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EDITORIAL

Cholangiocarcinoma, gone without the Wnt?

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Abstract

Cholangiocarcinoma (CCA) is a relatively rare malignancy of the intra- or extra-hepatic bile ducts that is classified according to its anatomical localization as intrahepatic, perihilar or distal. Overall, CCA has a dismal prognosis due to typical presentation at an advanced irresectable stage, lack of effective non-surgical treatments, and a high rate of disease recurrence. CCA frequently arises on a background of chronic liver inflammation and cholestasis. Chronic inflammation is accompanied by enhanced cell turnover with generation of additional inflammatory stimuli, and a microenvironment rich in pro-inflammatory mediators and proliferative factors that enable accumulation of mutations, transformation and expansion of mutated cells. A recent study by Boulter *et al* implicates the Wnt signaling cascade in cholangiocarcinogenesis. Wnt ligands Wnt7B and Wnt10A were found to be highly overexpressed in human CCA tissue. Wnt7B protein was present throughout the tumor stroma, and often co-localized with a subset of $CD68^{+}$ macrophages. To address in a direct manner whether Wnt signaling is engaged in development of CCA, Boulter *et al* explored the Wnt signaling pathway in an experimental model that recapitulates the multi-stage progression of human CCA. Wnt ligands found to be elevated in human CCA were also upregulated during the course of CCA development following thioacetamide treatment. Wnt10a increased during the (pre-cancerous) regenerative phase, while Wnt7b induction paralleled tumor growth. Along with upregulation of target genes, the findings demonstrate that the canonical Wnt pathway is progressively activated during cholangio-carcinogenesis. Macrophage depletion, eliminating a major source of Wnt7b, prevented activation of the canonical Wnt cascade, and resulted in reduced number and volume of tumors in this model. Moreover, specific inhibitors of the canonical Wnt pathway (ICG-001 and C-59) caused reduction of tumor area and number, in xenograft and thioacetamide models of CCA. The aggregated findings show that experimental, and presumably human CCA, is a Wnt-driven tumor. Modulation of Wnt signaling, alone or in combination with surgical

or chemotherapy approaches, holds promise in the management of this fatal malignancy.

Key words: Intrahepatic cholangiocarcinoma; Liver neoplasms; Carcinogenesis; Wnt signaling pathway; Wnt7B protein; Wnt proteins

Core tip: Cholangiocarcinoma (CCA) is a relatively rare malignancy of the intra- or extra-hepatic bile ducts with dismal prognosis. CCA frequently arises on a background of chronic liver inflammation and cholestasis, which creates a microenvironment rich in pro-inflammatory mediators and proliferative factors that enable accumulation of mutations, transformation and expansion of mutated cells. A recent elaborate study by Boulter *et al* (*J Clin Invest* 125:1269) has provided novel insights into the molecular pathogenesis of CCA. Involvement of the Wnt signaling pathway in cholangiocarcinogenesis, and effect of Wnt inhibitors on CCA development *in vivo* are discussed in this Editorial.

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INTRODUCTION

Cholangiocarcinoma (CCA) is a relatively rare malignancy of the intra- or extrahepatic bile ducts that is classified according to its anatomical localization as intrahepatic, perihilar or distal[1,2]. CCA accounts for 10%-20% of the primary liver malignancies, with perihilar (50%-67%) and distal extrahepatic tumors (27%-42%) comprising the majority of CCA cases[1]. Tumor biology (*e.g.,* growth pattern, mutation spectrum) and clinical presentation, management and outcomes are different for the three CCA types. Overall, CCA has a dismal prognosis due to typical presentation at an advanced irresectable stage, lack of non-surgical potentially curative treatments, and a high rate of disease recurrence. The five-year survival rate is 5%-10%.

Our understanding of the molecular pathogenesis of CCA is limited. CCA frequently arises on a background of chronic liver inflammation and cholestasis, as reflected by risk factors of cholangiocarcinogenesis (*e.g.,* liver cirrhosis, viral hepatitis, hepatolithiasis, liver fluke infestation, primary sclerosing cholangitis). Chronic inflammation is accompanied by enhanced cell turnover with generation of additional inflammatory stimuli, and a microenvironment rich in pro-inflammatory mediators and proliferative factors that enable accumulation of mutations, transformation and expansion of mutated cells[3,4]. Cholestasis may contribute to cholangiocarcinogenesis through effects of (conjugated) bile salts on proliferation and invasion of cholangiocytes[5,6].

The overall incidence of CCA has increased over the past decades and this is attributed to a global rise in the incidence of intrahepatic CCA. Liver transplantation is generally not considered for treatment of CCA due to frequent tumor recurrence and poor five-year survival rates after liver transplantation for intrahepatic CCA. Hence, resection is the only potentially curative treatment of CCA. The majority of patients with CCA, however, do not qualify for surgery and have to resort to palliative therapies. Molecular-targeted therapies hold potential for personalized treatment of malignancies including CCA[7]. A recent study by Boulter *et al*[8] implicates the Wnt signaling cascade in cholangiocarcinogenesis. Importantly, specific inhibitors of this pathway prevented tumor development in animal models of CCA.

THE WNT SIGNALING CASCADE

Wnt signaling is initiated by binding of membrane-bound Wnt ligand to a transmembrane receptor of the Frizzled family, and can operate in autocrine and paracrine modes[9-11]. Wnt ligands are a family of secreted glycoproteins that have undergone a lipid modification (Cys-palmitoylation) that is essential for biological activity. The Frizzled family are G-protein coupled receptors that, alone or in conjunction with co-receptors (*e.g.*, Lrp5/6), serve as binding sites for Wnt ligands. Canonical Wnt signaling results in a transcriptional response in which the transcription factor β-catenin plays a central role, whereas non-canonical Wnt signaling cascades control the cytoskeletal structure or intracellular Ca^{2+} content through β-catenin-independent non-genomic actions. The canonical Wnt signaling pathway is the focus of the studies of Boulter *et al*[8]. In the absence of Wnt signaling, β-catenin is targeted for proteasomal degradation by a multi-protein complex. Formation of this degradation complex is abrogated by activation of Wnt signaling, resulting in cytoplasmic accumulation and subsequent nuclear translocation of β-catenin. There, β-catenin acts in concert with other transcription factors (*e.g.*, TCF/LEF family members) to activate expression of target genes including cell cycle-related genes (*e.g.*, *CCND2*, *CDKN2A*).

Wnt signaling was identified through its role in carcinogenesis[10], but not surprisingly found to participate in normal development and adult tissue homeostasis as well. Mutations in downstream components of the Wnt signaling pathway have been identified in various types of human cancers[11,12]. For example, adenomatous polyposis coli (APC), a tumor suppressor that is part of the β-catenin degradation complex, is frequently mutated in colorectal and gastric cancers. Mutations in β-catenin that enhance protein stability, exemplifying a gain-of-function mutation, have been found in hepatocellular carcinoma[11]. Hepatic adenomas that are positive for β-catenin have a high risk for malignant conversion and are typically resected, whereas other adenoma types are generally left untreated[13]. Targeting of the Wnt

pathway is being explored as treatment of Wnt-driven malignancies[14].

