Teses. I. Dichi, Isaias. II. Simão, Andréa Name Colado. III. Universidade Estadual de Londrina. Centro de Ciências da Saúde. Programa de Pós-Graduação em Ciências da Saúde. IV. Título.

CDU 616.36-002

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Londrina, 29 de abril de 2014.

Dedico este trabalho a Deus e à minha familia, pilares indispensáveis ao meu viver.
Ao meu esposo Fernando, pelo amor e paciência infinitos.

AGRADECIMENTOS

Por todo o trabalho e paciência dedicados à realização desta dissertação, agradeço especialmente ao meu orientador, professor Isaías Dichi.

À professora Andréa Name Colado Simão, por todo empenho e dedicação, e principalmente por sempre acreditar na concretização deste estudo.

Aos doutores Jan Walter Stegmann e Edson T. Anzai, por permitir que acompanhasse seus pacientes no ambulatório de hepatites virais.

Ao enfermeiro Edvilson Cristiano Levine, do Centro de Testagem e Aconselhamento do município, pelo fornecimento das fichas de notificação do munícipio para busca ativa de pacientes.

A todos os alunos do Mestrado e Doutorado do setor de Imunologia do Hospital Universitário, pelo apoio para a realização dos exames laboratoriais.

À banca examinadora do processo de qualificação, profa. Edna Maria Vissoci Reiche e prof. Vinicius Daher Alvares Delfino, pelas valiosas correções.

Aos amigos infectologistas Zuleica Naomi Tanno e Flávio Jun Kazuma, pela imensa colaboração e sugestões.

“Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quer passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu.”

Fernando Pessoa.

BARROS, Fernanda Esteves Nascimento Barros. **Influência das adipocinas e da ferritina na resistência à insulina em pacientes infectados pelo vírus da hepatite C**. 2014. Dissertação (Mestrado em Ciências da Saúde) - Universidade Estadual de Londrina, Londrina, 2014.

RESUMO

A infecção crônica pelo vírus da hepatite C (HCV) é uma das causas mais prevalentes de doença hepática crônica, levando à cirrose e ao carcinoma hepatocelular em grande número de pacientes. A resistência insulínica (RI) é uma das características da infecção crônica pelo HCV, relacionada principalmente aos genótipos 1,3 e 4, e a altos níveis de carga viral sérica. No entanto, o mecanismo específico pelo qual o vírus leva à RI não é totalmente conhecido. Estudos indicam que esteatose hepática, presença de citocinas inflamatórias, adiponectina e sobrecarga de ferro estejam envolvidos no processo de desenvolvimento de RI nestes pacientes. Essas alterações metabólicas também contribuem para diminuir a resposta virológica sustentada ao tratamento e estão associadas à progressão da fibrose hepática. O objetivo do presente estudo é analisar a influência das adipocinas e da ferritina na RI e na fibrose hepática dos pacientes com HCV. Foi realizado um estudo caso controle, com 130 indivíduos: 80 controles livres da doença e 50 pacientes infectados com HCV (23 com RI e 27 sem RI). Os grupos foram pareados por idade, sexo, IMC, etnia e presença de tabagismo. Foi realizado entrevista e coleta dos seguintes exames: colesterol total e frações, glicose, aspartato aminotransferase, alanina aminotransferase, gama glutamiltransferase, insulina, ferritina, TNFA, IL-6, adiponectina, leptina. Foram calculados o HOMA-IR para determinação de resistência insulínica e teste APRI para determinação de fibrose hepática. 86% dos pacientes estavam infectados com genótipo 1 e 24% com genótipo 3. O grupo de pacientes com HCV apresentou menores níveis de colesterol total e LDL-colesterol comparado com o grupo controle ($p<0,0001$), e níveis de triglicerídeos do grupo HCV sem RI também foram menores ($p<0,05$) comparados ao grupo controle. Em relação ao metabolismo da glicose, os níveis de glicemia de jejum foram significativamente maiores nos pacientes HCV com RI quando comparados ao grupo controle ($p<0,0001$), e níveis de insulina e índice HOMA-IR foram mais elevados ($p<0,0001$) em pacientes com HCV com RI quando comparados ao grupo controle e ao grupo HCV sem RI. Os grupos com HCV apresentaram níveis mais elevados de TNFA e IL-6 quando comparados ao grupo controle ($p<0,001$ e $p<0,0001$ respectivamente), e o grupo com RI também diferiu do grupo sem RI, com níveis mais elevados no primeiro ($p<0,0001$ e $p<0,005$, respectivamente). Houve diminuição dos níveis de adiponectina nos grupos com HCV em relação ao grupo controle ($p<0,01$), enquanto pacientes com HCV com RI apresentaram níveis mais altos de leptina ($p<0,05$) quando comparados ao grupo HCV sem RI. Pacientes com HCV apresentaram diferença significativa nos níveis de ferro sérico e ferritina quando comparados ao grupo controle ($p<0,05$ e $p<0,0001$, respectivamente). Os níveis de ferritina foram mais elevados em pacientes com HCV com RI quando comparados ao grupo sem RI ($p<0,01$). O teste de correlação de Spearman demonstrou que o HOMA-IR estava positivamente correlacionado com os níveis de AST ($r=0,302$; $p<0,05$), IL-6 ($r=0,368$; $p<0,05$), leptina ($r=0,356$; $p<0,05$) e teste APRI ($r=0,457$; $p<0,01$). Os níveis de adiponectina estavam diretamente correlacionados com IL-6 ($r=0,495$; $p<0,05$) e teste APRI ($r=0,405$) e inversamente correlacionados com a carga viral ($r=-0,621$; $p<0,05$), enquanto leptina esteve

positivamente correlacionada com AST (r=0,507; p<0,05), ALT (r=0,357; p<0,05) e TNFA (r=0,406; p<0,05). A análise multivariada mostrou que o teste APRI foi o único parâmetro independente associado à RI (p<0,05) em pacientes com HCV. Os dados do presente estudo sugerem uma complexa relação entre adipocinas, ferritina, RI e progressão de fibrose hepática em pacientes com HCV. Os níveis baixos de adiponectina parecem estar mais relacionados à esteatose hepática e ao aumento de ferritina do que à resistência insulínica nos pacientes com HCV. O conhecimento do complexo mecanismo envolvido na progressão da doença hepática proporcionará melhora no tratamento do doente com hepatite C, associando drogas hipoglicemiantes aos tratamentos convencionais disponíveis.

Palavras-chave: Hepatite C. Adipocinas. Resistência à insulina. Ferritina.

BARROS, Fernanda Esteves Nascimento Barros. **Adipokines, Ferritin and Insulin Resistance in CHC**. 2014. Dissertation (Master in Health Sciences) - University of Londrina, Londrina. 2014.

ABSTRACT

Hepatitis C virus (HCV) is one of the most prevalent causes of chronic liver disease worldwide and leads to cirrhosis and hepatocellular carcinoma (HCC) in a high percentage of carriers. Insulin resistance (IR) is a specific feature of chronic HCV, associated with genotype 1, 3 and 4 and high serum HCV RNA level. However, the specific mechanism by which HCV leads to IR are still not fully understood. It has been postulated that steatosis, proinflammatory cytokines, adiponectin, and iron overload could play a crucial role in glucose abnormalities associated with HCV infection. In addition, metabolic derangements contribute to a decrease in sustained virological response and are associated with progression of liver fibrosis. The aim of the present study was to analyze the influence of adipokines and iron status parameters on IR and fibrosis index in patients with CHC. The study included 130 subjects: 80 healthy individuals selected and 50 HCV infected patients (23 with IR and 27 without IR). This study included 130 subjects: 80 healthy individuals selected from among blood donors of the University Hospital and 50 HCV infected patients (23 with IR and 27 without IR) from the outpatient clinic of Infectology of Londrina University Hospital. The groups were paired by age, gender, BMI, ethnicity and tabaccoism. The following blood parameters were evaluated after fasting for 12 hours: Aspartate aminotransferase (AST), alanine aminotrasferase (ALT), gamma glutamyltransferase (GGT), fasting glucose, total cholesterol, HDL- cholesterol, LDL-cholesterol, triacylglycerol, iron, ferritin, insulin. The homeostasis model assessment (HOMA) was used as a surrogate measurement of insulin sensitivity. TNFA, IL-6, adiponectin, and leptin were also assessed as well as aminotransferase-to-platelet index (APRI) to evaluate hepatic fibrosis. Eighty-six percent of patients were infected with HCV genotype 1 while 24% were infected with HCV genotype 3. Both HCV groups presented lower ($p<0.0001$) serum total cholesterol and LDL-cholesterol levels when compared to the control group, whereas triacylglycerol levels were lower in HCV patients without IR compared to the control group ($p<0.05$). With regard to glucose metabolism, plasma glucose levels were significantly higher in HCV with IR group when compared to the control group ($p<0.0001$), whereas insulin levels and HOMA-IR were significantly higher ($p<0.0001$) in HCV patients with IR compared to both HCV without IR and control groups. Both HCV groups presented significantly higher TNFA and IL-6 levels compared to the control group ($p<0.001$ and $p<0.0001$, respectively), whereas HCV patients had significantly higher TNFA and IL-6 levels when compared to HCV patients without IR ($p<0.0001$ and $p<0.005$, respectively). There was a decrease in adiponectin levels in both HCV groups in relation to the control group ($p<0.01$), whereas HCV patients with IR showed significantly higher ($p<0.05$) leptin levels compared to HCV patients without IR. HCV groups had significantly higher iron and ferritin levels compared to the control group ($p<0.05$ and $p<0.0001$, respectively). Additionally, ferritin levels were significantly higher in HCV patients with IR compared to HCV patients without IR ($p<0.01$). Spearman`s correlation verified that HOMA-IR was positively correlated with AST ($r=0.302$; $p<0.05$), IL-6 ($r=0.368$; $p<0.05$), leptin ($r=0.356$; $p<0.05$) and APRI ($r=0.457$; $p< 0.01$). Adiponectin levels were directly correlated with IL-6 ($r=0.495$, $p<0.05$) and

APRI ($r=0.405$) and inversely correlated with viral load ($r=-0.621$; $p<0.05$), whereas leptin was positively correlated with AST ($r=0.507$; $p<0.05$), ALT ($p=0.357$; $p<0.05$) and TNFA ($r=0.406$, $p<0.05$). Multivariate analysis showed that APRI index was the unique parameter independently associated to IR ($p<0.05$) in patients with HCV. In conclusion, data from the present study suggest a complex relationship between adipokines, ferritin, IR and progression of liver injury in patients with HCV. It is likely that hypoadiponectinemia is more related to liver steatosis and to increased ferritin levels than to IR in HCV. The understanding of the complex mechanism involved in the context of the liver disease progression will provide a more accurate picture of the phenomena and and development also more effective drugs.

