SECTION: LIVER AND HEPATOBILIARY SYSTEM

036

LIVER RESECTIONS FOR METASTATIC COLORECTAL CANCER

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Introduction

Liver resection for liver metastatic colorectal cancer (LMCC) is nowadays the only potentially curable treatment modality for patients with this diagnosis. Except resection procedures there are other LMCC treatment methods: ablation methods (RFA, cryodesturction, ethanolisation), chemoembolisation, adjuvant systemic and locoregional chemotherapy. Multimodal therapy of LMCC means combination of these treatment modalities.

Methods

This is a retrospective study examining our group of patients who underwent liver resections for LMCC. All records of 48 patients, who underwent this kind of treatment for metastatic colorectal cancer in IKEM from 4/98 to 6/03, were reviewed.

Results

Over this period we performed 51 liver resections in 48 patients (21 women, 27 men, mean age 59 years, range: 46–76 years). Intraoperative ultrasonography of the liver is absolutely necessary investigation in liver surgery, all our resected patients underwent this procedure, and it is the most sensitive investigation for assessing of disease stage. Based on this knowledge the modality of following treatment is decided. In our group of patients we performed 57 % anatomical resections (18 right or left lobectomies, 10 bi and segmentectomies), rest of the group was treated by nonanatomical resection (23 cases). In 14 patients we found liver metastasis during primary tumor resection, in 3 cases we performed combined liver and colon resection. For metachronous metastases were indicated 34 patients, mean interval from primary tumor resection was 3.2 months (range 1 – 7 months). In 19 cases we applied a combination of resection and ablation methods. Porcatether for HAIC we used in 18 cases, ablative methods in 9 cases (RFA 5x, cryo 2x, ethanolisation 2x).
Perioperative mortality was 2 % (1 patient died for gastrointestinal bleeding). 7 patients had second recurrence and re-operation (median interval 10 months after the first liver surgery). One patient had the second and one patient fifth recurrence; all these recurrences were surgically treated.

Conclusion

When the metastatic colorectal cancer is located only in liver, liver surgery (curable resection) seems to be the best benefit for the patient. Radical resection procedures offer statistically significant survival prolongation (low early mortality and low blood loss is necessary). Multimodal therapy, that means hepatic resection, radiofrequent ablation and HAIC for colorectal metastases, is worthwhile treatment. Early mortality of these treatment modalities is in our group acceptable and similar to the other centres.

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037

LOCALIZED DAMAGE OF RABBIT LIVER TISSUE INDUCED BY HIGH INTENSITY FOCUSED SHOCK WAVES

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Background

Focused shock waves (FWS) have been routinely used for treatment of kidney stone diseases for more than a decade. Great success of extra-corporeal shock wave lithotripsy stimulated studies of applications of the FSW in other branches of medicine, including cell damage that can be potentially applied to noninvasive treatment of cancer tissues. In the case of stone disintegration there is a strong acoustic non-homogeneity at the interface between the liquid and the stone where the shock is partially reflected and absorbed. However, acoustic characteristics of cancer tissue are the same as the surrounding healthy tissues. This means that the FSW should act locally (mechanically and/or chemically) in the predicted region on an originally acoustically homogenous medium.
A novel method for generation of high intensity focused shock waves (HIFSW) has been developed. We observed local injury of Rabbit tissues in vitro and in vivo induced by HIFSW.

Methods

A cylindrical pressure wave created by a high voltage multi-channel discharge in water with an increased electrical conductivity (5–20 mS/cm) is focused by a metallic parabolic reflector. The discharge is formed on a composite anode consisting of a cylindrical stainless steel electrode (60 mm in diameter, 100 mm long) covered by a thin (0.2–0.3 mm thick) porous (opened porosity 3–5 %) ceramic layer. In such arrangement at the applied voltage of 30 kV, the electric field on the metallic anode almost reaches the value of 1MV/cm

and a large number of short discharge channels distributed homogeneously on the anode surface are initiated. Each discharge channel creates a semi-spherical pressure wave, and by superposition of all of the waves a cylindrical pressure wave propagating from the anode is formed. A metallic parabolic reflector (cathode) focuses on the cylindrical pressure wave transforming it near the focus into a strong shock wave. Dimensions of the focal volume are of 8x30 mm. Such arrangement enables us to produce either a single shock wave that overcomes the cavitation threshold near the focal point, or two successive shock waves where the first one produces an acoustic nonhomogeneity on which the second – main shock wave dissipates.

Results

We found extensive necrosis of tissues in the liver in vitro and also in vivo near the shock wave focus. Very similar results were also found in renal and splenic tissues exposed to the focused shock waves. The surrounding hepatic parenchyma, 3–4 cm near the center of necrosis, was found changing from necrosis to steatosis. We demonstrated that the HIFSW destroy human red blood cells and white blood cells very efficiently. A simple method to assess the mechanical effects of the HIF shock waves is to study the interaction with fresh potatoes that have a high water content. It is known that mechanical damage to a potato results in a color change of the damaged mass. We exposed a 6 cm thick slab of a fresh potato to the shock waves. The slab was placed to the focal region and exposed to 10 shocks at 30 kV. The potato was then cut along the wave axis and after several hours the damaged region became dark. The experiment demonstrated that only the focal region (inside the potato) had been damaged. No damage was seen between the potato surface and the focal region.

Conclusion

Our results confirmed lesion of the tissue with HIF shock wave. In the literature it is a known fact that the use of high-intensive focused ultrasound (HIFU) damages tissue thermically. Our pre-clinical results show that even cavitation can cause the lesion (necrosis) of non-aired soft tissues. This shows another possible way of destroying inoperable tumor tissues using HIF shock waves.

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038

PROBIOTICS AND PREBIOTICS IMPROVE MILD CHRONIC HEPATIC ENCEPHALOPATHY

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Introduction

Hepatic encephalopathy exerts unfavourable influence on the condition of patients with cirrhosis of liver. The treatment must be complex, i.e. removing the precipitating causes and modifying the nutrition. Also prebiotics (nonresorbable disaccharides) and recently probiotics are administered. On this basis we can quantify possibilities of improving the mild chronic hepatic encephalopathy (CHE) with liver cirrhosis and portal hypertension.

Methods

In addition to the complex therapy without prebiotic, fifteen patients (9 male, 6 female) obtained intermittently the probiotic preparation Enterococcus faecium+selenium (IVAX, Opava-Komárov). One capsule daily was administered in three 28-day intervals, separated by two 14-day pauses. During their complex treatment 12 patients (10 male, 2 female) took the prebiotic lactitol (Importal, Zyma), 10 grams daily for 56 days. Two patients underwent the complex therapy without pre and probiotics (control). The degree of portal systemic encephalopathy (PSE) was quantified at the start and at the end of treatment, using the index PSEI (Conn, Liebertal, 1980). It included the status of consciousness, asterixis, number connecting test, serum ammonia and EEG. Data were evaluated by Student's t-test. Obtained results were transformed into measurements of the effect range and its probability, predicted for the mean (with 95 % confidence interval, Ci) and individual (95 % tolerance interval, Ti)

Results

In the group treated by probiotic, the value of PSEI decreased by 70 % (Ci – 100 % to – 48 %; Ti –100 % to +2 %) from the starting mean of 33 (Ci 26 to 40; Ti 6 to 60).
In the prebiotic group, the value of PSEI dropped from the starting mean of 26 (Ci 17 to 36; Ti 55 to 0) by 78 % (Ci –100 % to – 46 %; Ti – 100 % to – 31 %). All these decreases were statistically significant ($p < 0.05$). The replication probability approaches in either case the probability value of 1. In two controls, the decreases were smaller.

Conclusion

CHE with cognitive and extrapyramidal changes with alteration of intellect, personality, working ability and sleep is present in 25–48 % of treated, and as high as in 60–70 % non-treated patients. The present contribution points out the necessity to use not only the pre– but also the probiotics in the complex treatment of CHE. Obtained results prove their effectiveness very similar. They have either direct (probiotics) or non-direct (prebiotics) influence upon the pathologic gut flora, resulting in reduction of ammonia production in colon. Any specific effect of selenium cannot be evaluated from the results of the present contribution. Its outcome indicates that in some cases the complete normalisation of hepatic encephalopathy can be expected. Wide offer of probiotics, presently realised for general population, should be directed more intensively also towards prevention and therapy of chronic hepatic encephalopathy.

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039

STENOSIS OF INFERIOR VENA CAVA AND HEPATIC VEIN STENOSIS IN A PATIENT WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

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Background

Stenosis of the intrahepatic portion of the inferior vena cava (IVC) may take the clinical form of the Budd-Chiari syndrome. Etiologically, this is most probably due to transformation of a thrombus developed in this particular site. In terms of pathogenesis, clinical course and treatment, this condition is then different from the conventional occlusion of hepatic veins in the Budd-Chiari syndrome. Stenosis in this localisation is relatively often reported in Asian populations; in Europe it is rather rare.

Case Presentation

The case is presented on one patient with stenosis of the intrahepatic portion of inferior vena cava and terminal part of hepatic veins, a state manifesting itself in ascites resistant to treatment. The case was originally seen as decompensated cirrhosis of the liver. Once properly diagnosed, it was successfully treated with angioplasty and subsequent anticoagulation.

