Arch. Biol. Sci., Belgrade, 67 (1), 11-18, 2015 DOI:10.2298/ABS140505001S

DOPPLER ULTRASOUND STUDY OF PORTAL HEMODYNAMICS IN PATIENTS WITH GAUCHER DISEASE

Radmila Šarenac-Kovač[1], Mirjana Perišić[1], Dragan Tomić[2], Slađana Dimitrijević[3] and Vladimir Jurišić[4,*]

[1] *Clinic of Gastroenterology and Hepatology, Clinical Center of Serbia,* Belgrade, Serbia
[2] *University of Belgrade, Faculty of Medicine,* Belgrade, Serbia
[3] *University of Kragujevac, Faculty of Science,* Kragujevac, Serbia
[4] *University of Kragujevac, Faculty of Medical Science,* Kragujevac, Serbia

[*]Corresponding author: vdvd@mailcity.com

Abstract – Gaucher disease is a lysosomal storage disorder caused by a deficiency of the enzyme glucocerebrosidase and characterized by the presence of pathological macrophages laden with glucosylceramide. Hepatosplenomegaly is a common manifestation of Gaucher disease, but symptomatic portal hypertension is rarely seen. The study included 20 untreated adult patients with Gaucher disease (non-neuronopathic type 1) diagnosed with the presence of Gaucher cells in the bone marrow, and 20 healthy subjects as controls. The examination of patients included color Doppler ultrasonography (pulsed Doppler mode), resistive index (RI) and Doppler perfusion index (DPI) using a Toshiba Xario ultrasound machine and a convex array probe PVT-375AX (1.9-6 MHz) with the objective of analyzing portal hemodynamics. Results showed that all patients had enlarged liver and spleen, and their average sizes were significantly larger than those in the healthy controls (liver: 17.04 vs.14.02 cm; spleen: 22.2 vs. 10.74 cm). DPI values were significantly different between patients and controls (0.15 vs. 0.21). Considering DPI <0.15 indicates arterial liver hypoperfusion and hypoxia, it can be concluded that a number of patients had a problem with liver oxygenation, which may be linked to the high angiotensin-converting enzyme (ACE) levels obtained in the patients (339.42 U/L), 10 times greater than in control subjects. Since ACE is a potent vasoconstrictor produced by spleen macrophages in Gaucher disease, we can suppose that elevated ACE is associated with effects on the blood vessels of the liver and spleen.

Keywords: Gaucher disease; Doppler ultrasonography; portal flow velocity

Received May 5, 2014; **Revised** December 10, 2014; **Accepted** December 12, 2014

INTRODUCTION

Gaucher disease is a lysosomal storage disorder caused by mutations in the gene encoding acid glucocerebrosidase and, consequently, deficiency of the enzyme. The disease is characterized by the presence of pathological macrophages also known as "Gaucher cells" laden with glucosylceramide. Gaucher cells infiltrate the spleen, liver and bone marrow. Patients with type 1 Gaucher disease (the non-neuronopathic, most common form) have distinct splenomegaly and mild hepatomegaly. Hepatosplenomegaly is a common manifestation of Gaucher disease, but symptomatic portal hypertension is rarely seen. Case reports of portal hypertension, with liver fibrosis and cirrhosis in patients with

Gaucher disease have been sporadic (Morrison and Lane 1955; 42: Imperato, 1960; Javett et al., 1966; Sales and Hunt, 1970; Kozower et al., 1974; Aderka et al., 1982; Mazor et al., 1986; Glass et al., 1987; Henderson et al., 1991, Bandyopadhyay et al., 2011; Singla et al., 2011).

Zimran et al. (1992) reported only two cases of portal hypertension in a series of 53 patients. According to Lachmann et al. (2000), liver infiltration with Gaucher cells for most patients has no significant clinical consequences; these authors classify Gaucher patients in two groups: those with intact spleens in whom the portal hypertension can be increased by splenectomy, and those with intrahepatic portal blood flow obstruction due to massive liver infiltration by Gaucher cells, accompanied by fibrosis.

Based on the significance of portal hypertension, and bearing in mind color Doppler sonography as a non-invasive method for the estimation of blood flow, we analyzed the relation between clinical signs in Gaucher disease and hemodynamic parameters in the portal system.

MATERIALS AND METHODS

All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in the study. The local human ethics committee of the Clinical Center of Serbia approved the study protocol.

