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PREVALÊNCIA E FATORES DE RISCO PARA DOENÇA HEPÁTICA GORDUROSA NÃO ALCOÓLICA EM PACIENTES COM DOENÇA DE CROHN

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Prevalência e fatores de risco para doença hepática gordurosa não alcoólica em pacientes com doença de Crohn

Dissertação de mestrado apresentada ao Programa de Pós-Graduação: Ciências em Gastroenterologia e Hepatologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Mestre.

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Dedico essa monografia a todos os pacientes do Ambulatório de Doenças Inflamatórias Intestinais do HCPA que prontamente se dispuseram a participar do projeto. Sem a participação deles nada disso seria possível.

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Sumário

RESUMO

A Doença de Crohn (DC) é uma condição inflamatória crônica oriunda da predisposição genética e fatores ambientais associados a uma desregulação do sistema imune. A plausibilidade biológica faz com que esperemos que os pacientes com DC tenham emagrecimento e desnutrição tanto pelo aumento do consumo energético secundário ao processo inflamatório quanto por possíveis alterações absortivas. Apesar de haver evidências científicas para que estes pacientes apresentem baixo peso, atualmente se observa um número significativo de pacientes com sobrepeso e obesidade, sendo este um dos principais fatores de risco para o desenvolvimento de Doença Hepática Gordurosa Não Alcoólica (DHGNA) na população em geral. A DHGNA é caracterizada pelo acúmulo de gordura nos hepatócitos e pode progredir para esteato-hepatite, cirrose e carcinoma hepatocelular. Alguns autores relatam uma prevalência maior de DHGNA em pacientes com DC se comparado à população em geral. No entanto, os mecanismos pelos quais a DHGNA e a DC se relacionam são pouco conhecidos, sugerindo que as Doenças Inflamatórias Intestinais (DII) possuem fatores de risco independentes para DHGNA. Nosso objetivo foi avaliar a prevalência e os fatores de risco para DHGNA em pacientes com DC. Material e métodos: estudo prospectivo que avaliou sequencialmente os pacientes com DC em acompanhamento no ambulatório de Doenças Inflamatórias Intestinais do Hospital de Clínicas de Porto Alegre entre julho de 2021 e maio de 2022. Foram coletados os dados relacionados às características clínicas da DC, medicações de uso prévio e atual, doenças pré-existentes, exames bioquímicos, óxido de trimetilamina (TMAO) plasmático, dados antropométricos e ingestão alimentar. O diagnóstico e classificação da DHGNA foi feito através de ultrassonografia (US) de abdômen superior. Resultados: 103 pacientes com DC e idade média de 45,2±15,0

anos, sendo 35,9% (n=37) do sexo masculino. A prevalência de DHGNA foi de 40,8% (n=42). Entre os pacientes com este diagnóstico, 42,9% (n=18) tinham hipertensão arterial sistêmica (HAS) (p<0,001), 11,9% (n=5) tinham dislipidemia (p=0,040) e 19% (n=8) tinham diabetes (DM) (p=0,009). O excesso de peso esteve presente em 62,1% da amostra (p<0,001). O tempo médio de diagnóstico da DC foi de 12,3 anos e 77,7% da amostra estava em remissão da DC. Em análise univariada não foram observadas diferenças estatísticas entre idade, sexo, características e atividade da DC, tratamento prévio ou atual para DC e ingestão alimentar com DHGNA. A presença de comorbidades como HAS, DM e dislipidemia, marcadores antropométricos como índice de massa corporal (IMC), circunferência abdominal (CA), diâmetro abdominal sagital (DAS), resultados laboratoriais como proteína C reativa (PCR), triglicerídeos (TG) e enzimas hepáticas alanina aminotransferase (ALT), gama glutamiltransferase (GGT) e fosfatase alcalina foram positivamente associados com o diagnóstico de DHGNA. Os níveis plasmáticos de TMAO não demonstraram relação com a presença de DHGNA e com a atividade da DC, mas se correlacionaram com o DAS (p 0,047). Após o ajuste pelo modelo multivariado, as variáveis que permaneceram estatisticamente associadas com a DHGNA foram: HAS (p=0,046), DM (p=0,017), DC ativa (p=0,023), sobrepeso (p=0,025) e obesidade (p<0,001). A presença de DHGNA nesta amostra esteve relacionada aos fatores de risco metabólicos semelhantes para a população em geral (hipertensão arterial sistêmica, diabetes, dislipidemia, sobrepeso e obesidade). As características da DC e os níveis plasmáticos de TMAO não demonstraram relação com a presença de DHGNA. Ao contrário do que se poderia esperar a atividade da DC apresentou correlação positiva com a DHGNA.

PALAVRAS-CHAVE: Doença Hepática Gordurosa Não Alcoólica, Esteato-hepatite, Esteatose Hepática, Doença Inflamatória Intestinal, Doença de Crohn.

ABSTRACT

Crohn's Disease (CD) is a chronic inflammatory condition arising from genetic predisposition and environmental factors associated with a dysregulation of the immune system. The biological plausibility makes us expect that patients with CD will have weight loss and malnutrition, both due to increased energy consumption secondary to the inflammatory process and possible absorption changes. Although there is scientific evidence that these patients are underweight, a significant number of overweight and obese patients are currently observed, which is one of the main risk factors for the development of Non-Alcoholic Fatty Liver Disease (NAFLD) in the general population. NAFLD is characterized by the accumulation of fat in hepatocytes and can progress to steatohepatitis, cirrhosis, and hepatocellular carcinoma. Some authors report a higher prevalence of NAFLD in patients with CD compared to the general population. However, the mechanisms by which NAFLD and CD are related are poorly understood, suggesting that Inflammatory Bowel Diseases (IBD) have independent risk factors for NAFLD. Our objective was to assess the prevalence and risk factors for NAFLD in patients with CD. Material and methods: prospective study that sequentially evaluated patients with CD that have been followed at the Inflammatory Bowel Diseases outpatient clinic of Hospital de Clínicas de Porto Alegre between July 2021 and May 2022. The data were collected related to the clinical characteristics of CD, use of previous and current medications, comorbidities, biochemical tests, plasmatic trimethylamine oxide (TMAO), anthropometric data and food intake. The diagnosis and classification of NAFLD was made by an upper abdominal ultrasound (US). Results: 103 patients with CD and mean age of 45.2 ± 15.0 years, 35.9% (n=37) were male. The prevalence of NAFLD was 40.8% (n=42). Among patients with this diagnosis, 42.9% (n=18) had systemic arterial hypertension

(SAH) (p<0.001), 11.9% (n=5) had dyslipidemia (p=0.040) and 19% (n=8) had diabetes mellitus (DM). (p=0.009). Excess weight was present in 62.1% of the sample (p<0.001). The mean diagnosis CD time was 12.3 years and 77.7% of the sample was in CD remission. In univariate analysis, no statistical differences were observed between age, sex, characteristics, and activity of CD, previous or current treatment for CD and food consumption and NAFLD. The presence of comorbidities such as SAH, DM and dyslipidemia, anthropometric markers such as body mass index (BMI), abdominal circumference (CA), sagittal abdominal diameter (DAS), laboratory results such as C-reactive protein (CRP), triglycerides (TG), liver enzymes alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) and alkaline phosphatase were positively associated with the diagnosis of NAFLD. TMAO plasmatic levels showed no relation with the presence of NAFLD and with the activity of CD, but correlate with sagittal abdominal diameter. After adjusting for the multivariate model, the variables that remained statistically associated with NAFLD were: SAH (p=0.046), DM (p=0.017), active CD (p=0.023), overweight (p=0.025) and obesity (p=0.023). <0.001). The presence of NAFLD in this sample was related to similar metabolic risk factors for the general population (systemic arterial hypertension, diabetes, dyslipidemia, overweight and obesity). The characteristics of CD and plasma levels of TMAO showed no relation with the presence of NAFLD. Contrary to what might be expected, CD activity showed a positive correlation with NAFLD.

Keywords: Non-Alcoholic Fatty Liver Disease, Steatohepatitis, Hepatic Steatosis, Inflammatory Bowel Disease, Crohn's Disease.

Lista de Abreviaturas

AUDIT - *The Alcohol Use Disorders Identification Test*

ALT - Alanina Aminotransferase

Anti-TNF – Anti-fator de necrose tumoral alfa

Anti-IL23 – Anti-interleucina 23

AST – Aspartato Aminotransferase

CA – Circunferência abdominal

CEP – Comitê de Ética em Pesquisa

DAS – Diâmetro abdominal sagital

DC – Doença de Crohn

DHGNA - Doença Hepática Gordurosa Não Alcoólica

DII – Doença Inflamatória Intestinal

EH – Esteatose Hepática

EUA - Estados Unidos da América

EHNA - Esteato-Hepatite Não Alcoólica

GGT - gama glutamiltransferase

HCPA – Hospital de Clínicas de Porto Alegre

IADC – Índice de Atividade de Doença de Crohn

IHB – Índice de Harvey-Bradshaw

IMC – Índice de Massa Corporal

MAFLD – Metabolic dysfunction associated fatty liver disease

NASH - Nonalcoholic steatohepatitis

OMS – Organização Mundial da Saúde

PCR – Proteína C Reativa

RCU – Retocolite Ulcerativa

RI – Resistência à insulina

SM - Síndrome Metabólica

SPSS® – *Statistical Package for the Social Sciences*

TCLE – Termo de Consentimento Livre e Esclarecido

TMAO – Óxido de Trimetilamina

TG – Triglicerídeos

LDL – Lipoproteína de baixa densidade

HDL – Lipoproteína de alta densidade

US – Ultrassonografia

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1. INTRODUÇÃO

As doenças inflamatórias intestinais (DII) são enfermidades crônicas de etiologia multifatorial que envolvem fatores genéticos e ambientais associados a uma resposta imunológica alterada e a disbiose e são representadas pela Retocolite Ulcerativa (RCU) e pela Doença de Crohn (DC)[1] [2]. O processo inflamatório da DC é transmural e pode acometer qualquer segmento do tubo digestivo[3], sendo a região acometida com mais frequência é a ileocecal[4]. Dentre as manifestações clínicas mais comuns da DC estão a diarreia crônica, dor abdominal, anemia, perda de peso, febre e fadiga. Os pacientes podem apresentar manifestações extra-intestinais hepatobiliares classicamente descritas como colangite esclerosante primária e colelitíase, não sendo a doença hepática gordurosa não alcoólica (DHGNA) considerada como parte destas manifestações[5]. A DC é mais prevalente na Europa e América do Norte[6]. No entanto, sua incidência no Brasil vem aumentando. Um trabalho recentemente publicado revelou que a incidência de DII no Brasil aumentou de 9,4 em 2012 para 9,6 casos/100,000 habitantes em 2020. A prevalência aumentou de 30 em 2012 para 100 casos/100,000 habitantes em 2020[7]. Concomitantemente, a prevalência de excesso de peso entre pacientes com DC é crescente, podendo chegar a 42%, mesmo em pacientes com doença ativa[8–10]. A DHGNA aumentou rapidamente em todo o mundo. A prevalência global tem sido estimada em 25,24% e já ocupa o segundo lugar entre as indicações de transplante hepático em geral, e a primeira indicação entre as mulheres[11] [12]. A obesidade é um dos fatores de risco mais importantes para o desenvolvimento de DHGNA na população em geral[13] [14]. Além da obesidade, o desenvolvimento de DHGNA tem como principais fatores de risco o diabetes, a hipertensão, dislipidemia e resistência à insulina (RI)[15] [16] [17]. Existe uma forte associação bidirecional entre DHGNA e síndrome metabólica (SM)[15], inclusive,

fazendo com que a DHGNA seja reconhecida como a manifestação hepática da SM[18] e esteja associada à morte por doenças cardiovasculares independentemente de comorbidades metabólicas[15].