Conclusion

After all the relevant hypercoagulation states potentially leading to IVC and hepatic vein stenosis were ruled out, the cause of the patient‘s stenosis could be traced to polycythemia concomitant to severe pulmonary obstruction disease and simultaneous corticoid therapy. A case like this has not been described in the literature before.

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040

PROBLEMS WITH EVALUATION OF AUTOANTIBODIES IN AUTOIMMUNE LIVER DISEASES

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Introduction

Definitive diagnosis of autoimmune liver disease requires a presence of autoantibodies. The purpose of this study was to assess results of autoantibodies in patients with autoimmune liver diseases and to analyse results of ELISA tests in comparison to indirect immunofluorescence (IF).

Methods

Results of studies carried out from February 2001 to April 2003 were used for prospective evaluation. The sera of 172 patients with AIH (96) and PBC (76) were tested by different ELISA and evaluated on rat liver/kidney/stomach by IF. Anti-SLA, anti-M4 (Euroimmun) and anti-M2 (Pharmacia) were detected by ELISA.

Results

Anti-SLA positive results were detected in 23 sera (18 patients) with AIH and in 3 sera (3 patients) with PBC. From 18 sera with AIH and anti-SLA by ELISA there was the suspicion of anti-SLA by IF in 12 cases. Out of 96 sera with AIH 11 were false positive results by IF. Anti-SLA were falsely negative by IF in all cases with AMA. From 80 sera (48 patients) with PBC we found 74 positive by both ELISA (anti-M2) and IF (AMA), 1 serum was incorrectly evaluated as anti-LKM, 2 were AMA negative and 3 were anti-M2 negative. Anti-M4 was detected in 86 sera (61 patients) with PBC. 50 sera/36 patients were positive and 36/25 were negative.

Conclusion

We found anti-SLA by ELISA not detectable by IF in 3 patients from 58 (5.17 %) with PBC. In the suspicion of “low-level AMA” we recommend a combination of ELISA and lower titration of sera by IF. The suspicion of anti-SLA by IF must be confirmed by ELISA. In cases of AMA positive sera, anti-SLA are hidden and therefore must be detected by ELISA.

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041

LIVER BIOPSY AND ITS CONTRIBUTION TO THE FINAL CLINICAL DIAGNOSIS OF DIFFERENT TYPES OF HEPATOPATHY

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Objectives

Liver biopsy continues to be the indispensable method for examining diffuse as well as focal liver lesions, providing essential information concerning the etiology and disease progression degree, monitoring the treatment effect and participating in establishing its prognosis. However, at present, the diagnostic significance of liver biopsy has decreased as a result of expansion of a number of noninvasive examination methods (such as virological, immmunological, genetic, imaging methods etc.). Thus its contribution remains limited to establishing the grade, stage and prognosis of the respective liver disease. The particular stress should be therefore laid on the individual risks and, on the other hand, the patient's benefit in indicating liver biopsy.

Methods

The contribution of liver biopsy to the final clinical diagnosis was evaluated retrospectively based on a cohort of liver biopsies carried out at our Department from 1997 through to 2003 and comprising 275 patients, all of them examined by an experienced pathologist. The samples were evaluated according to their size and the number of portobiliary spaces (interpretability of the liver biopsy) and grouped according to their contribution to the clinical diagnosis. 1 – sample not interpreted because of its insufficient (small) size, 2 – awareness of clinical diagnosis prior to histological examination, 3 – clinical diagnoses presumptive and confirmed by histological examination. 4 – clinical diagnosis entirely unknown, diagnosis established by histological examination. 5 – clinical diagnosis unknown, neither supported nor established by histological examination.

Results

A total of 275 liver biopsies obtained from 53 % males and 47 % females were carried out. The patients' average age was 46 years (18–79 years). The average length of processed sample was 11.4 mm (2–38 mm), the average number of portobiliary spaces being 5.2 (1–17). Only one severe complication was noted, namely the intraabdominal haemorrhage requiring surgical revision and resulting in the patient's recovery. According to the above criteria, 17 biopsies (6 %) were included in the group 1.54 biopsies (19 %) in the group 2.73 biopsies (26 %) in the group 3.77 biopsies (28 %) in group the 4 and 54 biopsies (20 %) in the group 5.

The most frequently occurring bioptic diagnoses in the group 4 (contributive to the diagnosis) included haemato-oncological complications (GVHD or toxic damage from cytostatics), alcoholic hepatopathy and other toxic liver lesions.

More than 50 % of patients included in the group 5 (non-contributive to the diagnosis) were relatively young patients with slightly elevated transaminases, not exceeding 3–4 times their normal values, and with USG signs of liver steatosis. Another large portion of the group (15 %) comprised patients suffering from advanced liver cirrhosis, in whom the liver biopsy fails to contribute to the diagnosis. The average length of samples comprised in the group 5 was 12.3 mm and their average content of portobiliary spaces was 4.3.

Conclusion

We failed to notice any difference in the quality and interpretability of samples included in groups 4 and 5 (contributory and non-contributory liver biopsies). The most contributory from the diagnostic point of view was the liver biopsy in haemato-oncological patients and in patients with toxic (particularly alcoholic) liver damage. Diagnostically, the less contributory sign appears to be the liver biopsy in asymptomatic patients with slight transaminase elevation and a sonographic pattern of liver steatosis and in patients suffering from advanced hepatopathy. The cause of limited diagnostic contribution of liver biopsy in such patients may be the bioptic examination of a less representative area of the liver, progress in clinical diagnosis (the relatively new diagnosis NASH) and, naturally, proper indication of the liver biopsy. By contrast, the size of individual samples does not seem to influence the contribution of liver biopsy to the diagnosis. The method (one-second biopsy) and the instrument used (a needle 1.4mm across) suffice for a successful diagnostic liver biopsy.

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042

BACTERIAL INFECTION IN PATIENTS WITH ACUTE BLEEDING AS A RESULT OF PORTAL HYPERTENSION

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Introduction

Acute bleeding into the upper part of gastrointestinal tract is the most serious complication of portal hypertension, most frequently caused by liver cirrhosis, leading to death in 30 % of cases in the first episode. Bleeding stops spontaneously in about 40 % of cases. Early rebleeding occurs in 40 % of patients within 6 weeks; most of these episodes occur in the first week.

Variceal bleeding is the last step of the chain initiated by increased portal pressure and progressive dilatation of varices until they finally bleed. Primary causes of bleeding are not clear. However, it is expected that even completely independent factors, of which the highest importance has a bacterial infection, play a very important role.

Association of bacterial infections and acute variceal bleeding was reported recently. The aim of this prospective study was to evaluate the frequency of different bacterial infections in cirrhotic patients with presence or absence of acute variceal bleeding at the time of hospitalisation.

Patients and Methods

88 cirrhotic patients: 44 with variceal bleeding (17 women, 27 men, average age 56.6 years, range 35–79 years – Group A) and 44 with other forms of decompensation of liver cirrhosis (13 women, 31 men, average age 54.0 years, range 35–83 years – Group B) were admitted to gastroenterological ICU. Biological material (blood, urine, throat swab, ascitic fluid) was obtained from these patients and sent for microbiological evaluation.

Results

We found positive cultures in 24 patients of Group A and in 13 of Group B ($p < 0.01$) – 12 versus 4 positive samples from blood ($p < 0.05$), 9 versus 10 from urine (N.S.), 9 versus 3 from throat (N.S.) and 2 positive ascitic fluid in both groups. Bacterial spectrum of positive samples consisted of both Gram-negative and Gram-positive bacteria in approximately same ratio. We did not confirm any higher occurrence of Gram-negative bacterial flora in liver cirrhosis patients with portal hypertension as indicated in available literature.

Conclusion

Bacterial infections are more common in cirrhotic patients with acute variceal bleeding than in patients with other forms of decompensation of liver cirrhosis. These data support the hypothesis of bacterial infection as a reason of acute variceal bleeding.

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043

BENIGN RECURRENT INTRAHEPATIC CHOLESTASIS ASSOCIATED WITH A NOVEL MUTATION IN *FIC1/ ATP8B1* GENE CASE REPORT

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Background

Benign recurrent intrahepatic cholestasis is an autosomal recessive disease characterised by cholestatic episodes with increased serum levels of bilirubin and bile salts, yet by a normal gamma glutamyl transpeptidase and cholesterol, a normal liver structure in biopsy and benign course. Three mutations associated with BRIC phenotype and a number of mutations linked to Byler disease have been reported in a single gene FIC1 (Familiar Intrahepatic Cholestasis 1)[1, 2] recently renamed *ATP8B1*.