The study included 20 untreated adult patients with Gaucher disease, 12 females and 8 males, at the Clinic of Hematology and Clinic of Gastroenterology and Hepatology (GEH), Clinical Center of Serbia (CCS). The study also involved a control of 20 healthy subjects, 12 males and 8 females.

The diagnosis of Gaucher disease (non-neuronopathic type 1) was confirmed by the presence of Gaucher cells in bone marrow at the Department of Hematology. Serum tests of liver function were normal in all examined patients.

Color Doppler ultrasonography (pulsed Doppler mode) was conducted at the Department of Abdominal Ultrasonography. The analyses were made with a Toshiba Xario ultrasound machine, using convex array probe PVT-375AX (1.9-6 MHz). Arterial resistive index (RI) was automatically calculated (using auto trace option), as RI=(Vmax–Vmin)/Vmax. Blood flow volume in the portal vein and hepatic artery was calculated based on the mean velocity and vessel area: FV=area×Vmean×60 (mL/min), using the option of auto trace flow volume of the ultrasound machine.

Doppler perfusion index (DPI) was calculated using the formula DPI = $\frac{FVha}{FVha+FVpv}$, where FVha is the flow volume of the hepatic artery, and FVpv is the flow volume of the portal vein. DPI mostly depends on arterial pressure and indicates the percentage of oxygenated blood supplying the liver. A low DPI indicates hypoxia, and high DPI A-V shunts.

Estimation of angiotensin-converting enzyme

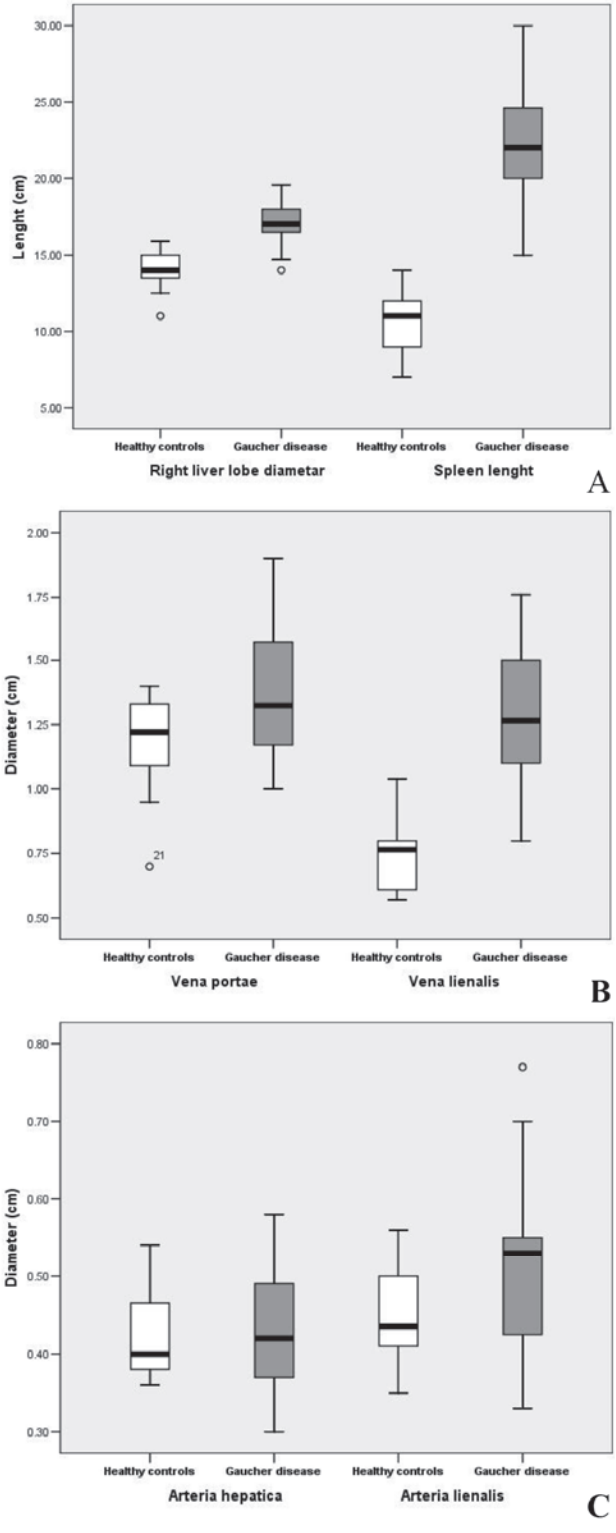
Serum ACE was determined by routine analyses using a biochemistry analyzer (Ilab 1800, Biochem diagnostics, Athens, Greece) in the biochemical laboratory of the Clinical Center of Serbia, Belgrade, and using a Trinity Biotech Kit (Wicklow, Ireland), in compliance with the manufacturer's instructions. The rate of decrease in absorbance at 340 nm is directly proportional to ACE activity in the sample.

