

ORIGINAL PAPER

Effect of Probiotics on Liver Function After Surgery Resection for Malignancy in the Liver Cirrhotic

Zijah Rifatbegovic¹, Deso Mesic¹, Farid Ljuca², Muharem Zildzic², Mustafa Avdagic², Kemal Grbic³, Mirha Agic¹, Bahrudin Hadziefendic¹
 Clinic for Surgery, University Clinical Centre Tuzla, Bosnia and Herzegovina¹
 The University of Tuzla, Faculty of Medicine, Bosnia and Herzegovina²
 General Hospital Sanski Most, Bosnia and Herzegovina³

Introduction: Hepatic resection is the accepted treatment for various liver tumors. Increasing evidence suggests that two factors significantly influence outcome and successfulness of the hepatic resection in patients with HCC in cirrhosis. There are liver function recovery and the degree of inflammation during early postoperative period. Objective: Aim of this study was to determine whether probiotic use influences on liver function recovery, degree of inflammation during early postoperative period, intraoperative risk, type and frequency of intraoperative and postoperative complications, morbidity, intraoperative and early postoperative mortality and a one-year survival rate in patients who have been used probiotic, and underwent the hepatic resection due to HCC in cirrhosis. Patients and methods: Study was conducted on 120 patients underwent the hepatic resection due to HCC in cirrhosis. This study has been done in University Clinical Centers Tuzla, Maribor and Strasbourg from October 2006 till February 2008. Patients were divided into 2 groups: 1) patients with liver cirrhosis and histologically verified HCC whom underwent liver resection surgery (segmentectomy/bisegmentectomy, right and left hemihepatectomy/extended hemihepatectomy) that used preoperatively and postoperatively probiotics (n = 60), 2) a control group of patients with liver cirrhosis and HCC, which did not use preoperative and postoperative probiotics (N = 60). Treatment with probiotics was conducted 3 days preoperatively and postoperatively with 7 day's oral supplementation. Results: This study have shown next: patients underwent to the hepatic resection due to HCC in cirrhosis who have been used preoperatively and postoperatively probiotic had liver function recovery better and faster, acute immune response better, serum level of tumor markers lower, intraoperative and postoperative complications were less frequent, and morbidity and mortality rates were lower than in those who have not been using probiotic. Conclusion: Probiotic use may make liver function recovery better and increases immune response in early postoperative period and positively influences outcome and successfulness of the hepatic resection in patients with HCC in cirrhosis. **KEY WORDS: HEPATIC RESECTION, CIRRHOSIS, HCC, PROBIOTIC**

Corresponding author: Ass prof Zijah Rifatbegovic, MD, PhD. University Clinical Center Tuzla, Department HBP surgery Tel. +387 35 30 31 25, GSM +387 61 89 45 55 Email: drzooro@gmail.com

1. INTRODUCTION

Cirrhosis is a diffuse liver disease characterized by fibrosis with conversion of normal liver architecture into

structurally abnormal nodules that have lost their normal lobular organization. Chronic liver damage resulting in inflammation, necrosis and eventu-

ally fibrosis. General characteristics of cirrhosis are the appearance of regenerative nodules separated by fibrous septum and loss of normal lobular architecture of the liver. The diagnostic procedures include tests of liver function (serum albumin and prothrombin time are the best indicators of liver function), biochemical tests (serum alkaline phosphatase and serum aminotransferases), serum electrolytes (sodium, low level indicator is difficult disease liver, which leads to a disorder of metabolism and water requiring diuretic therapy, increased serum alpha-fetoprotein above 400 ng/L indicates the premise for hepatocellular carcinoma (1,2). Hepatocellular carcinoma is the most common primary tumor of the liver in the world. Its incidence is about 1 million new cases per year, mostly in Southeast Asia and tropical Africa. Mortality is almost 100% except for a small number of early detected cases (3). Liver regeneration is altered in cirrhosis (4). Resection of the liver is sometimes extremely difficult tolerated by patients with cirrhosis, with a potential risk to a fatal outcome due to risk of liver failure, which dominates the clinical picture. The presence of portal hypertension may initiate postoperative complications such as rupture of esophageal varices and the development of ascites (5). In patients hospitalized for cirrhosis occurs mainly means malnutrition, 30-70% (6, 7). Factors associated with an increased risk of postoperative complications were: weight loss greater than 10% compared to preoperative body weight, serum albumin level lower than 30g/L. Therefore, the need for preoperative nutrition artificially