Os dados de prevalência da DHGNA entre os pacientes com DII são conflitantes e a associação etiológica não é clara. Alguns autores relatam um aumento na prevalência de DHGNA em pacientes com DII superior ao encontrado entre a população em geral, podendo chegar a 44%[11 19 20]. Metanálise recente observou que a prevalência global estimada foi de 32% (IC 95%, 24 a 40%). Dos 27 estudos selecionados, 13 utilizaram métodos de imagem para o diagnóstico de DHGNA evidenciando uma prevalência de 30% (IC 95%, 22-37%) e uma heterogeneidade alta (I^2=99%)[21]. Outra revisão sistemática demonstrou que os pacientes com DII possuem uma prevalência de 30,7% (IC 95%, 26.5-34.9) e um risco 2 vezes maior para desenvolver DHGNA do que indivíduos saudáveis[22].

2. REVISÃO BIBLIOGRÁFICA

2.1. Doença de Crohn

As DII são enfermidades crônicas de etiologia multifatorial, que envolvem fatores genéticos, imunológicos, ambientais além da desregulação da microbiota intestinal. As DII são representadas pela RCU e DC[1 2]. Os fatores ambientais podem ter grande influência sobre a etiologia da DC, conforme observado nas regiões onde a prevalência e incidência são maiores. A urbanização, mudanças na dieta, hábito de fumar, uso de antibióticos, exposição à poluição, stress e à microrganismos são os principais fatores ambientais relacionados à presença de DII[23].

Dentre as manifestações clínicas mais comuns da DC estão a diarreia crônica, dores abdominais, anemia, febre e fadiga. Além disso, algumas manifestações extra-intestinais podem estar relacionadas à doença ou ao tratamento medicamentoso e incluem artropatia periférica ou axial, eritema nodoso, pioderma gangrenoso, esclerite, episclerite, uveíte, fístulas, abcessos e anemia[5]. O processo inflamatório da DC é transmural e pode acometer qualquer segmento do tubo digestivo[3], a região acometida com mais frequência é a ileocecal[4]. Durante o curso da doença, pode ocorrer a evolução para comportamento penetrante ou estenosante, causando uma série de danos e complicações ao paciente[24]. Por esses motivos, a DC representa um grande desafio à saúde pública, uma vez que requer a disponibilidade de recursos assistenciais dispendiosos[25]. Barros et al., (2017)[24] observaram que um terço dos pacientes pode sofrer alteração do comportamento clínico da doença ao longo do tempo, sendo o padrão estenosante o que mais se altera.

Os dados nacionais para prevalência e incidência de DII são escassos. No ano de 2013, verificou-se incidência de 4,48 casos por 100.000 habitantes em São Paulo[26]. Estudo realizado no Piauí e que envolve vários estados do Nordeste demonstrou aumento na incidência anual de 0,08 a 1,53 casos de DII por 100.000 habitantes nos últimos 20 anos[27]. Estudo de revisão demonstrou aumento da incidência de DC no Brasil de 0,08 por 100.000 pessoas-ano em 1988 para 5,5 em 2015[28], colocando o Brasil entre os países com maior incidência de DII[25 29].

2.2 Diagnóstico e Classificação Clínica da Doença de Crohn

Para determinar o diagnóstico de Doença de Crohn é necessário analisar diversos fatores, tais como os achados clínicos, exame físico, exames laboratoriais, imagens radiológicas, exames endoscópicos e histopatológicos[5] [30].

A principal ferramenta diagnóstica na investigação das DII é a ileocolonoscopia, que deve ser solicitada diante da suspeita de DII[30]. Além do diagnóstico, este instrumento permite graduar a atividade da doença, monitorar a resposta à terapia, extensão, avaliação histológica e investigação de complicações infecciosas e neoplásicas[31].

A principal característica endoscópica da doença é o acometimento heterogêneo e salteado da mucosa intestinal, poupando áreas adjacentes às áreas acometidas. São observadas úlceras de diversos tamanhos e com profundidades variáveis dando suporte ao diagnóstico de DC. Durante a íleocolonoscopia é importante a coleta de biópsias para avaliar se os achados histopatológicos corroboram o diagnóstico de DC[1]. Para avaliação de extensão e comportamento da doença utiliza-se a classificação de Montreal (Tabela 1)[32]. Essa classificação é útil na sistematização e auxílio do aconselhamento ao paciente, na avaliação da progressão e, por consequência, na escolha da terapia mais adequada[33].

Tabela 1. Classificação de Montreal na DC

Termo	Descrição
Idade ao diagnóstico	
A1	Até os 16 anos
A2	Entre 17 e 40 anos
A3	Após os 40 anos
Localização da doença	
L1	Ileal
L2	Colônica

L3	Ileocolônica
L4*	Trato gastrointestinal alto isolado
Comportamento da doença	
B1	Não estenosante, não penetrante
B2	Estenosante
B3	Penetrante
p**	Doença perianal (modificador)

* L4 pode ser também um modificador que pode ser acrescentado à L1-L3.

** Doença perianal é um modificador que pode ser acrescentado à B1-B3.

Para avaliação e classificação da atividade da doença são utilizados os seguintes parâmetros: Índice de Harvey-Bradshaw (IHB), uma derivação simplificada e validada do tradicional Índice de Atividade de Doença de Crohn (IADC). O IHB é comumente utilizado por ser um índice de fácil aplicação na rotina assistencial e tem como objetivo avaliar a atividade da doença. Tem como vantagem utilizar registros do dia anterior à consulta, ao contrário do IADC que exige registro dos últimos 7 dias anteriores à avaliação. O IHB inclui a avaliação da sensação de bem-estar, dor abdominal, número de evacuações líquidas diárias, presença de massa abdominal e complicações da DC (Tabela 2). O IHB classifica a atividade da DC de acordo com o seguinte: pontuação <4 caracteriza doença em remissão, entre ≥5 e ≤7 doença leve a moderada e uma pontuação ≥8 indica doença moderada a grave[34].

Tabela 2. Índice de Harvey-Bradshaw

Variável	Descrição	Escore
1	Bem estar geral	0 = muito bom 1 = bom 2 = ruim

		3 = muito ruim 4 = péssimo
2	Dor abdominal	0 = nenhuma 1 = leve 2 = moderada 3 = severa
3	Nº evacuações líquidas ao dia	1 ponto para cada evacuação
4	Massa abdominal	0 = nenhuma 1 = duvidosa 2 = bem definida 3 = bem definida e dolorosa
5	Complicações	1 ponto por item • Artralgia • Uveíte • Eritema nodoso • Úlceras aftoides orais • Pioderma gangrenoso • Fissura anal • Nova fístula anal • Abscesso

Exames laboratoriais podem sinalizar a presença de atividade da doença, sendo úteis e de fácil utilização na monitorização do processo inflamatório, anemia, desidratação, desnutrição e deficiência de micronutrientes[1] [35]. A proteína C reativa (PCR) é uma proteína de fase aguda que tem sua produção estimulada por citocinas e fator de necrose tumoral. Seu nível sérico tende a se correlacionar com a atividade da doença, porém, em torno de 20% dos pacientes não são produtores de PCR apesar da existência de inflamação[1] [35]. A presença de alterações de exames laboratoriais hepáticos pode ocorrer com frequência devido à doença hepatobiliar, como é o caso da colangite esclerosante primária, que pode estar associada à DC e à hepatotoxicidade das medicações utilizadas no tratamento[1] [36]. Até 30% dos pacientes com DII apresentam alterações em enzimas hepáticas em algum momento da

evolução da doença de modo transitório ou flutuante sem significado clínico maior, no entanto, até 5% irão desenvolver doença hepática crônica[37].

2.3 Doença Hepática Gordurosa Não Alcoólica

A DHGNA é caracterizada pelo acúmulo de gordura nos hepatócitos na ausência de causas secundárias como ingestão de álcool significativa, uso prolongado de drogas esteatogênicas e desordens hereditárias monogênicas[15]. É principalmente proveniente da lipólise do tecido adiposo em adição da resistência à insulina e dieta hipercalórica[38]. A esteatose hepática (EH) acontece quando há presença de >5% de esteatose sem evidência de balonização, inflamação ou fibrose e pode progredir para EHNA, cirrose e carcinoma hepatocelular[15]. Quando há presença de balonização hepatocitária e processo inflamatório denomina-se esteato-hepatite não alcoólica (EHNA), a qual requer diagnóstico histológico. Também é comum encontrar na literatura os termos esteatose hepática e fígado gordo ou gorduroso. Ambos englobam as classificações DHGNA e EHNA sem distinguir o nível de dano ao tecido hepático[39].

Os fatores de risco para desenvolvimento de DHGNA foram classificados em 1) primários: obesidade e sobrepeso com obesidade central; diabetes; dislipidemia (aumento do colesterol e/ou triglicerídeos) e hipertensão arterial; 2) secundários: medicamentos como amiodarona, corticosteroides, estrógenos e tamoxifeno, toxinas ambientais de produtos químicos, esteroides anabolizantes e cirurgias abdominais; e 3) doenças associadas: hepatite crônica pelo vírus C, síndrome de ovários policísticos, hipotiroidismo, síndrome de apneia do sono, hipogonadismo, lipodistrofia, abetalipoproteina, deficiência de lipase ácida lipossomal[15 40].