Key words: Hepatitis C. Adipokines. Insulin resistance. Iron status.

LISTA DE ABREVIATURAS E SIGLAS

ALT	Alanina aminotransferase
APRI	*AST to platelet index ratio* (escore da razão entre AST e plaquetas)
AST	Aspartato aminotransferase
CHC	Carcinoma hepatocelular
GGT	Gama glutamil transferase
HAV	Vírus da hepatite A
HBV	Vírus da hepatite B
HCV	Vírus da hepatite C
HIV	Vírus da imunodeficiência humana
HOMA	*Homeostasis Model Assessment* (modelo de avaliação da homeostase)
IFNA	Interferon alfa
IFNG	Interferon gama
IL-6	Interleucina 6
IL-10	Interleucina 10
IMC	Índice de massa corpórea
NANB	Hepatite não-A não-B
OMS	Organização Mundial de Saúde
PAD	Pressão arterial diastólica
PAS	Pressão arterial sistólica
RI	Resistência insulínica
SM	Síndrome metabólica
TNFA	Fator de necrose tumoral alfa

SUMÁRIO

1 INTRODUÇÃO

Nas décadas de 1970 e 1980, desconheciam-se as causas etiológicas dos casos de hepatite pós-transfusão sanguínea. Na primeira metade da década de 1970, testes de triagem sorológicos direcionados à investigação dos vírus das hepatites A (HAV) e B (HBV) revelaram que 25% dos casos de hepatite associados às transfusões sanguíneas estavam relacionadas com o HBV. Os casos remanescentes, 75%, foram considerados como hepatites não-A e não-B (NANB). Aproximadamente 10 a 12% dos indivíduos que haviam recebido múltiplas transfusões desenvolveram hepatite NANB, com risco relativo de 0,45% por unidade transfundida (ALTER, 1982,1994; BRADLEY, 1990). Em 1989, pesquisadores da *Chiron Corporation* conseguiram sequenciar o genoma do vírus, denominando-o de vírus da hepatite C. Atualmente, está classificado como pertencente à família *Flaviviridae* e gênero *Hepacivirus*.

Desde sua descoberta, a hepatite por vírus C (HCV) passou a ganhar especial relevância entre as causas de doença hepática crônica no mundo (BRASIL, 2011; ROSEN, 2011). Estima-se que, aproximadamente, 3% da população mundial estejam infectadas pelo HCV, o que representa cerca de 170 milhões de indivíduos com infecção crônica e sob risco de desenvolver as complicações da doença. De acordo com a Organização Mundial da Saúde (WHO, 2011), o Brasil é considerado um país de endemicidade intermediária para a hepatite C, com prevalência de infecção em torno de 5%. Entretanto, estudos de base populacional e com doadores de sangue revelam prevalências inferiores às estimadas, colocando o Brasil como de baixa endemicidade (PALTANIN; REICHE, 2002; LAMPE et al, 2013; PEREIRA et al, 2013).

O HCV possui sete genótipos principais e numerosos subtipos (SCHEEL, RICE, 2013). O genótipo 1 é o mais prevalente, presente em 40 a 80% da população mundial infectada. A prevalência dos vários genótipos apresenta distribuição geográfica bastante variável, com genótipo 1 dominando nas Américas (70% dos casos), Japão (75%) e Europa (50-70%); genótipos 2 e 3 também são prevalentes nessas regiões. Genótipos 3 e 6 são frequentes no sul e sudeste da Ásia, e genótipos 4 e 5 na África, mas também na Europa. Genótipo 7 foi recentemente descrito na África Central (SCHEEL, RICE, 2013). No Brasil, há poucos estudos de soroprevalência de genótipos. De modo geral, predomina o genótipo 1 em 50 a 60% dos casos; o tipo 2 em 3 a 5%; e o tipo 3 em cerca de 35% (mais

frequente na região Sul). Os genótipos 4 e 5 são raros (NAGAYAMA et al, 2000; NEUMANN et al, 2000, BRASIL, 2011; LAMPE et al, 2013).

A resposta imune do hospedeiro determinará se o vírus será ou não erradicado do organismo. Estima-se que 15 a 30% dos pacientes que desenvolvem infecção crônica progredirão para cirrose em três décadas (THEIN et al, 2008).

Vários fatores, principalmente carga viral e genótipo, estão associados com risco aumentado de evolução para fibrose. Pacientes com cirrose devido ao HCV estão sujeitos a diversas complicações, incluindo carcinoma hepatocelular (CHC). (FATTOVICH et al, 1997; POYNARD; BEDOSSA; OPOLON, 1997; AFDHAL, 2008). O grau de fibrose está diretamente relacionado ao desfecho clínico do paciente (progressão para transplante hepático ou óbito) (DAVIS et al, 2010; ROSEN, 2011).

Cirrose por hepatite C crônica é responsável por cerca de 10.000 mortes a cada ano, e é a principal causa de indicação para transplante hepático no mundo ocidental, e principal fator de risco para CHC (McHUTCHINSON, 2004).

Há uma preocupação crescente com o impacto do aumento do índice de massa corpórea (IMC) no curso da infecção crônica pelo HCV. A interação entre a resposta imune e o sistema metabólico pode afetar a evolução da infecção crônica. Enquanto o papel dos adipócitos nas vias metabólicas é bastante conhecido, pouco se sabe sobre sua atuação na inflamação. Adipócitos e diversas células do sistema imune, como macrófagos e linfócitos T, desempenham funções semelhantes na ativação do complemento e na produção de citocinas (WELLEN; HOTAMISLIGIL, 2003; MATARESE; LA CAVA, 2004). O tecido adiposo produz hormônios e citocinas que influenciam a produção e armazenamento de energia pelo organismo e a resposta imune às agressões (MATARESE; LA CAVA, 2004).

Pacientes obesos desenvolvem hepatite por HCV mais agressiva e respondem menos à terapia antiviral, pois a ocorrência de sobrepeso, resistência insulínica e inflamação crônica pelo HCV representam um cenário de risco e de evolução desfavorável para o fígado (ROMERO-GOMEZ et al, 2006). A resposta imune anti-HCV ineficaz em pacientes obesos com hepatite C crônica está inversamente relacionado ao IMC e aos níveis séricos de adiponectina. Estes pacientes têm maiores níveis de alanina aminotransferase (ALT) e necroinflamação hepática, além de maior risco de evoluir para CHC. (PATTON et al, 2004; ZEKRY; MC HUTCHINSON; DIEHL, 2005; LEANDRO et al 2006; PALMER et al, 2008).

O risco cardiovascular está elevado em pacientes com hepatite C, mesmo em pacientes não obesos e não diabéticos, com escore de Framingham mais elevado do que no grupo sem infecção pelo HCV (KAKINAMI et al, 2013). A infecção por HCV é reconhecida como causa de estimulação imune crônica, levando a resposta inflamatória e produção de citocinas. Dois padrões distintos podem ocorrer: resposta tipo 1 é caracterizada por produção de IL-2 (interleucina 2), TNFA (fator de necrose tumoral alfa) e IFNG (interferon gama), responsáveis pela imunidade celular; resposta tipo 2 é definida pela produção de IL-4 (interleucina 4), IL-5 (interleucina 5), IL-6 (interleucina 6) e IL-10 (interleucina 10), promovendo resposta imune humoral. Essas alterações podem, potencialmente, levar a eventos cardiovasculares adversos, devido ao aumento de moléculas de adesão intracelular, expressão de anticorpos anti-endotélio, além de gerar estresse oxidativo, resistência insulínica e vasculite sistêmica (OLIVEIRA et al, 2013).

A Síndrome Metabólica (SM) é considerada um conjunto de fatores de risco cardiovascular caracterizada por adiposidade abdominal, resistência à insulina, dislipidemia e hipertensão arterial, gerando um estado pró-inflamatório sistêmico (REAVEN, 1988; MOLLER; FLIER, 1991). O vírus da hepatite C está associado com o desenvolvimento de resistência à insulina (RI) e, eventualmente, tipo 2, o que tem levado os pesquisadores a considerar a hepatite C crônica como uma doença metabólica. O aparecimento de diabetes ou intolerância à glicose nos pacientes com doença hepática crônica envolve alterações como hiperinsulinemia, RI e redução da captação de glicose (ABENAVOLI; ALMASO, 2011, GUTIERREZ-GROBE et al, 2011).