Patient and Methods

The male born in 1972 had first two episodes of cholestasis in 1973 and in 1975. The third episode in 1976 lasted for 12 months and was followed by an asymptomatic period. Since 1990 the patient had one attack annually on average. Serum bilirubin and bile salt concentrations were highly elevated but GGT and cholesterol remained in the normal range during the attacks. No evidence for metabolic, autoimmune or infectious liver disease has ever been found. Four subsequent liver biopsies performed within the last decade showed intrahepatic cholestasis with normal liver architecture. All attacks of cholestasis were successfully interrupted with administration of prednisone. Treatment with ursodeoxycholic acid was unsuccessful. The analysis of the ATP8B1 gene was performed by direct sequencing of the genomic DNA isolated from peripherial leucocytes. The presence of mutations in the patient and the family members was confirmed by restriction analysis. To confirm the scope of a deletion in exon 24 PCR products containing the deletion was cloned and each of the alleles was sequenced.

Results

The DNA analysis revealed the known missense point mutation T1982C (I661T) in exon 17 inherited from the father, and the yet unknown deletion 5 bp at the position 3122 in exon 24 inherited from the mother. The latter mutation results in frame shift and premature stop codon 73 amino acids downstream from the deletion (stop codon at the position 1114 instead of 1252). None of the three heterozygotes for the deletion in exon 24 (mother, her brother and her second son) has ever presented any signs of liver disease.

Conclusion

1. Our patient appeared to have the known point mutation T1982C in exon 17 and the yet unknown deletion in exon 24 of FIC1/ ATP8B1 gene being thus the first reported compound heterozygote affected with BRIC.
2. The deletion represents a novel mutation associated with the BRIC phenotype.
3. Administration of steroids should still be considered as an option for management of cholestatic episodes in subjects affected with BRIC.

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044

EFFECT OF PORTASYSTEMIC SHUNT ON HYPERSPLENISM

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Background

Secondary hypersplenism is a well-known complication of portal hypertension. Reduced platelet count is a more alarming sign for the physician than the risk for a patient. Improvement of thrombocytopenia is required when portal hypertension with splenomegaly and thrombocytopenia presents with life-threatening hemorrhage from the gastroesophageal varices. In this case, treatment aimed to stop the bleeding may be more beneficial than any intervention in the spleen.

Patients and Methods

In this study, the authors evaluated the long-term effects of the elective distal splenorenal shunt or small diameter H-shunt on splenomegaly and thrombocytopenia in 26 patients with portal hypertension, operated for re-bleeding from esophageal varices.

Results

25 patients had splenomegaly and 16 patients had thrombocytopenia before shunting. Surgery corrected splenomegaly in 16 patients (64 %), platelet counts increased in 13 of 16 patients with thrombocytopenia (81.2 %).

Conclusion

Selective or partial portal decompression is sufficient to alleviate the thrombocytopenia and splenomegaly associated with portal hypertension.

045

IS DIRECT SPLENOPORTOGRAPHY NECESSARY BEFORE SURGERY FOR PORTAL HYPERTENSION?

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Background

In 1998, the authors published an article advocating the significance of direct splenoportography in indications of shunt procedures in patients with haemorrhage being caused by the portal hypertension[1]. It has become necessary to reevaluate the attitude to this task, for today it is possible to visualize the portal system by means of magnetic resonance angiography. This method gives at least same results as classical direct splenoportography and in addition it has one important benefit – it is noninvasive. Therefore, apart from direct splenoportography and indirect splenoportography or the venous phase of contrast mesentericography and splenography, we now have a new type of examination, which provides a rather different image of the portal system and different spectrum of information. Before delicate surgery such as portocaval shunt is performed, all accessible data must be gained and properly evaluated to get the best information about the anatomy and topography of the portal system, its collaterals and the direction of blood–flow in them.

Patients Material and Results

The authors present several types of imaging of the portal system, describing their advantages and disadvantages. They comment on the amount and validity of gained information.

Conclusion

The results show that magnetic resonance angiography can replace classical direct splenoportography in many of its indications. Therefore, it may become a suitable alternative to the classical invasive examination with a high benefit for the patient.

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046

TECHNICAL DETAILS OF PERIPHERAL PORTOSYSTEMIC SHUNTS

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Background

Surgical shunts plays an important role as a long-term bridge to liver transplantation in potential transplant candidates with portal hypertension bleeding and well-preserved hepatic functional reserve (Child A), but in whom hepatic failure eventually develops. In patients, who are not transplant candidates and have a good hepatic reserve (Child A, B), the peripheral surgical shunt is an excellent and definitive alternative to TIPS in cases when sclerotherapy fails.

Patients and Method

The authors reviewed technical details of 26 peripheral portosystemic shunts, which had been performed at their institution since 1995.

Results

The long-term patency (from 2 to 5 years) of the 22 distal splenorenal shunts or 4 small-diameter mesocaval H-shunts was 81.8 % and 75 %, respectively. Overall operative mortality was 3.8 %; two cases of re-bleeding were successfully managed by emergency sclerotherapy.

Conclusion

The surgical portosystemic shunt still plays an important role in the treatment of selected patients with variceal bleeding who are not considered present or future transplant candidates. The distal splenorenal shunt has these advantages: 1. Selective decompression of gastroesophageal varices via splenic circulation maintains a prograde portal flow into the liver while causing the effective decompression of varices. 2. This shunt avoids extensive hepatic hilar mobilization that might significantly complicate any future transplantation. In cases of

technical difficulties the small-diameter mesocaval H-shunt is a good alternative with mildly higher risk of complications.

047

UNUSUAL SURGICAL APPROACHES TO MANAGE BLEEDING IN PORTAL HYPERTENSION

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Background

Sclerotherapy has become a method of the first choice in the treatment of acute variceal bleeding. However, re-bleeding within the five-year interval is all too common after this kind of management. TIPS is a new method which can resolve profuse haemorrhage from varices in patients with significantly reduced hepatic functional reserve. However, the high incidence of stenosis or occlusion (50 % during the first six months) reserves the use of this method for bridging the interval before definite therapy, i.e. transplantation of the liver.
The gold standard of surgical intervention for bleeding from esophageal varices is considered the splenorenal shunt according to Warren. It is, however, not always possible to set the surgical shunt on some main portal vein trunks. In such cases it is necessary to choose a solution, which is perhaps less typical however offers a chance of success.

Patients and Methods

The authors compare a group of 29 patients with splenorenal shunts with the group of 35 more or less individual cases of patients with devascularisations, splenectomies, atypical shunts, re-interventions and surgeries for bleeding from the distal part of the gastro-intestinal tract.

Results

The Warren shunt was performed in 29 patients, splenectomy was performed in seven patients with left-portal hypertension; other methods were indicated occasionally. Some procedures, which were performed, were unique and have not been published yet (i.e. left epiploic-renal shunt, inferior mesenteric-iliacal shunt).

Conclusion

Patients with sufficient functional hepatic reserve and those who are not candidates for transplantation and TIPS is not insertable, can be successfully protected from relapsing haemorrhages using either routine or unusual surgical procedures.

048

COLD ISCHEMIA – REPERFUSION INJURY OF RAT LIVER – HISTOLOGICAL STUDY

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Introduction

Liver transplantation has become a clinical method of therapy for patients with end-stage liver diseases. Cold ischemia – reperfusion injury is one of many problems that result in poor function or in primary non-function of the liver graft after transplantation[1]. The aim of this study was to describe histological changes of liver structure in isolated cold-stored and reperfused liver and to introduce some immunohistochemical methods into the study of this phenomenon.

Methods

Female Wistar rats weighing 230–290 g were used as liver donors. The livers (n = 4–5 in each group) were isolated, stored in University of Wisconsin solution for 3 h, 24 h or 48 h in 4 °C (cold ischemia), then warmed up to 37 °C for 30 min (warm ischemia) and subsequently reperfused for 90 min under a recirculation regimen (37 °C in heated box). The control livers were reperfused immediately after isolation. The recirculation perfusate was Krebs-Henseleit solution (100 ml) with bovine serum albumin, glucose, heparin and washed bovine erythrocytes. At the end of the reperfusion, the specimens of liver tissue were taken from a central region (nearby porta hepatis) and from peripheral lobes for histological examination, fixed in Baker's or Bouin's fixatives and paraffin-embedded. Sections (thickness of 5 μm) were stained with hematoxylin & eosin or methyl green – pyronin (MGP). Computer image analysis (software LUCIA, LIM, Prague) of light microscopy sections (MGP-stained) was used for the study and quantification (according to the morphological changes e.g., pyknosis, vacuolization, cytoplasm changes) of injured cells. Data were expressed as the means ± SE (approx. 10000 hepatocytes and 2500 SEC were assessed in each group). The sections were stained with mouse MAb against proliferating cell nuclear antigen – PCNA (clone PC10), vimentin – intermediate filaments (clone V9) using immunohistochemistry, and the TUNEL assay (In-situ Cell Death Detection Kit; Roche) was performed.