Existe uma forte associação bidirecional entre DHGNA e síndrome metabólica, sendo esta última definida como a presença de três ou mais fatores de risco, tais como circunferência da cintura maior que 102 cm em homens ou maior de 88 cm nas mulheres, nível de TG igual ou superior a 150 mg/dL, colesterol HDL inferior a 40 mg/dL em homens e inferior a 50 mg/dL em mulheres, pressão arterial sistólica >130 mm Hg ou pressão diastólica >85 mm Hg e nível de glicose plasmática em jejum maior ou igual a 110 mg/dL[15]. A DHGNA tem sido reconhecida como a manifestação hepática da síndrome metabólica[18] e está associada a morte por doenças cardiovasculares independente de comorbidades metabólicas[15].

Em virtude da influência metabólica sobre DHGNA uma nova nomenclatura foi sugerida para refletir os mecanismos atuais envolvidos com a esteatose hepática: a doença hepática gordurosa associada ao metabolismo ("*MAFLD", sigla em inglês*). A nova nomenclatura sugere que o diagnóstico de MAFLD seja baseado na presença de disfunção metabólica e não na exclusão de outros fatores, podendo ser definida mesmo na presença de outras doenças hepáticas, inclusive decorrentes do consumo excessivo de álcool[41].

Alterações de enzimas hepáticas como ALT, AST e GGT também podem estar presentes e devem ser investigadas em caso de suspeita de DHGNA, no entanto, não podem ser utilizadas isoladamente para determinação do diagnóstico[39].

A DHGNA é a doença hepática mais comum atualmente nos países ocidentais, afetando de 17–46% dos indivíduos adultos dependendo do método diagnóstico utilizado, idade, sexo e etinicidade[11 42]. A prevalência global de DHGNA é de 25,2%[11], entretanto estudo de revisão recente identificou aumento na frequência de DHGNA admitindo uma nova prevalência global de 29,8%[43]. Entre a população sul americana

essa prevalência é de 31% e entre os brasileiros é ainda superior, atingindo 35,2%[11] [44] [45].

2.4 Diagnóstico e classificação da Doença Hepática Gordurosa Não Alcoólica

De acordo com o "*Practice Guidance* da *American Association for the Study of Liver Diseases"*[15], o diagnóstico de DHGNA deve ser realizado mediante duas constatações: evidência de esteatose hepática por exame de imagem ou histologia e ausência de causas secundárias do acúmulo hepático de gordura, como consumo significativo de álcool (definido como >21 doses padrão por semana para homens e >14 doses padrão por semana para mulheres). Como causas secundárias de DHGNA são entendidas as doenças hepáticas tais como hepatites, hepatite autoimune, cirrose, hepatocarcinoma, ascite, colangite esclerosante primária, doença de Wilson, hemocromatose, síndrome dos ovários policísticos e uso de nutrição parenteral[15].

O exame teste padrão para diagnóstico da DHGNA é a biópsia. Porém, por se tratar de um método invasivo não é possível utilizá-lo frequentemente[15]. Os métodos mais utilizados são os de diagnóstico por imagem, como é o caso da ultrassonografia, a qual é mais acessível, menos invasiva e de baixo custo, sendo, portanto, amplamente utilizada[46] [47]. De acordo com uma metanálise, a ultrassonografia apresentou sensibilidade de 85% e especificidade de 94% para medir gordura hepática. Além disso, demonstrou ser tão sensível (94%) e específica (80%) quanto os exames de tomografia e ressonância magnética[48].

Alguns autores utilizam diferentes métodos diagnósticos para detectar DHGNA em um mesmo grupo, o que pode prejudicar a confiabilidade dos dados. Além disso,

algumas populações estudadas possuem doenças prévias que podem estar relacionadas ao desenvolvimento da DHGNA[49 50 51].

2.5 Doença de Crohn e Doença Hepática Gordurosa Não Alcoólica

Apesar de encontrar estudos que justifiquem a prevalência de baixo peso em pessoas com DC, estudos recentes mostram que nos últimos anos houve uma transição para o excesso de peso simultaneamente à população em geral[8 9]. O excesso de peso é um dos fatores de risco mais importantes para o desenvolvimento da DHGNA[13 14 52]. A prevalência de excesso de peso em pacientes com DII é variável, podendo chegar a 42% mesmo em pacientes com doença ativa[8 53]. Nosso grupo realizou uma avaliação nutricional nos pacientes com DC em remissão nesta mesma população constatando uma prevalência de 25,3% de sobrepeso e 12% de obesidade[54]. Segundo dados do Vigitel (2019), a prevalência brasileira de excesso de peso e obesidade em adultos é de 55,4% e 19,8%, respectivamente[55].

Em paralelo ao aumento das prevalências de DHGNA global e excesso de peso entre pessoas com DII, a DHGNA tem sido cada vez mais frequente entre essa população[11]. Estudo de revisão sistemática com metanálise encontrou prevalência de 27,5% de DHGNA em pacientes com DII[17]. Simon et al., (2018)[56] demostraram prevalência de 52% de DHGNA em pacientes com DII nos Estados Unidos da América (EUA). Em estudo retrospectivo, os autores analisaram uma amostra de 70 pacientes com DC atendidos em um hospital na cidade do Kansas, EUA, e encontraram uma prevalência de 44% de DHGNA e concluíram que existe forte associação entre DII e risco de desenvolver DHGNA (OR=4,53)[19].

Estudo realizado por Carrillo-Palau et al., (2021)[57] identificou que a resistência à insulina não está relacionada às características da doença em pacientes com DII, como o padrão de acometimento, atividade clínica e marcadores inflamatórios, mas mostrou-se positivamente associada à obesidade, circunferência abdominal, triglicerídeos (TG) e a presença de DHGNA.

Os mecanismos pelos quais a DHGNA se desenvolve no contexto das DII ainda são pouco conhecidos, no entanto, podem estar relacionados à má absorção ou comprometimento metabólico[37] e são mais prevalentes na DC do que na RCU[58]. Alguns estudos relacionam à hepatotoxidade do tratamento medicamentoso para DII, como os corticosteroides, imunomoduladores e anti-TNF[17 37 49 59], ao histórico de ressecções intestinais[17 49 59], ao tempo de duração da doença[50 59], às alterações na microbiota intestinal[60] e aos fatores de risco para SM[20 50 59].

Por outro lado, estudos tem demonstrado resultados controversos quanto aos fatores de risco para DHGNA em DII. Kang et al., (2019)[60] sugerem que o uso de anti-TNF pode ser um fator protetor para DHGNA em pacientes com DII, por estar relacionado à melhora da RI. Likhitsup et al., (2019)[19], Bessissow et al., (2016)[59] e Glassner et al., (2017)[50] não encontraram relação entre o IMC e o desenvolvimento de DHGNA em pacientes com DII, sugerindo que as DII possuem fatores de risco independentes para DHGNA.

2.6 Marcadores antropométricos e consumo alimentar

O IMC é extensamente utilizado para classificar o estado nutricional de indivíduos na prática clínica por ser uma medida simples, prática e sem custo, porém, não é capaz de refletir a composição corporal[61]. Apesar de ser um marcador

importante principalmente em condições metabólicas, a medida do percentual de gordura corporal não considera a distribuição da gordura, sendo necessária a associação de métodos de avaliação específicos, como por exemplo, a medida da circunferência abdominal (CA). A gordura visceral reflete de uma forma mais precisa os riscos envolvidos com doenças metabólicas e cardiovasculares[61]. Recentemente, verificou-se que a gordura visceral medida pelo diâmetro abdominal sagital (DAS) demonstrou correlação maior do que outras medidas antropométricas na avaliação de marcadores de risco cardiovascular[62] [63]. É conhecida a associação entre SM, percentual de gordura corporal e DHGNA[64], porém há escassez de estudos que avaliaram a relação da gordura corporal e visceral com o desenvolvimento de DHGNA em pacientes com DC[14].

Outro fator associado à DHGNA e à DII é o padrão de dieta ocidental caracterizado pelo consumo de maiores quantidades de alimentos de origem animal e laticíneos, açúcares refinados, alimentos ultraprocessados e a redução do consumo de fibras[25] [65]. Por outro lado, uma dieta rica em frutas e vegetais, rica em ácidos graxos ômega 3 e baixo teor de ácidos graxos ômega 6 está associada a uma diminuição do risco de desenvolver DII e DHGNA[65].

Apesar de não haver uma dieta que possa promover a remissão clínica de pessoas com DII ativa, é importante garantir o aporte nutricional adequado e monitorar as carências nutricionais comumente presentes devido às condições clínicas, endoscópicas e metabólicas presentes na DC[65] [66]. É comum encontrar deficiências de micronutrientes como ferro, cálcio, vitamina B12 e vitamina D que devem ser corrigidas através de suplementação e adequação da dieta simultaneamente[65] [66]. A vitamina C também é um antioxidante importante envolvido em diversos processos

metabólicos, incluindo a cicatrização e, também pode estar sendo subconsumida entre pessoas com DII devido ao baixo consumo de frutas e vegetais[67] [68].

O consumo de carboidratos refinados em excesso tem sido associado ao risco de DHGNA devido ao poder indutor de lipogênese hepática[69]. O consumo excessivo de açúcares aumenta o acúmulo de gordura visceral, aumentando também os TG plasmáticos, ambos fatores de risco para DHGNA[69]. Entre as gorduras, observa-se que o desequilíbrio entre as proporções de consumo de gorduras polinsaturadas, saturadas e trans está associado à piora da DHGNA[70] [71] [72]. A alimentação é uma característica importante no manejo da DHGNA e DC e sua qualidade pode estar relacionada com o prognóstico em ambas[66] [73].

Em pacientes com DII, também é comum a presença de disbiose devido à natureza inflamatória e acometimento do tecido intestinal[74]. Consequentemente, existe uma redução na diversidade da microbiota intestinal ao mesmo tempo em que há aumento na permeabilidade intestinal relacionada ao estado inflamatório sistêmico[74] [75]. É importante ressaltar que o intestino e o fígado são órgãos estreitamente ligados. Por essa razão, alterações na microbiota intestinal também afetam a saúde hepática, podendo agravar doenças pré-existentes e aumentar o risco de EH[75]. À medida em que ocorrem mudanças na composição da microbiota intestinal, é natural que também haja alterações na síntese de determinados metabólitos como ácidos graxos de cadeia curta, lipopolissacarídeos, ácidos bliares, amônia e óxido de trimetilamina (TMAO)[76].