Resistência insulínica, síndrome metabólica e eventos ateroscleróticos têm em comum a mesma base inflamatória. A presença de inflamação sistêmica é o principal mecanismo que leva à ação deficiente da insulina (SINGH; SAXENA, 2010). Além disso, evidências sugerem que proteínas do core do vírus lesam diretamente o tecido pancreático, induzindo disfunção de células β e alterando resposta insulínica à hiperglicemia. A presença da resistência insulínica *per se* prediz progressão mais rápida da fibrose, cirrose, insuficiência hepática e CHC, além de pior resposta à terapia antiviral contra o HCV (HSU et al, 2010; PETTA et al, 2008). Estudos indicam que vários distúrbios metabólicos, tais como a obesidade, RI, e esteatose hepática são importantes fatores de risco para a diminuição à resposta ao tratamento com interferon peguilado e a ribavirina (KHATTAB et al, 2010; ROMERO-GOMEZ et al, 2005). A RI e a o processo inflamatório gerado pela infecção pelo HCV contribuem não somente para o desenvolvimento de SM,

mas também para os outros distúrbios metabólicos verificados no decorrer da doença crônica (ESLAM et al, 2011; PETTA et al, 2011; CAMMA et al, 2009).

Os valores de HOMA IR >2 são preditores significativos de pior resposta do paciente ao tratamento. O mecanismo molecular que explica a associação entre resistência insulínica e baixa resposta ao tratamento ainda é desconhecido, mas estudos sugerem alteração na via da sinalização de citocinas 3 (SOCS-3). SOCS-3 é conhecida como inibidora da sinalização da insulina, causando RI, e parece estar aumentada em pacientes com hepatite C não respondedores ao tratamento. Inibe também a via interferon-induzida da Janus-kinase (JAK-STAT), suprimindo a expressão de proteínas antivirais (VANNI et al, 2009; KAWAGUCHI et al, 2004).

Na hepatite C crônica pelos genótipos 1 e 4, a resistência insulínica é associada à esteatose hepática e pode ser vírus-mediada ou relacionada a fatores metabólicos, como obesidade central (LO IACONO, 2007). Já nos pacientes com a infecção pelo genótipo 3, a esteatose hepática ocorre pelo efeito direto do vírus, mesmo na ausência de fatores de risco metabólicos (SANYAL, 2011).

Hiperinsulinemia e hiperglicemia estimulam diretamente as células hepáticas, levando à ativação do fator de crescimento de tecido conectivo e acúmulo de matriz extracelular. Leptina e TNFA podem ser as moléculas responsáveis por essas alterações, já que pacientes com RI têm níveis elevados de leptina e de TNFA, que estimulam as células hepáticas e levam à fibrogênese (PARADIS et al, 2001; DING et al, 2005, OTTE et al, 2004).

A incidência de CHC tem aumentado, e a RI é importante fator de risco, mesmo após ajuste de variáveis como gênero e sexo, independentemente de diabetes manifesto. A insulina tem efeito mitogênico e proliferativo, e está envolvida na progressão e recorrência do CHC. HOMA IR >2,3 é fator de risco independente para recorrência de CHC após ablação curativa por radiofrequência em pacientes com hepatite C, e HOMA IR >3,0 é fator prognóstico independente em pacientes homens com CHC (SUMIE et al, 2007; HUNG et al, 2011, IMAI et al, 2011).

Estudos epidemiológicos têm demonstrado altas taxas de incidência e prevalência de RI e diabetes tipo 2 em pacientes com hepatite C, se comparado a outras doenças hepáticas. Observa-se ainda que o *clearance* viral após tratamento adequado reduz a taxa de alterações glicêmicas no paciente. As consequências da RI no paciente com infecção viral crônica são traduzidas por progressão da fibrose e diminuição da

resposta aos antivirais, assim como aumento do risco de evolução para CHC. Um estudo prévio observou uma prevalência de 24,7% de SM em indivíduos residentes em Taiwan portadores do HCV contra apenas 13,2% de indivíduos controles sem infecção (p=0.02). Tem sido demonstrado também que a RI é mais frequente em pacientes infectados com HCV com os genótipos 1 e 4 (SERSTÉ et al, 2010; HUANG, 2011).

O estudo transversal de Mehta et col (2000), baseado no *Third National Health and Nutrition Examination Survey* (NHANES III), mostrou que pessoas acima de 40 anos, HCV positivas, têm uma prevalência três vezes maior de diabetes 2 em relação às pessoas HCV negativas, mesmo após ajuste de idade, etnia, IMC e nível socioeconômico. A prevalência de diabetes tipo 1 entre pessoas com infecção pelo HCV não se mostrou aumentada neste estudo. No Brasil, a relação entre hepatite C e diabetes 2 foi estudada por Cheinquer et col (1998), que demonstrou prevalência de 12% de diabetes em pacientes HCV e que, destes, 93% apresentavam cirrose hepática. Apenas 47% dos pacientes com HCV sem DM tinham cirrose. É interessante notar que o maior risco de desenvolver diabetes e resistência insulínica é uma característica inerente ao vírus C, uma vez que não foi relatada prevalência aumentada em pacientes infectados pelo vírus da hepatite B (MASON et al, 1999; MEHTA et al, 2000; RYU et al, 2001; OKAN et al, 2002).

Hepatite C crônica pode evoluir lentamente para fibrose hepática, e subsequente desenvolvimento de cirrose e insuficiência hepática ou carcinoma hepatocelular, mas a progressão pode ser rápida dependendo de fatores virais e do indivíduo. Alguns estudos sugerem prognóstico benigno para a infecção pelo HCV, com cerca de 2 a 3% dos indivíduos desenvolvendo cirrose nos 20 anos seguintes. Outros reportam prognóstico pior, com até 51% dos pacientes desenvolvendo cirrose em 22 anos (THEIN, 2008). A maioria dos pacientes têm doença hepática moderada a severa em biópsias (RYDER, 2004).

O padrão ouro para o diagnóstico de fibrose hepática ainda é considerado a biópsia hepática. A avaliação histológica fornece informações sobre a atividade necroinflamatória, além de esteatose e sobrecarga de ferro. O sistema mais utilizado para graduar a atividade inflamatória e o grau de fibrose é o METAVIR (SCHEUER, 1991; DESMET et al, 1994; BEDOSSA; POYNARD, 1996). No entanto, a biópsia hepática é uma técnica invasiva, com morbidade associada. Complicações menores são relativamente comuns, com cerca de um quarto dos pacientes evoluindo com dor no hipocôndrio direito após biópsia. Complicações severas são infrequentes, com sangramento significativo

variando de 0,05 a 5,3%, dependendo do serviço, com mortalidade abaixo de 0,15% (ROCHEY et al, 2009).

Apesar de ser considerado padrão ouro, os resultados de biópsia hepática têm sido questionados devido à possibilidade de variabilidade significativa entre a gradação de fibrose hepática entre diferentes observadores, além de que o espécime enviado para análise representa apenas cerca de 1/50000 do fígado, quando a amostra é adequada (SCHEUER, 2003; BEDOSSA; DARGERE; PARADIS, 2003).

Vários marcadores de fibrose hepática não invasivos têm sido propostos, sendo divididos em biomarcadores e técnicas de imagem. O APRI teste (*AST-to-platelet ratio index* – escore da razão entre AST e plaquetas) é calculado pela fórmula (AST/limite superior do valor de referência)/plaquetas (10^9/L) x 100. Foi proposto originalmente por Wai et al em 2003, e se tornou o marcador não invasivo de fibrose hepática mais estudado. O racional do teste APRI baseia-se no racional de que a piora da fibrose e o aumento da pressão portal estão associados com a redução de trombopoetina pelos hepatócitos, aumento do sequestro esplênico de plaquetas e redução do *clearance* de AST (SCHIAVON; NARCISO-SCHIAVON; CARVALHO-FILHO, 2014).

O teste APRI está validado para diferentes populações, inclusive coinfectados HIV/HCV e em pacientes dialíticos (SINGAL et al, 2011; CARVALHO-FILHO et al, 2008; SCHIAVON et al, 2007). Para pacientes com fibrose (METAVIR F≥2), apresenta sensibilidade média de 81%, especificidade média de 95%, sendo teste de fácil uso. Em pacientes com cirrose (METAVIR F≥4), sensibilidade média de 77% e especificidade de 94% (CHOU; WASSON, 2013).

O objetivo principal no tratamento dos pacientes com hepatite C crônica é atingir a resposta virológica sustentada, sendo o padrão ouro o tratamento com interferon peguilado associado à ribavirina, mas ainda com taxas de sucesso baixas, apesar da introdução dos novos inibidores de protease (ABENAVOLI; ALMASIO, 2011).

2 ADIPOCINAS, RESISTÊNCIA À INSULINA E HCV

O tecido adiposo libera diversas citocinas pró e anti-inflamatórias, incluindo as adipocinas adiponectina e leptina. Adiponectina, uma proteína plasmática de 30kDa, é a adipocina mais abundante. Ela funciona como um sensibilizador endógeno de insulina; tem efeito ainda sobre a diminuição dos depósitos de lipídios e ativação da via anti-inflamatória. O fígado é o principal órgão responsável por mediar os efeitos metabólicos gerados pela adiponectina através de ativação de receptores específicos. Nos hepatócitos, a adiponectina regula duas vias metabólicas, a via anti-inflamatória de proliferação de peroxissomos ativada pelo receptor alfa e a oxidação de ácidos graxos, que costumam estar reduzidas nos pacientes com hepatite C crônica (CORBETTA et al, 2011).

Os níveis de adiponectina estão diminuídos em pacientes com IMC elevado, resistência insulínica e diabetes (LEANDRO et al, 2006). É um importante regulador de resposta de citocinas, aumentando a liberação de IL-6. Os efeitos metabólicos e inflamatórios da adiponectina são mediados parcialmente por vias de sinalização MAPK (*mitogen-activated protein kinase)*, que desempenham papel fundamental na regulação de interferon α (IFNA) e interferon γ (IFNG) na resposta à infecção viral (GOH; HAQUE; WILLIAMS, 1999; LI et al, 2004).