Statistical analyses

Relevant collected data were processed by statistical methods (Original SPSS program at the University of Kragujevac) using the Mann-Whitney U-test and t-tests. The statistical significance was at $p < 0.05$.

RESULTS

Among 20 patients with type 1 Gaucher disease, the



male-female ratio was 1:1.5. The average patient age was 41.3±14.6 years (range 20-75), and the average age of healthy subjects was 44.2±1.44 years (range 21-64). At the time of diagnosis, the patients had multi-year symptoms. The common ultrasonographic finding was hepatomegaly and splenomegaly, with a typical "bright" liver and non-homogeneous, bright, nodulated spleen ("bright" liver and "bright" non-homogeneous spleen).

Average liver size, i.e. the right lobe length in the sagittal section, in healthy controls was 14.02±1.09 cm (range 11-15.9), median 14, and in patients 17.04±1.37 cm (range 14-19.6), median 17 (Fig. 1A). There was a significant difference between patients with Gaucher disease and healthy controls ($p<0.05$, Mann-Whitney U-test).

Average spleen size, i.e. craniocaudal length, in healthy controls was 10.74±2.15 cm (range 7-14), median 11, and in patients 22.20±4.51 cm (range 15-30), median 22 (Fig. 1A). There was a significant difference between patients with Gaucher disease and healthy controls ($p<0.05$, Mann-Whitney U-test).

Average venae portae diameter in healthy controls was 1.19±0.18 cm (range 0.7-1.4), median 1.22, and in patients 1.37±0.26 cm (range 1.0-1.9), median 1.32 (Fig. 1B). There was a significant difference between patients with Gaucher disease and healthy controls ($p<0.05$, t-test).

Average venae lienalis diameter (Fig. 1B) in healthy controls was 0.735±0.124 cm (range 0.57-1.04), median 0.765, and in patients 1.27±0.26 cm

Fig. 1. Liver parameters in patients with Gaucher disease in comparison to healthy controls. **A** –Liver size (right lobe) and spleen diameter (expresed in cm) in patients with Gaucher disease in comparison to healthy controls, measured by ultrasonography shows statistical significant difference ($p<0.05$). **B** – Vena portae and vena lienalis diameter (expressed in cm) in patients with Gaucher disease in comparion to healthy controls, measured by ultrasonography, shows statistical significant difference ($p<0.05$). **C** – Arteria hepatica and arteria lienalis diameter (expressed in cm) in patients with Gaucher disease in comparison to healthy controls, and measured by ultrasonography shows statistical significant difference ($p<0.05$).

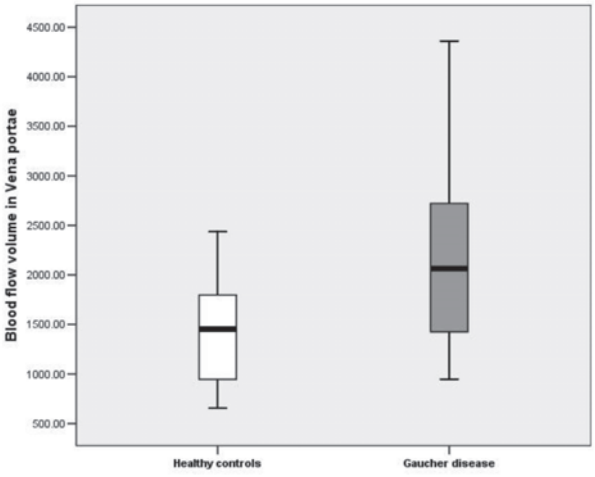


Fig. 2. Blood flow volume in vena portae (ml/min) in patients with Gaucher disease in compariosn to healthy controls, measured by Doppler ultrasonography, shows statistical significant difference (p<0.05)