O TMAO é o metabólito final da carnitina e colina, substâncias provenientes de alimentos de origem animal consumidos através da dieta, que após ingeridos são convertidos em trimetilamina pela microbiota intestinal (*Clostridium asparagiforme,*

Clostridium sporogenes, Clostridium hathewayi, Escherichia fergusonii, Anaerococcus hydrogenalis e Proteus penneri)[77] e logo metabolizados pelo fígado em TMAO[73]. O TMAO tem sido descrito como um marcador inflamatório e frequentemente relacionado à aterosclerose e doenças cardiovasculares[78]. Recentemente, descobriu-se que o TMAO pode ser um dos metabólitos associados com a patogênese da DHGNA[79], pois níveis aumentados de TMAO são encontrados em pacientes com DHGNA quando comparados com pessoas saudáveis[76]. O TMAO pode atuar reduzindo a tolerância à glicose e favorecendo o acúmulo de gordura nos hepatócitos[76]. Além disso, níveis plasmáticos de TMAO tem sido positivamente associados à obesidade, DM e mortalidade por todas as causas em pessoas com DHGNA, comprovando que existe uma forte relação entre TMAO e DHGNA[80 81]. Por esse motivo, o TMAO pode ser um novo marcador para SM e DHGNA[82]. Apesar de também haver envolvimento entre os níveis plasmáticos de TMAO e as DII[73], pouco se sabe sobre a relação do TMAO com a DHGNA em pacientes com DII diante da composição anormal da microbiota intestinal e marcadores inflamatórios semelhantes entre ambas as doenças[73 83 84].

3. JUSTIFICATIVA

Estudos demonstram aumento da ocorrência de DHGNA na população com DII sendo quase 2 vezes mais prevalente se comparada a população em geral. O crescimento da prevalência de sobrepeso e obesidade na população com DII assemelha-se à prevalência global e pode ser uma das explicações, no entanto, não parece ter uma correlação proporcional que explique completamente o aumento das taxas de DHGNA nestes indivíduos. A literatura cogita a possibilidade de correlação entre o tratamento medicamentoso para DII e hepatotoxicidade, como os

corticosteroides, imunomoduladores e anti-TNF, o histórico de ressecções intestinais, ao tempo de duração da doença, às alterações na microbiota intestinal e aos fatores de risco para SM, que além do excesso de peso, incluem RI, hiperlipidemia, hipertensão arterial, diabetes e CA aumentada. Os resultados dos estudos realizados até o momento são controversos quanto aos fatores de risco para DHGNA em pessoas com DII sugerindo que as DII possuem fatores de risco independentes para DHGNA. Existe uma necessidade de conhecer a prevalência da DHGNA entre os pacientes com DC no nosso meio e compreender melhor os aspectos relacionados ao desenvolvimento da DHGNA em pacientes com DII e o papel do TMAO neste contexto.

4. QUESTÃO DE PESQUISA

Qual é a prevalência da Doença Hepática Gordurosa Não Alcoólica em pacientes com Doença de Crohn? E quais fatores de risco estão relacionados com a etiologia da DHGNA nesta população?

5. OBJETIVOS

5.1 Objetivo Principal

Verificar a prevalência e os fatores de risco para Doença Hepática Gordurosa Não Alcoólica em pacientes com Doença de Crohn.

5.2 Objetivos Secundários

1) Avaliar a prevalência de Doença Hepática Gordurosa Não Alcoólica em pacientes com Doença de Crohn comparando com a prevalência global;

2) correlacionar as características e as medicações utilizadas na DC com o diagnóstico de DHGNA

3) Avaliar a correlação entre o diagnóstico de DHGNA e a avaliação antropométrica;

4) Avaliar a qualidade nutricional através da ingestão alimentar e sua correlação com o risco de desenvolver DHGNA;

5) Avaliar os níveis de Óxido de Trimetilamina e sua correlação com a DHGNA;

6) Avaliar os níveis de Óxido de Trimetilamina e sua correlação com a atividade da Doença de Crohn.

6. HIPÓTESE

A prevalência de DHGNA é maior e apresenta fatores de risco específicos na população com DC se comparada à população em geral.

7. ARTIGO ORIGINAL

O artigo intitulado “High prevalence and risk factors for nonalcoholic fatty liver disease in patients with Crohn's disease” será submetido à revista Journal of Gastroenterology and Hepatology, com um fator de impacto de 4,36 (2021) e classificada como B1 em Ciências Biológicas III (https://onlinelibrary.wiley.com/journal/14401746).

Title: High prevalence and risk factors for nonalcoholic fatty liver disease in patients with Crohn's disease

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Abbreviations: NAFLD: Non-alcoholic fatty liver disease, NASH: Nonalcoholic Steatohepatitis, IBD: inflammatory bowel diseases, CD: Crohn's disease, UC: ulcerative colitis, TMAO: trimethylamine N-oxide, DM: diabetes mellitus, SAH: systemic arterial hypertension, AC: abdominal circumference, SAD: sagittal abdominal diameter, TG: triglycerides, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyltransferase, anti-TNF alfa: alpha anti tumor necrosis factor, IR: insulin resistance, PCR: C-reative protein, BMI: body mass index, AUDIT: Alcohol Use Disorders Identification Test, LDL: low density lipoprotein, HDL: high density lipoprotein, US: ultrasonography.

ABSTRACT

There has been a significant increase in the prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) in people with Inflammatory Bowel Disease (IBD), particularly Crohn's Disease (CD). Despite this, the mechanisms and risk factors of NAFLD in IBD are still poorly understood and controversial. The aim of this study was to assess the prevalence and risk factors for NAFLD in patients with CD. Material and methods: prospective study conducted among consecutive patients diagnosed with CD treated at the Inflammatory Bowel Diseases outpatient clinic of Hospital de Clínicas de Porto Alegre between July 2021 and May 2022. Demographic, anthropometric and CD characteristics data were collected, food intake, previous and current medications, pre-existing diseases, laboratory tests, plasma levels of trimethylamine oxide (TMAO). All participants underwent ultrasound (US) of the upper abdomen for the diagnosis and classification of NAFLD. Results: 103 patients with a mean age of 45.2 ± 15.0 years, 35.9% (n=37) were male. The prevalence of NAFLD was 40.8% (n=42) and overweight (BMI>25kg/m²) was present in 62.1%. Other associated diseases were present among NAFLD patients: systemic arterial hypertension (SAH) 4.9% (p<0,001), dyslipidemia 11.9% (p=0,040) and diabetes (DM) 19% (p=0,009). There were no statistically significant differences between CD characteristics and activity, previous or current CD treatment and food consumption with NAFLD. The presence of comorbidities such as SAH, DM and dyslipidemia, anthropometric markers such as Body Mass Index (BMI), waist circumference (AC), sagittal abdominal diameter (SAD), laboratory tests such as C-reactive protein (CRP), triglycerides (TG) and Liver enzymes alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) and alkaline phosphatase were positively associated with the diagnosis of NAFLD. Plasma levels of TMAO showed no significant association with the presence of NAFLD and with CD activity, but correlated with SAD (p 0,047). After adjusting for the multivariate model, only the variables SAH (p 0,046), DM (p 0,017), active CD (p 0,023), overweight (p 0,025) and obesity (p<0,001) remained associated to the presence of NAFLD. Conclusion: The presence of NAFLD in this sample was related to similar metabolic risk factors for the general population (systemic arterial hypertension, diabetes, dyslipidemia, visceral fat by sagittal abdominal diameter, overweight and obesity). The characteristics of CD, nutrient intake and plasma levels of TMAO showed no relation with the presence of NAFLD. Contrary to what might be expected, Crohn's disease activity by IHB and PCR showed a positive correlation with NAFLD.
KEYWORDS: Non-Alcoholic Fatty Liver Disease, Hepatic Steatosis, Inflammatory Bowel Disease, Crohn's Disease.

INTRODUCTION

Crohn's disease (CD) is a chronic disease of multifactorial etiology that involves genetic, immunological and environmental factors, in addition to dysregulation of the intestinal microbiota[1] [2]. The incidence and prevalence of CD has been increasing in Brazil. A study recently revealed that the incidence of inflammatory bowel disease (IBD) in Brazil increased from 9,4 in 2012 to 9,6/100,000 inhabitants in 2020. The prevalence increased from 30 in 2012 to 100/100,000 inhabitants in 2020[3]. Concomitantly, the prevalence of overweight among patients with CD is increasing, reaching 42% even in patients with active disease[4] [5]. Obesity is one of the most important risk factors for the development of Non-alcoholic fatty liver disease (NAFLD) in the general population[6–9].

There is an increase in the prevalence of NAFLD among patients with CD reaching 44%[10–12]. The mechanisms involved between NAFLD in CD are still poorly understood[13]. Authors relate to the hepatotoxicity of drug treatment for IBD[8] [4–16], history of intestinal resections[8] [14] [15], the duration of the disease[8] [17], changes in the gut microbiota[18] and risk factors for metabolic syndrome (MS)[8] [11] [17]. In addition, some intestinal microbiota metabolites, such as trimethylamine (TMA), converted to trimethylamine oxide (TMAO) by the liver, may be involved[19]. Despite this, it is not entirely clear whether IBD have specific risk factors for NAFLD[8] [10] [17]. Our objective was to evaluate the prevalence and risk factors for NAFLD among outpatients with CD in a service specializing in inflammatory bowel diseases in southern Brazil.

MATERIALS AND METHODS

The sample consisted of patients over 18 years of age with a confirmed diagnosis of Crohn's disease for at least six months and who underwent regular follow-up at the Inflammatory Bowel Disease outpatient clinic of Hospital de Clínicas de Porto Alegre (a public referral hospital in southern Brazil). Considering the global prevalence of NAFLD of 25,2%[12], a confidence level of 95% and a confidence interval of 18% required a sample of 90 subjects. Adding 10% for possible losses and refusals, the minimum sample size was 100 people. Patients who used alcohol regularly were excluded, considering daily alcohol consumption ≥30g for men and ≥20g for women, patients who had a history of liver diseases, such as hepatitis, including autoimmune hepatitis, cirrhosis, hepatocarcinoma, primary sclerosing cholangitis, Wilson's disease, hemochromatosis, polycystic ovary syndrome, patients with a history of parenteral nutrition, malignancies or any transplants that characterized chronic use of corticosteroids and pregnant women. Demographic data, CD characteristics, comorbidities and alcohol consumption were collected.