patients undergoing liver resection, now indispensable for a better outcome (8, 9, 10). Probiotics, live bacteria that lead to the improvement of the patient through the established intestinal microbiological balance, today it is widely used as a dietary supplement. Prebiotics presents indigestible food ingredients that significantly influence the growth or activity of one or a limited number of different types of bacteria in the have potential to improve patient health in general. When used in combination, probiotics and prebiotics are called symbiotic. Bacterial translocation with cirrhosis was defined as migration of bacteria from the lumen to intestinal mesenteric lymph node. Most Active Bacterial translocation to lymph nodes mesenteric gram negative family members of entero-bacteria (*Escherichia coli* and *Klebsiella*) enterococci and other streptococci. These organisms are most commonly associated with infections in patients with cirrhosis that may be translocation even through the normal histological intestinal mucosa.

2. PATIENTS AND METHODS

The study was prospective, which began in October 2006 and lasted until February 2008 when we collected the required number of patients by choosing consecutive sample. It is conducted in the Tuzla, University Clinical Center, University Hospital of Strasbourg and Hautepierre, Maribor, University Clinical Center, Department of Abdominal Surgery. The study was conducted on 120 patients with HCC in liver cirrhotic selected for implementation resection procedures and were performed in university hospitals in Tuzla, Maribor and Strasbourg from October 2006 to February 2008.

Patients were divided into 2 groups: 1) patients with liver cirrhosis and histologically verified by HCC in whom underwent liver resection surgery (segmentectomy/bisegmentectomy, right and left hemihepatectomy/extended hemihepatectomy) that were used preoperatively and postoperatively probiotics (n=60), 2) a control group of patients with liver cirrhosis and HCC which did not use preoperative and postoperative probiotics (N=60). All patients underwent intestinal preparation from iso-osmotic solution (2L), which was given the day before surgery and received antibiotic prophylaxis, and (third-generation

cephalosporin's product) and the infusion of glucose solution 10% in 60 minutes before surgery. Patients with the size of HCC smaller than 3 cm are treated by resection procedure of volume segmentectomy or bisegmentectomies Resection surgeries and extended of the scope hemihepatectomy were performed in patients with tumor sizes of 2 or more adjacent segments. Treatment was not done in patients with diffuse liver cancer and patients with multivisceral resection. Treatment with probiotics is conducted 3 days preoperatively and postoperatively for 7 days of oral supplementation (probiotic and fiber ferment) preparation containing: *Lactobacillus plantarum* 2362, *L paracasei* subsp *paracasei* 19, *Pediococcus pentoseceus* 5-33:3 and 32-77:1 *L raffinolactis*. The composition has four specific LAB: 10 *Pediococcus pentosaceus* 5-33:3, 10 *Leuconostoc mesenteroides* 32-77, 1.10 *L paracasei* *L paracasei* subgroup *paracasci* 19:10 *L plantarum* alpha 362nd Treatment with probiotics is to start the preoperative oral administration, a day after surgery eneralnom nutrition through nasogastric transducer 100 kcal per day and gradually increased to 400 kcal / dando 3 or 4 days, gradually reducing where enteral feeding, in proportion to the increase of oral feeding. All patients preoperatively and seventh and fourteenth postoperative day were analyzed functional parameters of liver (total bilirubin, indirect bilirubin, the direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl trans-

