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# The effect of metformin on biochemical paremetres in patients with nonalcoholic fatty liver disease

Vesna Brzački[1,2], Marko Stojanović[2,3], Andrija Rančić[2,3], Milica Bjelaković[2,3]

[1]Univeristy of Niš, Faculty of Medicine, Niš, Serbia

[2]University Clinical Center Niš, Clinic of gastroenterology and hepatology, Niš, Serbia

[3]University of Niš, Faculty of Medicine, doctoral studies, Niš, Serbia

Contact: Vesna Brzački

48 Dr Zorana Djindjića Blvd., 1800 Niš, Serbia

E-mail: brzackiv@gmail.com



Nonalcoholic fatty liver disease (NAFLD) 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 aim of the study was to examine the effects of metformin on achieving positive biochemical responses in patients with NAFLD. The study included 146 patients, 96 men and 50 women, with NAFLD diagnosed by ultrasound. We performed biochemical analyzes. 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 750mg of metformin twice a day. There is a reduction of body weight, which was statistically significant after six months. The BMI decrease reach no statistical significance. Liver enzymes values showed a significant decrease in values relative to baseline after three and after six months of metformin therapy. Serum cholesterol and triglyceride levels are reduced during treatment with metformin and changes reached statistical significance at six months relative to baseline. There was a statistically significant decrease in HOMA IR value after

three and after six months compared to baseline. Our results show that metformin may be an appropriate addition to the diet, weight reduction and physical activity because it achieves the improvement in metabolic parameters, with almost no adverse events and good tolerance of therapy.

Key words: NAFLD, metformin,BMI

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# Uticaj metformina na biohemijske parametre kod pacijenata sa nealkoholnom masnom bolesti jetre

Vesna Brzački[1,2], Marko Stojanović[2,3], Andrija Rančić[2,3], Milica Bjelaković[2,3]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

[2]Univerzitetski klinički centar Niš, Klinika za gastroenterologiju i hepatologiju, Niš, Srbija

[3]Univerzitet u Nišu, Medicinski fakultet, student doktroskih studija, Niš, Srbija

Kontakt: Vesna Brzački

Bulevar dr Zorana Đinđića 48, 1800 Niš, Srbija

E-mail: brzackiv@gmail.com



Nealkoholna masna bolest jetre (NAFLD) je najčešći oblik hronične bolesti jetre u savremenom svetu. Može napredovati u nealkoholni steatohepatitis, što povećava rizik od razvoja ciroze jetre i hepatocelularnog karcinoma. Cilj studije je bio da se ispitaju efekti metformina na postizanje pozitivnih biohemijskih odgovora kod pacijenata sa NAFLD. Studija je obuhvatila 146 pacijenata, 96 muškaraca i 50 žena, sa NAFLD dijagnostikovanim ultrazvukom. Uradjene su biohemijske analize. Vrednosti svih parametara merene su na početku, nakon tri i nakon šest meseci terapije. Prilikom svake posete merena je telesna težina i indeks telesne mase (BMI). Svi pacijenti su na početku primali 750 mg metformina dva puta dnevno. Nakon šest meseci došlo je do statistički značajnog smanjenja telesne težine. Smanjenje BMI nema statistički značaj. Vrednosti enzima jetre su pokazale značajno smanjenje vrednosti u odnosu na početnu vrednost nakon tri i nakon šest meseci terapije metforminom. Nivoi holesterola i triglicerida u serumu su smanjeni tokom lečenja metforminom, sa statističkom značajnosti nakon šest meseci u odnosu na početnu vrednost. Došlo je do statistički značajnog smanjenja vrednosti HOMA IR nakon tri i nakon šest meseci u poređenju sa početnom linijom. Naši rezultati pokazuju da metformin može

biti adekvatan dodatak ishrani, jer doprinosi smanjenju telesne težine i fizičkoj aktivnosti, postiže poboljšanje metaboličkih parametara, gotovo bez neželjenih efekata i dobruse toleriše.

Ključne reči: NFLD, metformin, BMI

Introduction

There is an obvious increase in number of people with nonalcoholic fatty liver disease, NAFLD. Today NAFLD 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, NAFLD is a growing health problem which affects 20-30% of the population, while in diabetes mellitus type 2 may be as high as 75% (1). NAFLD is a state of accumulation of fat in the liver with no significant alcohol abuse. There is a wide range of changes of the liver, from the common hepatic steatosis to non-alcoholic steatohepatitis, liver damage which is similar to that of alcoholic liver disease.NAFLD is clearly associated with the risk of developing type 2 DM and cardiovascular disease (2). NAFLD is a common cause of asymptomatic elevation in liver enzymes, in particular alanine aminotransferase, ALT (3).

The pathogenesis of NAFLD 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 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 NAFLD. 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 may find mild to moderate elevations in ALT and AST enzymes with a ratio of AST: ALT less then 1. Hepatic enzymes can be considered normal in 75% of cases, so that the increase of enzyme is not sensitive for the diagnosis of NAFLD (6). For now, NAFLD ideal therapy do not exists. Changing lifestyle, diet and weight loss are difficult to achieve and maintained. As insulin resistance is a key pathogenetic mechanism of NAFLD 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 NAFLD.

The aim of the study was to examine the effects of metformin on achieving positive biochemical responses in patients with NAFLD.