The clinical data analyzed were systemic arterial hypertension, diabetes, dyslipidemia and hypothyroidism. Systemic arterial hypertension was considered according to the criteria established by the World Health Organization (2021)[20]. The diagnosis of diabetes was based on the Classification of diabetes mellitus published by the World Health Organization (2019)[21]. Dyslipidemia was classified according to criteria established by the Brazilian Guidelines on Dyslipidemias and Prevention of Atherosclerosis of the Brazilian Society of Cardiology (2017)[22]. Participants with fasting glucose >100mg/dL and <126mg/dL were included in a subcategory: pre-diabetes (pre-DM)[21]. Participants with a medical history of hypothyroidism undergoing treatment (hormone replacement) were classified as having hypothyroidism.
Laboratory tests for liver enzymes (AST, ALT, GGT, alkaline phosphatase), lipid profile, fasting glucose and C-reactive protein (CRP) were performed. Biochemical tests were considered altered according to local reference values. For the lipid profile, the reference values described in the Brazilian Guideline on Dyslipidemia and Prevention of Atherosclerosis Brazilian Society of Cardiology (2017)[22] were considered. These exams were performed under fasting conditions (minimum 8 hours).

Anthropometric assessment. Weight and height were measured according to established standards[23]. The classification of nutritional status was evaluated through the Body Mass Index (BMI), calculated through the equation: weight divided by height squared according to the criteria of the World Health Organization (2006)[24]. BMI was classified as underweight <18,5kg/m², normal weight if BMI between 18,5 and 24,9kg/m², overweight if BMI between 25 and 29,9kg/m², and obesity if BMI >30kg/m² [24]. Abdominal circumference (AC) was measured in an orthostatic position at the end of expiration at the midpoint between the lower costal margin and the iliac crest and classified according to the cutoff points proposed by National Cholesterol Education Program (NCEP) – Adult Treatment Panel III (ATP-III)[25]: >102 cm for men and >88 cm for women for increased risk of cardiovascular disease[26]. The sagittal abdominal diameter (SAD) was obtained with a portable Holtain-Kahn sliding-beam abdominal caliper with a 36 cm rod (HaB International Ltd) in the supine position with the knees slightly bent on a stretcher. The measurement was taken at the level of the navel[23] [27]. SAD was considered abnormal if >21,4cm for men (AUC=0,74, Sensitivity: 62,5% and specificity: 61,9%) (Figure 1) and >20,4cm for women (AUC=0,80, Sensitivity: 76,9% and specificity: 75%) (Figure 2) (cutoff points stipulated by the ROC curve).

Figure 1 and 2 - Cutoff point for sagittal abdominal diameter for male and female according to ROC curve (n=103).

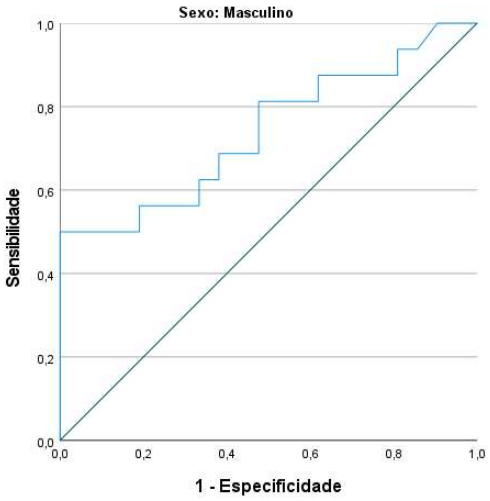

Figure 1. ROC curve. AUC=0,74; IC 95%: 0,57 a 0,91; Cutoff point>21,4: Sensibility: 62,5% and Specificity: 61,9%

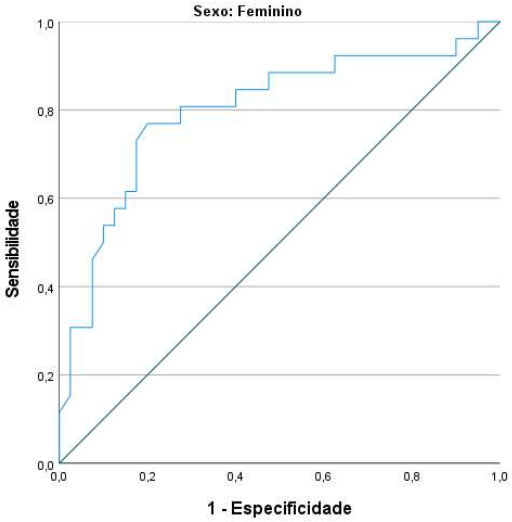

Figure 2. ROC curve. AUC=0,80; IC 95%: 0,68 a 0,92; Cutoff point>20,4: Sensibility: 76,9% and Specificity: 75%

The percentage of body fat was obtained using a tetrapolar bioimpedance scale model HBF-514C (Onron)[28–30]. Participants were previously instructed regarding preparation for the bioimpedance exam. All equipment used for anthropometric measurements was within the calibration period. Body fat percentage was considered high if >33% for women and >25% for men as suggested by the Latin American Consensus Document on Obesity, Abeso (1998)[31], also used by Soare et al., (2022)[32].

Diagnosis, activity and treatment of Crohn's disease. The diagnosis, extent and phenotype of CD were evaluated based on information from the electronic medical record and were classified according to the Montreal Classification[33]. Disease activity was classified according to the Harvey Bradshaw Index, adopting a score of <5 as disease in remission, ≥5 and ≤7 as mild to moderate disease, and ≥8 as moderate to severe disease[34]**.** Laboratory evaluation was also performed based on the CRP value and radiological and endoscopic evaluation based on the latest imaging tests available in the medical records (tomography, ultrasound and colonoscopy). Due to the fact that the study started during the COVID-19 pandemic, endoscopic data from the last 2 years were used. Participants with a history of bowel resections were included in the bowel resection classification. The use of drugs for the treatment of CD was classified as follows: immunosuppressants (Azathioprine and Methotrexate), Biologicals (Anti-TNF, Anti-IL23 and Anti-Integrin) and corticosteroids (prednisone and budesonide). The use of medications was unified according to their category due to the small sample size.

Current corticosteroid use was considered if the participant was using corticosteroids during the assessment. Previous use of corticosteroids was considered if the participant had used it during the last 12 months.

Diagnosis of Non-Alcoholic Fatty Liver Disease. For the diagnosis and classification of NAFLD, all participants underwent upper abdominal assessment with ultrasound examination, with a minimum fasting of 6 hours. Ultrasonography is able to detect the presence of steatosis if it is occurring in more than 10% of hepatocytes. Although it is not the gold standard instrument for the evaluation of hepatic steatosis, ultrasound is an accessible, non-invasive method that has high sensitivity[37]. All examinations were performed by the same radiologist with extensive experience and who was not aware of the clinical data of the participants. The diagnosis of Non-Alcoholic Fatty Liver Disease was defined according to the Practice Guidance of the American Association for the Study of Liver Diseases[35] according to the following criteria: imaging evidence of hepatic steatosis and absence of secondary causes of hepatic fat accumulation, such as significant alcohol consumption (daily alcohol consumption ≥30 g for men and ≥20 g for women) and other conditions as described in the exclusion criteria[36]. The degrees of hepatic steatosis were classified by ultrasound of the upper abdomen. Ultrasonography was performed with a convex, dynamic transducer with a frequency of 3.75MHz. Parenchyma texture was observed, whether heterogeneous or homogeneous, and hepatic steatosis was classified into degrees: grade 0, normal echogenicity; grade 1, mild steatosis, with visualization of fine echoes of the liver parenchyma, normal diaphragm and intrahepatic vessels; grade 2, moderate steatosis, with diffuse increase in fine echoes, impaired visualization of intrahepatic vessels and diaphragm; grade 3, marked steatosis, with marked increase in fine echoes and impaired or absent visualization of intrahepatic vessels[37]. Alcohol consumption was quantified using the AUDIT questionary[38], using only questions 1 to 3 and considering the content of 10g of pure alcohol per dose. The values used to calculate the dose were as follows: 1 beer (330ml) = 13g of pure alcohol; 1 glass of wine (140ml) = 13,3g of pure alcohol and 1 shot of distilled beverage (40ml) = 12,6g of pure alcohol[38].

Food Consumption. Food consumption was assessed through a non-consecutive 3-day food diary and the filling was reviewed together with each participant to minimize losses or forgetfulness by qualified nutritionist. The nutritional calculation of the food diaries was performed using the WebDiet nutrition software version 3.0[39] and the average of the 3 diaries was admitted for the values of final consumption of nutrients. Nutrients were grouped as follows: total calories, macronutrients (carbohydrates, proteins, total fats, mono and polyunsaturated fats, trans fats and cholesterol), micronutrients (iron, calcium, vitamins C and B12) and fiber. Carbohydrates, proteins and lipids were evaluated in grams, caloric percentage and adequacy of consumption. Adequacy of intake was considered according to acceptable

distribution ranges for macronutrient intake among adults over 18 years of age Dietary Reference Intakes[40]: carbohydrates between 45 and 65%, proteins between 10 and 35% and fats between 20 and 35% of the daily caloric percentage.

Trimethylamine Oxide. Blood samples were collected in 6ml EDTA tubes centrifuged at 14,000 rpm for 30 min at 4°C. One milliliter aliquots of plasma were stored at -80°C until analysis. Into a 20 µL aliquot of plasma, 60 µL of a methanolic solution containing the internal standard (100 ng/mL) and 240 µL of a cold acetonitrile were added. The mixture was shaken for 15 seconds and centrifuged at 12,000 rpm for 10 minutes. Afterward, 10 µL of the supernatant was injected into the LC-MS/MS. The analyses were performed in a Nexera-I LC-2040C Plus system coupled to an LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Japan). The chromatographic separation was achieved with a Syncronis HILIC column (100 mm × 4.6 mm, 5 µm, Thermo Scientific, Wilmington, DE, USA) eluted with a flow rate of 0.6 mL/min and 40 °C, with a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as follows: 0 - 2.5 min, 0 - 100% of B; 2.5 - 4.0 min, 100% of B; 4.0 - 4.2 min, 100-0% of B; and 4.2 - 10 min, 0% of B. The electrospray parameters were set in the positive ion mode as follows: capillary voltage, 4500 V; desolvation line temperature, 250 °C; heating block temperature, 400 °C; drying gas flow, 10 L/min; and nebulizing gas flow, 3 L/min. Collision-induced dissociation was obtained with 230 kPa argon pressure. The analysis was performed in multiple reaction monitoring (MRM) mode. MS-extracted ion chromatograms were generated using the following transitions: m/z 76.0 → 58.1 / 59.2 / 42.1 for detection of TMAO and m/z 85.1 → 66.1 / 68.2 / 46.2 for detection of TMAO-d9. One quantifier transition and two qualifier transition were selected for each compound. For data evaluation, LabSolutions® software (Shimadzu, Japan) was used for the data treatment. The calibration curve contained 10 – 10,000 ng/mL. The analyzes were carried out in the Analytical Center laboratory of the Federal University of Health Sciences of Porto Alegre, which has experience in this technique.