WNT SIGNALING IN CCA

By analyzing tumoral and matched unaffected liver tissue of patients with intrahepatic or perihilar CCA, Boulter *et al*[8] demonstrate that Wnt ligands Wnt7B and Wnt10A are highly overexpressed in tumor tissue. Wnt7B protein was present throughout the tumor stroma, often co-localizing with a subset of $CD68^+$ macrophages. Moreover, tumors displayed elevated levels of transcripts of known β-catenin targets (*e.g.*, *CCND2*, *CDKN2A, BIRC5),* and cancerous biliary epithelium showed increased immunohistochemical positivity for a number of β-catenin targets. These findings suggest that canonical Wnt signaling is activated in human CCA.

To address in a direct manner whether Wnt signaling is engaged in development of CCA, this pathway was further explored in an experimental model that recapitulates the multi-stage progression (*i.e.,* chronic cholangiocyte damage, inflammation, biliary repair/regeneration, tumorigenesis) of human CCA. For this purpose, rats were treated with thioacetamide (TAA) and sacrificed at pre-cancerous (0-16 wk of treatment) and cancerous (20-26 wk of treatment) stages. Mirroring end-stage CCA in humans, strong nuclear β-catenin staining was observed in cancerous epithelium. In the pre-cancerous stage, regenerating ductules showed a membranous staining pattern. Wnt ligands found to be elevated in human CCA were also upregulated during the course of CCA development following TAA treatment. Wnt10a increased during the (pre-cancerous) regenerative phase, while Wnt7b induction paralleled tumor growth. Along with upregulation of target genes, above findings demonstrate that the canonical Wnt pathway is progressively activated during cholangiocarcinogenesis.

Through an elegant set of experiments Boulter *et al*[8] demonstrate that Wnt7B in tumor stroma is largely derived from recruited bone marrow-derived macrophages rather than from resident Kupffer cells, and that these cells play a key role in CCA progression. The role of macrophages in CCA growth was initially assessed in mice xenografted with three different human CCA cell lines. Groups of mice with established palpable subcutaneous tumors received vehicle or treatments to deplete phagocytic macrophages or prevent differentiation of monocytes into macrophages. Xenograft characteristics were determined after a further growth period of 3 wk. Loss of macrophages by either of the two strategies, resulted in reduced number of $CD68^+$ macrophages and decreased Wnt7b expression in all xenografts. In two out of three xenografted CCA cell lines (*i.e.,* CC-LP-1 and SNU-1079, derived from intrahepatic CCA) this was accompanied by decreased expression of (human) proliferative genes, increased apoptosis and lowered tumor burden. The lack of functional consequences, despite loss of Wnt signal, in xenografts derived from the third cell line (*i.e.,* WITT-1) may relate to its different origin (distal extrahepatic CCA) and/or distinct growth requirements. As a model more representative in terms of tumor microenvironment (stroma) and disease progression, Boulter *et al*[8] then studied the consequences of macrophage depletion (liposomal clodronate) in TAA-induced CCA. Strikingly, macrophage ablation prevented activation of the canonical Wnt cascade (loss of tumoral Wnt7B signal) and resulted in reduced number and volume of tumors.

The aggregated findings show that experimental, and presumably human CCA, is a Wnt-driven tumor. Since general macrophage depletion is not feasible in clinical practice, Boulter *et al*[8] explored the impact of specific inhibitors of the canonical Wnt pathway in the xenograft- and TAA-model of CCA. For this, they chose two targets that were elevated in human CCA, namely *CTBP1* and *PORCN*. CTBP1 interacts with β-catenin to drive expression of growth-stimulating Wnt target genes, and their interaction can be targeted by ICG-001[15]. As mentioned above, Wnt ligands require palmitoylation for biological activity and this lipid modification can be prevented by the PORCN inhibitor C-59[16,17]. Both ICG-001 and C-59 were effective in reducing *in vitro* growth of five human CCA cell lines with presumed autocrine activation of canonical Wnt signaling (constitutive Wnt7B and β-catenin expression). Similar to macrophage ablation, ICG-001 and C-59 reduced tumor volume and mass in two CCA cell lines of intrahepatic origin when xenografted in mice, but did not affect WITT-1 xenograft growth. The reliance on Wnt signaling for proliferation and survival of CCA cells was confirmed in TAA-induced CCA, with ICG-001 and C-59 causing reduction of tumor area and number. Importantly, neither treatment affected body weight or caused liver test abnormalities, side effects observed with use of earlier generation Wnt inhibitors[14].

PERSPECTIVE

The work of Boulter *et al*[8] demonstrates that the canonical Wnt pathway is activated in intrahepatic and perihilar CCA. Inhibition of canonical Wnt signaling, either by depleting the macrophage source of Wnt ligand or *via* pharmacological blockage, reduces CCA formation in a rat model that closely resembles human CCA. This is achieved through stimulation of apoptosis and reduced cell cycle entry. Thus, Wnt signaling is important for proliferation and survival of CCA cells in the TAA-model. It remains to be determined whether human CCA growth/progression is Wnt-dependent, and hence amenable to targeting by Wnt pathway inhibitors. More detailed insight into the interaction of Wnt signaling with the complex cellular surrounding, and its integration with other cellular signaling cascades, is warranted. Time will tell if systemic or CCA-directed Wnt inhibition, alone or in combination with surgical or chemotherapy approaches, will improve clinical outcomes of this fatal malignancy.

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ORIGINAL ARTICLE

Retrospective Study

Role of epidural anesthesia in a fast track liver resection protocol for cirrhotic patients - results after three years of practice

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Abstract

AIM

To evaluate the potential benefits and risks of the use of epidural anaesthesia within an enhanced recovery protocol in this specific subpopulation.

METHODS

A retrospective review was conducted, including all cirrhotic patients who underwent open liver resection between January 2013 and December 2015 at Bologna University Hospital. Patients with an abnormal coagulation profile contraindicating the placement of an epidural catheter were excluded from the analysis. The control group was composed by patients refusing epidural anaesthesia.

RESULTS

Of the 183 cirrhotic patients undergoing open liver resections, 57 had contraindications to the placement of an epidural catheter; of the remaining 126, 86 patients received general anaesthesia and 40 combined anaesthesia. The two groups presented homogeneous characteristics. Intraoperatively the metabolic data did not differ between the two groups, whilst the epidural group had a lower mean arterial pressure (P = 0.041) and received more colloid infusions (P = 0.007). Postoperative liver and kidney function did not differ significantly.

Length of mechanical ventilation (P = 0.003) and hospital stay (P = 0.032) were significantly lower in the epidural group. No complications related to the epidural catheter placement or removal was recorded.

CONCLUSION

The use of Epidural Anaesthesia within a fast track protocol for cirrhotic patients undergoing liver resections had a positive impact on the patient's outcomes and comfort as demonstrated by a significantly lower length of mechanical ventilation and hospital stay in the epidural group. The technique appears to be safely manageable in this fragile population even though these results need confirmation in larger studies.