Results

We observed that the SEC were injured more and earlier than hepatocytes. Nearly unchanged liver structure was observed after 3 h of cold ischemia except for a high number of hepatocytes with two nuclei (9.06 ± 0.96 % while 4.46 ± 0.51 % in control group) was a remarkable finding. In the 24 h group, the SEC were detached from the hepatocytes, had a round shape and lost their function of covering sinusoids (32.59 ± 4.13 % of SEC while only 1.24 ± 0.64 % of hepatocytes showed characteristics of injury). In the 48 h group, we observed changed liver structure in most of the liver samples as a result of extended cold ischemia: sinusoids were wider than normal; the SEC were lost; the hepatocytes were detached one from another, formed spherical shapes and some regions looked like a culture of hepatocytes. In this group 51.47 ± 5.32 % of SEC and 2.86 ± 0.95 % of hepatocytes were damaged. In the control group, we observed a normal structure of liver parenchyma. Methyl green

– pyronin staining allowed a better view of morphological changes of the liver structure when compared with hematoxylin & eosin.
The PCNA staining showed the highest proliferation rate of hepatocytes in control and 3 h groups and its decrease with duration of cold ischemia while TUNEL positive cells were observed predominantly in the livers from 24 h and 48 h groups. Surprisingly, the injury of SEC and macrophage activation was well detected in vimentin-stained liver sections. In the control group, the vimentin staining was observed in the SEC and monocyte / macrophage population. With the duration of cold ischemia storage, the staining of SEC diminished as these cells detached from the hepatocytes. The monocyte / macrophage cell staining was maintained in all groups with the highest number of these cells in the livers from 3 h and 24 h groups of cold ischemia.

Conclusion

In conclusion, the cold storage of the livers in the University of Wisconsin solution for 48 h caused broad changes in the liver structure. The histological and immunohistochemical methods used in this work are useful tools for assessing the cold ischemia – reperfusion injury of the liver. Methyl green – pyronin staining is an advantageous staining for the study of these changes. The PCNA could be used as a proliferation marker and TUNEL assay for labeling the injured cells in this kind of experiments. Vimentin staining is a good tool for studying the SEC injury during cold ischemia – reperfusion injury.

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049

RECURRENCE OF PRIMARY SCLEROSING CHOLANGITIS AFTER LIVER TRANSPLANTATION

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Introduction

Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease characterized by chronic fibrosing inflammation involving intra and extrahepatic bile ducts. Although survival after liver transplantation (LTX) of patients without cholangiocarcinoma (CCA) is excellent, recurrence of PSC remains problematic.

Methods

Between April 1995 and June 2003, 287 patients underwent LTx at IKEM. We enrolled 29 patients with PSC transplanted between April 1995 and April 2002 (19 male, 10 female, mean age 45.0 years, resp. 46.7 years), into a retrospectively prospective study. Mean follow up was 921days, 1–2613 days). Protocol liver biopsy were performed yearly in all recipients, radiology investigation of biliary tree (ERCP, PTC) were done if clinically indicated. Recurrence of PSC was diagnosed based on Mayo Clinic criteria: 1. Confirmed diagnosis of PSC prior to LTx. 2. The cholangiogram showed nonanastomotic biliary strictures of the intra and/or extrahepatic biliary tree (occurring > 90 days after LTx) or 3. Fibro-obliterative lesions with/without ductopenia, biliary fibrosis or cirrhosis on histology. Patients and graft survival were calculated using the Kaplan-Meier technique. The Mantel-Cox test was done for comparison of survival curves.

Results

Eight-year survival of patients transplanted for PSC was 90.4 % compared to 78.8 % of other patients transplanted at IKEM (p = NS). From 29 patients in PSC cohort 3 patients died (9, 215 and 257 days after LTx due to primary allograft non-function, resp. recurrence of CCA diagnosed from explantated liver), 2 patients were followed abroad with incomplete data. Histological evidence of PSC recurrence was found in 2 patients (6.9 %) with a mean time to diagnosis > 4 years, one patient (3.4 %) had radiological features (nonanastomotic strictures) compatible with recurrent PSC. Acute cellular rejection was diagnosed in 48.3 % (14 patients), 13.8 % (4 patients) developed CMV hepatitis.

Conclusion

The frequency of recurrence of PSC found in our cohort corresponds to the published data. Despite the evidence of recurrent PSC after LTx, no patient required retransplantation. Long-term follow up will be necessary to assess the true impact of the recurrence of PSC on the long-term outcome of LTx.

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050

CHOLESTATIC HEPATITIS – MANIFESTATION OF TICLOPIDIN INDUCED LIVER INJURY- NEW INSIGHTS INTO MECHANISM OF LIVER DAMAGE

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Background

Cholestatic hepatitis is a rare complication of the antiplatelet agent ticlopidin. Several cases have been reported in the literature. However, the mechanism of ticlopidin induced liver damage remains un-

known, although the role of the immune system in its pathogenesis is suggested from previous studies.

Aim

The aim of the present study was to evaluate the role of the immune system in the pathogenesis of ticlopidin induced liver damage.

Patients

Three patients with cholestatic hepatitis with ticlopidin related liver injury, 3 patients with obstructive jaundice due to choledocholithiasis and 3 healthy individuals were involved in the present study. The diagnosis of ticlopidine induced cholestatic hepatitis was established in 3 patients who developed prolonged cholestatic hepatitis 2–12 weeks after receiving ticlopidin following percutaneous coronary angioplasty (PTCA). In one patient a liver damage developed 4 weeks after discontinuation of the drug. No dilatation or pathological changes of intrahepatic and extrahepatic bile ducts were observed in ultrasonography and ERCP. Other causes of liver injury were excluded. The canalicular cholestasis, infiltration of bile ducts with eosinophils, focal piecemeal necrosis, infiltration of portal tracts with lymfocytes and eosinophils and signs of apoptosis in liver biopsies were consistent with the diagnosis. The mean serum levels of liver function tests in this group were: bilirubin 386 ± 115 [μmol/l], ALT 3.89 ± 1.12 [μkat/l], AST 1.71 ± 0.96 [μkat/l], ALP 7.87 ± 2.68 [μkat/l], GMT 5.79 ± 2.11 [μkat/l]. The relative proportion of eosinophils in peripheral white blood cells was 7.9 ± 2.9 [%].

Methods

The serum levels of INF-γ, IL–2, IL–4, sFas (CD95+), sFas-L (CD95+ Ligand) and TNF-γ were measured by ELISA (Biosource International). The liver tissues obtained from liver biopsies in 3 patients with cholestatic hepatitis were immunostained with anti-CD4+, CD8+ and CD95+ antibodies (R&D). The TUNNEL assay to evaluate the grade of apoptosis was performed in liver tissue.

Results

The results are presented in following table:

	INF-γ [pg/ml]	IL–2 [pg/ml]	IL–4 [pg/ml]	sFas [pg/ml]	sFas-L [amol/ml]	TNF-α [pg/ml]
Ticlopidin induced	235	978	358	1732	211	955
Obstructive jaundice	215	718	277	815	86	418
Healthy	222	566	302	58	9	101

The serum levels of sFas, sFas-L and TNF-α were substantially higher among patients with ticlopidin-induced injury in comparison to patients with obstructive jaundice and healthy individuals (see table). There were no statistically different changes in serum levels of IL–4 and IFN-γ among these three groups of patients. The high proportion of CD8+ and CD95+ cells and positive reaction in TUNNEL assay suggests the role of enhanced apoptosis in the pathogenesis of ticlopidin related liver damage.

Conclusion

Cholestatic hepatitis is a rare adverse effect of ticlopidin treatment that may be immune mediated. The results of the present study suggest the key role of apoptotic pathways in drug induced liver damage. Patients receiving the drug should be monitored with liver function tests along.

051

LIVER TRANSPLANTATION IN PATIENTS WITH CHRONIC HEPATITIS B

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Background

Up to now the outcomes of liver transplantation in patients with chronic viral hepatitis B have not been very good because the recurrence of viral hepatitis in the graft has been high and often resulted in a premature mortality of liver transplant recipient. However, the administration of a combined therapy with lamivudine and hyperimmune anti-HBs globulin has led to a marked improvement in transplantation results and to an increase of the number of liver transplantations due to this indication.

Patients and Methods

Four men (aged 47 to 55 years) underwent the liver transplantation for cirrhosis, caused by chronic viral hepatitis B, at our centre. All were HbsAg carriers. They became the very first patients in the Czech Republic receiving this therapy with the combined immunoprophylactic regimen of lamivudine and hyperimmune anti-HBs globulin.

Results

HBV DNA negativity was achieved in all patients prior to transplantation; three of them were pretreated with lamivudine. At 4 to 17 months of follow-up, sustained suppression of HBV replication (HBV DNA negativity) was maintained in all four patients. No complications associated with this treatment were observed and no resistance to lamivudine was detected.

Conclusion

The combined therapy for chronic viral hepatitis B administered to liver transplant recipients at our centre showed very good outcomes. However, the development of lamivudine-resistant mutants during this therapy poses a problem, which may hopefully be overcome with the use of new antivirotics, such as adefovir or tenofovir.

052

CHRONOBIOLOGICAL ASPECTS OF 46 – YEARS' CASE REPORT OF ROTOR SYNDROME

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Background

The patient's story began in 1957 and continues to the present without any major incident. The chronobiological approach, adopted since the times of the world-renowned pioneer work by L. Dérer in Bratislava during the fifties and realized thanks to outstanding mathematical contributions by L. Kubáček, makes it possible to evaluate more exactly the time aspects of the pathological process in this

patient. Moreover, the time course of papers published about Rotor syndrome (RS) in the world literature will be analysed.