Statistical analysis. Quantitative variables were described as mean and standard deviation or median and interquartile range, depending on data distribution. Qualitative variables were described by absolute and relative frequencies. To compare means between groups, the t-student test was applied. In case of asymmetry, the Mann-Whitney test was used. In comparing proportions, Pearson's chi-square or Fisher's exact tests were applied. The Receiver Operating Characteristic (ROC) curve was used to verify cut-off points for SAD and TMAO. To control confounding factors, the Poisson Regression model was applied. The criterion for the entry of the variable in the multivariate model was that it presented a value of $p<0,20$ in the bivariate analysis and the criterion for permanence in the final model was that it presented a

value of p<0,10. The significance level adopted was 5% and the analyzes were performed using the Statistical Package for the Social Sciences (SPSS)® version 28.0.

Ethical aspects. The present study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (HCPA) under CAAE: 26817019.1.0000.5327. All participants signed the Free and Informed Consent Term.

RESULTS

During the period from July 2021 to May 2022, 203 patients were invited to participate in the study. 63 patients refused to participate, 33 patients did not return for the second interview, 1 patient was excluded due to alcohol use greater than permitted and 3 patients were approached but were not included due to ileostomy use, pregnancy and low educational level. The final sample consisted of 103 patients.

According to the ultrasound results, 40.8% (42) of the patients had NAFLD. Of these, 64.2% (27) were grade 1 (mild), 21.4% (9) grade 2 (moderate) and 14.2% (6) grade 3 (severe).

Demographic and Crohn's disease characteristics are described in Table 1. Females corresponded to 64.1% (n=66) of the sample and the mean age was 45.2±15.0 years. 50.5% (n=52) of patients were diagnosed with CD between 16 and 40 years of age and 29.1% (n=30) had at least one bowel resection surgery. The mean duration of CD was 12.3 years (SD ± 9.0). The most prevalent CD location was ileocolonic and the most frequent phenotype was non-stricturing and non-penetrating 48.5% (n=50), followed by stricturing 29.1% (n=30).

Current use of biologics was observed in 68 (66%) patients and was more frequent among patients without NAFLD, which is similar to the current use of immunosuppressants. Patients with hepatic steatosis were more frequently on current use of corticosteroids. However, no statistical value was assigned between the use of medication for the treatment of CD and the outcome. No patient was classified as steroid dependent.

Table 1 - Demographic and Crohn's Disease characteristics, medication use and relation with NAFLD (n=103).

Variables	**Total sample (n=103; 100%)**	**With NAFLD (n=42; 40,8%)**	**Without NAFLD (n=61; 59,2%)**	**p**
Age (years) – mean ± SD	45,2 ± 15,0	48,0 ± 14,6	43,3 ± 15,2	0,116
Gender – n(%)				0,863
Male	37 (35,9)	16 (38,1)	21 (34,4)	
Female	66 (64,1)	26 (61,9)	40 (65,6)	
Age at diagnosis – n(%)				0,450
Before 16 years	16 (15,5)	5 (11,9)	11 (18,0)	
Between 16 and 40 years	52 (50,5)	20 (47,6)	32 (52,5)	
After 40 years	35 (34,0)	17 (40,5)	18 (29,5)	
Resection – n(%)				1,000

No	73 (70,9)	30 (71,4)	43 (70,5)	
Yes	30 (29,1)	12 (28,6)	18 (29,5)	
DC (duration in years) mean ± SD	12,3 ± 9,0	13,0 ± 9,3	11,9 ± 8,7	0,061
Localization – n(%)				0,751
Ileum	32 (31,1)	14 (33,3)	18 (29,5)	
Colon	23 (22,3)	8 (19,0)	15 (24,6)	
Ileocolonic	47 (45,6)	20 (47,6)	27 (44,3)	
Isolated GI tract	1 (1,0)	0 (0,0)	1 (1,6)	
Phenotype – n (%)				0,725
Stricturing + Penetrant	13 (12,6)	4 (9,5)	9 (14,8)	
Non-stricturing non-penetrating	50 (48,5)	23 (54,8)	27 (44,3)	
Stricturing	30 (29,1)	11 (26,2)	19 (31,1)	
Penetrating	10 (9,7)	4 (9,5)	6 (9,8)	
Perianal disease– n(%)				0,970
No	75 (72,8)	30 (71,4)	45 (73,8)	
Yes	28 (27,2)	12 (28,6)	16 (26,2)	
Current treatment for CD – n(%)				
Immunosuppressants	64 (62,1)	24 (57,1)	40 (65,6)	0,509
Biologic	68 (66,0)	28 (66,7)	40 (65,6)	1,000
Corticosteroids	12 (11,7)	7 (16,7)	5 (8,2)	0,221
Prior treatment for CD – n(%)				
Immunosuppressants	44 (42,7)	17 (40,5)	27 (44,3)	0,858
Biologic	24 (23,3)	10 (23,8)	14 (23,0)	1,000
Corticosteroids	17 (16,5)	7 (16,7)	10 (16,4)	1,000

NAFLD: non-alcoholic fatty liver disease; CD: Crohn's disease; GI tract: gastrointestinal tract.
Quantitative variables described by mean and standard deviation (SD). Qualitative variables described by absolute and relative frequencies. *p value was considered statistically significant if <0.05.

The comorbidities and clinical activity of CD are described in Table 2. SAH, dyslipidemia and DM showed a positive relationship with NAFLD (p<0,05). Most patients (77.7%) were in clinical remission of CD according to the IHB classification. Even so, 40.8% of the sample had a CRP value above the laboratory reference value and the median CRP was higher in the NAFLD group (p<0,033) when compared to the group without NAFLD.

Table 2. Comorbidities and clinical activity of Crohn's Disease and relation with NAFLD (n=103).

Variables	**Total sample (n=103; 100%)**	**With NAFLD (n=42; 40,8%)**	**Without NAFLD (n=61; 59,2%)**	**p**
Comorbidities – n (%)				
SAH	24 (23,3)	18 (42,9)	6 (9,8)	<0,001
Dyslipidemia	6 (5,8)	5 (11,9)	1 (1,6)	0,040
Dyslipidemia without treatment	43 (41,7)	15 (35,7)	28 (45,9)	0,408
Hypothyroidism	5 (4,9)	1 (2,4)	4 (8,6)	0,646
DM groups				0,009
Without DM	88 (85,4)	32 (76,2)	56 (91,8)*	
Pre-DM	6 (5,8)	2 (4,8)	4 (6,6)	
DM	9 (8,7)	8 (19,0)*	1 (1,6)	
DC Activity Index - IHB				0,062
Disease in remission	80 (77,7)	28 (66,7)	52 (85,2)	
Mild to moderate disease	15 (14,6)	10 (23,8)	5 (8,2)	
Moderate to severe disease	8 (7,8)	4 (9,5)	4 (6,6)	
CRP – median (P25-P75)	3,5 (1 – 8,7)	5,1 (1,4 – 13,6)	2,1 (1,0 – 6,0)	0,033
CRP above range	42 (40,8)	21 (50,0)	21 (34,4)	0,169

NAFLD: non-alcoholic fatty liver disease; CD: Crohn's disease; SAH: systemic arterial hypertension;
DM: diabetes mellitus; CRP: C-reactive protein.

Quantitative variables described by mean and standard deviation (SD) or median. Qualitative variables described by absolute and relative frequencies. *p value was considered statistically significant if <0,05

As for the anthropometric characteristics described in Table 3, it appears that 35.9% of the sample were overweight and 26.2% were obese according to the BMI classification. The sagittal abdominal diameter indicated that 47 (45.6%) participants had the measurement above the cutoff points according to sex (>21,4cm for men >20,4cm for women). A positive relationship was found between AC and SAD with the presence of NAFLD (p<0,001).

Regarding body composition, 72.8% of patients had a body fat percentage above 33% for women and above 25% for men. Although there is no statistical significance between this variable and the presence of hepatic steatosis, it is observed that 83.3% of the sample with NAFLD have excess body fat compared to 65.5% of patients without the outcome.

The characteristics of food consumption are also described in Table 3. The mean energy consumption was 1875 ± 740 in kcal/day. No significant differences were observed between the percentage of consumption of carbohydrates, total fats and proteins between the groups. When analyzing the average distribution of carbohydrates and proteins, no relevant values of inadequate distribution were found that could be analyzed separately. Analyzing the results for micronutrients, very similar consumption averages were assumed for iron, calcium and vitamins C and B12. There is a low intake of fiber, calcium and vitamins C between both groups.

Table 3. Anthropometric data, food consumption and relation with NAFLD (n=103).