Key words: Anesthesia; Postoperative care; Analgesia; Epidural; Postoperative; Liver cirrhosis; Liver function tests; Complication

Core tip: This retrospective study evaluates the potential benefits and risks of the use of epidural anaesthesia within an enhanced recovery protocol in the subpopulation of cirrhotic patients undergoing liver resection. We included all cirrhotic patients who underwent open liver resection between January 2013 and December 2015 at our Unit. The study included 126 cirrhotic patients, 86 patients received general anaesthesia and 40 combined anaesthesia. The two groups presented homogeneous characteristics. The epidural group had a lower intraoperative mean arterial pressure (P = 0.041) and received more colloid infusions (P = 0.007). Postoperative liver and kidney function did not differ significantly. Length of mechanical ventilation (P = 0.003) and hospital stay were significantly lower (P = 0.032) in the epidural group. No complications related to the epidural catheter management were recorded.

Siniscalchi A, Gamberini L, Bardi T, Laici C, Gamberini E, Francorsi L, Faenza S. Role of epidural anesthesia in a fast track liver resection protocol for cirrhotic patients - results after three years of practice. *World J Hepatol* 2016; 8(26): 1097-1104 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i26/1097.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i26.1097

INTRODUCTION

Fast track surgery or Enhanced Recovery after Surgery (ERAS®) programmes have been first described in the 1990s in the field of colo-rectal surgery[1]. These programmes entail a number of evidence based actions aimed at reducing unnecessary perioperative stress and inflammation, and restoring as quickly as possible the normal preoperative physiology. Since their first introduction ERAS programmes are being implemented in different surgical specialties, and in more recent times also in the field of liver surgery[2-6]. A recent meta-analysis[7] evaluating five randomizedcontrolledtrials, has consolidated the evidence indicating that ERAS applied to liver resection surgery has a positive impact on post-operative complications and length of hospital stay.

The use of epidural anesthesia and analgesia is a vital part of any enhanced recovery program, mostly because it blunts the neuroendocrine response to surgical stress and allows better postoperative pain control and faster mobilization. Epidural analgesia has been widely applied in the field of open liver surgery with very positive results in terms of reduction in pain scores[8]. However cirrhotic patients undergoing liver resection represent a special subpopulation with a high risk of developing perioperative complications. In these patients the preoperative liver function, and the future remnant liver volume, are critical factors in determining perioperative morbidity[9] and the placement of an epidural catheter, and its management, could present potential risks, most of which related to coagulation disorders[10].

Another aspect to be taken into consideration is hemodynamics, in fact the cirrhotic hyperdynamic circulation could be particularly influenced by the sympathetic blockade produced by an epidural block, potentially leading to splanchnic malperfusion, which could be reflected in postoperative organ dysfunction.

In a previous study[11] we evaluated the incidence of post liver resection coagulopathy in cirrhotic patients, and discussed its hypothetic impact on the management of an epidural catheter (Figure 1). Following the results of this study, we have implemented a wider use of Epidural analgesia and anaesthesia also in a selected population of cirrhotic patients undergoing Liver resections. To date there are no studies considering the application of ERAS protocols to cirrhotic patients and the importance of epidural anesthesia within these protocols. Moreover most of the studies considering ERAS protocols applied to liver surgery populations have included patients undergoing liver resections for colorectal metastasis[3,5,6,12], in whom underlying liver function is expected to be normal.

The primary objective of this retrospective observational study was to evaluate the use of epidural analgesia in an ERAS program dedicated to cirrhotic patients undergoing liver resection for hepatocellular carcinoma (HCC) in terms of length of hospital stay, and incidence of complications.

Secondary objectives of the study were to evaluate the differences in terms of intraoperative hemodynamic stability, fluid management and postoperative liver and kidney function tests.

MATERIALS AND METHODS

Following the approval of our Hospital Ethics Committee (approval number: 100/2014/O/OssN), we conducted a retrospective observational review including all cirrhotic patients who underwent open liver resection between January 2013 and December 2015. Inclusion criteria

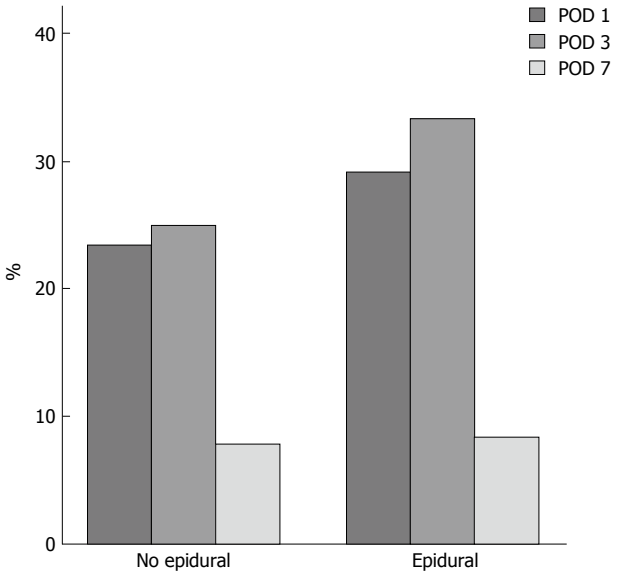

Figure 1 Course of post-operative coagulopathy. The figure displays the percentage of the patients in the two study groups presenting significant alterations of coagulation exams. Platelets count < 100000/μL or INR > 1.5 post operatively at day 1, 3, 7. INR: International normalized ratio; POD: Postoperative days.

were: Age > 18 years, histologically proven liver cirrhosis, open liver resection surgery for HCC. Exclusion criteria were: Abnormal preoperative coagulation profile contraindicating an epidural catheter placement [international normalized ratio (INR) values ⩾ 1.5 and/or platelet count < 100.000/μL[13]], laparoscopic liver resection.

Major hepatic resection was defined as a resection of three or more hepatic segments, whilst a minor hepatic resection was defined as a resection of two or fewer hepatic segments in accordance to the IHPBA classification[14]. All the liver resections were performed to achieve a tumor-free margin of at least 1 cm based on intraoperative examination and ultrasonography.

Patients were divided into two groups on the basis of the placement of an epidural catheter. The control group was composed by the patients who refused the placement of an epidural catheter, at the pre operative interview with the anesthetist. The same team of surgeons performed all of the surgical procedures. The ERAS protocol was applied to each patient included in this study. The main features of the ERAS protocol for cirrhotic patients used at our unit are described in Table 1. The anesthetic management for liver resection at our unit includes: General endotracheal anaesthesia, arterial line and central venous catheter placement for fluid infusions, hemodynamic monitoring (EKG, arterial blood pressure, CVP), and acid-base parameter measurement (blood gas analysis data).

General anesthesia is induced with propofol (2-2.5 mg/kg), fentanyl (1-2 mcg/kg) and rocuronium (0.6 mg/kg), while Sevoflurane 0.7-1.0 MAC and boluses of rocuronium and fentanyl are used for maintenance.

For combined anesthesia a T8-T9 epidural catheter is positioned before anesthesia induction. Anesthesia is induced with propofol, fentanyl and rocuronium, at the same dosages mentioned above, the epidural anesthesia is induced with an initial bolus of L-bupivacaine 7.5-10 mg and 10 mcg sufentanil, followed by a continuous infusion at 5 to 7 mL/h of L-bupivacaine 2.5 mg/mL. Narcosis is maintained with Sevoflurane at a concentration of 0.5-0.7 MAC, adequate muscle paralysis is maintained with boluses of rocuronium.