	Without the use probiotics (%)	With the use probiotics (%)	P
Early complications	33,33	13,89	<0001
Late complications	19,44	8,33	<0,001
Intraoperative mortality	2,78	0	<0,0001
Early mortality postoperative	8,33	2,78	<0,02
Late mortality postoperative	13,89	5,56	<0,01
Survival rates	66,67	86,11	<0,05

TABLE 1. Postoperative complications, mortality and survival rate of patients with and without the use of probiotics

ferase (GGT), alkaline phosphatase (ALP), overall protein, serum albumin, cholinesterase (HE), cholesterol test, and retention indocyanin green (ICG) at 15 min) and the parameters of the acute phase immune response: CRP (4 mmol / l), IL-1 (15.6 pg / ml), IL-6 (12.5 pg / mL) and tumor-necrosis factor (TNF- α to 3.91 pg / ml), the value of tumor markers alpha-fetoprotein (AFP) and cancerembrioantigen

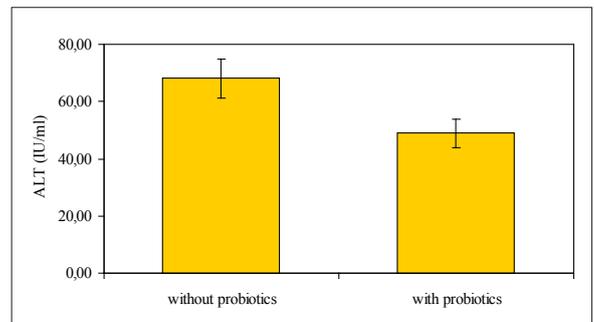


FIGURE 1. Density ALT in serum 14th postoperative day with and without the use probiotics

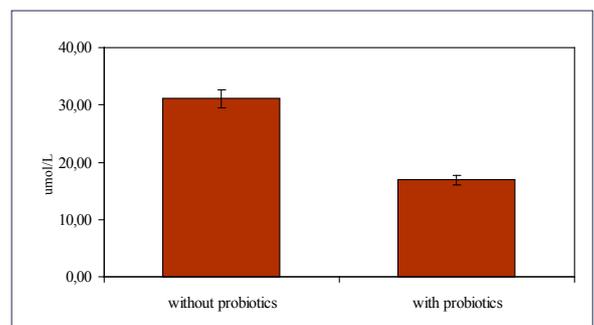


FIGURE 2. Density total bilirubin in serum 14th postoperative day with and without the use probiotics

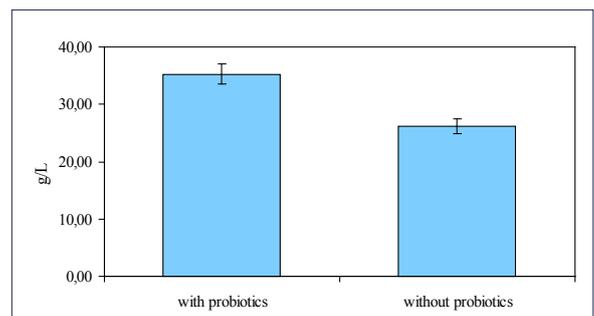


FIGURE 3. Density albumin in serum 14th postoperative day with and without the use probiotics

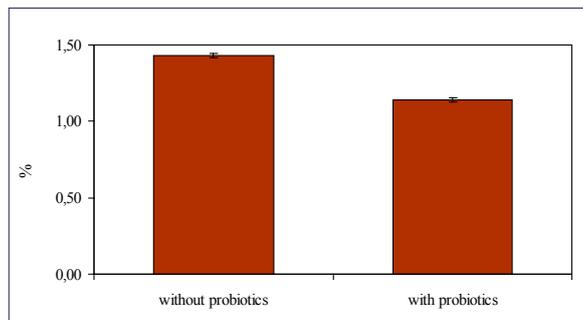


FIGURE 4. Test retention indocyanin green 14th postoperative day with and without the use probiotics