A atividade da adiponectina diminui a produção hepática de glicose e aumenta a entrada de glicose e oxidação de ácidos graxos no músculo esquelético; os seus níveis diminuem na obesidade e estão inversamente relacionados ao estado de resistência insulínica e aos níveis de proteína C reativa ultrassensível. Adiponectina tem correlação negativa com HOMA em indivíduos com síndrome metabólica (HIGASHIURA et al, 2004; SING; SAXENA, 2010). Há evidências crescentes de que a interação entre o sistema imune e metabólico afeta potencialmente o curso da infecção crônica pelo vírus C (PALMER et al, 2008).

Leptina, adipocina também produzida pelo tecido adiposo, desempenha importante papel na regulação e no metabolismo da gordura total do organismo e induz resistência insulínica, aumento de ácidos graxos livres no fígado e aumento da peroxidação de lipídios (ADINOLFI et al, 2001; GIANINNI et al, 2001; UYGUN et al, 2000). Desempenha também um papel imunomodulador na imunidade inata e adquirida, atuando como proteína de fase aguda durante a inflamação, e promovendo esteatose hepática e

fibrose (GIANINNI et al, 2001). Seus níveis encontram-se elevados em paciente com hepatite C crônica.

Palmer e col (2008), em estudo de coorte realizado em Sidney, na Austrália, com trinta e cinco pacientes com infecção crônica pelo HCV, avaliaram os níveis séricos de adiponectina e leptina. Os níveis totais de adiponectina estiveram relacionados à resposta imune anti-HCV - nível total de adiponectina de 6 (±2)µg/mL naqueles com resposta imune específica de células T anti-HCV *versus* 4.2 (±2)µg/mL em pacientes sem resposta (p=0.01). Os pacientes que apresentavam resposta imune celular anti-HCV positiva também tiveram menor índice de massa corpórea comparados com aqueles sem resposta imune efetiva [23 (±2,5) kg/m^2 *versus* 27 (±4) kg/m^2 – p=0,004]. O IMC se correlacionou negativamente com os níveis séricos totais de adiponectina (r= -0,5, p=0,004), e foi o preditor de resposta imune mais importante (p=0,02, *odds ratio* 1,54, 95% IC 1.1-2.2), seguido dos níveis de adiponectina total (p=0,05, *odds ratio* 2,4, 95% IC 0.95-3.8).

3 FERRITINA, RESISTÊNCIA À INSULINA E HCV

O fígado é o principal órgão de depósito de ferro, e cerca de um terço de todo o ferro corporal está depositado nos hepatócitos, no trato portal, nas células do mesênquima sinusoidal e nas células retículo-endoteliais. Desempenha também importante papel no metabolismo do ferro, pois ferritina e transferrina são produzidas no fígado. Diversos estudos demonstram aumento nos marcadores de metabolismo do ferro (ferritina, ferro, saturação de transferrina) e acúmulo de ferro em pacientes com hepatite C crônica, levando à piora do quadro inflamatório hepático. (MITSUYOSHI et al, 2013; VAGU; SULTANA; RUTA, 2013).

A ferritina é a principal proteína de depósito de ferro intracelular. Tem-se sugerido que, quando os marcadores do metabolismo do ferro estão elevados, a incidência de síndrome metabólica aumenta. A ferritina aumentada está associada com hiperinsulinemia e hipertrigliceridemia. Depósito de ferro em vários tecidos afeta a sensibilidade e a função da insulina, levando à resistência insulínica e à inflamação (VARI et al, 2007; JEHN; CLARK; GUALLAR, 2004; FUMERON et al, 2006).

Altos níveis de ferritina e transferrina estão associados com síndrome metabólica, hiperinsulinemia e HOMA IR elevado (VARI et al, 2007). A ferritina sérica foi proposta também como fator de risco cardiovascular. Sua concentração tem correlação positiva com glicose sérica, triglicerídeos e apolipoproteína B e correlação inversa com HDL colesterol. Correlaciona-se também com ácido úrico, um marcador de resistência à insulina.

Depósitos elevados de ferro podem aumentar a oxidação de lipídios, especialmente de ácidos graxos livres, pela produção de radicais livres, agindo o ferro como potente catalisador. No fígado, podem aumentar a resistência insulínica pela interferência na habilidade da insulina de suprimir a produção hepática de glicose (REAVEN, 1988; VUORINEN-MARKKOLA; YKI-JARVINEN, 1994).

Considerando que a hepatite C é uma doença de prevalência elevada, com resultados ainda desapontadores mesmo após tratamento adequado, esperamos, com o presente estudo, elucidar alguns pontos no metabolismo das adipocinas e da resistência insulínica nestes pacientes, para que o tratamento seja mais individualizado e abrangente do que o disponível atualmente, com taxas de resposta virológica sustentada mais elevadas.

4 OBJETIVOS

4.1 OBJETIVOS GERAIS

- ✓ Avaliar a influência das adipocinas e da ferritina na resistência insulínica em pacientes com HCV.
- ✓ Verificar se a presença de resistência insulínica está associada ao grau de fibrose hepática em pacientes com HCV.

4.2 OBJETIVOS ESPECÍFICOS

- ✓ Avaliar o perfil metabólico e a presença de resistência insulínica em pacientes com HCV atendidos em Londrina;
- ✓ Determinar os níveis de adiponectina, leptina, TNFA e IL-6 em pacientes com HCV atendidos em Londrina;
- ✓ Determinar os níveis séricos de ferritina em pacientes com HCV atendidos em Londrina;
- ✓ Comparar as alterações metabólicas dos pacientes com hepatite C, com e sem resistência insulínica, com controles saudáveis;
- ✓ Verificar a correlação entre os níveis de adipocinas e de ferritina com a resistência à insulina avaliada pelo HOMA IR em pacientes com HCV;
- ✓ Verificar se a adiponectinemia e leptinemia estão associadas à presença de resistência à insulina em pacientes com hepatite C;
- ✓ Verificar se a presença de resistência insulínica está associada ao grau de fibrose hepática avaliada pelo APRI em pacientes com hepatite C crônica.

5 CASUÍSTICA, MATERIAIS E MÉTODOS

5.1 DELINEAMENTO DO ESTUDO: Este estudo foi realizado na cidade de Londrina, norte do estado do Paraná. Trata-se de um estudo caso-controle, no qual foram selecionados 80 indivíduos sem hepatite C (grupo controle) entre doadores de sangue do Hemocentro Regional de Londrina, convidados a participar voluntariamente deste projeto, e conforme autorizado pela chefia médica do hemocentro; e 50 pacientes com diagnóstico de hepatite C (grupo HCV) acompanhados no ambulatório de Hepatites Virais do Ambulatório do Hospital das Clínicas da Universidade Estadual de Londrina, no Centro de Testagem e Aconselhamento do município de Londrina (CTA) e no Consórcio Intermunicipal de Saúde do Médio Paranapanema (CISMEPAR), controlados por sexo, etnia, idade, índice de massa corpórea (IMC) e presença de tabagismo.

Foram realizadas entrevista e coleta de dados em um único momento e, a seguir, os pacientes foram encaminhados para coleta de sangue.

O trabalho foi aprovado pelo Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, parecer n. 035/2012.

Critérios de inclusão grupo controle: idade entre 18 e 67 anos, ter assinado o termo de consentimento informado livre e esclarecido, ser apto à doação de sangue, segundo critérios estabelecidos pelo Ministério da Saúde (BRASIL, 2013).

Critérios de inclusão grupo hepatite C: ter entre 18 e 67 anos, ter duas sorologias reagentes para anticorpos anti-HCV e carga viral qualitativa ou quantitativa positiva, ter assinado o termo de consentimento informado livre e esclarecido.

Critérios de exclusão grupo controle: inaptidão para doação de sangue conforme critérios estabelecidos pelo Ministério da Saúde (BRASIL, 2013).

Critérios de exclusão no grupo hepatite: ter abaixo de 18 ou acima de 67 anos; recusa em assinar o termo de consentimento; carga viral negativa para hepatite C apesar da sorologia reagente para anticorpos anti-HCV; gestantes; presença de câncer; insuficiência cardíaca grave – classe funcional III e IV; diabetes tipo 1; doença renal crônica; presença de sorologia reagente para HIV 1/2 ou para o vírus da hepatite B.

5.2 COLETA E PREPARO DAS AMOSTRAS: As amostras de sangue foram obtidas por punção venosa após jejum de 12 horas.

5.3. AVALIAÇÃO DO ÍNDICE DE MASSA CORPORAL (IMC), CIRCUNFERÊNCIA ABDOMINAL E DETERMINAÇÃO DA PRESSÃO ARTERIAL: os pacientes foram pesados (kg) e medidos (m) e a partir destes dados calculou-se o IMC (peso/altura^2). A circunferência abdominal foi aferida por fita métrica, sempre pelo mesmo avaliador. A pressão arterial sistólica (PAS) e diastólica (PAD) foi determinada com o indivíduo sentado após 5 minutos de repouso, com esfigmomanômetro aneróide.

5.4 AVALIAÇÃO DO CONSUMO DE ÁLCOOL: foi considerado etilista o paciente que referiu consumo de álcool maior que 30g/dia, de acordo com os critérios da Organização Mundial de Saúde (OMS).

5.5 AVALIAÇÃO DO TABAGISMO: foi considerado tabagista o paciente que fumava, à época da avaliação, e que havia fumado pelo menos 100 cigarros na vida, de acordo com a OMS.

5.6 SOROLOGIA PARA DOENÇAS INFECCIOSAS: Foram realizadas sorologias para HIV e Hepatite B. Foi empregada a metodologia de quimioluminescência em micropartículas (ARCHITECT®, Abbott Laboratory, Abbot Park, IL, USA).