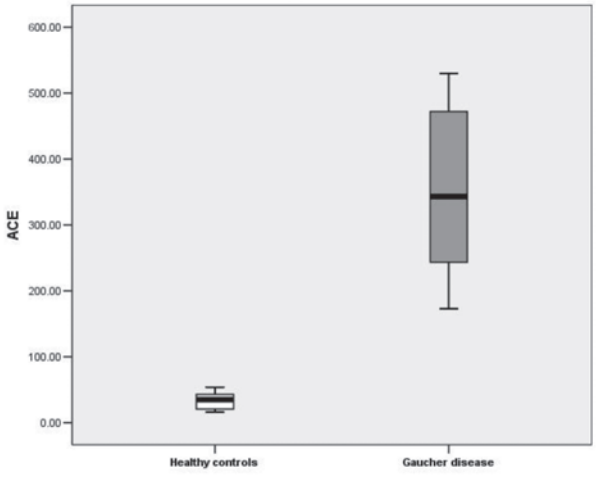


Fig. 4. Significant difference in the ACE value (expressed in U/L) between patients with Gaucher disease and healthy controls (p<0.05).

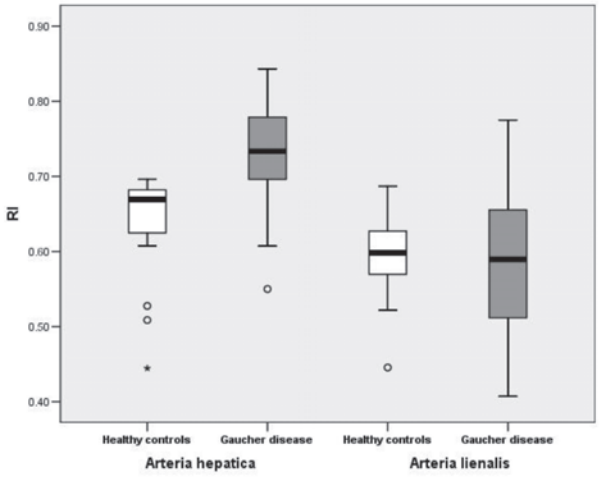


Fig. 3. Resistive index in arteria hepatica and arteria lienalis in patients with Gaucher disease in comparison to healthy controls shows statistical significant difference (p<0.05).

(range 0.8-1.76), median 1.26. This diameter was analyzed parametrically. There was a significant difference between patients with Gaucher disease and healthy controls (p=0.011<0.05, t-test).

Average arteriae hepaticae diameter (Fig. 1C) in healthy controls was 0.42±0.057 cm (range 0.36-0.54), median 0.40, and in patients 0.43±0.076 cm (range 0.30-0.58), median 0.42. There was a significant difference between patients with Gaucher disease and healthy controls (p=0.924>0.05, Mann-Whitney U-test).

Average arteriae lienalis diameter (Fig. 1C) in healthy controls was 0.45±0.06 cm (range 0.35-0.56), median 0.43, and in patients 0.52±0.13 cm (range 0.33-0.77), median 0.53. There was a significant difference between patients with Gaucher disease and healthy controls (p=0.047<0.05, t-test).

Blood flow velocity in the portal system

Vena portae: mean maximal velocity of blood in healthy controls was 36.7±11.57 cm/sec, range 22-63, median 35, and in patients 42.85±12.09 cm/sec, range 22-70, median 41.5. There was no significant difference between patients with Gaucher disease and healthy controls (p=0.108>0.05, t-test).

Vena lienalis: the mean maximal velocity of blood in healthy controls was 37±11.51 cm/sec, range 21-70, median 35.50, and in patients 42.38±13.64, range 22-60, median 48. There was no significant difference (p=0.221>0.05, Mann-Whitney U-test).

Comparison of blood, vena portae and vena lienalis velocities in healthy controls indicated that there was no significant difference in blood velocities in vena portae and vena lienalis in healthy controls (median VP=35.00, mean VP=36.70±11.57, median VL=35.50 mean VL=37±11.51; p=0.86>0.05, Mann-Whitney U-test).

Arteria hepatica: systolic velocity of blood in healthy controls was 79.35±2927 cm/sec on average, range 42-119, median 79, and in patients 72.8±23.76, range 40-153, median 67. Systolic velocity of the hepatic artery was non-parametrically analyzed. There was no significant difference between patients and healthy controls (p=0.142>0.05, Mann-Whitney U-test). Diastolic velocity in healthy controls was 28.95±1.41 cm/sec on average, range 16-57, median 25, and in patients 19.5±7.10, range 10-36, median 18.5 (Fig. 2c). The velocity was non-parametrically analyzed. There was significant difference in diastolic velocity in the hepatic artery (p=0.001<0.05, Mann-Whitney U-test).