Table 1 Fast track protocol for cirrhotic patients undergoing liver resection

Fast track protocol for cirrhotic patients undergoing liver resection
Preoperative counseling
Regular diet on the day before surgery
No bowel preparation
Intraoperative CVP target < 6 mmHg, restricted fluids administration
ICU admission for at least the first post-operative night (or on POD 0)
Maintenance fluids discontinued on POD 3
Nasogastric probe removal on POD 1
Liquid diet on POD 1
Regular diet on POD 3
Urinary catheter discontinued on POD 3
Drains removal on POD 3
Ambulation on POD 3
Discharge criteria: Liver and kidney function tests compatible with preoperative data or decreasing, able to tolerate food intake, able to ambulate, good pain control (NRS < 3)

ICU: Intensive care unit; CVP: Central venous pressure; POD: Postoperative days; NRS: Numerical rating scale.

Postoperative pain control in patients without epidural is maintained with a PCA system with intravenous morphine (1-2 mg/h continuous infusion, bolus 1 mg, lockout 15 min, maximum dose in 4 h 18 mg) and boluses of paracetamol (1 g intravenous, max 3 g per day), when oral intake is possible, morphine is substituted with oxycodone.

Postoperative pain control in patients without epidural is maintained with a PCA system with intravenous morphine (1-2 mg/h, bolus 1 mg, lockout 15 s, maximum dose in 4 h 18 mg) and boluses of paracetamol (1 g intravenous, max 3 g/d), when oral intake is possible, morphine is substituted with oxycodone.

In patients with the epidural catheter, postoperative analgesia is maintained with a continuous epidural infusion of L-bupivacaine 1.25 mg/mL and sufentanil 0.5 mcg/mL at a rate of 5-7 mL/h. After the first 36 hour post operatively only the local anesthetic infusion was maintained and the opioid stopped. Intravenous paracetamol (1 g iv, max 3 g/d) is added if more analgesia is needed.

Fluid infusions during hepatic dissection follow the units protocol and target a low central venous pressure (⩽ 6 mmHg). Red blood cells in cirrhotic patients are transfused when hematocrit is lower than 24% and/or hemoglobin is lower than 8 g/dL. The occurrence of hypothermia was prevented by infusion of warm fluids, forced-air warming and the use of warm water on the surgical field.

Table 2 Preoperative data

	Group no epidural (n = 86)	Group epidural (n = 40)	P
Sex male (%)	69 (80.2%)	30 (75%)	0.655
Age (yr)	63.28 ± 11.38	62.8 ± 11.92	0.832
BMI	26.65 ± 4.36	25.23 ± 5.50	0.155
Cirrhosis etiology			
HBV	19	10	0.947
HCV	49	27	0.434
Alcohol	9	2	0.501
Other	13	3	0.341
Type of resection			
Major	19	12	0.461
Minor	67	28	
Preoperative data			
AST (UI/L)	50.8 ± 47.4	46.9 ± 29.1	0.586
ALT(UI/L)	47.4 ± 43.1	52.3 ± 38.5	0.541
Bilirubin (mg/dL)	0.80 ± 0.43	0.77 ± 0.40	0.710
INR	1.14 ± 0.12	1.10 ± 0.79	0.092
Creatinine (mg/dL)	1.08 ± 1.24	0.87 ± 0.26	0.164
Urea (mg/dL)	37.25 ± 13.74	36.84 ± 11.79	0.867
Platelet count	180134 ± 83856	211079 ± 94262	0.088

HBV: Hepatitis B virus; BMI: Body mass index; HCV: Hepatitis C virus; AST: Aspertate aminotransferase; ALT: Alanine transaminase; INR: International normalized ratio.

Perioperative coagulation alterations are corrected according to POC coagulation testing using a thromboelastograph (TEG®).

All patients at end of surgery were admitted to the intensive care unit (ICU). The routine ICU admission for at least the first post-operative night is a part of the ERAS protocol for cirrhotic patients used at our unit (Table 1).

Data collected preoperatively included patient characteristics, underlying surgical pathology, etiology of cirrhosis, MELD score, baseline coagulation profile and blood tests.

Intraoperative data analyzed included type of hepatic resection, fluid infusions and transfusion of blood products, while hemodynamics and blood gas analysis data were registered at the beginning of the intervention, after resection and at the end of surgery.

Postoperative blood tests collected were liver and kidney function tests on postoperative days (POD) 1, 3 and 7. Postoperative complications were also evaluated using Clavien-Dindo classification, acute kidney injury was classified following AKI network criteria.

Statistical analysis

Statistical analysis was carried out with IBM SPSS 21. Categorical data were expressed as numbers (percentages), continuous variables as mean and standard deviation. Differences in perioperative data between groups were evaluated with t-test for continuous variables and χ^2 test or Fisher exact test for nominal variables. A general linear model for repeated measures was used to compare postoperative function tests and intraoperative measures of arterial pressure, central venous pressure and blood gas analysis data. For Clavien-Dindo classification and postoperative kidney injury evaluated with AKIN score, Mann-Whitney test was used.

RESULTS

From January 2013 to December 2015, 183 cirrhotic patients underwent elective open hepatic resection for hepatocellular carcinoma at the Department of Surgery and Transplantation of Bologna University. Fifty-six of these were excluded because their preoperative coagulation profile was incompatible with the placement of an epidural catheter. The remaining 126 patients were included in the study and divided into two groups on the basis of the presence of an epidural catheter during surgery; 86 patients received a general endotracheal anesthesia (group no epidural) while 40 patients received a combined anesthesia (group epidural). All of the patients who received epidural anaesthesia, could effectively control post-operative pain with the epidural protocol and did not require intravenous opioids, also no catheter displacement occurred. The two groups were homogeneous for the demographic aspects, and etiology of cirrhosis, Table 2 shows preoperative data. Intraoperative data showed a significantly lower mean arterial pressure during resection and higher hypotension time and colloids infusions in the epidural group (Table 3), whilst central venous pressure (CVP) and metabolic data in terms of pH, lactate and base excess were not significantly different. Postoperative liver and kidney function tests, as well as platelet count did not significantly differ between the two groups (Table 4).

The course of postoperative coagulopathy is shown in Graph 1, we have to highlight that on POD 7, 6 patients out of 126 still had a measurable coagulopathy (INR > 1.5 and/or Plt < 100000/μL). Amongst these patients 3 had undergone a minor resection and one a major resection under general anaesthesia. The remaining 2 patients with coagulopathy had undergone a major liver resection with a combined anesthesia and had to have their coagulations profiles corrected before a safe removal of the epidural catheter could be performed. The correction was performed with the infusion of FFP and there were no complications after the removal of the catheter.

The length of ICU stay did not significantly differ between the two groups. The duration of mechanical ventilation and length of hospital stay were significantly lower in the epidural group (Table 5).

The rate of complications and their severity classified following Clavien-Dindo score and postoperative acute kidney injury did not differ, however 9 cases of postoperative delirium were recorded, all of which occurred in the general anesthesia group.

In the epidural group no complications related to epidural catheter placement or removal were recorded. Epidural catheters were usually removed between POD 3 and 5 and there was no need for major analgesics adjuncts in these patients.