Material and Methods

Over the period from 1957 to 2002, altogether 36 measurements of total bilirubinemia were plotted versus time as chronogram, to assess the total trend during the whole period of observation, as well as plexogram, to study possible seasonal fluctuation. Chronogram was also constructed from 0–7 yearly numbers of published papers between 1963–2002. Data were processed by Halberg's cosinor regression, using originally modified software.

Results

The seasonal plexogram revealed lower plasma bilirubin levels in summer compared to winter due to changing light intensity from winter to summer. Slight increases in bilirubinemia over the years are an artefact: samples in last decade were always withdrawn in winter when the patient was not busy with agricultural work. The frequency of publications was low. It reached its maximum around 1982. There was a strange 5.5-year periodicity in the data. However, to speculate about the 2nd harmonics of the dominating 11-years-long cycle of solar activity would be too courageous.

Conclusion

The principal finding is the stability of plasma bilirubin levels, fluctuating around 80 μM/l, during almost one half of the century. The syndrome remains extremely rare; there are only a few descriptions available. Alarming should perhaps be the decline of overall interest in this pathological-clinical unit in the resent years, despite its unresolved basis.

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053

ROTOR SYNDROME – A WHITE SPOT IN THE WORLD TEXTBOOKS OF MEDICINE

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Background

Rotor syndrome (RS), described by Rotor, Manahan and Florentin in 1948, represents, along with Dubin-Johnson syndrome (DJS), the two main forms of benign conjugated hyperbilirubinemia. While there are several mutations in MRP2 gene identified as the curable cause of the disease in the latter, the etiopathogenesis of RS, apparently on a molecular basis, remains obscure to date. The present contribution aims at confronting various opinions as published in renowned textbooks and by the present author.

Materials and Methods

The present author observed one of the first cases of RS from 1958. He published the case report in detail 43 years ago. Many biochemical examinations were performed. Sulfobromophthalein plasma clearance (measurements by Charbonnier) was analysed with the aid of an original mathematical model based on the system of differential equations and resulting in estimates of liver uptake, storage and excretory function.

Results

New information was added concerning the impaired hemocoagulation (explained later by Seligsohn *et al.* as inborn defect), increased porphyrinuria and particularly the extremely pronounced decrease in sulfobromophthalein plasma clearance – for liver uptake by 86 %, for liver storage by 75 % and for liver excretion by 89 % (point estimates for the sample only).

Discussion

Non-invasive differential diagnosis of RS and DJS are difficult, apart form cases with the presence of typical intralysosomal pigment in centrolobular liver cells in DJS. Unfortunately, the sulfobromophthalein test with clearly differing clearance curve cannot be considered as non-invasive. The leading defect in RS is assumed in decreased hepatic storage of bilirubin (Harrison's Textbook) or – obviously inappropriately – in its decreased canalicular secretion (Cecil's Textbook).

Conclusion

In the era of gene therapy the differentiation between both syndromes becomes practically important. Maybe, this possibility, feasible for Dubin-Johnson syndrome, will be also attained for the Rotor syndrome. To this purpose the author can supply DNA samples from his well-defined patient observed almost for a half of the century. Perhaps the patient will be willing to be subjected to a direct examination, to obtain e.g. liquid nitrogen frozen sample of liver tissue.

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054

TREATMENT OF EARLY SURGICAL COMPLICATIONS AFTER LIVER TRANSPLANTATION

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The results of liver transplantation have improved dramatically over the past 20 years. This has produced a huge increase in demand for this procedure. Improvements in outcome are multifactorial and include identification of patients who are likely to benefit from liver transplantation, earlier referral of such patients, improved surgical technique and perioperative care, and better immunosuppressive regiments. In the last decade 1-year survival of patients after liver

transplantation has reached more than 90 % in many transplant centres and the survival curve for other years is relatively flat[1]. On the other hand, the liver transplantation is a procedure with high morbidity. Nearly 100 % patients have at least one complication of any kind[2]. Complications of liver transplantation occur in patients who often suffered from serious medical problems before transplantation. Complications affecting liver transplant recipients prior to discharge or death can be classified as 'immediate' in intensive care phase or 'early':

Immediate – postoperative bleeding, PNF (primary non-function of the graft) and acute renal failure.

Early – primary poor or delayed function of the graft, HAT (hepatic artery thrombosis), portal vein thrombosis, bile leakage or biliary obstruction (cholangitis), bacterial infection, AR (acute rejection), opportunistic infection (viral, fungal).

Immediate Complications after Surgery

Complications following the liver transplantation within immediate period are mostly influenced by the transplant operation itself and the quality of newly inserted liver graft. Excessive intraoperative bleeding poses a major risk for the development of postoperative complications. In liver transplantation the surgeon is most often confronted with patients with portal hypertension with fragile venous collaterals and coagulopathy. Pharmacological therapy like administration of aprotinin can reduce the fibrinolysis inherent in liver transplantation. Surgical skill and experience are probably still the most important predictors of blood loss during surgery and immediate postoperative bleeding[3]. The incidence of postoperative haemorrhage with the need of operative revision is about 10–15 %.

PNF is the failure of the new liver graft. This may be caused due to pre-existing factors in the donor (i.e. fat liver), poor preservation or reperfusion injury[4]. After the surgery the patient develops a progressive acidosis, renal failure and all of the other features characterising fulminant hepatic failure. Hemodynamic instability and death ensues unless retransplantation is urgently undertaken. Fortunately PNF is rather rare although poor or delayed function of the liver graft occurs in about 5–10 % of cases.

Renal impairment is observed in many liver transplant recipients. It is characterised by a period of oliguria and an elevation of creatinine for several days after the surgery. It is usually responded to correction of any fluid deficit and an infusion of dopamine. Acute renal failure requiring haemofiltration or dialysis is uncommon in stable patients transplanted for chronic liver disease. Patients with acute liver failure are often dialysis-dependent before and after liver transplantation. This is best performed as continuous arterio-venous haemodialysis. Most patients will produce a diuresis within 2 weeks. Nephrotoxicity of CyA and FK506 is well known and in some cases in early postoperative period a drug regimen should be tailored according to creatinine.

Early Complications after Surgery

Causes of PNF in this period are displayed on Table 1.

Table 1. Causes of PNF early after liver transplantation[5]

	Incidence	Time	Coagulopathy	Graft survival
PNF	6 %	48 hours	Yes	10 %
HR	rarely	6–24 hours	Yes	not mentioned
AR	50–70 %	5–7 days	No	90 %

The incidence of HAT early after transplantation reaches about 3–5 %, more in paediatric recipients. Early detection and urgent surgical treatment can be crucial for the graft and patient survival. Fortunately the portal thrombosis is a rather rare complication.

An early biliary leakage or obstruction as the cause of cholangoitis can be detected by ERCP and mostly treated by minimally invasive approaches (endoscopically or percutaneously). All patients receive prophylactic broad-spectrum antibiotics for 3 days following surgery and this period is extended in patients requiring prolonged ventilation. Bacterial infections are common and most frequently involve the respiratory and biliary tract, especially in the case of biliodigestive anastomoses (hepaticojejunostomy – HJA).

High incidence of pleural effusions contributes to impaired respiratory function, larger ones should be tapped. All immunosuppressed patients are at risk of developing opportunistic infections and after liver grafting CMV and fungal infection are common as well. Risk factors include fulminant hepatic failure as an indication for liver transplantation, prolonged ventilation, PNF or delayed graft function and high dosage immunosuppression[6].

The introduction of CyA in the early 1980s and other immunosupressants in 1990s and induction triple therapy led to minimisation of problems with acute rejection. Nevertheless an episode of acute rejection especially in HCV patients can start the infection complication and following deterioration of patient condition including the liver function. Therefore this is the place for tapering of immunosuppression regiment in each patient as a result of experience of transplant team. Nevertheless acute rejection after liver transplantation as a cause of graft failure is extremely rare. Complications leading to indications for early retransplantation are very similar to the causes of PNF.

IKEM Experience

Within the period of 4/1995 till 5/2002 in Institute for clinical and experimental medicine (IKEM) 246 liver transplantations were provided. The incidence of HAT was 3.3 % (9 cases) with the incidence in 4,1 day (1–15 days). After selective angiography urgent thrombectomy in 3 patients and reanastomosis in 5 patients were done with mortality reached 22.2 %. Incidence of biliary complications was in the first 280 liver transplantation 27.8 % (78 pts.). In 2002 this was only 12 %. Transduodenal endoscopy was successful in 36 and percutaneous approach in 9 patients. Due to the failure of miniinvasive approach, HJA was done in 21 patients (3 anastomosis reconstructions). 4 patients were indicated to re-transplantation. Deaths related to biliary complication were mentioned in 2 patients after re-LTx (0.7 %).

Conclusion

There are a few areas in surgery that can match the major advances in outcome after liver transplantation for the patients. Nevertheless complications developing after the liver transplantation are common and vary which produces a lengthy learning curve for the liver transplant team. Many problems can often be pre-empted by experience transplant team by the early recognition of clinical patterns. Potential disasters can be averted.

