SERUM ACTIVITY OF ENZYMES AND BILIRUBIN IN PATIENTS WITH CIRRHOSIS AND LIVER CANCER

Nafija Serdarević[1,2], Marina Ćurlin[3], Monika Franković[2,4], Sabina Šegalo[2], Nermina Klapuh-Bukvić[1,2], Ivana Vujica[4]

[1]Institute for Clinical Biochemistry with Immunology, Clinical Center of the University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina

[2]Faculty of Health Studies, University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina

[3]Faculty of Health Studies, University of Mostar, 88000 Mostar, Bosnia and Herzegovina

[4]Hospital "Dr. Fr. Mato Nikolić", Laboratory, 72276 Nova Bila, Republic of Croatia

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ABSTRACT

Introduction: The most important biochemical reactions of the human organism take place in the liver, and therefore it represents one of the most important organs for life. Parameters that play an important role in the diagnosis and monitoring of patients are the enzymes ALT, AST, GGT, ALP, and bilirubin.

Objective: To evaluate the serum activity of enzymes and bilirubin in patients with liver cirrhosis and liver cancer. Materials and methods: The study included 120 patients aged over 50 years. Among them, 40 patients had liver cirrhosis, 40 had cancer and liver metastases, and 40 patients were apparently healthy (control group). The concentrations of AST, ALT, GGT, and ALP were determined on Abbott Architect i2000sr biochemical analyzer and Dimension analyzer.

Results: The study showed that the mean values of the studied parameters were significantly higher in subjects with liver cirrhosis and cancer with liver metastases than in the control group. A statistically significant difference ($p < 0.05$) was found in ALP and bilirubin concentrations between the studied groups. In addition, the study revealed a statistically significant difference ($p < 0.05$) in ALT, AST, and GGT activity between subjects with cancer and the control group and subjects with cirrhosis and the control group.

Conclusions: The results confirm that the activities of enzymes ALT, AST, GGT, ALP, and bilirubin were increased in subjects with cirrhosis and cancer compared to the control group.

Keywords: enzymes, bilirubin, liver cirrhosis, liver cancer

Corresponding author: Nafija Serdarević, PhD, Full Professor

e-mail: serdarevicnafija@yahoo.com

INTRODUCTION

The liver is responsible for several functions, including primary detoxification, protein synthesis, and digestive enzyme production. It plays an important role in metabolism, in the regulation of red blood cells, and in the synthesis and storage of glucose. Liver function tests include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), 5'nucleotidase, total bilirubin, conjugated (direct) bilirubin, unconjugated (indirect) bilirubin, prothrombin time (PT), international normalized ratio (INR), lactate dehydrogenase, total protein, globulins, and albumin (1).

Chronic liver injury leads to inflammation and fibrosis of the liver. The occurrence of fibrosis completely alters the structure of the liver and disrupts the liver parenchyma and vascular architecture. Progressive fibrosis and cirrhosis then lead to decreased metabolic and synthetic function of the liver, causing increased bilirubin levels and decreased production of clotting factors and platelets. Cirrhosis can be the result of chronic liver injury from any cause. Factors associated with an increased risk of progression to cirrhosis include older age, mela gender, and comorbidities (especially in patients coinfected with HIV and HCV) (2, 3). Recent research has shown that liver fibrosis is a dynamic process, and that early cirrhosis is even reversible (4).

Primary liver cancer accounts for 6 % of all cancers and 9 % of all cancer deaths, is the sixth most common cancer, and the second leading cause of cancer death. Major primary liver cancers include hepatocellular carcinoma (HCC), which accounts for about 75 %, and cholangiocarcinoma (6 %). Although either surgical resection or liver transplantation can be used to treat liver cancer, there are limitations due to the high recurrence rate and the low acceptance of transplantation, as this cancer is often detected at a late stage (5, 6).

Aminotransferases, including aspartate transaminase (AST) and alanine aminotransferase (ALT), which are known circulating blood-based biomarkers, have historically been used to demonstrate liver injury. However, elevated aminotransferases have also been found to be associated with human disease and systemic dysregulation of metabolic function (7).