Meterials and methods

The study included 146 patients, 96 men and 50 women, of the Clinic for Gastroenterology and Hepatology, Clinical Center Nis, Nis, in the period from 2022. to 2024., with the signed consent of the patient. The study has approval of local ethics committee. The study included patients with NAFLD diagnosed by ultrasound. Ultrasound parameters for diagnosis were presence two of the four ultrasound criteria. 1. Liver echogenicity exeeding that on renal cortex, 2. loss of definition of the diapragm, 3 poor delineation of the intrahepatic arhitecture, 4. attenuation of ultrasound wave. Ultrasound examinations were performed by one ultrasonografist on camera

Including criteria were abstinence from alcohol or alcohol use no more than two drinks a week, less than 20g / day, increase in serum ALT and AST greater than 1.5 x ULN, negative viral markers, HBsAg, and HCV, no data of chronic liver disease, Wilson, hemochromatosis. The study did not included patients with diabetes mellitus, the use of certain drugs, methotrexate, amiodarone, Cushing's disease, liver cirrhosis, chronic renal failure, heart failure NYHA III and IV, previous usege of metformin, lactate values over 2.2 mmol / l. All patients underwent ultrasound examination of the liver, when they was diagnosed with NAFLD. We performed biochemical analyzes of ALT, AST, gamma glutamyl transferase, alkaline phosphatase enzyme, glucose, cholesterol, triglycerides.

We also determined insulin, C-peptide levels and HOMA-IR. 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 analyzes were performed at the Institute of Biochemistry, Clinical Center Nis, and fasting insulin and C-peptide concentrations were determined in Center for nuclear medicine, Clinical Center Nis. All patients at baseline received 750mg of metformin twice a day. All data is expressed in mean±SD. Students t test and chi square test were used to determine the differences between groups. A p value of $< 0.05$ was taken as significant.

Results

The basal values of observed parameters, as well after three and after 6 months from the inclusion of metformin are given in Table 1. There is a reduction of body weight, which was statistically significant after six months, as compared to baseline, but not in relation to the value

after 3 months. The BMI decrease reach no statistical significance. There are no significant changes in fasting blood glucose value. Serum cholesterol and triglyceride levels are reduced during treatment with metformin and changes reached statistical significance at six months relative to baseline. Impairment of AST has statistical significance after three and six months compared to baseline, as well as impairment after 6 months compared to value after three months. ALT level showed a statistically significant reduction after three and aftersix months compared to baseline, no significant differences after 6 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, no significant differences after 6 months compared to 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 6 months compare to baseline, $p<0.01$

** statistical significance after 6 months compare to 3 months, $p<0.01$

Discussion

Metformin was introduced as a first-line treatment of diabetes mellitus type 2 for more than half a century. The effect of metformin lowering blood glucose is explained by reducing hepatic gluconeogenesis, stimulation of 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 by decreasing synthesis.

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

It was observed that even smaller reduction in body weight can lead to improvements in markers of NAFLD, in particular ALT and imaging markers of liver fat (9). There is also no significant differences in blood glucose value during follow-up. Cholesterol and triglyceride levels are reduced after three and after six months, with a statistically significant change after 6 months compared to baseline values. Metformin significantly reduces the percentage of patients with NAFLD in impaired fasting glucose, IFG, compared to patients treated with diet alone. Metformin therapy also significantly lowered the percent of patients who meet the diagnostic criteria for metabolic syndrome (10). There is data of different lipid changes during treatment NAFLD with metformin, from mild to moderate decrease, also that during the follow-up of 12 months there was no changes in lipid levels, even with the increase during that period (11). Our results show a reduction of lipids values after three and after six months of therapy,

The role of insulin resistance in the development of NAFLD is complex, so that both hepatic and peripheral insulin resistance are clearly associated with the emergence of NAFLD. There is the diminished ability of insulin to suppress lipolysis, which increases the inflow of the free fatty acids from adipose tissue to the liver. There is 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 NAFLD 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 NAFLD. Reducing the levels of insulin using metformin reduces the risk that becomes similar to the risk in people who have had low or normal basal insulin levels. High insulin levels probably results in primary insulin resistance rather than decrease hepatic extraction of insulin in any liver disease (12, 13).

Liver enzymes values showed a significant decrease in values relative to baseline after three and after six months of metformin therapy. There is statistical significance of changes in AST after six months compared to baseline and after three months, while ALT level after six months shows value similar to those after three months of treatment. Most studies show a significant reduction in ALT and AST by metformin therapy with normalization of ALT as much as in 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 NAFLD, but because of possible complications, cost and inconvenience for patients is often replaced by ultrasound and CT diagnostics. Percentage of patients with NAFLD was higher if the diagnosis was performed by liver biopsy. In this way we can get the data if there is NAFLD or NASH. Studies investigating the histological changes of liver biopsy showed no significant difference in the histological findings in the course of metformin. Despite the good response and the improvement in metabolic parameters, only about 30% of the patients showed noticeable improvement in the level of steatosis, and only 20% of patients show an improvement in the degree of inflammation after one year of metformin (11, 14). There is a question of treatment duration and daily dose of merformina. There are a variety of study duration and administered dose of metformin. Duration ranges from 4-12 months, a total daily dose of metformin from 0.85gr to 3gr, average

1.5gr. Because of, for now, a limited number of studies the optimal dose of metformin and duration of treatment has not yet been defined (15).

## Conclusion

The specific drug therapy of NAFLD is not yet defined and available, and no drug can be a substitute for lifestyle modification. Our results show that metformin may be an appropriate addition to the diet, weight reduction and physical activity because it achieves the improvement in metabolic parameters, with almost no adverse events and good tolerance of therapy, although not lead to significant histological changes in the liver. It is important to reduce the risk of metabolic syndrome and cardiovascular risk.



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