Arteria lienalis: systolic velocity of blood in healthy controls was 94.55±20.64 cm/sec on average, range 68-130, median 89, and in patients 81.75±26.14, range 40-120, median 88.5. Systolic velocity of arteria lienalis was parametrically analyzed. There was no significant difference (p=0.164>0.05, t-test). Diastolic velocity in healthy controls was 38.15±9.50 cm/sec on average, range 25-61, median 34, and in patients 34.92±16.28, range 9-67, median 35.5. The velocity was parametrically analyzed. There was no significant difference in diastolic velocity, Vmin (p=0.540>0.05, t-test).

Blood flow volume (FV)

Vena portae: Mean blood flow volume in healthy controls was 1386.87±492.56 ml/min, range 655.09-2435.56, median 1453,31, and in patients 2275.61±935.36, range 1139.82-4420.81, median 2126.96 (Fig. 2). There was a significant difference between healthy controls and patients (p=0.001<0.05, t-test).

Arteria hepatica: Mean blood flow volume in healthy controls was 388.55±143.74 ml/min, range 146.13-778.56, median 350.53, and in patients 389.53±243.53, range 131.55-1197.77, median 352.31. There was no significant difference between healthy controls and patients (p=0.547>0.05, Mann-Whitney U-test).

Doppler perfusion index (DPI) in healthy controls was 0.23±0.11 on average, range 0.09-0.53, median 0.21, and in patients 0.17±0.10, range 0.03-0.34, median 0.15. There was no significant difference between patients and healthy controls (p=0.060>0.05, Mann-Whitney U-test).

Resistive index (RI) of arteria hepatica in healthy controls was 0.64±0.07 on average, range 0.43-0.70, median 0.67, and in patients 0.73±0.08, range 0.55-0.81, median 0.75 (Fig. 5). There was a significant difference between patients and healthy controls (p<0.05, Mann–Whitney U-test).

Resistive index (RI) of arteria lienalis in healthy controls was 0.60±0.06 on average, range 0.44-0.69, median 0.59, and in patients 0.59±0.10, range 0.41-0.78, median 0.59. There was no significant difference between healthy controls and patients (p=0.739>0.05, t-test).

Comparison of RI arteria hepatica and arteria lienalis values: in patients with Gaucher disease significant difference was found in the RI values of these arteries (mean HA=0.73±0.08 Mean LA=0.59±0.10; p=0.001<0.05, t-test) (Fig. 3). In healthy controls, significant difference was found in the RI values of these two arteries (median of HA=0.67, mean values of HA=0.64±0.07, median for LA=0.60, mean values of LA=0.60±0.07 p=0.006<0.05, Mann-Whitney U-test) (Fig. 3).

Level of serum angiotensin-converting enzyme (ACE) in healthy controls was 32.82±12.13 U/L on average, range 16-54, median values of 34.95, and in patients 339.42±134.76, median values of 321.50 (Fig. 4). There was a significant difference in the ACE

value between healthy controls and patients ($p<0.05$, t-test).

DISCUSSION

Gaucher disease is rare, so the group of 20 patients in the present study correlates with the general prevalence of 1:200.000 (Grabowski, 2000). We found a higher incidence in our cohort women (1.5:1), which differed from the incidence reported in Kenya (1:1.25 in males). (Murila et al., 2008). There have been reports that splenomegaly is usually the earliest sign in Gaucher disease. Hepatomegaly is another frequent sign of Gaucher disease, but is usually milder than splenomegaly (Barranger and Rice, 1993), which is also reported in our study. However, in the available literature, there are no data on Doppler flow measurements in patients with Gaucher disease and insufficient data on portal hypertension in this disease.

In accordance with liver and spleen size, we found that their veins were dilated. Although the vena portae diameter in patients was significantly larger in comparison to healthy controls, it was within the normal value range (up to 1.5 cm), so this parameter does not indicate portal hypertension. The vena lienalis diameter in patients was significantly larger in comparison to healthy controls, more than 50% of the normal diameter, a typical finding in splenomegaly.