5.7 ANÁLISE DOS MARCADORES BIOQUÍMICOS E INFLAMATÓRIOS: Todos os participantes foram submetidos à coleta de sangue após 12 horas de jejum. Foram dosados os níveis séricos de colesterol total e frações, triacilgliceróis, glicose, aspartato aminotransferase (AST), alanina aminotransferase (ALT) e gama-glutamil transferase (GGT) e ferro sérico em um autoanalisador bioquímico (Dimension RxL Max Integrate Chemistry System, Newark, New Jersey, USA). As dosagens de insulina e ferritina foram realizadas por imunoensaio de micropartículas por quimioluminescência (ARCHITECT®, Abbott Laboratory, Abbot Park, IL, USA). O *Homeostasis Model Assessment* (HOMA) foi utilizado para avaliar a resistência à insulina e calculado de acordo com a fórmula: HOMA-IR = insulina jejum (uU/mL) X glicose jejum (mmol/L) / 22,5. O ponto de corte do índice HOMA-IR (2,5) para presença de resistência insulínica em pacientes com hepatite C foi determinado a partir de vários estudos descritos por Eslam et cols (2011) e Corbetta (2011).

5.8 DETERMINAÇÃO DA CARGA VIRAL E GENÓTIPO: a carga viral (HCV-RNA) dos pacientes foi realizada pela metodologia de reação em cadeia da polimerase (PCR) por transcriptase reversa quantitativa utilizando o equipamento COBAS AMPLICOR (Roche Diagnostics, Branchburg, NJ). A genotipagem foi realizada por PCR pela amplificação da região do core viral. Esses dados foram obtidos pela consulta ao prontuário médico.

5.9 DETERMINAÇÃO DAS ADIPOCINAS: os níveis plasmáticos de citocinas TNFA, IL-6, adiponectina e leptina foram mensurados usando um teste de enzimaimunoensaio (*sandwich enzyme-linked immunosorbent assay*) – ELISA - com reagentes comercialmente disponíveis (ELISA Ready-SET-Go! Set, eBioscience, San Diego, California, USA). Os resultados foram expressos em pg/mL ou mg/mL de acordo com as orientações do fabricante.

5.10 TESTE APRI: O APRI teste (*AST-to-platelet ratio index* – escore da razão entre AST e plaquetas) foi calculado pela fórmula (AST/limite superior do valor de

referência)/plaquetas (10^9/L) x 100, conforme descrito por Wai et al (2003), a partir de dados laboratoriais de cada paciente. Valores acima de 0,88 predizem fibrose (IC 95%, 0,80-0,96) e acima de 0,94 predizem cirrose (IC 95%, 0,89-1,0).

6 ANÁLISE ESTATÍSTICA

As variáveis categóricas foram avaliadas pelo teste exato de Fisher ou teste do Qui-quadrado conforme apropriado. Idade, IMC, carga viral foram analisados pelo teste não paramétrico de Mann-Whitney para comparar pacientes com HCV com e sem resistência insulínica. Comparação entre os 3 grupos foi realizada usando o teste não paramétrico de Kruskal-Wallis com análise post teste de Dunn. Os resultados foram expressos em mediana e intervalos interquartis (25-75%). Os resultados foram considerados significantes quando $p<0,05$. Para avaliação das correlações foi utilizado o teste de correlação de Spearman. Para análise estatística foi utilizado o programa Graph Pad Prism versão 3.0. A análise multivariada, para verificar quais parâmetros estavam associados à presença de resistência à insulina em pacientes com HCV, foi realizada utilizando-se aquelas variáveis cuja análise univariada apresentou $p<0.10$. Para esta análise foi utilizado o programa GraphPad Instat version 3.0 (GrahpPad Software, San Diego, CA).

7 ARTIGO DESENVOLVIDO

Para o cumprimento dos objetivos propostos inicialmente, foi desenvolvido um artigo científico intitulado:

INFLUENCE OF ADIPOKINES AND FERRITIN ON INSULIN RESISTANCE AND FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C

Adipokines, Ferritin and Insulin Resistance in chronic hepatitis C.

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ABSTRACT

Background: It has been postulated that steatosis, proinflammatory cytokines, adiponectin, and iron overload could play a crucial role in glucose abnormalities associated with HCV infection

Aim: Analyze the influence of adipokines and ferritin on insulin resistance (IR) and fibrosis index in patients with CHC. **Methods:** The study included 130 subjects: 80 healthy individuals selected and 50 HCV chronically infected patients (23 with IR and 27 without IR). **Results:** Both HCV groups presented higher TNFα ($p<0.001$) and IL-6 ($p<0.0001$) levels compared to the control group. HCV patients with IR had higher TNFα ($p<0.0001$) and IL-6 ($p<0.005$) levels when compared to the group without IR. There was a decrease in adiponectin levels in both HCV groups in relation to the control group ($p<0.01$). HCV patients with IR showed higher ($p<0.05$) leptin levels compared to the group without IR. Ferritin levels were higher in HCV patients with IR compared to the group without IR ($p<0.01$). Adiponectin levels were directly correlated with IL-6 ($r=0.495$, $p<0.05$) and APRI ($r=0.405$) and inversely correlated with viral load ($r=-0.621$; $p<0.05$). Leptin was positively correlated with AST ($r=0.507$; $p<0.05$), ALT ($p=0.357$; $p<0.05$) and TNFα ($r=0.406$, $p<0.05$). **Conclusion:** Data from the present study suggest a complex relationship between adipokines, ferritin, IR and progression of liver injury in patients

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with HCV. It is likely that hypoadiponectinemia is more related to liver steatosis and to increased ferritin levels than to IR in HCV. The present study reinforces the need of careful attention to reduce the risk of insulin resistance in HCV patients.

Keywords: Chronic hepatitis C. Insulin resistance. Adipokines. Ferritin. Liver fibrosis

Abstract word count: 250

Manuscript word count: 3702

INTRODUCTION

Hepatitis C virus (HCV) is one of the most prevalent causes of chronic liver disease worldwide and leads to cirrhosis and hepatocellular carcinoma (HCC) in a high percentage of carriers (1). Insulin resistance (IR) is a specific feature of chronic HCV, associated with the genotypes 1, 3 and 4 (2-4) and high serum HCV RNA level (3). However, the specific mechanism by which HCV leads to IR are still not fully understood. It has been postulated that steatosis, proinflammatory cytokines, adiponectin, and iron overload could play a crucial role in glucose abnormalities associated with HCV infection (4-7). In addition, metabolic derangements contribute to a decrease in sustained virological response and are associated with progression of liver fibrosis (8).

Proinflamatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNFA) appear to be the major regulators, which can provoke IR in adipose tissue, skeletal muscle and liver by inhibiting insulin signal transduction (9-10). It has been suggested that the blockade of insulin receptor substrates (IRS) 1 and IRS-2 signaling via proinflammatory cytokines is involved (11). However, the association of IL-6 and TNFA with HCV-induced IR has been controversial in the literature (6, 12, 13).

The adipokines (adiponectin and leptin) are a family of white adipose tissue-derived serum proteins that influence the glucose and lipid metabolism (14). To date, several studies have shown the potential of adiponectin, an anti-inflammatory adipokine, to improve insulin sensitivity, to reduce lipid deposition, and to activate the anti-inflammatory pathway (14). Adiponectin antagonizes both production and activity of TNFA. On the other hand, TNFA itself inhibits adiponectin (15). Some studies have investigated the role of adiponectin in HCV, but it remains controversial. Some authors suggest that HCV increases adiponectin (16) whereas others have not found these results (17, 18). Leptin has been reported in experimental models to have pro-inflammatory,

proangiogenic, pro-fibrogenic, and pro-mitotic effects. However, leptin has been found to be increased in some studies (17, 19) studies conducted in patients with CHC. A study reported that high serum leptin concentrations correlated with more severe steatosis, low viremia, and a low antiviral response, mainly in patients infected with HCV genotype 1, which constituted 71% of the study population (20).

Hyperferritinemia is a common finding in chronic hepatitis C (CHC) and, thus, it was suggested that iron overload plays a decisive role in the pathophysiology of glucose abnormalities and could be the link between HCV and glucose abnormalities (21). In addition, serum ferritin values may predict hepatic iron deposition and severity of fibrosis in patients with CHC (21). Proinflammatory cytokines up-regulate ferritin expression (22). On the other hand, serum adiponectin is inversely associated with ferritin levels. A previous study has demonstrated that serum ferritin is among the best predictors of serum adiponectin (22). In addition, ferritin and iron homeostasis have been implicated in the pathogenesis of many diseases, including liver disease, IR and metabolic syndrome. It has been hypothesized that iron excess may interfere with hepatic insulin extraction and catalyses the formation of free radicals, both leading to the development of hyperinsulinemia and IR (23). Therefore, hyperinsulinemia may be directly responsible for the accumulation of iron in the liver because insulin can stimulate cellular iron uptake through transferrin receptor externalization (24).

Therefore, we hypothesized that inflammatory status caused by HCV could increase serum ferritin and provoke IR. Nevertheless, IR could also contribute to enhance serum ferritin and leptin levels worsening liver injury mediated by iron in patients with CHC. Iron overload and IR, besides HCV, could also influence adiponectinemia. We are not aware, to date, of any study, which has evaluated all aforementioned factors concomitantly. Thus, the aim of the present study was to analyze the influence of adipokines and iron status parameters on IR and fibrosis index in patients with CHC.

SUBJECTS AND METHODS

Subjects

The study included 130 subjects: 80 healthy individuals selected from among blood donors of the University Hospital and 50 HCV (23 with IR and 27 without IR) infected patients from the outpatient clinic of Infectology of Londrina University Hospital. All HCV patients were seropositive for HCV antibodies and HCV RNA viral load. Patients with concurrent hepatitis B virus or human immunodeficiency virus (HIV) infections, autoimmune hepatitis or HCC evidence were excluded. The control group comprised of 80 healthy subjects controlled by sex, age, ethnicity, and body mass index (BMI). They were considered healthy based on history, physical examination and laboratory tests. No control subjects were seropositive for HCV antibody.

