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IS FECAL CALPROTECTIN DETERMINATION USEFUL FOR PATIENTS WITH METABOLIC ASSOCIATED FATTY LIVER DISEASE?

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ABSTRACT

Aim: To investigate the possible relationship between fecal calprotectin (FC) level and ultrasound indicators of steatosis and fibrosis wich defined by attenuation coefficient (AC) and liver stiffness (LS) from two-dimensional (2D) shear-wave elastography (SWE) in patients with metabolically associated fatty liver disease (MAFLD).

Materials and methods: The study included 110 persons with MAFLD; mean age 51.3±4.8 years, 65 (59.1%) men. There were used laboratory, sonography and statistical methods.

Results: Stage S1 of steatosis was diagnosed in 42 (38.2%), S2 - in 56 (50.9%), S3 - only in 12 (10.9%) MAFLD patients. The carbohydrate metabolism disorders were found in 62 (56.4%); 38 (34.5%) patients among them suffered from type 2 diabetes. The lipid metabolism disorders were diagnosed in the vast majority of patients included in this study. The minimal excess of fecal calprotectin (FC) was detected in 72 MAFLD patients (65.5%), the moderate increase of FC was found in 12 persons, the FC more than 10-fold excess of the norm was observed in only 8 MAFLD patients. FC levels were significantly elevated in MAFLD patients with a S2-S3 compared to those with a S1 (75.8 [42.9–112.1] vs. 46.3 [28.2–65.4], $p<0.01$).

Conclusions: Fecal calprotectin levels are significantly elevated in patients with MAFLD. Future studies are warranted to establish the definitive role and clinical utility of FC as a potential biomarker of probably liver steatosis as well as other diseases associated with methabolic syndrome and its complications.

KEY WORDS: fecal calprotectin, inflammation biomarker, steatosis, two-dimensional shear-wave elastography, ultrasound steatometry.

INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is quite common in the modern population. It is currently the most common liver disease worldwide, affecting over one-third of the population [1]. This disease is most often caused by insulin resistance, is one of the components of the metabolic syndrome and is combined with various disorders of substances in the body. That is why it was called MAFLD instead of NAFLD. The course of MAFLD is most often asymptomatic or mildly symptomatic, and the complaints of the vast majority of such patients are nonspecific and vague, such as heaviness (rarely muffled non-intense pain) in the right hypochondrium or discomfort in the abdomen as a result of bloating and excessive gas formation.

Flatulence is the symptom that makes the vast majority of such patients consult a doctor. Subsequently, during the examination of most of such persons, it turns out, as an accident, that the person's diagnosis is MAFLD. For patients with abdominal distension, it is important to investigate the cause of this condition, as flatulence can occur due to a variety of reasons. Among them are: pancreatic enzyme insufficiency, biliary dysfunction, inflammatory bowel diseases, helminthiasis and others. For such a cohort of patients, multiparametric ultrasound examination of the liver is important, but currently there is a lack of data from multicenter studies on the role of this instrumental examination in the diagnosis of MAFLD.

It is known about the existence of the "intestine-liver" pathogenetic axis [1]. Accumulating evidence points to a significant role of inflammation as a one of pathophysiological driver of MAFLD progression [2]. In the pathogenesis of MAFLD, hepatocytes suffer from cellular stress. Neutrophils granulocytes play a significant role in the pro-inflammatory cascades [3]. Calprotectin is a calcium- and zinc-binding protein dimer (also known as the S100A8/S100A9 complex) that is present in the cytosol of neutrophilic granulocytes [4]. Upon inflammation, calprotectin is actively secreted by neutrophils as part of their stress response [5, 6]. Fecal calprotectin (FC) is clinically used as a biomarker of active intestinal inflammation in patients with inflammatory bowel disease (IBD) [7]. Given the role of hepatic infiltration of neutrophils and systemic inflammation in MAFLD, calprotectin levels may also reflect the inflammation in the pathogenesis of MAFLD [1]. However, only a limited number of clinical studies have been performed examining calprotectin as biomarker in subjects with MAFLD or NAFLD, and conflicting results have been reported [8-10]. Neutrophilic inflammation

plays a known role in the pathogenesis of MAFLD [11].

But does fecal calprotectin change in patients with MAFLD? Does FC correlate with indicators of steatosis and fibrosis in case of presence of MAFLD?

AIM

Therefore, the purpose of our work was to investigate the possible relationship between fecal calprotectin level and ultrasound indicators of steatosis and fibrosis which defined using the attenuation coefficient (AC) from attenuation imaging (ATI) and liver stiffness (LS) and dispersion slope (DS) from two-dimensional (2D) shear-wave elastography (SWE) in patients with metabolically associated fatty liver disease.