When artery diameters were examined, the width of the arteria hepatica was almost the same in patients and healthy controls, while the lumen of arteria lienalis is significantly wider in patients compared to healthy controls, as a result of splenomegaly. On the one hand, flow velocities in the vena portae and vena lienalis are not significantly different between patients and healthy controls, athough the flows are somewhat more rapid in patients. A somewhat increased blood velocity and somewhat larger diameter of the vena portae influenced the blood flow volume (FV) in this vein to be significantly larger in patients than healthy controls. Blood flow volume (FV) in the hepatic artery was almost the same in patients and healthy controls, which is in accordance with almost the same diameter of the arteria hepatica in both groups.

DPI (Doppler perfusion index) of the liver, as the ratio of the FV hepatic artery and sum of portal FV and hepatic artery FV, was not significantly different in patients and healthy controls, while the median was significantly lower in patients. As DPI <0.15 indicates arterial liver hypoperfusion and hypoxia, we can conclude that a number of patients have a problem with liver oxygenation.

The measurement of blood flow velocity in the hepatic artery demonstrated a significantly lower diastolic velocity in patients than in healthy controls. Systolic blood velocities in the hepatic artery were not significantly lower in patients and healthy controls, but low diastolic velocity significantly raised the arterial resistive index (RI) in patients, indicating liver fibrosis. In the lienal artery, neither systolic nor diastolic blood velocity were significantly different in patients and healthy controls, so there was no significant difference in the RI of arteria lienalis, either.

The mechanism of hepatic fibrosis in Gaucher disease is obscure and may be multifactorial. Lachmann et al. (2000) presented only 4 patients with Gaucher disease showing massive hepatic fibrosis associated with portal hypertension and consequently with esophageal varices. James et al. (1981) found a pericellular fibrosis in 23 liver biopsies in Gaucher patients. Lichtenstein et al. (1997) and Hollak et al. (1997) suggested that the initial fibrosis may be induced by several inflammatory mediators, including M-CSF and IL-8 from the activated macrophages in Gaucher disease. Since macrophages are the main cell types affected in Gaucher disease, it is now apparent that the pathology is caused by the macrophage activation. Previous data indicated that levels of interleukin-1b (IL-1b), interleukin-1 receptor antagonist, IL-6, tumor necrosis factor-a (TNF-alpha) and soluble IL-2 receptor (sIL-2R) are elevated in the serum of Gaucher patients (Barak et al., 1999). We particularly stress that tissue macrophages of the liver (Kupffer cells) can be affected in Gaucher disease (Zimran, 1997).

ACE is a non-specific indicator of lipid storage. Here we made a comparison of our values with Doppler spectra, considering that an elevated level of ACE can indicate an excess of lipids in macrophages (Gaucher cells). The results we obtained show a 10-fold higher level of ACE blood values in all 20 patients when compared to healthy controls (339.42 vs. 32.82 U/L). Based on the obtained results it is possible that this potent vasoconstrictor has an effect on the altered regimen of flow in the portal system in this disease.

Based on the measured Doppler parameters we did not observe portal hypertension in the 20 patients. A wider portal vein lumen is due to hepatomegaly, while larger vena lienalis and arteria lienalis lumens are due to significant splenomegaly. Blood flow volume (FV) in the dilated portal vein is increased, while liver arterial perfusion is correspondingly decreased, and the RI resistance in the non-dilated hepatic artery is greater. On the other hand, in the dilated splenic artery, resistance to blood flow is normal.

The comparison of RIs indicates that the RI of the arteria hepatica is significantly higher than that of the arteria lienalis both in patients and healthy controls, which indicates a specially regulated regimen of splenic arterial flow, with lower resistance to blood flow than in the liver. In our previous study, we demonstrated that there was specificity of splenic blood flow in liver cirrhosis (Perišić et al., 2005, Glišić et al., 2013).

Acknowledgments - This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 175056.

Authors' contribution

Radmila Šarenac-Kovač colected patients and performed analyses. Mirjana Perišić, Dragan Tomić and Vladimir Jurišić designed the study. Sladjana Dimitrijević performed the statistical analysis.

Conflict of interest disclosure

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

Aderka, D., Garfinkel, D., Rothem, A. and *J. Pinkhas* (1982). Fatal bleeding from esophageal varices in a patient with Gaucher disease. *Am. J. Gastroenterol* **77**, 838-839.

Bandyopadhyay, R., Bandyopadhyay, S. and *P. K. Maity* (2011). Gaucher disease presenting with portal hypertension. *Indian Pediatrics* **48**, 801-802.