None of the participants in the study presented heart, renal, oncological diseases or iron deficiency anemia, and none were receiving oestrogen replacement therapy or drugs for iron deficiency. All patients gave written informed consent, and the study protocol was fully approved by the Ethical Committee of the University of Londrina (Paraná, Brazil).

Anthropometric and Blood Pressure Measurements

Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2), and the waist circumference (WC) was measured with a soft tape on standing subjects midway between the lowest rib and the iliac crest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. The mean of two blood pressure measurements taken with a minute interval between them after the subjects had been seated was used for the statistical analysis.

Metabolic Parameters

Peripheral blood samples were collected with EDTA as anticoagulant and without anticoagulant after fasting for 12 hours. All samples were immediately centrifuged at 3,000 rpm for 15 min, and plasma and sera aliquots, respectively, were stored in -80^0 C freezer until use. The samples were consecutively identified by number to

guarantee confidentiality. Aspartate aminotransferase (AST), alanine aminotrasferase (ALT), gamma glutamyltransferase (GGT), fasting glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, and iron levels were evaluated using commercial reagent in a biochemical autoanalyzer (Dimension TM Dade AR, Dade Behring, Deerfield, IL, USA). Fasting insulin and ferritin levels were determined using chemiluminescence microparticle imunnoassay (Architech™, Abbott Laboratory, Abbott Park, IL, USA). The homeostasis model assessment (HOMA) was used as a surrogate measurement of insulin sensitivity. HOMA for IR (HOMA-IR) was calculated using the insulin fasting (μU/mL) x glucose fasting (nmol/L)/22.5. It was considered IR when subjects exhibited HOMA-IR≥ 2.5.

Evaluated Adipokines

Plasma adipokines, such as TNFA, IL-6, adiponectin, and leptin, were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) with commercially available reagents (ELISA Ready-SET-Go! Set, eBioscience, San Diego, California, USA). The results were expressed as pg/mL, except adiponectin that was expressed as mg/mL, according to the manufacturers' instructions.

Aminotransferase-to-platelet ratio index (APRI)

APRI is a simple index, consisting of 2 readily available laboratory results aspartate aminotransferase (AST) level and platelet count that can predict significant fibrosis (with area under ROC = 0.88) and cirrhosis (with area under ROC = 0.94) in treatment-naïve CHC patients with a very high degree of accuracy (25).

The APRI is calculated as (AST/upper limit of normal range)/platelet count (10^9/L) x 100.

Viral Load and Genotyping of HCV RNA

Viral RNA was extracted from 200μL of each sample using the High PureViral Nucleic Acid reagent set (Roche Molecular Biochemicals, Mannheim, Germany). HCV RNA was detected and quantified in serum with the COBAS AMPLICOR system

according to the manufacturer's instruction (Roche Diagnostics, Branchburg, NJ). HCV genotyping was carried out by PCR amplification of the core region.

Statistical Analysis

A statistical analysis program (Graph Pad Prism version 4.0) was used for evaluations. Distribution of gender, ethnicity and clinical history were analyzed using the Fisher's Exact test or chi-square test. Disease duration, viral load, and APRI were analyzed using the Mann-Whitney test to compare patients infected with HCV with or without IR. Comparisons between groups were done using the Kruskal-Wallis test with Dunn's post test and data were expressed as the median and interquartile range (25-75 percentiles). Correlations were evaluated by Spearman's rank correlation. To determine which factors were independently associated with IR, the variables that presented $p < 0.10$ in the univariate analyses were included in the multivariate logistic regression model, and were evaluated by the GraphPad Instat version 3.0 (GrahpPad Software, San Diego, CA). The results were considered significant when $p<0.05$.

RESULTS

Clinical and laboratory data of all patients are described in Table 1. Eighty-six percent were infected with HCV genotype 1 while 24% were infected with HCV genotype 3. There were no differences in relation to disease duration, genotype and viral load between those with IR and without IR, whereas APRI was significantly higher ($p=0.002$) in HCV patients with IR.

HCV patients with IR did not differ from the other groups with regard to age, gender, ethnicity, smoking, SBP, DBP, and HDL-cholesterol levels. However, BMI and WC were significantly higher in HCV patients with IR when compared to HCV patients without IR ($p<0.05$). Both HCV groups presented lower ($p<0.0001$) serum total cholesterol and LDL-cholesterol levels when compared to the control group, whereas triacylglycerol levels were lower in HCV patients without IR compared to the control group ($p<0.05$) (Table 2).

With regard to glucose metabolism, plasma glucose levels were significantly higher in HCV with IR group when compared to the control group

($p<0.0001$), whereas insulin levels and HOMA-IR were significantly higher ($p<0.0001$) in HCV patients with IR compared to both HCV without IR and control groups (Table 2).

Liver enzymes AST, ALT and GGT showed significantly higher ($p<0.0001$) values in both HCV groups compared to the control group. In addition, serum GGT levels were significantly higher in HCV patients with IR compared to HCV patients without IR ($p<0.05$) (Table 2).

Pro inflammatory adipokines TNFA and IL-6 showed similar results. Both HCV groups presented significantly higher TNFA and IL-6 levels compared to the control group ($p<0.001$ and $p<0.0001$, respectively), whereas HCV patients had significantly higher TNFA and IL-6 levels when compared to HCV patients without IR ($p<0.0001$ and $p<0.005$, respectively). There was a decrease in adiponectin levels in both HCV groups in relation to the control group ($p<0.01$), whereas HCV patients with IR showed significantly higher ($p<0.05$) leptin levels compared to HCV patients without IR (Figure 1).

Regarding iron metabolism biomarkers, HCV groups had significantly higher iron and ferritin levels compared to the control group ($p<0.05$ and $p<0.0001$, respectively). Additionally, ferritin levels were significantly higher in HCV patients with IR compared to HCV patients without IR ($p<0.01$) (Figure 2).

Spearman`s correlation verified that HOMA-IR was positively correlated with AST ($r=0.302$; $p<0.05$), IL-6 ($r=0.368$; $p<0.05$), leptin ($r=0.356$; $p<0.05$) and APRI ($r=0.457$; $p< 0.01$). Adiponectin levels were directly correlated with IL-6 ($r=0.495$, $p<0.05$) and APRI ($r=0.405$) and inversely correlated with viral load ($r=-0.621$; $p<0.05$), whereas leptin was positively correlated with AST ($r=0.507$; $p<0.05$), ALT ($p=0.357$; $p<0.05$) and TNFA ($r=0.406$, $p<0.05$) (Table 3). Multivariate analysis showed that APRI index was the unique parameter independently associated to IR ($p<0.05$) in patients with HCV (data not shown).

DISCUSSION

To our knowledge, this is the first study to report a simultaneous increase in TNFA, IL-6, ferritin, and leptin levels in CHC patients with IR when compared to CHC patients without IR, and a reduction in adiponectin levels in both CHC groups.

Conceivably, while IR does not seem to be involved in adiponectin decrease, it seems to favor ferritin increase in CHC patients.

Metabolic abnormalities are common in patients with HCV infection. The current study is consistent with previous experimental and clinical studies that have examined the association between CHC and lipid metabolism (18, 26, 27). HCV core protein reduces microssomal triglyceride transport protein function, leading to impairment of VLDL, triglycerides and apolipoprotein B (APO-B) secretion, which in turn contributes to hepatic lipid accumulation and reductions in plasma total cholesterol (28).

HCV may promote IR irrespective of the severity of liver disease and this effect appears to be genotype specific (31), especially HCV genotypes 1, 3 and 4 (2, 29, 30). It has been shown that HCV genotype 1b diminishes insulin receptor substrate (IRS-1) levels and causes IR (30). In addition, it has been suggested that IR promotes fibrotic progression (29, 31).

Our data showed that increased IR evaluated by HOMA-IR was directly correlated with APRI index, which predicts fibrosis in HCV infected patients (25). Hyperinsulinism directly activates stellate cells and in association with hyperglycemia increases connective tissue growth factor, a key cytokine in hepatic fibrogenesis (32). In addition, IR as evaluated by HOMA-IR was independently associated with virally mediated portal inflammation and an increased rate of fibrosis progression, suggesting that IR is a cause rather than a consequence of hepatic fibrosis in HCV infection (29).

It has been postulated that steatosis, proinflammatory adipokines and iron overload could play a crucial role in glucose abnormalities associated with HCV infection (4-7). HCV patients with RI had an increase in pro inflammatory adipokines (TNFA, IL-6 and leptin) which can directly affect glucose metabolism. Our data are in accordance with previous studies, which showed that HCV patients had increased TNFA, IL-6 and leptin levels (6, 13, 16, 33) and it is likely a consequence of virus-mediated inflammatory response (13). TNFA is released from Kupffer cells, B cells, adypocytes and hepatocytes. One produced, TNFα can modulate adipocytes and bring about changes in the production of cytokines, adiponectin and leptin (4). TNFA inhibits the expression of glucose transporter (GLUT-4) and lipoprotein lipase in peripheral tissues, which leads to impaired insulin action on peripheral tissue and hepatic glucose uptake (4, 6, 33). In addition, IL-6 also is secreted from Kupffer cells, adipocytes, B cells, and hepatocytes. Increased IL-6 derived from adipocytes leads to an ongoing acute-phase response that

acts on hepatocytes and promotes hepatic IR (4). Therefore, in the present study, HOMA-IR was directly correlated with IL-6 and leptin. Leptin plays an important role in the regulation and metabolism of body fat and may induce IR, increase fatty acid concentrations in the liver, and enhance lipid peroxidation (35–37). Leptin may act as an immunomodulator, inducing the release of cytokines, such as TNFA, interferon γ (IFNG), interleukin 18 (IL-18), and tumor growth factor (TGF-B1), thus promoting liver steatosis and fibrosis (38). Previous studies have shown that leptin levels are increased in CHC patients (17; 33). Our results demonstrated that HCV patients with IR had increased leptin levels and that they were directly correlated with the inflammatory process, HOMA-IR and with liver injury markers. Therefore, it is conceivable to suggest that leptin also participates in IR occurrence. However, differently from the present results, some reports did not find correlation between these adipokines and IR (13, 16) suggesting that HCV associated IR was not mediated by these adipokines (16). Taken all together, our data reinforce the hypothesis that pro inflammatory adipokines are involved in the development of IR in HCV patients.