Gamma-glutamyl transferase (GGT) is an enzyme in the liver located mainly on the capillary side of liver cells and the membrane of bile duct epithelial cells. Hypersynthesis in the liver, obstruction of bile secretion, and injury and hyperplasia of the bile duct epithelium can cause elevated serum GGT. Elevated GGT is found in many liver diseases and understanding the characteristics of GGT in different liver diseases may be helpful to better understand the pathogenesis of liver diseases and select therapeutic targets (8).

Intestinal alkaline phosphatase (IAP) is a subtype of the alkaline phosphatase family and is produced exclusively in the intestine, with the highest expression in the duodenum (9). Endogenous IAP levels have been shown to be lower in inflammatory bowel diseases such as

inflammatory bowel disease and diabetes. IAP levels have not yet been studied in the context of liver fibrosis (10, 11). The aim of this study is to investigate serum enzyme and bilirubin activity in patients with liver cirrhosis and liver cancer.

MATERIALS AND METHODS

The retrospective study was conducted in the Hospital "Dr. fra Mato Nikolić", Nova Bila, and included data from medical records collected from the beginning of 2014 to the end of 2018. The study included 120 patients aged over 50 years. Among them, 40 patients had liver cirrhosis, 40 patients had cancer and liver metastases, and 40 patients were apparently healthy (control group). Medical documentation was used for data on laboratory, clinical, and demographic characteristics: sex, age, and enzyme activity of ALT, AST, GGT, ALP, and total bilirubin levels.

The activities of enzymes AST, ALT, GGT, and ALP were determined on the biochemical analyzer Abbot Architect i2000sr and Dimension analyzer. The basis of all methods for measuring enzyme activity is based on the same principle - the enzyme acts on its specific substrate, whereupon the concentration of the resulting reaction product or the decrease in the concentration of the substrate is usually measured, and rarely the concentration of the enzyme itself. The measurement of the concentration of the resulting product actually determines the rate of the enzymatic reaction, i.e., the catalytic concentration.

Microsoft Excel was used to prepare and store the data for statistical analysis. The software package used for data processing was IBM SPSS Statistics for Windows, version 21.0 (Armonk, NY: IBM Corp) and MedCalc. Qi Macros 2019 program was used for the graphical layout. The data obtained are presented in tables and figures. The tested results were statistically processed for $p < 0.05$.

RESULTS

A total of 120 subjects participated in the study. Of these, 33% of subjects were diagnosed with liver cirrhosis, 33 % had cancer, and 33 % of subjects were in the control group. The group with liver cirrhosis consisted of 65 % men and 35 % women, and in the group with cancer were 55% of men and 45 % of women. In the control group, we had the same number of males and females. The average age of the subjects in the whole sample was 68.1 +/-8.5 years, with the youngest subject being 53 years old and the oldest 94 years old. In the group of subjects with liver cancer, 68 % were at the stage of metastasis, and 33% had already been diagnosed with cancer. The results show that the group with liver cancer had the highest mean ALT values, followed by the group with liver cirrhosis, and the lowest in the control group, as shown in Figure 1. ALT enzyme activity in the control group had the lowest value of 10 U/L, and the highest value was 39 U/L. In the disease groups of patients with liver cirrhosis, the lowest value was 20 U/L, and the highest was 118 U/L, while in the group with cancer, the lowest activity was 12 U/L and the highest was 422 U/L.

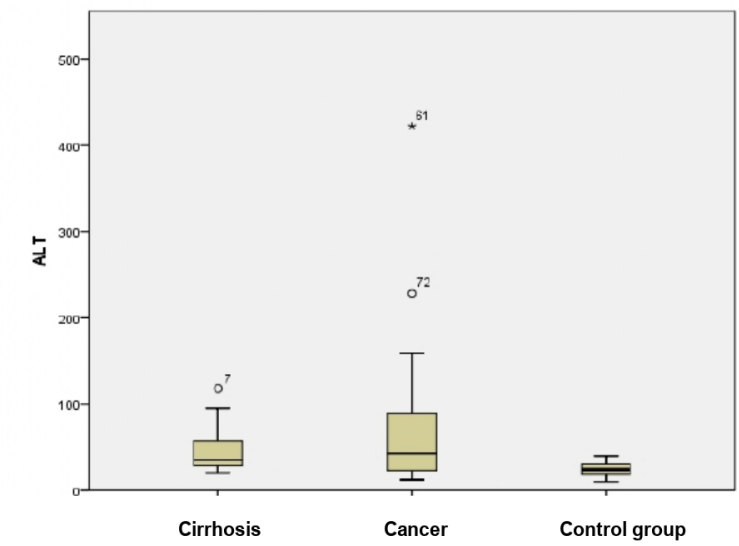

Figure 1. Graphic representation of the average value for ALT.

