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THE EFFECT OF METFORMIN ON BIOCHEMICAL PARAMETERS IN PATIENTS WITH METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common form of chronic liver disease in the modern world. The importance of this condition is that it can progress to nonalcoholic steatohepatitis, which increases the risk of developing liver cirrhosis and hepatocellular carcinoma. The study aimed to examine the effects of metformin on achieving positive biochemical responses in patients with MASLD. The study included 146 patients, 96 men and 50 women, with MASLD diagnosed by ultrasound. Biochemical analyses were performed as well. The values of all parameters were measured at baseline, after three and after six months of therapy. On each visit, the body weight and body mass index (BMI) were obtained. All patients at baseline received 750 mg of metformin twice a day. There was a reduction in body weight, which was statistically significant after six months. The BMI decrease reached no statistical significance. Liver enzyme values showed a significant decrease in values relative to baseline after three and six months of metformin therapy. Serum cholesterol and triglyceride levels were reduced during treatment with metformin, and changes reached statistical significance at six months relative to baseline. There was a statistically significant decrease in the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) value after three and six months compared to baseline. Results showed that metformin may be an appropriate addition to diet, weight reduction, and physical activity, as it led improvements in metabolic parameters, with minimal adverse events and good tolerance of therapy.

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Key words: *metabolic dysfunction-associated steatotic liver disease metformin, body mass index*

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Introduction

There is an obvious increase in the number of people with metabolic dysfunction-associated steatotic liver disease (MASLD). Today, MASLD is the most common form of chronic liver disease in the modern world. The importance of this condition is that it can progress to nonalcoholic steatohepatitis (NASH), which increases the risk of developing liver cirrhosis and hepatocellular carcinoma. Therefore, MASLD is a growing health problem which affects 20–30% of the population, while in type 2 diabetes mellitus (DM), it may be as high as 75% (1). MASLD is a state of accumulation of fat in the liver with no significant alcohol abuse. There is a wide range of changes in the liver, from the common hepatic steatosis to nonalcoholic steatohepatitis, liver damage which is similar to that of alcoholic liver disease. MASLD is clearly associated with the risk of developing type 2 DM and cardiovascular disease (2). MASLD is a common cause of asymptomatic elevation in liver enzymes, in particular, alanine aminotransferase (ALT) (3).

The pathogenesis of MASLD is not well understood. It is assumed that insulin resistance is a major pathogenetic disorder that leads to liver steatosis. Steatosis itself further increases the sensitivity of the liver to metabolic damage, leading to the progression of hepatic steatosis to steatohepatitis and fibrosis (4). Ultrasound examination of the abdomen is the most common diagnostic tool for MASLD. If the percentage of fat

in the liver is 20–30%, the sensitivity for detection of hepatic steatosis is 85%, a specificity of 94% (5). You mayfind mild to moderate elevations in ALT and AST enzymes with a ratio of AST:ALT less than 1. Hepatic enzymes can be considered normal in 75% of cases, so that the increase in enzyme is not sensitive for the diagnosis of MASLD (6). For now, ideal therapy for MASLD does not exist. Changing lifestyle, diet and weight loss are difficult to achieve and maintain. As insulin resistance is a key pathogenetic mechanism of MASLD use of insulin sensitizers may be an answer to medical therapy. Metformin, as a representative of this group of drugs, may play an important role and exert a positive effect on biochemical parameters and histological changes in patients with MASLD.

The study aimed to examine the effects of metformin on achieving positive biochemical responses in patients with MASLD.

Meterials and Methods

The study included 146 patients, 96 men and 50 women, treated at the Clinic of Gastroenterology and Hepatology, Clinical Center Niš, in the period from 2022 to 2024, with the patient's signed consent. The study has been approved by a local ethics committee. The study included patients with MASLD diagnosed by ultrasound. Ultrasound parameters for diagnosis were the presence of two of the four ultrasound criteria: i) liver echogenicity exceeding that of the renal cortex, ii) loss of definition of the diaphragm, iii) poor delineation of the intrahepatic architecture, vi) attenuation of the ultrasound wave. Ultrasound examinations were performed by one ultrasonographer on the camera.

The inclusion criteria for the study required participants to be either abstinent from alcohol or to consume no more than two drinks a week, with a daily alcohol intake of less than 20 g/day. Participants also needed to show an increase in serum ALT and AST levels greater than 1.5 x ULN, and to have negative viral markers for HBsAg and HCV. Additionally, there should be no data of chronic liver disease, Wilson's disease, or hemochromatosis. The study did not include patients with DM, Cushing's disease, liver cirrhosis, chronic renal failure, heart failure III and IV according to New York Hecrt Associotian Classification. Patients receiving certain drugs such as methotrexate, amiodarone or those who had previously used metformin as well as patients with lactate values exceeding 2.2 mmol/l were not considered for the study. All patients underwent ultrasound examination of the liver upon diagnosis with NAFLD. Biochemical analyses measuring ALT, AST, gamma-glutamyl transferase, alkaline phosphatase enzyme, glucose, cholesterol, and triglycerides were conducted as well.