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055

HEPATOCELLULAR CARCINOMA – DIAGNOSTIC PROBLEMS

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Introduction

Hepatocellular carcinoma (HCC) makes up 5 % of all worldwide known malignancies. Half a million die every year from the disease. HCC doesn't occur uniformly in the world population. It depends on prevalence of HBV and HCV infection, abuse of alcohol and contact with aflatoxin B1. HCC is mostly based on hepatocirrhosis. Similar to other malignits advanced diagnosis is important to its therapy.

Aim

The object of this study was to determine the quality of HCC diagnostic procedure in retrospective processing of clinical and sectional specimens from those patients who died at II IK due to HCC.

Methods

Between the years 2000–2002 66 cases were examined by section at UAP LF UP on hepatocirrhosis, and HCC was diagnosed in 12 cases, i.e. 20 %. No case of HCC was confirmed without hepatocirrhosis. In the group of 12 patients only 50 % of the cases were diagnosed with the HCC tumor. This study included 3 women and 9 men with an average age of 68.6 – the youngest patient was 54 and the oldest was 85. The results of clinical and laboratory determinations and results of investigative viewing methods, in some case even histological determinations, have been entered in details in this study. Circumstances of HCC diagnostic are mentioned only in a few cases. At the end of this study two unusual case reports are noticed with infrequent metastasis localization.

Conclusion

The diagnostic methods of HCC seemed to be quite elementary. However, according to our experience, some methods had failed in diagnosis of HCC (e.g. USG). According to our opinion, it will be necessary to establish a procedure of diagnostic methods and concept of residency in order to achieve an advanced diagnosis of HCC followed by a successful therapy.

056

SURGICAL TREATMENT OF PRIMARY AND SECONDARY LIVER TUMORS – A SINGLE CENTRE STUDY

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The purpose of this study was to evaluate single centre results of surgical treatment of hepatic tumors. Over the period from January 1999 to July 2003, 132 patients with liver tumors were admitted at our Department and were indicated for open resection /130/ or laparoscopic procedure (2). Various malignant liver lesions were diagnosed preoperatively by ultrasound
(USG), computed tomography (CT), elevated tumours markers (CEA, AFP, CA19–9) or magnetic resonance imaging (MRI). The ultimate diagnosis was confirmed by postoperative histological examination of specimens and included hepatocellular carcinoma (HCC), cholangiocellular carcinoma, secondary liver tumors such as metastases of colorectal carcinoma (CRC), breast carcinoma and others. The group of 132 patients was analysed with regard to diagnostic algorithm, indication to surgery, and the follow-up period. The diagnosis included CRC metastases in 82 patients, metastases of breast carcinoma in 13 patients, hepatocellular carcinoma in 7 patients, cholangiocellular carcinoma in 3 patients, and others in 27 patients. Postoperative morbidity was 24.2 % and mortality 1.5 %. Surgical resection is the treatment of choice for patients with liver tumors. 47 patients with unresecable lesions without extrahepatic spreading underwent the Radiofrequency ablation (RFA). Altogheter 89 lesions were treated. The 30-days postprocedure morbidity was 21.5 %, mortality was 0 %. Laparoscopic resection was used in two cases and was the method of choice for small lesions in the left liver lobe.

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BILIARY TRACT LESION DURING LAPAROSCOPIC CHOLECYSTECTOMY IN THE HOSPITAL PROSTĚJOV – RESULTS OF 10 YEARS

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Background

It is not possible to avoid a lesion of biliary tract completely during the laparoscopic cholecystectomy (LCE) of biliary tract even in contemporary minimum invasive surgery. In theory we may expect the highest number of lesions committed by operators during their practicing of the laparoscopic operation. Our study does not support this theory; it brings comparison of our results with other studies. In addition, there is one example of the lesion of biliary tract becoming paradoxically a benefit for the patient.

Patient and Methods

From 1992 to 2002 we accomplished 3403 laparoscopic operations, 1430 of that were LCE. In all we caused 6 iatrogenic biliary lesions meaning 0.4 % occurrence. We solve such complications

in our department by Hepatic Jejuno Anastomosis (HJA) of Roux-Y or Volker's drain without any requirement for patient's transport into a special surgical clinic.

Results

The patients were six women of the average age 45 years. Three lesions were identified preoperatively and 3 post. There were 4 large and 2 small lesions. All were discovered during laparoscopic operations performed in our department between the 3rd and 10th year of the study. The large lesions were solved by HJA. One small lesion was operated as T-drain and the other as nasobiliary probe.
The biliary tract lesion was paradoxically beneficial to one woman. During the conversion and HJA a tumour of the lienal flexure was found. Therefore, sinistral hemicolectomy was carried out at the same time. The woman died 5 years later as a result of another diagnosis. Remaining five patients still live and continue to lead their life in full. During the last dispensational medical examination all displayed good state of health. Two of them had low elevations of GMT and ALP.
In comparison to similar studies that have the range of lesions 0.27–1.0 % the result of our department (0.4 %) is very good.

Conclusion

The authors introduce a list of biliary tract lesions and their medical solutions in comparison with other studies. One interesting causal example is presented. Authors also present a complete view of safety precautions during these operations. The complications are solved on a case basis at the department.

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ORTHOGRADE LAPAROSCOPIC CHOLECYSTECTOMY WITH ACUTE CHOLECYSTITIS

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Objective

This project evaluates the significance of orthograde laparoscopic cholecystectomy performed on patients with acute or chronic cholecystitis or patients after ERCP. The total number of orthograde cholecystectomies from the beginning of the year 2000 to the end of the year 2002 have been evaluated.

Methodology

Authors evaluated the total number of orthograde laparoscopic cholecystectomies performed at the 2nd Clinic of Surgery, Olomouc, from the beginning of the year 2000 to the end of the year 2002. The surgeries were performed on patients with acute or chronic cholecystitis, pericholecystitis, and on patients who underwent preoperative ERCP and were diagnosed with ductus cysticus amputation, aberrant bile duct or demimonstrosity of biliary arbor caused by the pressure of cholecyst during ERCP.

Results

The total number of orthograde cholecystectomies using laparoscopy was 54. 31 patients with acute cholecystitis were operated on. 13 patients in total underwent preoperative ERCP: cystic duct was not visualized in four patients during ERCP, five patients were diagnosed with aberrant bile duct and four patients with cholecyst pressing upon the bile duct. 49 cholecystectomies were completed using laparoscopy. There were five cases of conversion always related to acute cholecystitis. Preoperative biliography was carried out during 24 operations: five patients with aberrant bile duct were diagnosed during ERCP, four patients with cystic duct amputation during ERCP, four patients with the Mirizzi syndrome, six patients with acute cholecystitis, and five patients with chronic cholecystitis. We used a harmonious scalpel for the operations. During 42 operations, argon plasmatic coagulation was used to stop bleeding.
We did not encounter any peroperative complications. There was a total number of six postoperative complications. In two cases postoperative pancreatitis developed and was dealt with conservatively. One re-operation had to be performed due to postoperative hepatic bleeding with the bleeding not identified. There were three cases of abscess in a wound created after insertion of the port under the gladiolus – the place of extraction of cholecyst.

Conclusion

With increasing surgeon's erudition in laparoscopic surgery techniques, even seemingly mini-invasively insolvable cholecyst diseases can be dealt with by orthograde laparoscopic cholecystectomy.

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RESULTS OF LIVER TRANSPLANTATION FOR SEQUELA OF HCV OR HBV INFECTION

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Introduction

Liver cirrhosis C is the most common indication for liver transplantation (LTx) worldwide. Long-term outcome is sub-optimal due to universal recurrence of viral infection and development of fibrosis and cirrhosis of the liver allograft in approximately 50 % of transplant recipients in 5-years from LTx. A minority of recipients develop an aggressive form of recurrence with rapid development of graft failure and signs of fibrous cholestatic hepatitis. High replication level, acute rejection episodes and steroid administration are predictive of poor outcome.
The sequel of hepatitis B viral infection is currently a standard indication for liver transplantation in most programs. Since introduction of combined chemo- and immunoprophylaxis the long-term results are excellent.

Aim

Aim of the study was to present long-term results of transplantation therapy of patients with HCV- and HBV-infection in comparison with other liver transplant recipients.

Materials and Methods

In the time period from April 1995 till August 2002, 55 LTx for HCV-related cirrhosis were performed in our centre. Of these 55 cases 8 were re-transplantation: 4 for HCV recurrence, 2 for de-novo HCV infection post transplantation, and 2 for technical complications in HCV-positive patients. Liver biopsies were done 1, 2, 3, 5 and 7

years after LTx and whenever clinically relevant. In total 259 liver biopsies were studied and classified by an experienced transplant pathologist using the Ishak modification of the Knodell score. Graft and patient survival and cirrhosis- free survival were calculated according to Kaplan-Meier technique, survival curves were compared by the Mantel-Cox test.