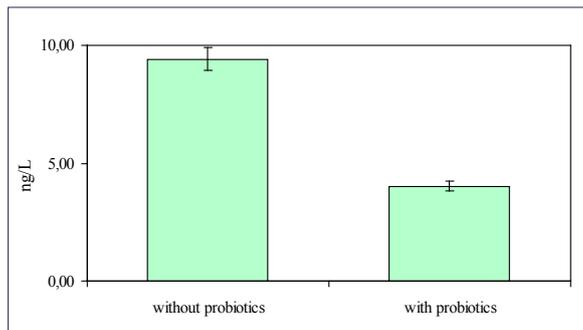


FIGURE 5. Density TNF in serum 14th postoperative day with and without the use probiotics

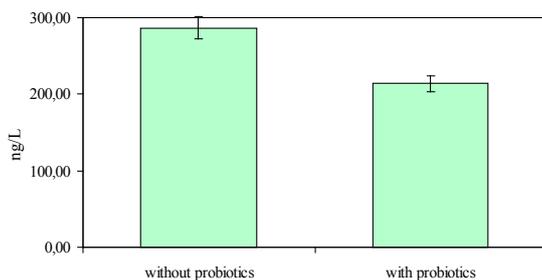


FIGURE 6. Density AFP in serum 14th postoperative day with and without the use probiotics

(CEA), the type and number of operative complications. The results were analyzed using Chi-square, Student t-test, Kaplan-Meier's test and ANOVA test for analysis of variance between groups. Statistical results were considered significant if the difference between the values of parameters were analyzed with statistical probability of $p < 0.05$.

3. RESULTS

The concentration of serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) preoperatively, seventh and fourteenth postoperative day in patients who used the probiotic was significantly lower than in those who are not used ($p < 0.05$, $p < 0.02$, $p < 0.02$) (Figure 1). Serum gamma-glutamyl transferase (GGT) preoperatively, on the sev-

enth and the fourteenth postoperative day were higher in both groups but in the group that used probiotics was significantly lower than that which it is used ($p < 0.05$). Preoperatively, the seventh and the fourteenth postoperative day the concentration of cholinesterase and total cholesterol in serum of patients who had and who did not use probiotics did not differ significantly. Preoperatively, the seventh and the fourteenth postoperative day concentration of bilirubin (total and conjugated) in patients who underwent liver resection, using the probiotic significantly lower than in those who did not use probiotics ($p < 0.05$, Figure 2). Comparing the preoperative, the seventh and the fourteenth postoperative day values for total protein and albumin in patients who underwent liver resection surgery has been found that it was significantly higher in those who used the probiotic than in those who did not use ($p < 0.05$, Figure 3). Preoperatively, on the seventh and the fourteenth postoperative retention test day values of indocyanin green in patients undergoing liver resection was found that its value was significantly lower in those patients who used the probiotic than in those who have not used ($p < 0.01$, Figure 4). Concentrations of protein acute phase responses (CRP, IL-1, IL-6 and TNF) in serum of patients who had and who did not use probiotics were elevated but between their values, no statistically significant differences preoperatively ($p < 0.05$), the seventh and the fourteenth postoperative day the concentration of acute phase proteins responses were significantly lower in patients who have used probiotics ($p < 0.02$, Figure 5). Concentrations of tumor markers CEA and alpha fetoprotein in serum patients who had and who did not use probiotics were elevated but between their values,