In contrast, adiponectin, the most abundant of all the adipokines, increases insulin sensitivity, and exerts anti-steatotic, anti-inflammatory and antifibrotic effects (14, 39-41). However, the action of adiponectin in CHC is still controversial. Previous studies have shown that serum adiponectin levels were not altered (42) or decreased (18) in CHC. Other studies showed that adiponectin levels were significantly higher in CHC (16, 43-45). Some studies reported that adiponectin correlated with stage of liver cirrhosis, aminotransferase activity, and inflammatory markers (16, 45). Therefore, it was suggested that in HCV patients with fibrosis and/or necroinflammation, adiponectin levels might increase due to the decreased excretion probably leading to accumulation in the serum (16, 46). Corbetta et al. (47) demonstrated that moderate to severe fibrosis in chronic hepatitis is associated with hyperadiponectinemia. The further finding of reduced adiponectin receptor 1 (ADIPOR1) expression in HCV-infected hepatocytes was consistent with a condition of adiponectin resistance occurring in progressed HCV. However, Cua et al. (13) did not find any difference in serum leptin and adiponectin levels between HCV and control groups, suggesting that these adipokines do not mediate the increased IR observed in HCV-infected persons. In contrast, Chen et al. (18) demonstrated that compared to BMI-matched, age-matched, and sex-matched healthy controls, CHC-infected patients had lower levels of adiponectin, similarly to the

results found in the present study, independently of their IR status. Our data are in agreement with Sumie`s et al. (48) study, which also verified a positive correlation between adiponectin and APRI index. These conflicting results might be associated with our small sample size and the different cohorts of patient's collection as well as different grading systems for steatosis.

Unexpectedly, in the present work, there was a direct correlation between adiponectin and IL-6. The current understanding of the role of IL-6 in the context of obesity is ambiguous. Although obesity-associated induction of adipose IL-6 production induces CRP secretion, and thus IL-6 has been classically considered an important pro-inflammatory marker. There is growing evidence that IL-6 has also anti-inflammatory activities mediated by classic signaling, whereas pro-inflammatory responses of interleukin-6 are rather mediated by trans-signaling (49).

Hypoadiponectinemia is considered a good predictor of hepatic steatosis in HCV infection (50-52). Although hepatic steatosis was not measured in the current study, it is known that hypoadiponectinemia combined to high TNFA levels could result in steatosis (4, 15). Korah et al (53) demonstrated that adiponectin was decreased in the presence of steatosis in patients with HCV genotype 4 infection. This is in accordance with others studies conducted in patients with HCV genotype 4 and HCV of different genotypes (50-52). In addition, steatosis is an important cofactor in HCV because it is associated with fibrosis and reduces early and sustained virologic response.

The present study is in accordance with other report, which showed that HCV patients had higher ferritin levels than healthy subjects (22). In addition, this study verified for the first time, to our knowledge, that, besides HCV infection *per se*, IR proportioned an additional increase in ferritin levels. The increase in ferritin levels, a positive acute phase protein, concomitantly to the increase in TNF-α and IL-6 can be explained by the increased inflammatory activity verified in HCV patients with IR. It can be suggested that enhancement in ferritin levels verified in the later group contributes to the increase in liver injury evaluated by the APRI index, thus worsening liver metabolism and favoring IR in a vicious circle.

Of note, previous studies have demonstrated that ferritin was inversely associated with plasma adiponectin, (54-56). The potential inhibitory role of iron on adiponectin production and secretion is still unclear. Wlazlo et al. (54) observed associations of ferritin with both adipocyte IR and low adiponectin

levels. These authors suggested the hypothesis that iron metabolism influence adipose tissue function. Gabrielsen et al. (22) demonstrated in humans that the association between serun ferritin and adiponectin is independent of inflammation and that serum ferritin, even within its normal ranges, is among the best predictors of serum adiponectin. Insulin has been shown to up-regulate iron uptake and ferritin levels (57-58). In addition, elevated ferritin may interfere with hepatic insulin extraction leading to peripheral hyperinsulinaemia and IR. It is known that liver-mediated IR is an early consequence of iron-dependent damage. The fact that adipocytes use iron levels to regulate adiponectin suggests a role for adipocytes in coordinating organism-wide metabolic responses to iron availability, as they do for responses to overall macronutrient status (22). The present study seems to be in line with these previous studies, which found an inverse association between adiponectin and ferritin.

The present study has some limitations and strengths. First, the small number of participants, due to the rigorous inclusion criteria, which were followed; second, liver biopsies were not performed in all patients. However, this analysis could be at least partially substituted by the APRI index. This study evaluated adipokines and iron status parameters in HCV patients with and without IR. This design allowed inferring IR participation in several physiopathological mechanisms, which accompany liver injury in HCV patients. Thus, the original design can be considered the main strength of this study.

In conclusion, data from the present study suggest a complex relationship between adipokines, ferritin, IR and progression of liver injury in patients with HCV. It is likely that hypoadiponectinemia is more related to liver steatosis and to increased ferritin levels than to IR in HCV. The present study reinforces the need of careful attention to reduce the risk of insulin resistance in HCV patients. When needed, the use of insulin resistance reducing agents, such as metformin or thiazolidinediones, with current HCV standard treatment may benefit chronic hepatitis C patients. The understanding of the complex metabolic mechanisms involved in the context of the liver disease progression will provide a more accurate picture of the phenomena.

Acknowledgments

This work was supported by the University of Londrina Research Funds (FAEPE).

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Table 1: Clinical, laboratory and histopathologic characteristics in HCV patients with insulin resistance (HOMA ≥ 2.5) or without (HOMA < 2.5)

	HCV HOMA <2.5 (n = 23)	HCV HOMA ≥2.5 (n = 27)	p
Disease Duration (years)*	3.0 (2.0-10.0)	4.0 (2.25-10.0)	NS
Genotype (n/%)			
1	21 (91.3)	22 (81.5)	NS
2	0	0	
3	2 (8.7)	5 (18.5)	
4	0	0	
Anti-Hypert drugs (n/%)			
No	21 (91)	21 (77.7)	NS
Yes	2 (7)	8 (22.3)	
Antilipemic drugs (n/%)			
No	22 (95.6)	24 (88.8)	NS
Yes	1 (4.4)	3 (11.2)	
Hypoglicaemic agents (n/%)			
No	21 (91)	20 (74)	NS
Yes	2 (7)	7 (26)	
APRI	0.621 (0.408-0.991)	1.720 (0.901-3.767)	0.0019
VL (copies)*	943900 (264400-16900000)	770400 (158500-2853000)	0.9151

Chi-square test or Fisher exact test. *Mann-Whitney test. Data are expressed as median (25-75%).

HCV, hepatitis C virus; HOMA , homeostasis model assessment; APRI, aminotransferase-to-platelet ratio index; VL, viral load.

Table 2: Demographic, Clinical and laboratory characteristics in controls (cont) and patients with HCV infection with insulin resistance (HOMA ≥ 2.5) or without (HOMA < 2.5)

Parameters	**Control (n = 80)**	**HCV HOMA < 2.5 (n = 23)**	**HCV HOMA ≥ 2.5 (n = 27)**	**CONTROL *vs* HOMA < 2.5**	**CONTROL *vs* HOMA ≥ 2.5**	**HOMA < 2.5 *vs* HOMA ≥ 2.5**
Age (years)	48.0 (45.0-53.0)	53.0 (39.0-59.0)	54.5 (46.8-60.0)	NS	NS	NS
Gender (F/M)	57/23	12/11	16/11	NS	NS	NS
Caucasian / not Caucasian	60/20	14/9	16/11	NS	NS	NS
Smoking / not smoking	7/74	6/17	5/22	NS	NS	NS
BMI (kg/m^2)	25.39 (22.94-28.21)	25.36 (22.04-26.86)	27.72 (25.47-30.35)	NS	NS	**<0.05**
WC (cm)	92.0 (87.0-102.0)	90.0 (75.0-97.0)	96.5 (90.0-103.6)	NS	NS	**<0.05**
SBP (mmHg)	118.0 (106.0-130.0)	110.0 (100.0-130.0)	120.0 (110.0-120.0)	NS	NS	NS
DBP (mmHg)	75.0 (69.0-80.0)	70.0 (60.0-80.0)	80.0 (60.0-80.0)	NS	NS	NS
Triacylglycerol (mg/dL)	108.0 (79.0-149.0)	84.0 (52.0-122.0)	95.5 (64.25-188.5)	**<0.05**	NS	NS
Total cholesterol (mg/dL)	207.0 (184.0-237.0)	165.0 (138.0-194.0)	158.0 (119.0-173.5)	**<0.0001**	**<0.0001**	NS
Parameters	**Control (n = 80)**	**HCV HOMA<2.5 (n = 23)**	**HCV HOMA≥2.5 (n = 27)**	**CONTROL *vs* HOMA<2.5**	**CONTROL *vs* HOMA ≥2.5**	**HOMA<2.5 *vs* HOMA≥2.5**