A statistical difference between groups was determined using the Mann-Whitney U test for ALT and a statistical difference of $p < 0.0001$ was found between the control group and the cancer group and the control group and cirrhosis. Using the same test, no statistical difference was found between the liver cirrhosis and liver cancer groups ($p = 0.6100$). The results are shown in Table 1.

Table 1. Comparison of the catalytic activity of the enzyme ALT between the tested groups.

Comparative groups	Mann-Whitney U	Z	p
ALT Cancer/Control group	360.50	4.23	<0.0001
ALT Cirrhosis/Control group	264.00	5.15	<0.0001
ALT Cancer/Cirrhosis group	747.00	0.51	0.601

The study showed that the enzymatic activity of AST in the control group had the lowest value of 11 U/L and the highest value of 33 U/L. In the other groups studied, the lowest enzymatic activity of AST in patients with liver cirrhosis was 18 U/L and the highest was 114 U/L, while in the group with cancer, the lowest activity was 10 U/L and the highest was 576 U/L. The results are shown in Figure 2.

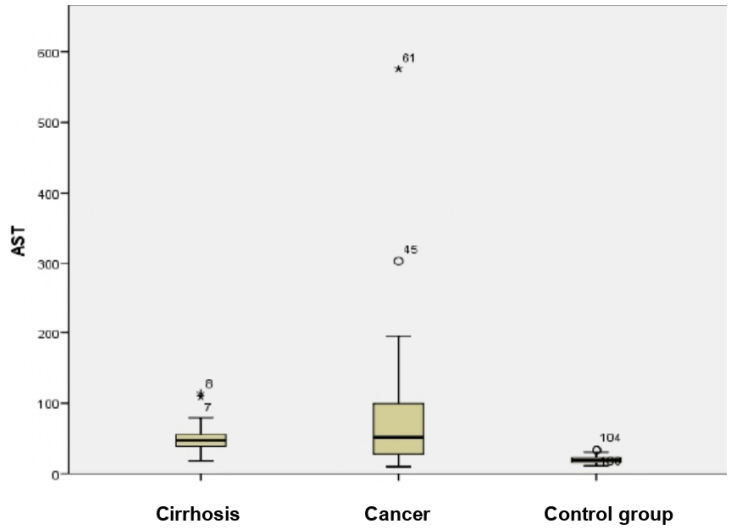

Figure 2. Graphic representation of the average value for AST.

A statistically significant difference (p <0.0001) was found with the Mann-Whitney U test for AST between the control group and the cancer group, as well as between the control group and the liver cirrhosis group. No statistically significant difference was found between the liver cirrhosis and liver cancer groups using the same test (p = 0.358). The results are shown in Table 2.

Table 2. Comparison of the catalytic activity of the AST enzyme between the tested groups

Comparative groups	Mann-Whitney U	Z	p
AST Cancer/Control group	200.00	5.77	<0.0001
AST Cirrhosis/Control group	43.00	7.28	<0.0001
AST Cancer/Cirrhosis group	704.50	0.91	0.358

The enzyme activity of GGT had the lowest value of 7 U/L in the control group, and the highest value was 50 U/L. The liver enzyme activity in patients with liver cirrhosis had the lowest value of 70 U/L and the highest value of 743 U/L, while in the group with cancer, the lowest activity was 36 U/L and the highest was 1892 U/L. The results are shown in Figure 3.