Variables	**Total sample (n=103; 100%)**	**With NAFLD (n=42; 40,8%)**	**Without NAFLD (n=61; 59,2%)**	**p**
BMI (kg/m^2) – mean ± SD	27,4 ± 5,7	31,1 ± 5,9	24,8 ± 3,9	<0,001
BMI classification – n(%)				<0,001
Underweight	4 (3,9)	0 (0,0)	4 (6,6)	
Normal weight	35 (34,0)	5 (11,9)	30 (49,2)	
Overweight	37 (35,9)	15 (35,7)	22 (36,1)	
Obesity	27 (26,2)	22 (52,4)	5 (8,2)	
AC classification – n(%)				<0,001
Normal	58 (56,3)	13 (31,0)	45 (73,8)	
High	45 (43,7)	29 (69,0)	16 (26,2)	
SAD classification – n(%)				<0,001
Normal	56 (54,4)	12 (28,6)	44 (72,1)	
High*	47 (45,6)	30 (71,4)	17 (27,9)	
Excess body fat – n(%)				0,077
No	28 (27,2)	7 (16,7)	21 (34,4)	
Yes	75 (72,8)	35 (83,3)	40 (65,6)	
Energy consumption (Kcal)	1875 ± 740	1783 ± 758	1938 ± 726	0,299
Carbohydrate %	50,9 ± 7,1	50,5 ± 6,8	51,2 ± 7,4	0,604
Carbohydrate (g)	242,9 ± 76,3	238,5 ± 76,4	245,9 ± 76,8	0,628
Fiber (g)	17,2 ± 7,0	17,0 ± 7,4	17,3 ± 6,8	0,835
Protein %	18,1 ± 4,1	18,8 ± 4,2	17,7 ± 4,1	0,157
Protein (g)	85,9 ± 32,1	87,7 ± 33,1	84,7 ± 31,6	0,652
Total fat %	30,9 ± 5,6	30,7 ± 4,9	31,1 ± 6,1	0,705
Total fat (g)	66,9 ± 25,6	65,7 ± 26,2	67,7 ± 25,4	0,690

Above the RDA – n(%)	18 (17,5)	4 (9,5)	14 (23,0)	0,209
Saturated fat (g)	23,3 ± 9,7	22,7 ± 9,5	23,7 ± 9,9	0,606
Monounsaturated fat (g)	20,0 ± 8,9	19,3 ± 8,5	20,4 ± 9,2	0,515
Polyunsaturated fat (g)	11,9 ± 5,5	12,0 ± 5,3	11,9 ± 5,8	0,914
Trans fat (g)	0,93 (0,63 – 1,37)	0,93 (0,59 – 1,55)	1,00 (0,62 – 1,35)	0,872
Cholesterol (mg)	326,7 ± 157,0	316,2 ± 148,8	334,0 ± 163,2	0,574
Iron (mg)	13,0 ± 4,4	13,0 ± 4,7	13,0 ± 4,2	0,991
Calcium (mg)	409 (306 – 675)	443 (330 – 757)	409 (286 – 576)	0,090
C Vitamin (mg)	59,3 (26,7 – 104,9)	58,7 (32,9 – 129,6)	59,3 (21,1 – 98,1)	0,489
B12 Vitamin (µg)	4,07 (2,33 – 6,33)	4,92 (2,33 – 6,45)	3,53 (2,24 – 5,94)	0,183

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; AC: abdominal circumference; SAD: sagittal abdominal diameter. Quantitative variables described by mean and standard deviation (SD) or median. Qualitative variables described by absolute and relative frequencies. p value was considered statistically significant if <0,05.

Laboratory tests are described in Table 4. The mean results for total, LDL and HDL cholesterol tests were higher in the NAFLD group (p>0,05). Triglycerides (expressed in median) were positively associated with the presence of hepatic steatosis (p<0,002). The medians for alkaline phosphatase, gamma glutamyltransferase and alanine aminotransferase were significantly higher in the NAFLD group (p<0,05).

Table 4. Laboratory tests and relation between NAFLD in patients with Crohn's Disease.

Variables	**Total sample (n=103; 100%)**	**With NAFLD (n=42; 40,8%)**	**Without NAFLD (n=61; 59,2%)**	**p**
Lipid profile				
Total cholesterol – mean ± SD	178,9 ± 48,3	188,5 ± 43,8	172,4 ± 38,4	0,052
Above range – n(%)	37 (35,9)	20 (47,6)	17 (27,9)	0,065
LDL – mean ± SD	105,7 ± 34,3	112,4 ± 36,9	101,1 ± 31,9	0,099
Above range – n(%)	25 (24,3)	13 (31,0)	12 (19,7)	0,281
HDL – mean ± SD	50,8 ± 12,9	48,7 ± 11,5	52,3 ± 13,7	0,158
Below range – n(%)	23 (22,3)	8 (19,0)	15 (24,6)	0,672
Triglycerides – median (P25-P75)	94,5 (67,3 – 150,8)	120 (80,5 – 174,5)	86 (61 – 127)	0,002
Above range – n(%)	19 (19,0)	10 (24,4)	9 (15,3)	0,375
Liver enzymes				
Alkaline phosphatase – mean ± SD	78,4 ± 24,8	84,4 ± 28,1	74,2 ± 21,5	0,040
Above range	2 (1,9)	2 (4,8)	0 (0,0)	0,164
GGT – median (P25-P75)	21 (13 – 33)	24 (15,8 – 36)	17 (12,5 – 27,5)	0,012
Above range	17 (16,5)	10 (23,8)	7 (11,5)	0,165
AST/TGO – median (P25-P75)	19 (16 – 25)	21 (17 – 28)	19 (16 – 24,5)	0,145
Above range	6 (5,8)	4 (9,5)	2 (3,3)	0,222
ALT/TGP – median (P25-P75)	16 (12 – 27)	19 (14 – 27,3)	15 (11 – 25)	0,012
Above range	1 (1,0)	1 (2,4)	0 (0,0)	0,408

NAFLD: non-alcoholic fatty liver disease.
Quantitative variables described by mean and standard deviation (SD) or median. Qualitative variables described by absolute and relative frequencies. p value was considered statistically significant if <0,05

Table 5 describes the plasma TMAO values. The median plasma TMAO value of this sample was 4.31 ug/mL. The minimum TMAO value found was 0.77 ug/mL and the maximum was 44.92 ug/mL. Slightly higher TMAO values were observed in patients with NAFLD and clinical

CD activity, however, without statistical significance (p>0.05). SAD was the only variable that was related to higher TMAO levels (p 0,047).

Table 5. Blood levels of Trimethylamine Oxide (TMAO) and relation with other variables. (n=103).

Variable	TMAO ug/mL median (P25-P75)	p
Total sample	4,31 (2,46 – 7,99)	
NAFLD		0,629
Yes	5,00 (2,75 – 7,53)	
No	4,14 (2,33 – 8,66)	
DC activity		0,529
Yes	5,23 (2,86 – 8,74)	
No	4,17 (2,44 – 7,65)	
SAH		0,085
Yes	5,35 (3,69 – 7,96)	
No	4,07 (2,23 – 7,99)	
DM		0,400
Yes	4,40 (3,27 – 8,80)	
No	4,28 (2,43 – 7,80)	
Dyslipidemia		0,088
Yes	8,31 (3,95 – 13,1)	
No	4,20 (2,43 – 7,42)	
Excess body fat		0,348
Yes	4,65 (2,46 – 8,00)	
No	3,86 (2,47 – 6,64)	
High SAD		0,047
Yes	5,71 (2,84 – 8,75)	
No	3,57 (2,23 – 6,99)	

NAFLD: non-alcoholic fatty liver disease; CD: Crohn's disease; TMAO: trimethylamine oxide; SAH: systemic arterial hypertension; DM: diabetes mellitus; DAS: sagittal abdominal diameter. Quantitative variables described by median. p value was considered statistically significant if <0,05.

After adjusting for the multivariate model (Table 6), the variables that remained statistically associated with NAFLD were: SAH (p=0,046), DM (p=0,017), active Crohn's disease (p=0,023), overweight (p=0,025) and obesity (p<0,001). Due to the possible multicollinearity effect between BMI, AC and SAD, two other models were tested. Both models showed the same associated factors. The model with AC instead of BMI demonstrates that a patient with increased AC has a 132% higher prevalence of NAFLD than those with adequate AC (PR=2,32; 95% CI: 1,35 to 4,02; p=0,002). Also, the model with SAD instead of BMI shows that a patient with increased DAS has a 118% higher prevalence of NAFLD than those with adequate SAD (PR=2,18; 95% CI: 1,26 to 3,79; p =0,005).

Table 6 - Poisson regression for NAFLD.

Variables	Prevalence Ratio	CI 95%	P
SAH	1,43	1,01 – 2,02	0,046
DM groups			
Without	1,00	-	-
Pre	0,75	0,40 – 1,44	0,391
DM	1,83	1,11 – 3,00	0,017

DC activity			
Yes	1,63	1,07 – 2,49	0,023
No	1,00	-	-
BMI			
Underweight/ Normal weight	1,00		
Overweight	2,82	1,14 – 6,98	0,025
Obesity	4,47	1,92 – 10,4	<0,001
Excess body fat			
Yes	1,77	0,94 – 3,30	0,075
No	1,00		

NAFLD: non-alcoholic fatty liver disease; CD: Crohn's disease; SAH: systemic arterial hypertension; DM: diabetes mellitus. Criteria for the entry of the variable: p<0.20 in the bivariate analysis and p<0.10 for permanence in the final model.

DISCUSSION

The 40,8% prevalence of NAFLD found in the sample was higher than most studies available in the literature for patients with IBD and higher than the global (25.2%) and Brazilian (35.2%) prevalences for NAFLD[12 41 42]. In a retrospectively evaluated sample of 421 patients with IBD, the authors described a frequency of 13.3% (n=56) of NAFLD (ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), with 50 patients with CD and 6 patients with UC[17]. In a North American population of 140 patients (70 cases with IBD and 70 controls without IBD) treated at a hospital in Kansas City, a retrospective case-control study showed a 44% prevalence of NAFLD (CT diagnosis) in the group with IBD compared to 16% in the control group, concluding that the presence of IBD increases the risk of developing NAFLD (OR=4,53)[10]. Simon et al., (2018)[43] analyzed 462 patients with IBD in a prospective cohort in the US and found a 52% prevalence of NAFLD assessed by CT. The data found in this study corroborate the literature regarding patients with IBD presenting a higher prevalence of NAFLD when compared to the population without IBD.

Regarding the characteristics of CD, the average time (12.3 in years) of CD diagnosis was higher than that found in previous studies[8 44], however it was not related to NAFLD. Contrary, Saroli and Palumbo et al., (2018)[45] and Zou et al, (2019)[15], found that CD time was significantly longer in patients with NAFLD. 29.1% of the analyzed sample underwent at least 1 intestinal resection surgery, but this finding was not associated with the presence of NAFLD, which differs from that found by other authors who describe a strong relation between the history of intestinal resection and NAFLD, which may increase at 3.7 times the risk of NAFLD among these patients[8 15 44]. No associations were found between the characteristics of CD and the presence of NAFLD. In a study that evaluated the relation between MS, IBD severity and NAFLD severity in patients with a dual diagnosis of IBD and NAFLD, the authors did not find significant differences between phenotype, extent and activity of CD with worsening of liver fibrosis, indicating that the severity of NAFLD is associated with MS and not with IBD severity[46].