Results

In total 13 deaths in HCV-positive patients were recorded. Four, because of graft failure due to HCV recurrence, 4, due to cancer (2x HCC, 2x PTLD), 1 for primary non-function, 3 for sepsis, 1 tragically. Viral replication was found in all those surviving 1 year. One- and five-year graft survival was 71 % and 64 %, respectively, in HCV-positive group, and 88 % and 82 %, respectively, in HCV-negative patients ($p < 0.006$). One- and five- year patient's survival in HCV-positive patients was 81 % and 72 %, respectively; one- and five-year patient's survival in HCV negative was 89 % and 82 %, respectively, (p = NS). The probability of development of cirrhosis 3 and 5 years after liver transplantation for HCV infection was 33 % (95 % CI: 16.50 %), and 41 % (CI: 20.63 %).
The first liver transplantation for HBV-related acute liver failure was performed in 1999, 5 other cases were indicated for HBV- related liver cirrhosis. In all cases standard prophylaxis with Lamivudine (both pre- and post-transplant) and intravenous anti-HBs immunoglubulin during and after LTx was instituted. We recorded no recurrence during the follow-up period from 3–33 months and all patients are alive.

Conclusion

Although patient survival shows no significant difference between HCV-positive and HCV-negative recipients during the 5-year follow up; the probability of liver allograft cirrhosis was high (41 % with 95 % CI 20–63 %), rendering a significant impact on graft survival. Disease recurrence prophylaxis and treatment are urgently needed. Chronic and acute liver failure caused by hepatitis B virus is a minor indication for liver transplantation with excellent results when the proper prophylaxis is performed.

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060

ALPHA–2-MACROGLOBULIN AS A BIOCHEMICAL MARKER OF FIBROSIS IN PATIENTS WITH CHRONIC VIRAL HEPATITIS

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Background

Fibrosis is one of the important pathobiochemical processes participating in the development of chronic liver diseases. Liver biopsy is the standard method for assessing fibrosis. This procedure is recommended before the initiation of antiviral therapy and is important for monitoring the progression of fibrosis. Unfortunately, liver biopsy is invasive and prone to complications. The use of biochemical parameters as fibrosis markers could substantially reduce the number of biopsies performed for the management of chronic hepatic viral infections. Recently, several studies reported rather very good correlation of alpha–2-macroglobulin (AMG) to the activity of liver fibrosis[1]. AMG is secreted by tissue macrophages and fibroblasts and functions in the environment of extracellular matrix macromolecules.

Aim

The determination of AMG levels, as potential fibrogenesis markers, in correlation to histological staging and to another potential serum fibromarker – hyaluronic acid[2] (HA), in patients with chronic viral hepatitis.

Patients and Methods

64 blood samples from patients with chronic hepatitis (23 hepatitis B and 41 hepatitis C) and 20 blood samples from healthy controls were assayed for AMG. Fibrosis in liver biopsy specimens were staged on scale of 0–4: 0- no fibrosis, 1- portal fibrosis without septa, 2- few septa, 3- numerous septa without cirrhosis, 4- cirrhosis. AMG was estimated immunochemically with monospecific antibodies and hyaluronic acid with enzym-immunoassay.

Results

The levels of AMG were elevated in patients with chronic hepatitis and liver cirrhosis. After dividing patients according to the results of histological grading of fibrosis, there was no difference between group without fibrosis and healthy controls.

Parameters	AST (μmol/l)	ALT (μmol/l)	Albumin (g/l)	Alpha–2-MG (mg/l)	CHE (U/l)
Hepatitis – fibrosis grade 0	0.62	1.11	37.8	1375	4730
Hepatitis – fibrosis grade 1–2	0.78	1.42	37.9	1820	4471
Hepatitis – fibrosis grade 3	1.80	1.98	36.5	2810	3572
Cirrhosis – Child-Pugh A	0.75	0.61	40.9	2280	4067
Cirrhosis – Child-Pugh B	1.15	0.78	29.9	3479	1793
Cirrhosis – Child-Pugh C	1.18	1.05	21.6	2305	1220
Healthy controls	0.37	0.32	39.3	1308	3961

The patients with fibrosis 1–3 had a significantly higher level of AMG than controls. The group 3 also showed significantly higher levels of AMG compare to groups 1–2. The group of patients with liver cirrhosis had higher level of AMG than patients with chronic hepatitis (2723 mg/l vs. 1954 mg/l). There was significant correlation between levels of HA and AMG ($r = 0.43$, $p < 0.001$).

Conclusion

Based on the results of our study, we can conclude that the estimation of AMG significantly correlated with the presence and activity of liver fibrosis. The findings also support our opinion that AMG could be helpful in diagnosis and monitoring of liver fibrosis.

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061

TRACE ELEMENTS METABOLISM IN PATIENTS WITH ALCOHOLIC LIVER STEATOSIS AFTER TREATMENT WITH ESSENTIAL PHOSPHOLIPIDS

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Background

Zinc is one of the essential trace elements. This element is ubiquitous in subcellular metabolism. It is, for example, required for RNA and DNA synthesis and for the function of over 200 zinc metalloenzymes. There are many gastrointestinal diseases, which are connected with altered zinc metabolism. In this group we can find also alcoholic liver diseases. Copper is another essential trace element. In the blood plasma copper is transported in the form of ceruloplasmin. Ceruloplasmin is not only a simple transport protein but it is a multifunctional protein with various other physiological functions (role in iron metabolism, scavenger of reactive radicals)[1]. At least a part of these functions is connected with ceruloplasmin enzymatic activity. Recent studies in humans suggest that the specific enzymatic activity of ceruloplasmin (activity per unit mass of enzyme protein) is a more sensitive indicator of copper status than either serum copper and ceruloplasmin or erythrocyte superoxide dismutase[2].

Aim

In the study we aim our effort to estimate the effect of administration of essential phospholipids on the metabolism of zinc and copper in patients with alcoholic liver steatosis.

Patients and Methods

An open clinical trial was performed in patients suffering from alcoholic liver steatosis. Two capsules of Essentiale forte (Rhone-Poulenc Rorer) were administered 3 times daily for 3 months. Individual biochemical parameters were determined every month. The serum levels of zinc and copper were determined by the atomic absorption spectrophotometry (Varian AA–475). The amount of ceruloplasmin protein was estimated immunochemically and the enzymatic activity of ceruloplasmin was estimated as polyphenoloxidase activity.

Results

There was no difference between zinc level in patients with liver steatosis and in healthy controls (15.56 μmol/l vs. 15.33 μmol/l). There was neither any difference between zinc levels before and after therapy (15.56 μmol/l vs. 15.65 μmol/l). The copper level in patients with liver steatosis was moderately decreased in comparison to healthy controls (15.45 μmol/l vs. 17.44 μmol/l). The therapy with essential phospholipids had no significant effect on copper level (15.45 μmol/l vs. 15.43 μmol/l). The differences in ceruloplasmin levels between patients with liver steatosis and healthy controls were neither significant (348 mg/l vs. 343 mg/l), but the specific activity of ceruloplasmin in patients was substantially decreased in comparison to controls (0.57 vs. 0.82). The specific activity of ceruloplasmin after therapy was moderately increased in comparison to level before therapy (about 18 % higher after therapy).

Conclusion

The decreased specific activity of ceruloplasmin in spite of normal levels of copper and ceruloplasmin in patients with alcoholic liver steatosis suggested some problems in copper metabolism in these patients. The results of our study showed positive effect of therapy with essential phospholipids on copper metabolism in patients with alcoholic liver steatosis.

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062

PERITONEOVENOUS SHUNT – MODIFICATION WITH THE USE OF LONG SAPHENOUS VEIN

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Introduction

Peritoneovenous shunt (PVS) plays a major role in surgery of intractable ascites in patients with liver cirrhosis. A permanent peritoneal cavity drainage with recirculation of ascitic fluid into the vascular space based on a positive pressure gradient between peritoneal cavity with ascites and venous pressure is the principle. Over the years many drainage systems have been developed. Nowadays the systems with possibility of active flow management (Denvers shunt) enabling to keep a long-term cumulative function are the optimum. The only disadvantage is their high price. An attention is therefore focussed on some modifications using “own resources“ as described by H. J. Vadeyar *et al.* Long saphenous vein is used as a drainage system. A natural valve in saphenous orifice ensures the one-way ascites flow.

Methods

When deciding for the PVS formation, present criteria for indication and contraindication have been leading for us. Presumptions for sapheno – peritoneal modification of PVS are patent femoro-iliaco-caval portion of the deep venous system and suitable long saphenous vein with sufficient orifice valve.
The procedure was performed under general anaesthesia and all patients received perioperative antibiotics and LMWH.
The long saphenous vein is exposed through the vertical incision down, its branches are ligated and it is divided at 20 cm. In simple mechanic manner (catheterization with a saline solution flush) we check central patency of the saphena and sufficiency of its orifice valve (no backflow from the femoral vein). The inguinal canal is exposed through an oblique incision and we get to the parietal peritoneum after dividing the internal oblique muscle fibre laterally from the spermatic cord (funiculus) in the internal ring. This is the place for the incision in peritoneum. The proximal cut end of the long saphenous vein is turned upwards and pulled through the subcutis above the inguinal ligament. We form a slight curve in the venous

orifice to prevent a sharp bend. We cut the peritoneum and perform a watertight anastomosis with obliquely cut saphenous end using a continuous prolene 6.0 suture. The pressure of ascites on the suture can be reduced by Trendelenburg's position. The wounds are closed in layers wihout any drain. Subsequent care of the patient with a mobilised ascites and of the shunt is the same as in other types of PVS.