MATERIALS AND METHODS

The study included 110 subjects with MAFLD who underwent multiparametric ultrasound examination of the liver with steatometry (measurement of attenuation coefficient) and two-dimensional shear wave elastography (2D-SWE) (measurement of liver stiffness). Each patient, in addition to ultrasound, underwent a general clinical examination, which included some biochemical blood parameters (ALT, AST, ALP, GGT, total and direct bilirubin, total protein, cholesterol, triglycerides, glucose, glycosylated hemoglobin, thyroid-stimulating hormone), calculation of the body mass index (BMI), atherogenicity coefficient (AC) and the insulin resistance coefficient (IR-HOMA)

The mathematical apparatus of the study included the following formulas:

1. BMI = m / h2, where BMI – body mass index, m – weight (kg), h – height (m)
2. AC = (total cholesterol - HDL) / HDL, where AC – atherogenicity coefficient, HDL - high-density lipoproteins
3. IR-HOMA = fasting blood glucose * fasting blood insulin) / 22.5

Multiparametric ultrasound investigation of the liver was carried out using the SONEUS P7 (Ultrasign, Kharkov, Ukraine) and a C1-5 convex sensor with frequencies of 1-5 MHz. We prospectively investigated patients with MAFLD by two-dimensional shear-wave elastography (2D-SWE) with associated controlled attenuation parameter (CAP) as part of a routine clinical investigations. MAFLD was defined as CAP ≥ 2.20 dB/cm. Significant liver fibrosis (stage 2 or higher out of 4) was defined as LS measurement ≥7.0 kPa.

The presence of fibrosis in the liver tissue was assessed using two-dimensional shear wave elastography, measuring the Young's modulus of the liver (liver stiffness, LS) in kilopascals (kPa) and the speed of the shear wave during the passage of ultrasound through the liver tissue (attenuation coefficient) in m/s. Staging of fibrosis was carried out according to the following criteria:

- F0 - absence of fibrosis: LS 6.5 kPa and less; shear wave speed 1.47 m/s and less
- F1-F2 – fibrosis without septa formation: LS 6.6-6,9 kPa; shear wave speed 1.48-1.52 m/s

vF2 - fibrosis with single septa: LS 6,9-7,5 kPa; shear wave speed 1.52-1.58 m/s

- F2-F3 - LS 7,5 -8,2 kPa; shear wave speed 1,58-1.65 m/s
- F3 – fibrosis with multiple septa, without cirrhosis: LS 8.2-9.3 kPa; shear wave speed 1.65 - 1.76 m/s
- F4 – fibrosis with multiple septa, with cirrhosis: LS is more than 9.3 kPa; the speed of the shear wave is more than 1.76 m/s

To assess the degree of steatosis, we performed ultrasound liver steatometry and measured controlled attenuation parameter (CAP). Staging of steatosis was carried out according to the following criteria:

- stage S0 (no steatosis) – when CAP less than 2.2 dB/cm
- stage S1 (mild steatosis) – when the fatty infiltration in 5 – 33% of liver cells and CAP from 2.20 to 2.25 dB/cm
- stage S2 (moderate steatosis) – when the fatty infiltration in 33-66% of liver cells and CAP from 2.3 to 2.90 dB/cm
- stage S3 (severe steatosis), when more than 66% of liver cells are infiltrated by fat occupies, CAP in this stage is more than 2.9 dB/cm

Fecal calprotectin (FC) was determined in the laboratory by the solid-phase enzyme-linked immunosorbent assay method. A calprotectin level in feces less than 50 μg/g was considered as normal.

Statistical processing of materials was carried out using the "Statistica 10.0" application program package.

This prospective open clinical study was conducted at Uzhhorod National University from December 2022 to February 2023. Each patient involved in it signed an informed agreement for examination and treatment. This study was conducted in accordance with ethical principles in medicine. It is part of the scientific topic of the therapy and family medicine department of the faculty of postgraduate education (Uzhhorod National University) 36A-2021 "Innovative methods of diagnosis and treatment of internal organs pathology in obese patients", state registration number 0121U111773.

RESULTS

All 110 patients (mean age 51.3±4.8 years, 65 (59.1%) men) denied drinking alcohol and had negative laboratory test results for viral hepatitis B and C. In all 110 people included in the study, ultrasound signs of liver steatosis were detected. Diagnosis of MAFLD was detected according to CAP more than 2.2 dB/cm. It was steatosis of liver that was the criterion for the inclusion of patients in this study. Stage S1 was diagnosed in 42 (38.2%) patients with MAFLD, stage S2 was detected in 56 (50.9%) examined persons. Stage S3 was diagnosed only in 12 (10.9%) MAFLD patients. 72 patients (65.5%) were overweight or obese, but the morbid obesity (BMI≥40) was determined in only three (2.73%) of them. The carbohydrate metabolism disorders were found in 62 persons (56.4%). 38 (34.5%) patients among them suffered from type 2 diabetes. In 24 patients (21.8%) the compensated diabetes was detected. 14 (12.7%) persons suffered from the subcompensated T2DM. There was no decompensated diabetes among the examined. The lipid metabolism disorders were detected in the vast majority of patients included in this study. There was the presence of atherogenic dyslipidemia in the vast majority of patients

with MAFLD. Their lipid profiles were characterized by an isolated decrease of HDL level (14 persons, 12.7%), an isolated increase of LDL level (17 patients, 15.5%), a combined disorder (decrease in HDL + increase in LDL, 67 patients, 60.9%), hypertriglyceridemia (34 patients, 30.9%) and increased atherogenicity ratio (82 persons, 74.5%)

MAFLD in the stage of steatohepatitis (nonalcoholic steatohepatitis, NAFLD) was verified in 42 examined patients (38.2%). The other 68 persons (61.8%) had MAFLD in the stage of steatosis, without inflammation signs. The vast majority of cases of steatohepatitis (32 out of 42, 76.2%) was with minimal activity of the inflammatory process, as their ALT level did not exceed 3 norms.