Table 3 Intraoperative data

		Group no epidural (n = 86)	Group epidural (n = 40)	P
Hemodynamic parameters				
MAP	Baseline	94.4 ± 12	89.5 ± 11.8	0.035
(mmHg)	Post-resection	77.3 ± 16.9	71.2 ± 9.7	0.041
(P = 0.004)	End of surgery	74.9 ± 10.7	74.9 ± 10.7	0.048
CVP	Baseline	8.26 ± 3.4	8.92 ± 3.15	0.323
(mmHg)	Post-resection	6.0 ± 3.25	5.76 ± 3.13	0.704
(P = 0.991)	End of surgery	7.35 ± 3.13	6.92 ± 2.57	0.466
Metabolic parameters				
pH	Baseline	7.44 ± 0.043	7.44 ± 0.055	0.717
(P = 0.627)	Post-resection	7.40 ± 0.053	7.39 ± 0.053	0.608
	End of surgery	7.38 ± 0.573	7.39 ± 0.062	0.258
Lac (mmol/L)	Baseline	2.07 ± 3.15	1.98 ± 3.11	0.925
(P = 0.894)	Post-resection	4.22 ± 6.11	4.68 ± 7.40	0.800
	End of surgery	2.59 ± 2.20	2.62 ± 1.88	0.958
BE (mEq/L)	Baseline	1.27 ± 2.10	1.6 ± 1.94	0.563
(P = 0.343)	Post-resection	-1.93 ± 2.37	-1.31 ± 2.61	0.354
	End of surgery	-2.72 ± 2.88	-2.56 ± 2.92	0.499
Other data				
Lenght of surgery (min)		250.4 ± 93.48	267.6 ± 88.97	0.326
Hypotension duration (min)		2.28 ± 4.52	5.43 ± 6.68	0.006
Cristalloids infusions (mL)		2768 ± 1213	2574 ± 1022	0.354
Colloids infusions (mL)		259 ± 320	428 ± 312	0.007
RBC transfusions (U)		0.06 ± 0.239	0.01 ± 0.304	0.470
Total diuresis (mL)		467 ± 376	552 ± 384	0.248

MAP: Mean arterial pressure; RBC: Red blood count; Lac: Lactate; BE: Base excess; CVP: Central venous pressure.

Table 4 Post-operative data

		Group no epidural (n = 86)	Group epidural (n = 40)	P
Hepatic function tests				
AST (UI/L)	POD 1	205 ± 141	238 ± 168	0.239
(P = 0.451)	POD 3	97 ± 66	96 ± 54	0.334
	POD 7	49 ± 29	52 ± 26	0.636
ALT (UI/L)	POD 1	195 ± 161	229 ± 210	0.144
(P = 0.605)	POD 3	157 ± 126	157 ± 106	0.391
	POD 7	67 ± 47	73 ± 40	0.884
Bilirubin (mg/dL)	POD 1	1.60 ± 1.0	1.60 ± 0.88	0.994
(P = 0.557)	POD 3	1.8 ± 1.05	1.57 ± 0.81	0.306
	POD 7	1.38 ± 1.06	1.26 ± 1.31	0.636
INR	POD 1	1.34 ± 0.18	1.31 ± 0.20	0.593
(P = 0.544)	POD 3	1.31 ± 0.16	1.30 ± 0.25	0.899
	POD 7	1.26 ± 0.14	1.12 ± 0.15	0.319
Platelet count	POD 1	163649 ± 78332	148015 ± 72007	0.647
(P = 0.532)	POD 3	148015 ± 72007	132275 ± 43514	0.277
	POD 7	191073 ± 74978	187586 ± 63602	0.827
Kidney function tests				
Creatinine (mg/dL)	POD 1	0.96 ± 0.87	0.87 ± 0.42	0.579
(P = 0.417)	POD 3	0.98 ± 1.04	0.79 ± 0.29	0.331
	POD 7	0.96 ± 1.15	0.78 ± 0.28	0.410
Urea (mg/dL)	POD 1	33.37 ± 13.87	32.86 ± 12.17	0.866
(P = 0.315)	POD 3	38.0 ± 21.17	33.79 ± 14.27	0.332
	POD 7	35.57 ± 20.13	29.76 ± 11.67	0.151

For repeated measures, the P value expressed under the variable is referred to the between subjects effect test. AST: Aspertate aminotransferase; ALT: Alanine transaminase; INR: International normalized ratio; POD: Postoperative days.

DISCUSSION

The results of this study suggest that the use of epidural anaesthesia and analgesia in the context of ERAS® protocols for cirrhotic patients undergoing liver surgery is feasible. In fact none of the patients in the epidural group had complications related to the positioning or the removal of the epidural catheter. However, the incidence of an epidural complication requiring an elective surgical treatment varies between 1 event in 22189 and 1 event in 4330 epidural placements in the general population[15]. Hence to consistently rule out the potential

Table 5 Hospital length of stay and complications

	Group no epidural (n = 86)	Group epidural (n = 40)	P
Post operative MV lenght (h)	7.34 ± 18.11	1.29 ± 1.74	0.003
ICU stay (d)	2.78 ± 2.35	2.43 ± 1.57	0.183
Total PO hospital stay (d)	11.49 ± 7.95	8.65 ± 3.26	0.032
AKIN (grade)			
0	81	40	0.121
1	3	0	
2	1	0	
3	1	0	
DINDO (grade)			
1	30	15	0.262
2	19	9	
3	10	1	
4	0	0	
5	0	0	

MV: Mechanical ventilation; PO: Postoperative; AKIN: Acute kidney injury network classification; DINDO: Clavien dindo classification of surgical complications.

safety issues relating to epidural catheters in the specific subpopulation of cirrhotic patients, a larger sample should be considered.

Postoperative coagulopathy is considered another great risk in cirrhotic patients, often limiting the use of regional anesthesia techniques in this subpopulation. However the incidence of the postoperative coagulopathy, especially in minor resections, appears to be compatible with the safe management of an epidural catheter.

It also must be underlined that hemostasis alterations in cirrhotic patients are more complex than a simple increase in hemorrhagic risk due to coagulation factors deficiency[16]. Hence laboratory values such as the INR and platelet count do not describe entirely the wide array of alterations, which constitute the hemorrhagic risk of these patients. Probably in the near future thromboelastometry will have a major role in better defining the individual coagulation profile. Moreover, neuraxial blocks are safely undertaken even in patients assuming platelets inhibitors such as ASA and undergoing surgical interventions in which systemic anticoagulation is prescribed in the postoperative period, such as peripheral vascular surgery[17].

Combined anesthesia had significant intraoperative hemodynamic effects in terms of lower mean arterial pressure and longer hypotension duration, which required more colloid infusions but had no metabolic effects on base excess and lactate concentration, even CVP was not significantly affected by the sympathetic blockade.

Postoperative data showed slightly higher AST and ALT values in the epidural group, however it must be noted that, in this group, major resections were more frequent than minor resections, hence these data are difficult to interpret. Finally these differences in postoperative transaminase levels did not have any clinical impact, as no cases of postoperative liver failure were observed, and the postoperative courses of INR bilirubin and kidney functions were substantially comparable between the two groups.