no statistically significant differences ($p < 0.05$) preoperatively, and the seventh postoperative day, the fourteenth postoperative day the values were still elevated, but were statistically significantly lower in Patients who have used probiotics ($p < 0.02$, Figure 6). The incidence of early and late post-operative complications after hepatic resection in patients who used the probiotic was significantly smaller than in those who did not use (13.89 vs. 33.33%, $p < 0.0001$ and 8.33 vs. 19.44%, $p < 0.001$, Table 1). Rates of intraoperative, early postoperative and late postoperative mortality after liver resection were statistically significantly lower in patients who used the probiotic than those who did not (0 vs. 2.78%, $p < 0.0001$; 8.33 vs. 2.78, $p < 0.02$, 5.56 vs. 13.89 $p < 0.01$, Table 1). Survival rates of patients after surgery resection the liver in patients who used the probiotic was significantly greater than in those who did not use (86.11 vs. 66.67%, $p < 0.05$, Table 1). There was no statistically significant difference between the value of Child-Pough and MELD score and type of cirrhosis in patients who are not used probiotic, and they underwent liver resection for hepatocellular carcinoma in cirrhosis.

4. DISCUSSION

Serum levels of AST and ALT are markers of hepatocyte damage, since these enzymes when the damaged, hepatocytes come from them in the extracellular fluid and appear in serum. The analysis of serum AST and preoperative. No statistically significant difference in both groups of patients, seventh and fourteenth postoperative day was found statistically significant differences in patients who used the probiotic and the concentrations were significantly lower. Similar results were found and other studies (8,9,10). These results indicate a slower recovery and the recovery of liver function in patients with resection procedure who did not use probiotics. Concentrations of GGT in serum of patients postoperatively and seventh fourteenth day were significantly lower in patients who used the probiotic. Similar results were found in other studies (8,11). Postoperative concentrations of ALP in serum of patients who underwent resection surgery preoperatively were not significantly different. These results indicate that cholestasis is more intense in pa-

tients who underwent liver resection surgery in cirrhosis without treatment probiotic. Relative cholestasis reflects the regeneration process in the liver. These results are consistent with results of other studies (8,10). The use of probiotics improves erythropoiesis and oxygenation tissue, accelerates the elimination of harmful products of metabolism improving so create intense blood elements required by frankie stimulants erythropoiesis, primarily erythropoietin. Similar results were published (8,11,12). Postoperatively fourteenth day the values of serum total, conjugate, unconjugated statistical bilirubin were significantly closer to the reference group of patients who have used probiotic. These results are consistent with results of other studies (4,8). After resection surgery, IL-6, IL-1 and CRP concentrations were significantly decreased in the group of patients who used preoperative and postoperative seventh and fourteenth day probiotics. Preoperative use of probiotics enhances natural immunity, particularly increasing the resistance to infection, weakening the possible postoperative inflammatory reaction and reducing postoperative infectious complications, thus shortening the time patients stay in hospital, and the time of use of antibiotic therapy (13). In patients with obstructed jaundice in whom made external biliary drainage has been reduced markers of liver necrosis, conversely an increase in activity of membrane stability as a consequence of treatment changes of bile from which the conclusion is that changes in bile during external biliary drainage can re-establish the function of intestinal wall (intestinal barrier), the primary recovery of physical damage the intestinal mucosa (8,11,14,15). Early and late postoperative complications, intraoperative mortality, early and late postoperative mortality were significantly lower in patients who used the probiotic. Survival rate was statistically significantly higher in patients who used the probiotic preoperatively, seventh and fourteenth days postoperatively. One of the important results of our study is certainly the conclusion that preoperative use of probiotics improves the result of resection of the tumor in the liver and enhances the effect of cell activity during treatment.

5. CONCLUSION

The concentration of serum aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, conjugated bilirubin, the test for retention of green indocyanin seventh and fourteenth postoperative day in patients who used the probiotic was significantly lower than in those who did not use. The seventh and the fourteenth postoperative day the concentration of cholinesterase and serum cholesterol in patients who had and who did not use probiotics did not differ significantly. Comparing the amount of total protein and albumin seventh and fourteenth day postoperatively in patients who underwent liver resection surgery, it was found that it was significantly higher in those who used the probiotic than in those who did not use. Seventh and fourteenth postoperative day the concentration of protein acute phase response (CRP, IL-1, IL-6 i TNF) in serum of patients who did not use probiotic were elevated but were significantly lower in those patients who used the probiotic. The incidence of early and late postoperative complications after liver resection in patients who used the probiotic was significantly lower than in those who did not use. Rates of intraoperative, early postoperative and late postoperative mortality after liver resection were statistically significantly lower in patients who used the probiotic. Overall survival rate after surgery resection was statistically higher in the patients who used the probiotic.