HDL	54.0	52.0	50.0	NS	NS	NS
(mg/dL)	(44.0-62.0)	(39.0-67.0)	(39.5-62.0)			
LDL	127.0	93.0	71.80	**<0.0001**	**<0.0001**	NS
(mg/dL)	(106.7-150.6)	(71.6-112.0)	(52.8-98.3)			
Fasting glucose	92.0	93.0	101.0	NS	**<0.01**	NS
(mg/dL)	(86.0-98.0)	(87.0-104.0)	(90.0-116.5)			
Fasting insulin	7.7	7.1	16.0	NS	**<0.0001**	**<0.0001**
(mg/dL)	(5.3-11.7)	(5.1-8.8)	(13.6-27.4)			
HOMA-IR	1.603	1.887	4.170	NS	**<0.0001**	**<0.0001**
	(1.059-2.393)	(1.240-2.128)	(3.109-8.671)			
AST (UI/L)	22.0	51.0	74.0	**<0.0001**	**<0.0001**	NS
	(16.0-26.0)	(39.8-61.8)	(49.0-124.8)			
ALT (UI/L)	34.0	89.9	98.0	**<0.0001**	**<0.0001**	NS
	(28.0-42.0)	(64.5-128.5)	(58.3-168.0)			
GGT (UI/L)	25.0	45.0	110.0	**<0.0001**	**<0.0001**	**<0.05**
	(20.0-38.0)	(31.0-90.0)	(72.0-197.0)			

Chi-square test or Fisher exact test. Kruskal-Wallis test with post-hoc Dunn test. Data are median (25% – 75%). NS, non-significant; HCV, hepatitis C virus; HOMA-IR, homeostatic model assessment – insulin resistance; F, female; M, male; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

Table 3: Spearman's correlation between Insulin Resistance (HOMA-IR), adiponectin and leptin with liver enzymes, iron status, APRI and viral load in HCV patients

	HOMA-IR	Adiponectin	Leptin
AST	**0.302***	-0.096	**0.507***
ALT	0.073	0.194	**0.357***
GGT	0.337	-0.101	0.136
Ferritin	0.103	0.166	-0.125
Iron	0.040	0.001	0.058
TNFA	0.183	0.115	**0.406***
IL-6	**0.368***	**0.495***	0.111
Leptin	**0.356***	-0.124	---
Adiponectin	-0.185	---	-0.124
APRI	**0.457***	**0.405***	0.049
HCV-RNA	-0.0826	**-0.621***	-.018

HCV, hepatitis C virus; HOMA-IR, homeostatic model assessment – insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT – gamma-glutamyl transferase; APRI, aminotransferase-to-platelet ratio index. *$p<0.05$

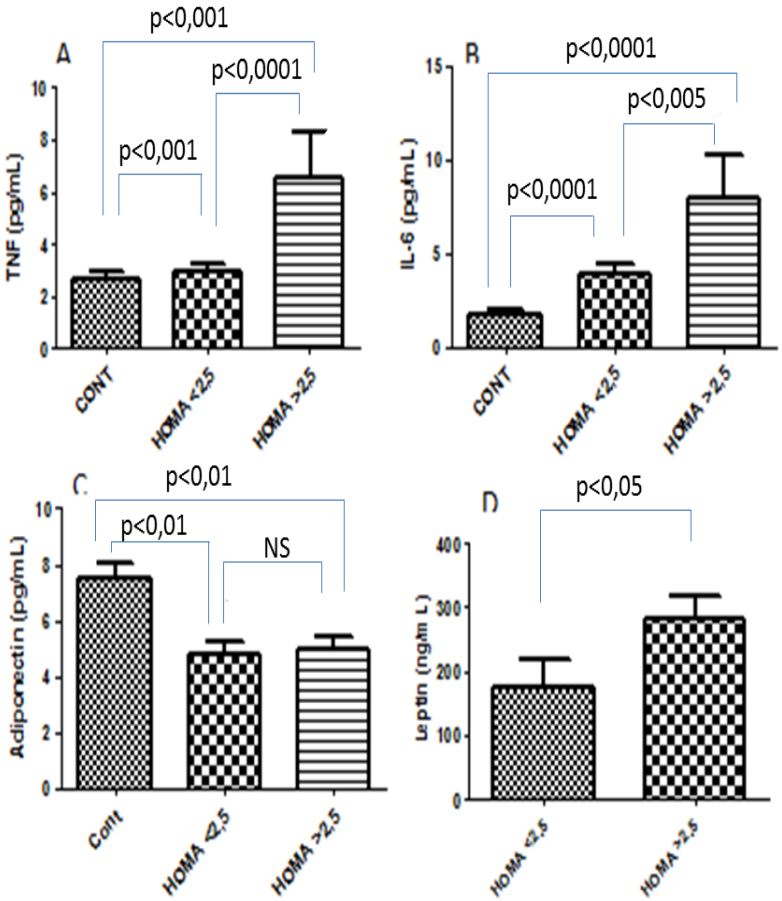

Figure 1: Adipokines levels in control subjects (cont), HCV patients with resistance insulin (HOMA ≥ 2.5) or without (HOMA<2.5). HOMA, homeostasis model assessment.

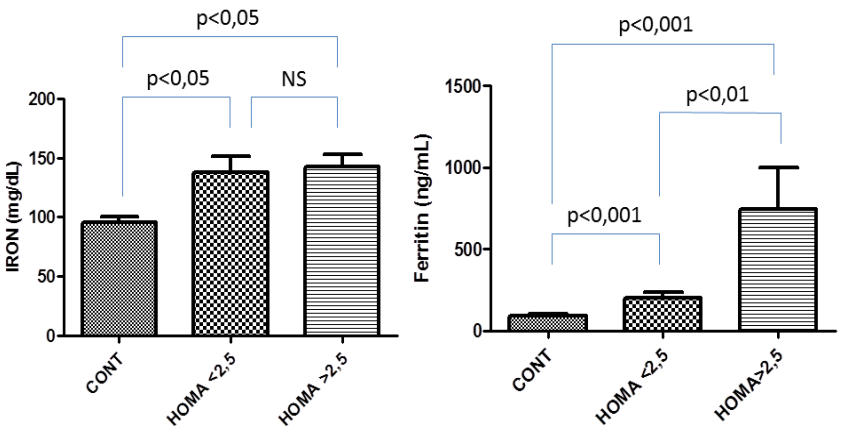

Figure 2 – Iron and ferritin in HCV patients and in control subjects. (cont), HCV patients with (HOMA ≥ 2.5) or without (HOMA<2.5) insulin resistance.

8 CONCLUSÃO

Pacientes com hepatite C apresentam maior HOMA-IR que indivíduos saudáveis. Sendo que a RI avaliada por este índice foi diretamente correlacionada ao índice de fibrose APRI em acordo com estudos prévios que demonstram que a RI prejudica a progressão da doença.

Os níveis séricos de ferro e ferritina são significativamente maiores em indivíduos infectados pelo HCV quando comparados a indivíduos saudáveis. A presença de RI em pacientes com HCV proporciona um aumento adicional nos níveis de ferritina destes pacientes.

Os níveis séricos de adipocinas pró-inflamatórias (TNFA, IL-6 e leptina) são significativamente maiores em indivíduos com HCV quando comparados a controles saudáveis. No entanto, indivíduos com HCV e RI apresentam aumento adicional destas adipocinas, o que demonstra o possível envolvimento destas moléculas na fisiopatologia da RI.

Pacientes com HCV apresentam menores níveis de adiponectina quando comparados a indivíduos controles. Isso parece estar relacionado à presença de esteatose e ao aumento de ferritina e não a presença de RI propriamente dita.

Houve uma correlação direta entre a adiponectina e a IL-6. O papel da IL-6 no contexto da obesidade é ambíguo. Embora a produção de IL-6 associada à obesidade leve à produção de proteína C reativa, há evidências crescentes de seu papel antiinflamatório mediado pela via clássica.

9 CONSIDERAÇÕES FINAIS

O presente estudo apresenta algumas limitações, como o pequeno número de participantes, devido aos rigorosos critérios de inclusão. Ainda, fibrose hepática foi avaliada pelo teste APRI, ao invés do padrão ouro que é a biópsia hepática, devido aos dados não estarem disponíveis no prontuário de todos os pacientes avaliados.

O estudo avaliou as adipocinas e a ferritina em pacientes HCV com e sem resistência insulínica. Este desenho permitiu avaliar a participação da RI em diversos mecanismos fisiopatológicos, que fazem parte do processo de agressão hepática nos pacientes HCV.

A avaliação de pacientes em diferentes serviços permitiu também conhecer melhor a realidade e a fragilidade do atendimento ao paciente HCV em Londrina, Paraná.

O presente estudo sugere uma complexa relação entre adipocinas, ferritina, resistência insulínica e progressão de fibrose hepática em pacientes HCV. Reforça a necessidade da atenção cuidadosa e multidisciplinar a estes pacientes, no intuito de reduzir/prevenir a RI. O uso de hipoglicemiantes orais, quando indicados, pode beneficiar os pacientes HCV, com ou sem tratamento anti-viral específico.

O entendimento dos complexos mecanismos metabólicos no contexto da doença hepática levará a melhoria do atendimento e tratamento destes pacientes.

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ANEXO

ANEXO A

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2. Luscombe C, Pedersen J, Bowden S, Locarini S. Alterations in intrahepatic expression of duck hepatitis B viral markers with ganciclovir chemotherapy. Liver 1994; 14: 182-92.

3. Gines A, Salmeron J M, Gines P, et al. Effects of somatostatin on renal function in cirrhosis. Gastroenterology 1992; 103: 1868-84.

4. Demetris A J, Kakizoe S, Oguma S. Pathology of liver transplantation.
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