A recent large retrospective study by Kambakamba *et al*[18] postulated that epidural anesthesia could have a role in jeopardizing postoperative kidney function in major, but not in minor liver resections. The difference in our results could be explained primarily by the fact that cirrhotic patients were excluded from the analysis in the Kambakamba study; also our sample is much smaller in size and we did not register a use of vasoactive drugs to correct intraoperative hypotension as extensive as the one in their study group. Postoperative complications were not significantly different between the two groups, however it is interesting to note that in the group without epidural anesthesia we observed 9 cases of postoperative delirium, while none was observed in the group receiving epidural anesthesia.

Also respiratory complications were observed only in patients treated with general anesthesia and postoperative systemic opiates. Patients receiving epidural analgesia in 50% of the cases were extubated at the end of surgery in the operating theatre, and in general required fewer hours of mechanical ventilation. These results indicate a beneficial role of epidural anesthesia with regard to the respiratory system function and its possible postoperative complications.

The shorter postoperative hospital length of stay observed in the epidural group could be related to a better analgesia, faster ambulation and a better postoperative intestinal function. We registered a longer mean hospital length of stay than the one enounced in other studies; the composition of our study population considering only cirrhotic patients has contributed in altering our results in this sense.

Another important aspect to underline is the large number of patients which were considered not eligible for neuraxial analgesia (57 out of 183 patients), in which other analgesic techniques to reduce postoperative opiates use, such as continuous wound infusion of local anesthetics[19], intercostal nerve blocks[20], intrathecal morphine administration[21] and TAP block[22] could find an indication. In a recent review by Hughes *et al*[23] these techniques appear to be in some cases even superior to epidurals in terms of reduction of postoperative complications, despite providing less relief from pain. Another recent RCT from Hughes *et al*[24] has compared epidural anaesthesia and analgesia with a combination of TAP and rectus sheath block with continuous wound infiltration, confirming the superiority of this alternative technique to TEA in terms of post operative complications and recovery and also achieving comparable pain scores. These results are particularly promising especially because to our knowledge this is the first trial achieving comparable pain scores with a technique alternative to TEA, and need to be confirmed by larger multicenter trials. Finally it is our belief that, based on the most solid evidence available at the moment, the use of TEA still represents the technique providing the most comfort to the patient whilst accelerating post operative

recovery compared to standard general anesthesia and opiate analgesia; alternative analgesic techniques find their correct indication in those patients not eligible for an epidural catheter positioning making a complete avoidance of systemic opiates in this population achievable.

The main limitations of the present study lay in its retrospective design, and the limited numerosity of the sample, which originated from a single center.

In conclusion, the main results of this study show that the known benefits of thoracic epidural anaesthesia and analgesia within an ERAS protocol for perioperative management, seem to be reproducible in a subpopulation including only cirrhotic patients undergoing open liver surgery. Epidural anaesthesia plays a major role in accomplishing many of these benefits, and its systematic use has important effects on patient outcomes and comfort. Our results also show that, in a selected population of cirrhotic patients, the technique can be performed safely without complications even if this aspect needs to be confirmed in larger populations.

COMMENTS

Background

Enhanced recovery after surgery is a solid reality in most surgical specialties and has been successfully applied to liver surgery. The subpopulation of cirrhotic patients undergoing liver resections has been poorly studied and represents a challenge for the application of such protocols. Moreover the use of epidural anaesthesia and analgesia in this subpopulation is still a matter of debate.

Research frontiers

Defining the possible benefits of using epidural anaesthesia within an Enhanced Recovery after Surgery (ERAS) protocol for cirrhotic patients undergoing liver resection surgery is of great relevance in order to further implement the use of such protocols.

Innovations and breakthroughs

This is the first retrospective study showing improved post operative outcomes using an ERAS protocol and epidural anaesthesia in a population including only cirrhotic patients undergoing liver resection surgery.

Applications

These data suggest that the implementation of an ERAS protocol for cirrhotic patients using epidural anaesthesia is feasible, safe and provides positive clinical outcomes. This could be of great value in spreading the implementation of ERAS protocols to this particular subpopulation of patients.

Peer-review

The manuscript describes the findings of a retrospective review to determine if there are benefits with the use of ERAS and epidural during liver resection surgery. The study is reasonably large and could provide useful information to the readers.

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ORIGINAL ARTICLE

Prospective Study

Immune response to hepatitis B virus vaccine in celiac subjects at diagnosis

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Abstract

AIM

To evaluate hepatitis B virus (HBV) vaccine response and correlation with human leukocyte antigens (HLA) and/or gluten intake in celiac patients at diagnosis.

METHODS

Fifty-one patients affected by celiac disease, diagnosed at the Department of Pediatrics of the University of Catania (Italy), were recruited. All patients were tested at admission for immunization against HBV, according to findings from analysis of quantitative HBV surface antibody (anti-HBs). The anti-HBs titer was measured by enzyme-linked immunosorbent assay. Following the international standards, subjects with antibody titer < 10 IU/L were defined as non-responders. The prevalence of responders and non-responders among celiac subjects and the distribution of immunization for age were examined. In addition, the prevalence of responders and non-responders was assessed for correlation to HLA and clinical features at diagnosis of celiac disease.

RESULTS

The entire study population was divided into three groups according to age: 24 patients aged between 0

to 5.5 years (48.9%, group A); 16 aged between 5.5 and 9.5 years (30.61%, group B); 9 aged between 9.5 and 17 years (18.75%, group C). Comparison of the percentage of responders and non-responders between the youngest and the oldest age group showed no significant difference between the two groups (P > 0.05). With regard to the HLA haplotype, comparison of the distribution of vaccination response showed no statistically significant difference between the different genotypes (homozygosity for the HLADQ2 haplotype compared with HLADQ2/DQ8 heterozygosity or other haplotypes; P > 0.05). Moreover, distribution of the responders according to clinical features of celiac disease showed no statistically significant differences (P > 0.05).

CONCLUSION

This prospective study confirmed the lower percentage of response to HBV vaccine in celiac subjects. However, the underlying mechanism remains unclear and further studies are needed.

Key words: Celiac disease; Hepatitis B virus vaccination; Human leukocyte antigens; Gluten; Poor response

Core tip: Correlation between celiac disease and lower response to hepatitis B virus (HBV) vaccine has been demonstrated, but the causes remain unclear. The lack of prospective data represents an extensive gap between the time of vaccination and development of the immune response, contributing to select "false non-responders" (*i.e.*, those who are destined to lose the antibody titer over time). The originality of our prospective study is that of analyzing the response to HBV vaccine in a group of celiac patients at the time of diagnosis in an attempt to nullify the percentage of error related to confounding factors.