Further, insulin, C-peptide levels and HOMA-IR were determined. The values of all parameters were measured at baseline, after three and six months of therapy. On each visit, the body weight and body mass index (BMI) were obtained. The biochemical analyses were performed at the Institute of Biochemistry, Clinical Center Niš, and fasting insulin and C-peptide concentrations were determined at the Center of Nuclear Medicine, Clinical Center Niš.

At baseline, all patients received 750 mg of metformin twice daily. Data are presented as mean ± standard deviation (SD). Student's t-test and chi-square test were used to determine differences between the groups, and a p-value < 0.05 was taken as significant.

Results

The basal values of observed parameters, as well as values obtained after three and six months from the inclusion of metformin, are given in Table 1. There was a reduction in body weight, which was statistically significant after six months as compared to baseline, but not in relation to the value after three months. The BMI decrease reached no statistical significance. There were no significant changes in fasting blood glucose value. Serum cholesterol and triglyceride levels were reduced during treatment with metformin, and changes reached statistical significance at six months relative to baseline. The impairment of AST had statistical significance after three and six months compared to baseline, as well as the impairment after six months compared to the value after three months. ALT level showed a statistically significant reduction after three and six months compared to baseline, and no significant differences after six months compared to value after three months. There was a statistically significant decrease in HOMA-IR value after three and after six months compared to baseline, and no significant differences after six months compared to the value after three months.

Table 1. Changes in biochemical parameters and HOMA-IR

	Baseline	Three months	Six months
Body weight kg	88.7 ± 12.4	86.8 ± 10.7	84.6 ± 10.5*
BMI	29.7 ± 3.3	29.3 ± 2.7	28.7 ± 3.0
Fasting glucose mmol/l	5.1 ± 1.3	5.0 ± 1.1	5.0 ± 1.2
Cholesterol mmol/l	6.24 ± 2.73	5.53 ± 2.26	5.19 ± 2.59*
Triglycerides mmol/l	4.01 ± 1.85	3.69 ± 1.45	3.12 ± 1.73*
AST IU/L	79.63 ± 10.48	62.25 ± 14.68*	50.07 ± 9.55* **
ALT IU/L	85.12 ± 11.17	69.73 ± 17.44*	59.36 ± 14.33*
HOMA-IR	7.2 ± 2.4	4.0 ± 1.4	4.1 ± 1.7

*statistical significance after six months compared to baseline, $p < 0.01$
**statistical significance after six months compared to three months, $p < 0.01$

Discussion

Metformin was introduced as a first-line treatment for type 2 DM for more than half a century. The effect of metformin lowering blood glucose is explained by reducing hepatic gluconeogenesis, stimulating glucose taking into muscle and an increase in fatty acid oxidation in the adipose tissue (7). The final result is improving peripheral insulin sensitivity. Activation of AMPK by metformin has beneficial effects on lipid metabolism. The mechanism of loss of body fat is not only through direct inhibition of adipogenesis, but also by changing the synthesis and secretion of adipokines. Under the action of metformin, adiponectin stimulates AMPK and prevents hepatic lipid accumulation by increasing the β-oxidation of free fatty acids as well as decreasing synthesis.

The study included 146 patients, 96 men and 50 women. Maruti et al. suggest that the prevalence of in MASLD men is 31% and in women 16%, which means that the male sex is a risk factor for this disease. In our patients, there is a decrease in body weight and BMI after three and six months of the introduction of metformin therapy. All patients were on a prescribed diet and nutrition before monitoring. No statistically significant changes in body weight and BMI were observed after three and six months of treatment with metformin. Most authors observed similar changes, weight loss, with no statistically significant changes (8).

It was observed that even a smaller reduction in body weight can lead to improvements in markers of MASLD, in particular ALT and imaging markers of liver fat (9). There is also no significant difference in blood glucose value during follow-up. Cholesterol and triglyceride levels are reduced after three and six months, with a statistically significant change after six months compared to baseline values. Metformin significantly reduces the percentage of patients with MASLD in impaired fasting glucose (IFG) compared to patients treated with diet alone. Metformin therapy also significantly lowered the percentage of patients who met the diagnostic criteria for metabolic syndrome (10). There is data on different lipid changes during the treatment of MASLD with metformin, from mild to moderate decrease. Additionally, during the follow-up of 12 months, there were no changes in lipid levels, even with the increase during that period (11). Our results show a reduction in lipid values after three and six months of therapy.