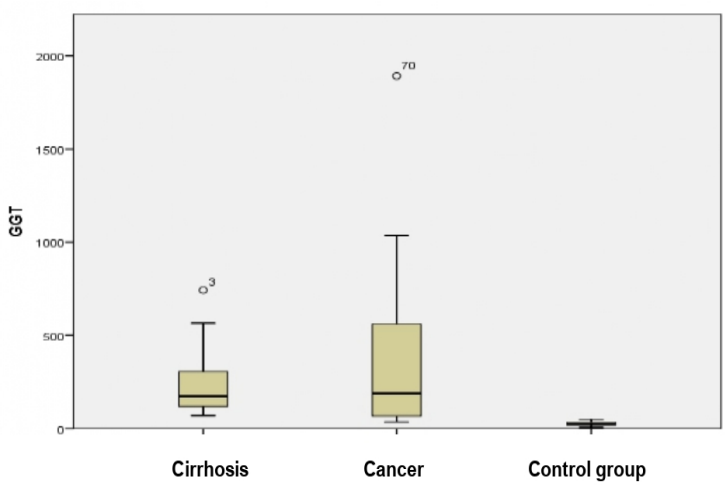

Figure 3. Graphic representation of the average value for GGT

Using the Mann-Whitney U test, statistically significant differences for GGT were found between the control group and the cancer group and between the control group and liver cirrhosis ($p < 0.0001$). No statistically significant difference ($p = 0.613$) was found between the liver cirrhosis group and the liver cancer group. The results are shown in Table 3.

Table 3. Comparison of the catalytic activity of the GGT enzyme between the tested groups.

Comparative groups	Mann-Whitney U	Z	p
GGT Cancer/Control group	16.50	7.54	<0.0001
GGT Cirrhosis/Control group	0.00	7.69	<0.0001
GGT Cancer/Cirrhosis group	747.5	0.505	0.613

The control group had the lowest value of ALP enzyme activity at 35 U/L and the highest at 114 U/L. Patients with liver cirrhosis had the lowest ALP value of 65 U/L and the highest of 235 U/L, whereas in the group with cancer, the lowest activity was 47 U/L and the highest was 929 U/L. The results are shown in Figure 4.

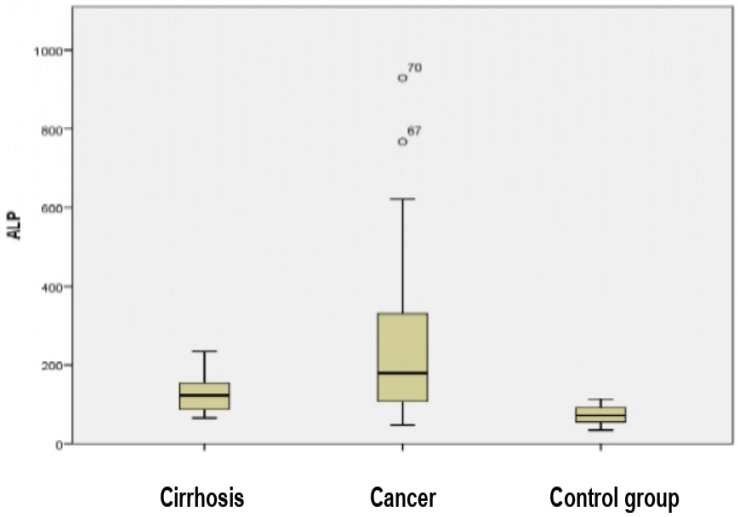

Figure 4. Graphic representation of the average value for ALP.

A statistically significant difference for ALP using the Mann-Whitney U test was found between the control group and the cancer group and the control group and the liver cirrhosis ($p < 0.0001$). Using the same test, a statistical difference of $p = 0.0009$ was found in the liver cirrhosis and liver cancer groups, and the results are shown in Table 4.

Table 4. Comparison of the catalytic activity of the ALP enzyme between the tested groups.

Comparative groups	Mann-Whitney U	Z	p
ALP Cancer/Control group	152.50	6.23	<0.0001
ALP Cirrhosis/Control group	251.50	5.27	<0.0001
ALP Cancer/Cirrhosis group	454	3.39	0.0009

Serum bilirubin concentration in the control group had the lowest concentration of 4 µmol/L and the highest of 18 µmol/L. Patients with liver cirrhosis had the lowest bilirubin concentration of 9.90 µmol/L and the highest concentration of 48.30 µmol/L, while in the group with cancer the lowest concentration was 5 µmol/L and the highest was 578.20 µmol/L. The results are shown in Figure 5.