Principi et al., (2018)[11] evaluated a sample of 465 patients with IBD (CD=258) and found no relation between activity, phenotype and extent of CD and NAFLD.

Regarding the clinical activity of CD by the IHB, 77.7% of the patients were in clinical remission of CD. Of these, 52 (85.2%) had NAFLD grade 0 at US. However, PCR results demonstrate that the median value was higher among patients with hepatic steatosis. Li et al., (2017)[13] correlated high CRP values with a higher rate of NAFLD in patients with CD. Surprisingly, multivariate regression showed that patients with active CD had a 63% higher prevalence of NAFLD when compared to patients in remission in our study. (PR=1,63; CI 95%: 1,07 – 2,49). In a retrospective cohort study that followed 321 patients with IBD, the authors concluded that the underlying disease activity was a predictor of NAFLD[8]. We know that patients with metabolic syndrome are in a state of systemic inflammation that also influences CRP levels[15].

It was not possible to find a relation between the use of drug treatment for CD and NAFLD. Although without statistical significance, it is possible to notice that 62.5% and 58.8% of the patients who were using immunosuppressants and anti-TNF, respectively, did not have NAFLD, which may suggest a direct protective factor for these two classes drugs on hepatic steatosis, or even a lower rate of NAFLD due to a better control of the inflammatory process derived from CD[14]. Others autors[17] [46] also found similar results when comparing the use of treatments for CD between the IBD+NAFLD group and IBD only, as they found that there were no differences between the use of corticosteroids, biologicals and immunosuppressants between the groups. A review study conducted to assess the use of corticosteroids and the risk of developing NAFLD among patients with IBD concluded that the use of corticosteroids does not pose a potential risk for the development of NAFLD in this population[47].

In the CD patients studied, we found an association between SAH, DM and dyslipidemia and the presence of NAFLD. After adjusting the results by Poisson regression, it was concluded that hypertensive patients have a 43% higher prevalence of NAFLD than non-hypertensive patients. (PR=1,43; CI 95%: 1,01 – 2,02) and patients with DM are 83% more likely to have NAFLD than those without DM (PR=1,83; CI 95%: 1,11 – 3,00). The findings of the present study are similar to those of the general population with regard to comorbidities related to the presence of NAFLD[35] [48].

Our results demonstrate a high prevalence of overweight (62.1%). After multivariate regression, overweight subjects were 182% more likely to have NAFLD than those with low weight or normal weight (PR=2,82; 95% CI: 1,14 – 6,98) and obese subjects has an increased probability of NAFLD by 347% when compared to those who are underweight or normal weigh (PR=4,47; CI 95%: 1,92 – 10,4). A review study demonstrated that obesity is one of the main markers related to the development of HE in patients with IBD (OR=2,1)[49]. The results

demonstrate that the high frequency of overweight and obesity among patients with IBD is similar to the general population[5]. The literature relates this finding to the advancement of treatments for IBD, which make patients stay longer in remission, favoring weight gain[50].
Increased abdominal circumference was also related to NAFLD. 45 (43.7%) patients had AC measurement above the sex cut-off point. This result is also observed in patients with IBD according to a study conducted by Principi et al, (2018)[11] who found an independent relation between the presence of NAFLD and AC (OR=1,68). AC is positively associated with IR regardless of the characteristics of IBD[51].

The SAD is an accessible anthropometric marker for the assessment of visceral fat. Our results showed cutoff points for SAD similar to those found in other studies (>21,4cm for men and >20,4cm for women)[27] [52]. In a prospective Brazilian study, 824 adult women from 3 Brazilian states recruited from diabetes clinics were included, metabolic syndrome and obesity or through local advertisements, which were compared with healthy controls. The authors found an ideal cut-off point of 21 cm for the SAD and verified a correlation with IR (HOMA-IR), and in women with increased SAD there were 3.2 more chances of IR. When compared to AC, SAD showed reliability independently of BMI, which does not occur in AC, as it reflects together with subcutaneous fat[27]. Li et al. (2021)[53] found a relation between SAD >21.3cm in women and >22.6cm in men with a BMI above 25kg/m² in a representative North American population. Risérus et al., (2010)[54] described an association between cardiometabolic risk and SAD cutoff points (ROC curve) >22cm for Swedish men and >20cm for women. To the best of our knowledge, this is the first study to use SAD measurement in patients with CD.

Importantly, after the multivariate analysis, it was found that the anthropometric assessments BMI, SAD and AC overlapped. In this study, there was no superiority of one form of anthropometric assessment over the other to assess the association with NAFLD.

Observing the results of laboratory tests for the lipid profile, it is clear that only the median of triglycerides showed a positive relationship with NAFLD. Similar results were found in a prospective study that collected data on the lipid profile of a sample of patients with IBD and found a positive relationship with the presence of HE and triglycerides[45].

Regarding liver enzymes, the means and/or medians for ALT, alkaline phosphatase and GGT were higher among patients with NAFLD (p<0,05). Similarly, Principi et al, (2018)[11] admitted a relation between GGT levels and the risk for NAFLD in patients with IBD (OR=2,77) and Sourianarayanane et al, (2013)[14] found a higher frequency of altered ALT between NAFLD and IBD patients when compared to the IBD group. Veltkamp et al., (2022)[55] described a positive association between AST, ALT and GGT and hepatic steatosis. A study also observed a

relation between AST and ALT between patients with IBD and MS when compared to patients who had IBD but did not have MS[46]. Changes in liver enzymes may be present in up to 30% of patients with IBD[16], and it is not possible to conclude that the changes assumed in this sample are caused by NAFLD. In our study, we did not analyze the presence of liver fibrosis, and it was not possible to reach a conclusion regarding liver damage at the cellular level.

We did not find important differences for the nutrients analyzed between the groups with and without NAFLD. A cohort study involved 150 patients with IBD in remission compared to a control group composed of healthy people and aimed to assess differences in dietary habits. The authors found that there were no differences between the consumption of carbohydrates and proteins between the groups. They found caloric intake (88 kcal difference) and fat consumption were positively associated with the IBD group[11].

In both groups we found insufficient fiber intake (g) (below 25g for women and 38g for men)[40], which seems to be common among CD patients due to fear of disease complications, such as intestinal obstruction, since 41.7% of the sample has a stricturing phenotype of CD (isolated or associated with the penetrating phenotype), causing patients to restrict fiber consumption[56]. In turn, fiber consumption by the Brazilian population in general is considered insufficient[57]. Low fiber intake represents a low consumption of fresh foods, such as fruits, vegetables and whole grains[58].

It was not possible to find a relation between plasma levels of TMAO with the presence of NAFLD and clinical activity of CD, but with SAD. Higher levels of TMAO have been related to MS, DM, SAH, dyslipidemia, cardiovascular diseases and worsening NAFLD[59]. Schiattarella et al., (2017)[60] found that for every 10 mol/L of TMAO there is a 7.6% increase in the risk of all-cause mortality. In a study of 106 patients with IBD compared to 373 controls without IBD, Wilson et al., (2015)[61] found lower levels of TMAO in the population with IBD compared to controls. However, it is important to note that the authors found low frequencies of disease activity both in patients with CD (29.4%) and in those with UC (17.9).

Our study is the first to assess the prevalence and risk factors for NAFLD in a sample from a reference outpatient clinic for the treatment of IBD in southern Brazil. We emphasize as qualities of our study the variety of investigated and unpublished factors in relation to the risk for NAFLD in patients with CD, the standardization of diagnostic methods for the outcome and variables, the reduction of biases regarding inter-rater variability during data collection and the prospective design that allows us to increase reliability and reduce information losses[62].

We also assume some limitations in our study. The first is related to the need for patients to return to the hospital for study evaluations, which may have caused patients with active

Crohn's disease to refuse to participate or not return to complete their participation in the study due to clinical symptoms of the disease. Second, the sample size may have influenced the results found. Finally, our study has a cross-sectional design that only allows us to generate hypotheses regarding the risk factors for NAFLD[45].

CONCLUSION

The prevalence of NAFLD in patients with CD was 40.8%, higher than the global prevalence of NAFLD. The main risk factors found for NAFLD in this population of patients with CD were SAH, diabetes, dyslipidemia, visceral fat per SAD, overweight and obesity. The characteristics of CD, nutrient intake and plasma levels of TMAO showed no relation with the presence of NAFLD. Contrary to what might be expected, CD activity by IHB and PCR showed a positive correlation with NAFLD.

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8. CONCLUSÃO

Encontramos alta prevalência de DHGNA (40,8%) nessa amostra de pacientes com DC, superior à população em geral. A presença de DHGNA nesta amostra esteve relacionada aos fatores de risco metabólicos semelhantes para a população em geral (hipertensão arterial sistêmica, diabetes, dislipidemia, gordura visceral pelo diâmetro abdominal sagital, sobrepeso e obesidade). As características da DC, a ingesta de nutrientes e os níveis plasmáticos de TMAO não demonstraram associação com a presença de DHGNA. Ao contrário do que se poderia esperar, a atividade da doença de Crohn pelo IHB e pelo PCR apresentou correlação positiva com a DHGNA. Valores mais elevados de triglicerídeos, fosfatase alcalina, gama glutamiltransferase e alanina aminotransferase foram encontrados entre o grupo que apresentou DHGNA. O consumo alimentar foi muito semelhante entre os pacientes com e sem esteatose hepática e não demonstrou associação com a DHGNA. Nessa amostra, atribuiu-se relação semelhante entre os aspectos metabólicos já estabelecidos para o desenvolvimento de DHGNA entre a população em geral, que parecem ser agravados pela atividade da doença de Crohn.

9. CONSIDERAÇÕES FINAIS

Os fatores de risco para DHGNA já estabelecidos para a população em geral podem estar agravados na doença de Crohn devido à complexidade de processos metabólicos envolvidos com a atividade da doença. São necessários mais estudos longitudinais que possam confirmar os aspectos envolvidos entre a presença de doenças inflamatórias intestinais e o risco de desenvolver DHGNA.

PERSPECTIVAS

Dados os resultados preocupantes de nosso estudo com relação ao excesso de peso e prevalência de DHGNA, sugere-se que pacientes com DII sejam monitorados quanto à presença de DHGNA, principalmente na presença de fatores de risco como obesidade, dislipidemia e diabetes.

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