Filippelli M, Garozzo MT, Capizzi A, Spina M, Manti S, Tardino L, Salpietro C, Leonardi S. Immune response to hepatitis B virus vaccine in celiac subjects at diagnosis. *World J Hepatol* 2016; 8(26): 1105-1109 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i26/1105.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i26.1105

INTRODUCTION

Celiac disease (CD) is a permanent immune-mediated enteropathy, triggered by gluten in genetically predisposed individuals. The genetic predisposition consists of the presence of alleles encoding for the molecules DQ2 or DQ8 of the human leucocyte antigen (HLA)[1]. A significant correlation between CD and a lower response to the hepatitis B virus (HBV) vaccine was demonstrated several years ago, but the causes of this phenomenon remain unclear. Many authors have postulated the role of HLA molecules (DQ2 and DQ8) in affecting an impaired immune response to HBV vaccine in CD[2]. On the other hand, it has been theorized that gluten intake could represent the main factor involved, because according to some studies the percentage of responders among celiac patients who are compliant with a gluten-free diet (GFD) is similar to that among healthy subjects[3,4].

Despite the many hypotheses, the debate on poor response to hepatitis B vaccination in CD remains largely open. It could be hypothesized that many confounding factors in some of the previous studies have contributed to maintaining this uncertainty. First of all, the lack of prospective data determines a more extensive gap between time of vaccination and development of the immune response, contributing to select "false non-responders" (*i.e.*, those who are destined to lose the antibody titer over time)[5]. Moreover, it could be easier to evaluate the effective role of HLA in influencing HBV vaccine response when CD has just been diagnosed and no other factors have yet intervened.

For all these reasons, the aim of our prospective study was to eliminate or reduce such confounding factors and to evaluate hepatitis B vaccination response in celiac patients at diagnosis of the disease and its possible correlation with HLA and/or gluten intake.

MATERIALS AND METHODS

In this prospective study we recruited 51 patients affected by CD, diagnosed at the Department of Pediatrics of the University of Catania (Italy). The diagnosis of CD was made according to the European Society for Paediatric Gastroenterology, Hepatology and Nutrition criteria updated in 2012[6]. The total serum IgA levels were measured in all patients in order to exclude the presence of a selective deficit of IgA. Inclusion criteria required that subjects must have completed obligatory vaccinations, including the HBV vaccine. All patients where tested at admission for immunization against HBV, according to finding from quantitative analysis of the HBV surface antibody (anti-HBs). The anti-HBs titer was measured by enzyme-linked immunosorbent assay. Following the international standards, subjects with antibody titer < 10 IU/L were defined as non-responders[7].

Two of the 51 celiac patients were excluded because of insufficiency of their serum samples for analysis of the anti-HBs titer.

We examined the prevalence of responders and non-responders among celiac subjects and the distribution of immunization for age. For this, all patients were divided into three groups on the basis of their age at diagnosis: Group A children were aged between 1.5 and 5.5 years; group B children were aged between 5.5 and 9.5 years; group C children were aged between 9.5 and 17 years.

Moreover, we divided all 49 patients on the basis of clinical features at diagnosis of CD and distinguished them in the following three groups: Group 1 patients had typical form (onset with diarrhea, abdominal pain, cramping or distension, dyspepsia, vomiting or failure to

Table 1 Serologic and histologic findings of the duodenal biopsies for celiac disease diagnosis

	TTG IgA (μA/mL)			Marsh score	
	70-200	201-300	> 300	3C-B2	3B-B1
Patients, *n*	20	10	19	26	23

thrive); group 2 patients had atypical form (onset with other symptoms such as deficiency iron-anemia, chronic fatigue, behavior change, dermatitis and joint pain); group 3 patients had silent form (asymptomatic onset). The prevalence of responders and non-responders was assessed for correlation to HLA and the clinical features at diagnosis of CD (typical or atypical onset).

At the end, we compared the results obtained by the present observational study with the results of a retrospective study previously conducted in our Department of Pediatrics.

Statistical analysis

The statistical analysis of data was performed with the use of SPSS version 21.0 software (SPSS Inc. Chicago, IL, United States). The results for quantitative variables were expressed as mean ± SD, and those of qualitative variables were expressed as frequencies and percentages. Differences between groups were compared using the Mann-Whitney *U* test for two independent samples. The Fisher's exact test was used to compare frequencies. For all analyses, statistical significance was defined as $P < 0.05$.

RESULTS

Data for the serologic and histologic findings of duodenal biopsies (according to Marsh classification) used for the diagnosis of CD are summarized in Table 1, while characteristics of the 49 patients included in the study (sex, age, percentage of responders, HLA haplotype) are summarized in Table 2.

When we divided the entire study population into the three age groups, we found 24 patients were aged between 0 to 5.5 years (48.9%, group A), 16 were aged between 5.5 and 9.5 years (30.61%, group B) and 9 were aged between 9.5 and 17 years (18.75%, group C). The responders were distributed into the three age groups as follows: 19 (38.77%) in group A; 11 (22.44%) in group B; 4 (8.16%) in group C. Comparing the percentage of responders and non-responders between the youngest and the oldest group, no significant difference was found ($P > 0.05$).

With regard to the HLA haplotype, comparison of the distribution of vaccination response showed no statistically significant difference between the different genotypes (Table 2). Moreover, the distribution of responders according to clinical features of CD was as follows: 20 out of 26 patients in group 1; 11 out of 17 in group 2; 3 out of 6 in group 3. The typical form showed significant association with the presence of HLADQ2 ($P < 0.05$). Comparison of the immunological vaccine response between the three groups showed no statistically significant differences related to the clinical features (Table 2).

Table 2 Patient characteristics and distribution of human leukocyte antigens and clinical features

	Responders	Non-responders	*P* value
HBV vaccination	34 (69.4%)	15 (30.6%)	
Female sex	22 (66.7%)	11 (33.3%)	
Male sex	12 (75%)	4 (25%)	
Median age	5.55 (± 3.25 SDS)	8.04 (± 4.3 SDS)	> 0.05
HLA			
DQ2/DQ2	8	4	
DQ2/DQ8	6	1	
Other HLA[1]	19	10	
Distribution according HLA			> 0.05
Clinical form of CD			
Typical form	20	6	
Atypical form	11	6	
Silent form	3	3	
Distribution according clinical form			> 0.05

[1]Includes heterozygosis for HLA DQ2, heterozygosis for HLA DQ8 and homozygosis for HLA DQ8 patients. HBV: Hepatitis B virus; CD: Celiac disease; SDS: Standard deviation score; HLA: Human leukocyte antigens.

Finally, we found a statistically significant difference in the vaccination response for patients in the present observational study as compared to patients analyzed in the previous retrospective study. In the present study, 34 out of 49 patients were responders compared to 30 out of 60 patients in the retrospective study ($P < 0.01$).

DISCUSSION

CD is defined as an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically-susceptible individuals and is characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLADQ2 or DQ8 haplotypes and enteropathy[6].

The reasons why CD could be related to an inadequate response to hepatitis B vaccination have long been discussed. Some previous studies have suggested a genetically-related failure of response, attributed to particular HLA antigens, mainly the DQ2 haplotype, which is also involved in autoimmunity[8,9]. In fact, while DQ2 is present in onlyapproximately 40% of the general population, it is expressed in up to 81% of CD patients. The HLADQ2 status would induce an inadequate Th2 response, leading to inefficient B cell differentiation and formation of memory T cells[8,10,11]. In 2007, a study by Park *et al*[2] demonstrated that more than 50% of the enrolled children with CD did not show a response to standard vaccination regimens for HBV, in contrast to a physiological response that was observed with other vaccinations (tetanus, rubella, and *Haemophilus influenzae* type b). This finding supported the hypothesis that HLA haplotype played a specific role in response

to HBV vaccine. One year later, a subsequent study conducted by Ahishali *et al*[12] confirmed this theory by finding responsiveness to hepatitis B vaccination in 68% of celiac patients, in contrast to the 100% response observed for the controls, emphasizing the genotypic co-incidence.