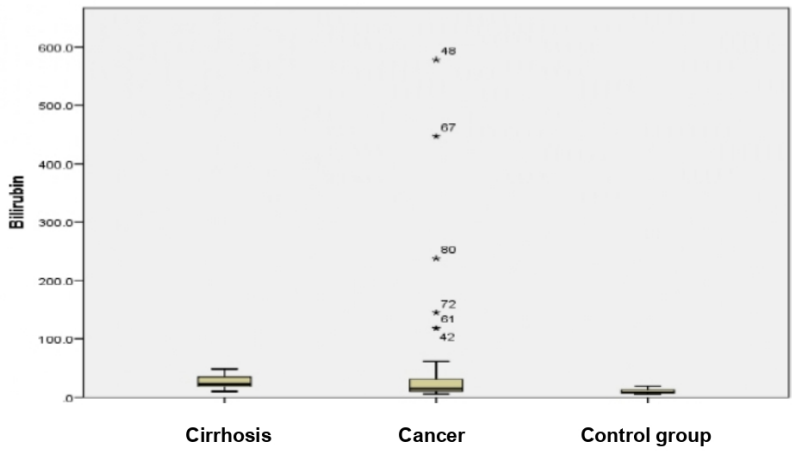

Figure 5. Graphic representation of average values for bilirubin.

A statistically significant difference for bilirubin was found using the Mann-Whitney U test between the control group and the cancer group and the control group and the liver cirrhosis at p <0.0001. Using the same test, a statistically significant difference of p = 0.0078 was found in the groups with liver cirrhosis and liver cancer. The results are shown in Table 5.

Table 5. Comparison of serum bilirubin concentration between the examined groups.

Comparative groups	Mann-Whitney U	Z	p
Bilirubin cancer/Control group	363	4.08	<0.0001
Bilirubin cirrhosis/Control group	60	7.06	<0.0001
Bilirubin cancer/Cirrhosis group	523.50	2.66	0.0078

DISCUSSION

The aim of our study was to analyze the activities of ALT, AST, GGT, ALP, and bilirubin in patients diagnosed with liver cancer, i.e., with liver metastases, and in patients diagnosed with liver cirrhosis. We also determined the values of the same parameters in the control group of subjects and compared the obtained results to determine the difference between the studied groups. The study included three groups of patients, namely 40 patients with cirrhosis, 40 patients with cancer, i.e., liver metastases (68 % were at the stage of metastases and 33% had cancer), and a control group of also 40 subjects.

Determination of aspartate transaminase (AST) and alanine aminotransferase (ALT) activity, which is used to measure liver damage, has been associated with some chronic diseases and mortality. The highest value of average enzyme activity was found in the group with cancer, 65.5 U/L, followed by the group of subjects with cirrhosis, 45.2 U/L, while the subjects in the control group had the lowest activity, 24.6 U/L.

The minimum in the control group was 10 U/L, while the group with cancer had the highest value of 422 U/L (Figure 1).

For the parameter AST, the highest average values of 82.5 U/L were recorded in the group with cancer, followed by the group of subjects with cirrhosis at 49.0 U/L, while the lowest values of 19.5 U/L were obtained in the control group. The lowest value is in the group with cancer and is 10 U/L, while the highest value of 576 U/L was recorded in the group with cancer (Figure 2).

The highest mean values of the GGT parameter were found to be 345.4 U/L in the group with cancer, followed by the group with cirrhosis, 225.1 U/L, and the expected lowest values of 26.2 U/L were obtained in the control group. The minimum activity for GGT in the control group of subjects corresponds to 7 U/L, whereas the group with cancer had the highest value of 1892 U/L (Figure 3).

The average enzyme activity for ALP, ie, the highest activity was measured in the group of subjects with cancer with a value of 245.2 U/L, followed by the group with cirrhosis at 123.0 U/L, and the lowest values of 73.7 U/L were obtained in the control group. The lowest value was found in the control group of subjects and was 35 U/L, while the highest value of 929 U/L was found in the group with cancer (Figure 4).