Barak, V., Acker, M., Nisman, B., Kalickman, I., Abrahamov, A., Zimran, A. and *S. Yatziv* (1999). Cytokines in Gaucher's disease. *Eur. Cytokine Net.* **10**, 205-210.

Barranger, T. A. and *E. Rice* (1993). An overview of Gaucher disease. *Gaucher Clin. Perspect* **1**, 1-5.

Glass, R. B., Poznanski, A. K., Young, S. and *M. A. Urban* (1987). Gaucher disease of the liver: CT appearance. *Pediatr. Radiol.* **17**, 417-418.

Glišić, T. M., Perišić, M. D., Dimitrijevic, S. and *V. Jurišić* (2014). Doppler assessment of splanchnic arterial flow in patients with liver cirrhosis: correlation with ammonia plasma levels and MELD score. *J. Clin. Ultrasound* **42**, 264-269

Grabowski, G. A. (2000). Gaucher disease: Lessons from a decade of therapy. *J. Pediatr.* **144**, 515-519.

Henderson, J. M., Gilinsky, N. H., Lee, E. Y. and *M. F. Greenwood* (1991). Gaucher disease complicated by bleeding esophageal varices and colonic infiltration by Gaucher cells. *Am J. Gastroenterol.* **86**, 346-348.

Hollak, C. E., Evers, L., Aerts, J. M. and *M. H. van Oers.* (1997). Elevated levels of M-CSF, sCD14 and IL8 in type 1 Gaucher disease. *Blood Cells Mol. Dis.* **23**, 201-212.

Imperato, A. M. (1960). Gaucher disease with ascites. *Ann. Surg.* **151**, 431-436.

James, S. P., Stromeyer, F. W., Chang, C. and *J. A. Barranger.* (1981). Liver abnormalities in patients with Gaucher disease. *Gastroenterology* **80**,126-133.

Javett, S. N., Kew, M. C. and *D. Liknaitsky* (1996). Gaucher disease with portal hypertension: case report. *J. Pediatr.* **68**,810-812.

Kozower, M., Kaplan, M. M., Kanfer, J. N., Norton, R. A. and *H. J. Wolfe* (1974). Esophageal varices in a 60-year-old man with Gaucher disease. *Am. J. Dig. Dis.* **19**, 565-570.

Lachmann, R. H., Wight, D. G, Lomas, D. J., Fisher, N. C., Schofield, J. P., Elias, E. and *T. M. Cox* (2000). Massive hepatic fibrosis in Gaucher disease: Clinico-pathological and radiological features. *QJM* **93**, 237-244.

Lichtenstein, M., Zimran, A. and *M. Horowitz* (1997). Cytokine mRNA in Gaucher disease. *Blood Cells Mol. Dis.* **23**, 395-401.

Mazor, M., Wiznitzer, A., Pinku, A., Katz, M. and *J. R. Leiberman* (1986). Gaucher disease in pregnancy associated with portal hypertension. *Am. J. Obst. Gynae.* **154**, 1119-1120.

Morrison, A. M. and *M. Lane* (1955). Gaucher disease with ascites: a case report with autopsy findings. *Ann. Intern. Med.* **42**,1321-1329.

Murila, F., Rajab, J. A. and *J. M. Ireri* (2008). Gaucher disease at a national referral hospital. *East Af. Med.l J.* **85**, 455-458.

Perišić, M. D., Ćulafić, Dj. M. and *M. Kerkez* (2005). Specificity of splenic blood flow in liver cirrhosis. *Rom. J. Intern. Med.* **43**, 141-151.

Sales, J. E. H. and *A. H. Hunt* (1970). Gaucher disease and portal hypertension. *Br. J. Surg.* **57**, 225-228.

Singla, R., Singla, A., Kumar, S., Agarwal, A. and *P. Kar* (2011). Gaucher's disease with portal hypertension and hypersplenism. *Trop. Gastroenterol.* **32**, 68-71.

Zimran, A. (ed.) (1997). Gaucher's Disease. Bailliere Tindall, Cambridge UK.

Zimran, A., Kay, A., Gelbart, T., Garver, P., Thurston, D., Saven, A. and *E.* Beutler (1992). Gaucher disease. Clinical, laboratory, radiologic, and genetic features of 53 patients. *Medicine (Baltimore)* **71**, 337-353.