The role of insulin resistance in the development of MASLD is complex, so that both hepatic and peripheral insulin resistance are clearly associated with the onset of MASLD. There is a diminished ability of insulin to suppress lipolysis, which increases the inflow of free fatty acids from adipose tissue to the liver. There is a reduced ability of insulin to inhibit gluconeogenesis, which leads to hyperglycemia and increased insulin resistance. Metformin has a positive effect on all of these processes by improving insulin sensitivity. Patients with MASLD have significantly higher levels of insulin and HOMA-IR index. Our results show a significant reduction in HOMA-IR after three months, with the maintenance of those values without significant changes after 6 months. It has been observed that people with higher levels of insulin and HOMA-IR have a higher risk over five years to develop MASLD. Reducing the levels of insulin using metformin reduces the risk that becomes similar to risk that in people who have had low or normal basal insulin levels. High insulin levels probably result in primary insulin resistance rather than decreased hepatic extraction of insulin in any liver disease (12, 13).

Liver enzyme values showed a significant decrease in values relative to baseline after three and six months of metformin therapy. There is a statistical significance of changes in AST after six months compared to baseline and after three months, while ALT level after six months shows a value similar to those after three months of treatment. Most studies indicate that metformin therapy significantly reduces ALT and AST, with normalization of ALT in as many as 56% of patients (12). This can be important because there is a greater risk of disease progression with higher values of transaminases (3).

Liver biopsy is the gold standard for the diagnosis of MASLD, but because of possible complications, cost and inconvenience for patients is often replaced by ultrasound and CT diagnostics. The percentage of patients with MASLD was higher when assessed by liver biopsy, allowing for accurate data on the presence of MASLD or NASH. Studies investigating the histological changes of liver biopsy showed no significant difference in histological findings during the course of metformin treatment. Despite the good response and the improvement in metabolic parameters, only about 30% of patients showed noticeable improvement in the level of steatosis, and only 20% of patients showed an improvement in the degree of inflammation after one year of metformin use (11, 14). There is a question of treatment duration and the daily dose of metformin. There are various study durations and doses of metformin that have been administered. Duration ranges from 4–12 months, a total daily dose of metformin from 0.85 gr to 3 g, average 1.5 g. Because of this, for now, there is limited number of studies, and the optimal dose of metformin and duration of treatment have not yet been defined (15).

Conclusion

The specific drug therapy of MASLD is not yet defined and available, and no drug can be a substitute for lifestyle modification. Our results indicate that metformin may be an benefitial addition to a regimen that includes diet, weight reduction and physical activity. It improves metabolic parameters, with almost no adverse events and good tolerance of therapy. However, it does not lead to significant histological changes in the liver. Reducing the risk of metabolic syndrome and cardiovascular disease is crucial.

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UTICAJ METFORMINA NA BIOHEMIJSKE PARAMETRE KOD BOLESNIKA SA NEALKOHOLNOM MASNOM JETROM

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Metabolička disfukcija povezana sa masnom bolešću jetre (engl. *metabolic dysfunction-associated steatotic liver disease* – MASLD) predstavlja najčešći oblik hronične bolesti jetre u savremenom svetu. Može napredovati u nealkoholni steatohepatitis, što povećava rizik od razvoja ciroze jetre i hepatocelularnog karcinoma. Cilj ove studije bio je da se ispitaju efekti metformina na postizanje pozitivnih biohemijskih odgovora kod bolesnika sa MASLD-om. Studija je obuhvatila 146 bolesnika (96 muškaraca i 50 žena) sa MASLD-om, koji je dijagnostikovan ultrazvukom. Urađene su biohemijske analize. Vrednosti svih parametara merene su na početku, posle tri meseca terapije i posle šest meseci terapije. Prilikom svake posete lekaru mereni su telesna težina i indeks telesne mase (engl. *body mass index* – BMI). Svi bolesnici su na početku primali 750 mg metformina dva puta dnevno. Nakon šest meseci terapije došlo je do statistički značajnog smanjenja telesne težine. Ispostavilo se da smanjenje BMI-ja nema statistički značaj. Pri poređenju početnih vrednosti enzima jetre sa vrednostima posle tri meseca i posle šest meseci terapije metforminom, zabeleženo je njihovo značajno smanjenje. Nivoi holesterola i triglicerida u serumu opali su u toku lečenja metforminom. Statistički značajna razlika u vrednostima primećena je pri poređenju početnih vrednosti sa vrednostima nakon šest meseci lečenja. Došlo je do statistički značajnog smanjenja vrednosti HOMA-IR (engl. *homeostatic model assessment for insulin resistance*) indeksa posle tri meseca i posle šest meseci lečenja u odnosu na početnu liniju. Rezultati ove studije pokazuju da metformin može biti adekvatan dodatak ishrani, s obzirom na to da doprinosi smanjenju telesne težine i fizičkoj aktivnosti, postiže poboljšanje metaboličkih parametara, skoro bez neželjenih efekata, i da se dobro podnosi.

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***Ključne reči**: metabolička disfukcija povezana sa masnom bolešću jetre metformin, indeks telesne mase*