The results of a study by Lopez JB. et al (12) showed that the values of the parameters ALP [U/L], GGT [U/L], and AST [U/L] were abnormal in about 90% of patients with HCC. Elevated ALP values with normal bilirubin were a more common feature of HCC than in healthy subjects, although this association was not statistically significant.

The mean bilirubin concentration in the cancer group recorded the highest values, 55.1 µmol/L. This was followed by the group of subjects with liver cirrhosis, in which the bilirubin was 26.1 µmol/L, and finally the control group, in which the bilirubin was 9.4 µmol/L. The high values of the standard deviation in the group of subjects with cancer indicate that the bilirubin levels in this group vary considerably. The lowest value of this parameter was 4.4 µmol/L and was found in the control group, while the highest value of 578.2 µmol/L was found in the group of cancer patients (Figure 5).

In a study by Whitfield J. et al (13), the results of laboratory tests in 1578 patients were compared between cases (with alcoholic cirrhosis, 753 men, 243 women) and controls (with concomitant alcohol consumption during life but without liver disease; 439 men, 143 women). The mean value of the parameter bilirubin in the control group of subjects was 9.3 µmol/L, whereas its mean value in alcoholic cirrhosis was 88.7 µmol/L. The mean value for the parameter ALT [U/L] in this study was 38.0 U/L in the control group and 45.0 U/L in the case of alcoholic cirrhosis. The mean value of the parameter AST [U/L] was 41 U/L in the control group and 83.4 U/L in the case of alcoholic cirrhosis. The mean value of the GGT parameter in the control group was 113.6 U/L, and in alcoholic cirrhosis, the mean value was 424.0 U/L (13). The analysis showed that the mean values of the measurements of all five tested parameters in the control group were within the

limits. The highest values of the parameters ALT, AST, GGT, ALP, and bilirubin are in the group of patients with cancer, i.e., liver metastases.

Biomarkers of liver function (gamma-glutamyl transferase, GGT; alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; total bilirubin) are used in the clinical diagnosis of various diseases, including those associated with liver function damage and impairment. Higher levels of specific combinations of these liver function biomarkers have been shown to be independently associated with liver cirrhosis, hepatitis infection, biliary obstruction (14), and risk of diabetes (15, 16), which in turn is also associated with increased risk of HCC (17). Previous case-control studies found that GGT, ALT, and AST were elevated in approximately 90% of diagnosed HCC cases, while half of the cases also had elevated liver-specific alkaline phosphatase (ALP) or bilirubin levels (18). In our study, the differences between the serum levels of ALP and bilirubin were statistically significant at $p < 0.05$ in the group of patients with liver cirrhosis and cancer (Tables 4 and 5). Using the Mann-Witney test, no statistical significance was found for $p < 0.05$ when examining enzymes AST, ALT, and GGT in the groups with liver cirrhosis and cancer (Tables 1-3). Statistical significance was found for $p < 0.0001$ by examining the statistical significance between the control group and the group with cirrhosis and with cancer for the parameters AST, ALT, GGT, ALP, and bilirubin (Tables 1-5).

Fifty patients with cirrhosis and fifty patients with HCC were included in the study by Mehinovic L. et al. Significant differences were observed between these two groups for the parameters AST, GGT, and ALP ($p < 0.05$), while there were no statistically significant differences for the parameter's bilirubin and ALT ($p > 0.05$) (19). Authors Xu Liu et al. studied markers of liver function and compared them in cirrhosis and HCC. There was a total of 339 patients and 162 women with a mean age of 52 years. They were 185 patients with cirrhosis and 166 patients with hepatocellular carcinoma. The Mann-Whitney U test was used to compare the group with HCC and cirrhosis. Statistical significance was found for all parameters except ALP (0.220) and GGT (0.529) (20).

In a recent systematic review, GGT, but not ALT, was associated with an increased risk of liver cancer, but geographic differences were observed for ALT (21). Existing prospective observational studies that examined the association between biomarkers of liver function and liver cancer were mostly based on Asian patients (22, 23) and/or limited to specific enzymes (either transaminases, ALT, and AST or GGT) (16, 17). A cohort study based on predominantly hepatitis-negative markers measured only transaminases and found that both enzymes were good independent predictors of HCC development (24). Studies in the hepatitis-infected population found positive associations of HCC risk with many liver enzymes but not with bilirubin (23, 24). In a Swedish study, higher GGT levels were prospectively associated with an increased risk of cancer, including liver cancer, suggesting that this

single enzyme is not specific to liver and biliary tract disease (25, 26).