To perform a better peritoneal drainage we have suggested a modification with a silastic catheter whose fenestrated peritoneal end (25 cm) is placed in the peritoneal cavity and saphenous end reaching vein just bellow the valve.

Results

Between 1999 and 2001 we performed in all 12 shunts of this type in 6 patients with tension intractable ascites in liver cirrhosis, Child-Pugh-Turcott classification from 7 to 12.

3 shunts in 2 pts were performed using a peritoneal catheter.

There was no function in 2 shunts; only intraoperative ascites drainage was evident. 8 shunts failed in 36 hours after a transient reduction of tension ascites. 1 patient with a functional shunt (with a peritoneal catheter) died 3 weeks later due to a concomitant disease.

Only in l patient (after primary shunt failure and contralateral secondary one formation) we can claim a long-term (meaning 3 months) successful function. The patient has only residual ascites.

We reoperated (from the groin access and venotomy) due to an early failure of the shunt in 6 pts 15 times in all. We claimed inflow failure in 9 cases – omentum stuck the anastomosis very probably. We found no technical mistake in the anastomosis. We detected a bent in the venous orifice in 1 shunt. In 5 cases we claimed saphenous thrombosis.

In case of primary shunt failure (repeated) we performed a shunt of the same type contralaterally.

When not successful with the contralateral shunt, we performed a fully functional Denver shunt.

We had no relevant hemorrhage or infection or ascitic leakage. No patient died in relation to either primary surgery or surgery for shunts complications.

Conclusion

At first sight saphenoperitoneal shunt is indisputably elegant and easy type of permanent drainage of intractable.

Our experiences – results are puzzled. The advantage of autologous material using, a possibility of formation of the same shunt contralaterally and the low price balanced in our group the frequency of shunt occlusion with problematic service.

Comparison of various departments' experience as well as evaluation of long-term results is missing. References about this type of shunt in our and the world literature have been still sporadic.

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063

EFFECTIVENESS OF DIAGNOSTICS OF ICTERUS (ICTERIC STATE)

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Introduction

Currently, when economics affects all spheres of medicine, it is necessary to respect rational diagnostic and therapeutic procedures, not only for economic reasons, but also for the benefit of the patient.

To this purpose it is necessary to know all current possibilities. We need to chart the present situation for elaborating guidelines.

Data, which we would like to present in our retrospective study, express a measurement of the rationality of diagnostic procedures.

Methods

In our II. Department of Internal Medicine we are engaged in gastroenterology, hepatology and diabetology. The majority of patients, who are included in this part of Central Moravia (about 200,000 inhabitants), come for diagnosis of icterus (with the exception of acute virus hepatitis).

We express here the logic of diagnostic procedure in points (Insurance companies pay acc. to such points). We divided patients according to diagnostic conclusion into 3 groups.

1) cirrhosis
2) lithiasis
3) others

Results for diagnosis: 1/ cirrhosis:

2) lithiasis
3) the others

Results

In spite of the fact that retrospective study can be erroneous, our results show, that the time for assessment of cause of icteric state is

1) 318
2) 516
3) 290

It will be useful to continue with this study and compare it with other centres.

Conclusion

It would be useful to continue with this study in specialized centres in university hospitals and also in district hospitals, and on the basis of these experiences propose guidelines.

These guidelines facilitate their use in medical centres having the necessary personnel and equipment.

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064

ZINC SALTS EFFICIENTLY DECREASE SERUM BILIRUBIN LEVELS IN HYPERBILIRUBINEMIC RATS

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Background

Enterohepatic circulation (EHC) of bilirubin is implicated in the pathogenesis of several conditions such as neonatal jaundice or black pigment gallstone formation. Inhibition of the EHC of bilirubin might lead to a substantial decrease in serum as well as biliary levels. In the present study we investigated the effect of oral administration of Zn sulphate and Zn methacrylate on serum bilirubin levels in naturally hyperbilirubinemic Gunn rats as well as Wistar rats with artificially induced elevation of serum bilirubin levels.

Methods

Six male Gunn or Wistar rats weighing 250–270 g were used in each experiment. Hyperbilirubinemia in Wistar rats was induced by a 14-day injection of unconjugated bilirubin (UCB) into peritoneal cavity (4 mg/100 g b.wt/day). All the animals were fed a normal diet for one week. After that Zn methacrylate or sulphate were admixed into pulverized food in a final concentration of 1% w/w and the rats were fed the Zn diet for two weeks. Serum bilirubin and Zn levels, and faecal excretion of bile pigments were determined at the beginning of the study and after 2 weeks of Zn diet. Simultaneously, an in vitro study was performed to assess bilirubin binding capacity of the Zn salts used.

Results

In Wistar rats the intraperitoneal injection of UCB resulted in elevation of serum bilirubin levels from 0.8 ± 0.1 to 102 ± 10 μmol/l ($p < 0.0001$). By contrast, oral administration of Zn methacrylate to Gunn rats and artificially hyperbilirubinemic Wistar rats resulted in a significant decrease of serum bilirubin levels (166 ± 53 μmol/l vs. 123 ± 38 μmol/l, $p < 0.05$, and 102 ± 10 μmol/l vs. 14 ± 3.8 μmol/l, $p < 0.0001$, respectively). Simultaneously, the faecal urobilinoid output also decreased in both animal models (from 160 ± 107 to 111 ± 26 nmol/100 g b.wt, $p < 0.05$, vs. 1528 ± 856 to 415 ± 355, $p < 0.0001$) as well as faecal bilirubin output (from 299 ± 36 nmol/100 g b.wt to 258 ± 19, $p < 0.05$ vs. 2533 ± 653 to 1226 ± 820, $p < 0.0001$). The drop in faecal bilirubin excretion was due to formation of Zn precipitates of bilirubin in the gut lumen. The decrease in faecal urobilinoid excretion was caused presumably by the toxic effect of Zn on gut microflora reducing bilirubin. The effect of Zn sulphate on serum bilirubin in Gunn rats was even higher than that of Zn methacrylate (decline from 206 ± 34 to 131 ± 31 μmol/l, $p < 0.05$, vs. 166 ± 53 μmol/l to 123 ± 38 μmol/l, $p < 0.05$, respectively). Both Zn salts in 1 % concentrations in artificial micellar phase were found to be strong bilirubin binders (Zn methacrylate and Zn sulphate bound 98.1 % and 94.2 % of UCB, respectively).

Conclusion

In the present study, oral administration of Zn salts effectively decreased serum bilirubin levels in hyperbilirubinemic rats presumably as a result of inhibition of EHC of bilirubin. This approach might be useful in the treatment of severe unconjugated hyperbilirubinemias including neonatal jaundice as well as in the prevention of black pigment gallstone formation in predisposed subjects.

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065

LIVER TRANSPLANTATION FOR ALCOHOLIC LIVER CIRRHOSIS IN IKEM

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Background

Alcoholic liver cirrhosis (ALC) is an accepted indication for liver transplantation (OLT). The recurrence of alcohol abuse (RAA) after OLT for ALC is reported in 10–50 % of recipients. We have retrospectively analysed RAA after OLT in our centre.

Methods

From X/1995 to II/2002, 42 of total 246 patients, underwent OLT for ALC. We administered a structuralized questionnaire to all patients surviving at least 1-year post OLTx for ALC. Liver biopsy was performed annually (with exception of year 4). The result of questionnaires was compared with laboratory parameters and graft histopathology. Socioeconomic status and cumulative survival rate (CSR) (at 1, 3, 5 years) were compared between RAA and sobriety groups.

Results

Sixteen (38 %) patients admitted alcohol consumption on the questionnaire. Serious graft damage was diagnosed in 4 recipients, 2 of them died due to graft failure, one for another reason. Twelve subjects returned to mild alcohol abuse. We found more steatosis: 56.3 %, and 31.1 %, and more Mallory hyaline: 18.75 % and 0 % ($p < 0.05$) and no difference in fibrosis between RAA and "sobriety" groups respectively. The RAA and "sobriety" groups differed in BMI (25.40 ± 3.33 vs 27.83 ± 3.85, $p < 0.04$), serum triacyglycerols 3.73 ± 2.07 vs 2.36 ± 1.76 mmol/l, $p < 0.04$), MCV 97.49 ± 7.02 vs 87.69 ±

3.14 fl, $p < 0.0004$), GMT (4.48 ± 6.96 vs 0.79 ± 1.17 µkat/l, $p < 0.05$). CSR (1, 3, 5 years) was lower in RAA than in sobriety group (100 %, 83 %, 74 % vs 100 %, $p < 0.05$).

Conclusion

Patients after OLT for ALC with RAA had a lower survival rate than the sober group and recurrence may have a fatal course. Considering the differences in histopathology and laboratory parameters in patients with and without RAA after OLT, the questionnaire method seems to be a valid tool for RAA detection.