The level of fecal calprotectin was examined (quantitatively) in all 110 patients with MAFLD. It was found that only 18 of all the examined had this indicator within the normal range. The minimal excess of FC (no more than 3 norms) was detected in 72 MAFLD patients (65.5%). The moderate increase of this indicator was found in another 12 MAFLD patients, in which fecal calprotectin level was more than 3 norms, but did not exceed a 10-fold increase. The fecal calprotectin level more than 10-fold excess of the norm was observed in only 8 MAFLD patients.

There were detected that independent predictors of steatosis were older age (adjusted odds ratio [aOR], 1.43; 95% confidence interval [CI], 1.13-1.78), higher BMI (aOR, 1.30; 95% CI, 1.19-1.40) and higher triglycerides (aOR, 1.46; 95% CI, 1.10-2.19). Patients who were classified as S2-S3 were older ($p < 0.01$) and were more often male ($p < 0.01$) than the persons with S1. In addition, participants with S2-S3 more frequently had metabolic syndrome, a history of diabetes ($p < 0.01$) and more frequently used antihypertensive, antidiabetic, and lipid-lowering drugs (all $p < 0.01$). BMI was higher in patients with a S2.

Fecal calprotectin levels were significantly elevated in MAFLD patients with S2-S3 compared to those with S1 (75.8 [42.9–112.1] vs. 46.3 [28.2–65.4], $p < 0.01$). The increasing age in MAFLD patients was not associated with a significant change of the level of fecal calprotectin.

Conducting two-dimensional shear wave elastography made it possible to detect fibrosis in 52 (47.3%) of the examined patients with MAFLD. In 37 (33.6%) of them, fibrosis was detected in stage F1, and in the remaining 15 (13.6%) patients, fibrosis was detected in stage F2. F3 was not detected among examined MAFLD patients. No signs of fibrosis were detected (stage F0) in 58 patients included in this study.

DISCUSSION

This study demonstrated that fecal calprotectin levels are increased in persons with MAFLD. The diagnosis of MAFLD in the patients included in this study was based on the results of ultrasound investigation by two-dimensional shear-wave elastography (2D-SWE) with associated controlled attenuation parameter (elastography and steatometry of the liver). Statistical analyses showed that fecal calprotectin levels were independently associated with steatosis stages after adjustment for relevant confounding factors, including comorbidities (diabetes, hypertension), cholesterol levels, insulin resistance. Statistical analyses demonstrated that there were significantly differential associations of calprotectin levels by BMI. Our results indicate that level of fecal calprotectin may be a promising biomarker for the presence of liver steatosis and the development of MAFLD.

We proved that the level of fecal calprotectin can reflect the involvement of neutrophils in the pathogenesis of MAFLD, similar to the results [12] regarding the level of calprotectin in the blood. Hepatic neutrophil infiltration is a salient feature of MAFLD progression, and several mechanisms have been proposed to explain how these neutrophils may accelerate disease progression [12]. We obtained convincing data on the association of fecal calprotectin only with the severity of steatosis, in contrast to other works [11], which described the correlation between inflammatory cytokines and the severity of fibrosis in the liver. The inflammatory cytokines inflict damage to hepatocytes and perpetuate inflammation and fibrosis [11]. Although the exact contribution of calprotectin to MAFLD pathogenesis is not well understood, serum levels of S100A8/S100A9 play a critical role in modulating the inflammatory response [5, 13]. Similarly, S100A8/S100A9 proteins are upregulated in experimental animal models of NAFLD, as well as in adipose tissue of patients with NAFLD [14]. Considering the role of calprotectin in other inflammatory diseases, it has been suggested that it serves a prominent role in innate immunity in the context of MAFLD-associated gut microbial dysbiosis [15]. In the present study, there were relations for the association between fecal calprotectin levels and steatosis stages. We cannot definitely explain these findings, but it seems that fecal calprotectin as inflammation biomarker may be a marker not only of the early pathogenetic MAFLD stages, but also the metabolic syndrome. Similar data were obtained [7] regarding the plasma level of calprotectin. Calprotectin is also deeply involved in the development of atherosclerosis mainly via the inflammatory process [16].

Taking into consideration the small group of patients with MAFLD involved in this study (only 110 people) and its short duration (only 3 months), the obtained results may probably not be transferred to the general population without additional research yet. However, the clinical significance of the data obtained in this study undoubtedly deserves attention and research in additional studies.

CONCLUSIONS

In conclusion, fecal calprotectin levels are significantly elevated in patients with MAFLD. Future studies are warranted to establish the definitive role and clinical utility of fecal calprotectin as a potential biomarker of probably liver steatosis as well as other diseases associated with methabolic syndrome and its complications.

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