CONCLUSION

The highest mean values for the biomarkers ALT, AST, GGT, ALP, and bilirubin were recorded in the group with liver cancer and liver metastases, then in the group of subjects with liver cirrhosis and the lowest values in the control group of subjects. The highest mean value was recorded for the parameter GGT in the group with cancer. The results show that there is a statistically significant difference between the values of the parameters ALP and bilirubin in the groups of subjects with cancer and the control group. Subjects with cancer had statistically significantly higher values for the parameter ALP, while subjects with cirrhosis had statistically significantly higher values for bilirubin. The study shows that the catalytic activity of enzymes and bilirubin is significantly higher in the group with cancer compared to subjects with cirrhosis as well as in the control group.

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SERUMSKA AKTIVNOST ENZIMA I BILIRUBINA U PACIJENTA S CIROZOM I TUMOROM JETRE

Nafija Serdarević[1,2], Marina Ćurlin[3], Monika Franković[2,4], Sabina Šegalo[2],
Nermina Klapuh-Bukvić[1,2], Ivana Vujica[4]

[1]Institut za Kliničku biohemiju sa imunologijom, Klinički centar Univerziteta u Sarajevu, 71000 Sarajevo, Bosna i Hercegovina

[2]Fakultet zdravstvenih studija, Univerzitet u Sarajevu, 71000 Sarajevo, Bosna i Hercegovina

[3]Fakultet zdravstvenih studija, Sveučilišta u Mostaru, 88000 Mostar, Bosna i Hercegovina

[4]Bolnica „Dr. fra Mato Nikolić“, Laboratorija, 72276 Nova Bila, Republika Hrvatska

SAŽETAK

UVOD: U jetri se odvijaju glavne biokemijske reakcije čovjekovog organizma, te samim tim ona predstavlja jedan od najvažnijih organa za život. Parametri koji imaju značajnu ulogu u dijagnosticiranju i praćenju oboljena jesu enzimi ALT, AST, GGT, ALP, te bilirubin.

CILJ: Istražiti serumsku aktivnost enzim i bilirubina u pacijenata s cirozom i karcinomom jetre.

MATERIJALI I METODE: Ispitivanjem je obuhvaćeno 120 pacijenata, starosti iznad 50 godina, odnosno 40 pacijenata s cirozom jetre, 40 pacijenata s karcinomom i metastazama na jetri, te 40 pacijenata kontrolne skupine. Koncentracije AST, ALT, GGT, ALP-a u ovom radu određene su na biokemijskom analizatoru Architect i2000sr, marke Abbott, te analizatoru Dimension.

REZULTATI: Istraživanje je pokazalo da su srednje vrijednosti ispitivanih parametara bile znatno više kod ispitanika s dijagnosticiranom cirozom jetre i karcinomom, odnosno metastazama na jetri, u odnosu na kontrolnu skupinu ispitanika. Ispitivanjem razlike parametra ALP i bilirubina između ispitivanih grupa, došli smo do zaključka da postoji statistički značajna razlika između ispitivanih parametara ($p<0,05$) kod naših skupina ispitanika. Istraživanje je pokazalo i da postoji statistički značajna razlika ($p<0,05$) za ALT, AST i GGT između ispitanika sa karcinomom i kontrolne skupine kao i ispitanika s cirozom i kontrolne skupine.

ZAKLJUČCI: Rezultati su potvrdili da su povišene aktivnosti enzima ALT, AST, GGT, ALP i bilirubina kod ispitanika s cirozom jetre i karcinomom u odnosu na kontrolnu skupinu ispitanika.

Ključne riječi: enzimi, bilirubin, ciroza jetre, karcinom jetre

Osoba za razmjenu informacija: dr.sc. Nafija Serdarević, red. prof.

E-mail: serdarevicnafija@yahoo.com