REFERENCES

1. Fan ST. Methods and related drawbacks in the estimation of surgical risks in cirrhotic patients undergoing hepatectomy. *Hepato-gastroenterology*, 2002; 49 (43): 17-20.
2. Jain PK, McNaught CE, Anderson AD, MacFie J, Mitchell CJ. Influence of synbiotic containing *Lactobacillus acidophilus* LA5, *Bifidobacterium lactis* BP12, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and oligofructose on gut barrier function and sepsis in critically ill patients: a randomized controlled trial. *Clin Nutr*, 2004; 23: 467-75.
3. Ercolani G, Grazi GL, Gian Luca, Vetrone G, Aramaki O, Cescon M et al. Liver Resection for Hepatocellular Carcinoma on Cirrhosis: Univariate and Multivariate Analysis of Risk Factors for Intrahepatic Recurrence *Annals of Surgery*, 2003; 237(4): 536-43.
4. Shoup M, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH et

- al. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. *Gastrointest Surg*, 2003; 7(3): 325-330.
5. Ezaki T, Yamamoto K, Yamaguchi H, Sasaki Y, Ishida T, Mori M et al. Hepatic resection for hepatocellular carcinoma existing with liver cirrhosis. *Hepatogastroenterology*, 2002; 49(47): 1363-6.
6. Kalliomäki M, Salminen S, Poussa T, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*, 2003; 361: 1869-1992.
7. Kanazawa H, Nagino M, Kamiya S, Komatsu S, Mayumi T, Takagi K et al. Antibiotics reduce postoperative infectious complications: a randomized controlled trial in biliary cancer patients undergoing hepatectomy. *Langenbecks Arch Surg*, 2005; 390:104-113.
8. Bengmark S. Synbiotics to strengthen gut barrier function and reduce morbidity in critically ill patients. *Clin Nutr*, 2004; 23: 441-5.
9. Clavien PA, Petrowsky H, Deoliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. *N Engl J Med*, 2007; 356, 1545-59.
10. Clavien PA, Selzner M, Rüdiger HA, Graf R, Kadry Z, Rousson V et al. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. *Ann Surg*, 2002; 238(6): 843-850.
11. Bengmark S. Gut and the immune system: enteral nutrition and immunonutrients. In: SIRS, MODS and MOF—Systemic Inflammatory Response Syndrome, Multiple Organ Dysfunction Syndrome, Multiple Organ Failure. In: Pathophysiology, Prevention and Therapy. Baue E A Faist E, Fry D, eds. Springer, New York, 2000; 420-36.
12. Nanashima A, Yamaguchi H, Shibasaki S, Morino S, Ide N, Takeshita H. Relationship between indocyanine green test and technetium-99m galactosyl serum albumin scintigraphy in patients scheduled for hepatectomy: Clinical evaluation and patient outcome. *Hepato Res*, 2006; 28(4): 184-190.
13. Rayes N, Seehofer D, Theruvath T, Mogl M, Langrehr JM, Nüssler NC et al. Combined perioperative enteral supply of bioactive pre- and probiotics abolishes postoperative bacterial infections in human liver transplantation: a randomised, double-blind clinical trial. *Am J Transplant*, 2005; 5: 125-30.
14. Sheih YH, Chiang BL, Wang LH, Liao CK, Gill HS. Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. *J Am Coll Nutr*, 2001; 20:149-56.
15. Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, Nomoto K, Nimura Y. Perioperative Synbiotic Treatment to Prevent Postoperative Infectious Complications in Biliary Cancer Surgery: A Randomized Controlled Trial. *Ann Surg*, 2006; 244 (5):706-14.