In 2009, Leonardi *et al*[13] published a case control retrospective study about the prevalence of HBV vaccine non-responders among celiac and healthy subjects. The anti-HBs titer was measured after a successful period of time on a GFD, as demonstrated by the normalization of serum markers of CD. The study confirmed that celiac patients have a lower percentage of response to hepatitis B vaccination than healthy controls. However, the authors also found a significantly higher number of responders among the celiac patients that were younger than 18-mo-old at diagnosis and a significantly lower number of responders in adolescent patients older than 14-year-old at diagnosis. The drawback of the study was that the HLA typing was performed in few patients, so that the study could not demonstrate the correlation of the phenomenon observed with HLADQ2 or HLADQ8, and that there was a long interval between the time of hepatitis B vaccination and the time of collecting samples for analysis of the anti-HBs titer. In this regard, a recent case control retrospective study by Zanoni *et al*[11] investigated the serological response to HBV and measles-containing vaccines in three groups of individuals: Diabetes mellitus type 1 (T1DM) patients, celiac patients and controls. No significant differences were found in the percentage of responders to HBV and measles vaccines among the T1DM and CD patients and the control group, and there was also a lack of correlation between HBV vaccine response and DQ2. According to the authors, these conflicting results between their findings and the data reported in the literature may be due to differences in ages of the examined subjects at time of vaccination and in time intervals between vaccination and blood sample collection for testing. They concluded that prospective studies of pathological and healthy groups, with same age at hepatitis B vaccination and same time interval for blood sample collection to determine antibody levels, are necessary to provide more conclusive data.

For these reasons, the originality of our prospective study is that of analyzing the response to hepatitis B vaccination in a group of 49 celiac patients at the time of diagnosis, helping us to nullify the percentage of error related to a long interval from time of hepatitis B vaccination to time of serum anti-HBs analysis. In fact, when we compared the results of our prospective study (based upon patients at time diagnosis of CD) with those retrospectively obtained by Leonardi *et al*[13] in 2009 (based upon celiac patients on a GFD), we found a higher percentage of responders among the celiac subjects, probably due to our study design having eliminated more of the potential confounding factors related to loss of immunity over the time, which have been documented extensively in the literature[14-16].

Meanwhile, we also observed that whereas more than half of our celiac population represented responders (69.39%), the percentage still remained lower than in the general population (90%), suggesting a role of genetic predisposition. However, comparison of the distribution of vaccination response showed no statistically significant difference between the different genotypes, providing an argument against the theory that homozygosity for the HLADQ2 haplotype could act in isolation to negatively influence the response to vaccination, in comparison with the HLADQ2/DQ8 heterozygosity or other haplotypes.

In this regard, several studies hypothesized that gluten intake at the time of vaccination could influence immune response, *via* competition of both gliadin peptides and hepatitis B surface antigen protein fragments for binding to HLADQ2 molecules, which could result in defective antibody production[3,17,18]. In support of this hypothesis, Nemes *et al*[19] showed that seroconversion after hepatitis B vaccination was 95.5% in CD patients vaccinated during dietary treatment; in contrast, in a second group of CD patients that were either untreated or with a diet status ranging from strict to non-strict, the response was 50.9%. The HLA DQ alleles did not seem to playa primary role because all of the patients carried the HLADQ2. In our study, patients were enrolled at diagnosis of CD, when their diet contained gluten; although, we do not know the exact period of exposure. It could be of interest to administer a booster dose of HBV vaccine in these subjects after a period of GFD and to subsequently evaluate the effects on the immune response. However, since our study did not reveal a significant correlation between HBV vaccine response and HLA alone, we now question whether it is possible that impaired immune response in CD is the result of a combination of several factors. Indeed, it could be possible that genetic predisposition, gluten intake and phenotype of the disease interact to influence a lower HBV vaccine response in CD.

In conclusion, our study is the first prospective study on HBV vaccine response in CD. The findings confirm the lower percentage of response to hepatitis B vaccination in the celiac population, as compared with healthy subjects. The mechanism that causes this phenomenon, however, remains unclear. According to our results, the mechanism does not appear to be related to HLA haplotype alone but could result from several variables working in combination. Further studies are needed to support this hypothesis and to establish the best surveillance program of response to HBV vaccine in CD.

COMMENTS

Background

The correlation between celiac disease and a lower response to the hepatitis B virus (HBV) vaccine has been demonstrated, but the causes remain unclear. Many confounding factors identified by previous studies have contributed to this uncertainty; moreover, the lack of prospective data represents a more extensive gap between the time of vaccination and the development of an immune response, contributing to select false non-responders (*i.e.*, those who are destined to lose the antibody titer over time). The originality of the authors' prospective

study lies in the authors' analysis of the response to hepatitis B vaccination in a group of celiac patients at the time of diagnosis, which allowed the authors' study to nullify the percentage of error related to these confounding factors.

Research frontiers

In this study, there is suggestion that genetic predisposition, gluten intake and phenotype of celiac disease could work in conjunction to influence a lower HBV vaccine response.

Innovations and breakthroughs

This study is the first prospective study in literature on the topic of lower HBV vaccine response in patients with celiac disease. This study confirms the lower percentage of response to hepatitis B vaccination in the celiac population, as compared with healthy subjects. According to the authors' results, the mechanism that causes this phenomenon is unlikely to be related to human leukocyte antigens haplotype alone but could be a result of several variables together.

Applications

This study provides additional evidence that, along with the collective data in the literature, will help to establish an optimal surveillance program of response to HBV vaccine in celiac disease.

Terminology

Non-responders are all subjects with a titer of hepatitis B surface antibody < 10 IU/mL after the primary vaccination cycle.

Peer-review

The authors have studied antibody response to HBV vaccine in celiac patients. This is an interesting study, well designed and performed.

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CASE REPORT

Contrast-enhanced ultrasonographic findings of serum amyloid A-positive hepatocellular neoplasm: Does hepatocellular adenoma arise in cirrhotic liver?

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Abstract

Hepatocellular adenoma (HCA) was recently classified into four pathological subtypes. There have been few studies describing the findings of contrast-enhanced ultrasonography (CEUS) of each type. Our case concerns a 78-year-old man who had undergone routine medical check-ups for hepatitis C for 11 years. Abdominal ultrasonography showed a 28 mm, hypo-echoic mass in the segment 4 of the liver. His integrating amount of drinking was 670 kg convert into ethanol. CEUS with Sonazoid demonstrated mild uniform hypo-enhancement with inflow of microbubbles from the periphery of the tumor in the arterial phase, and heterogeneously hypo-enhancement in the post vascular phase. Because the mass increased in size within 3 mo, a well differentiated hepatocellular carcinoma was suspected, and hepatic resection was performed. Microscopic findings showed homogeneous cell proliferation with low grade atypia, infiltration of inflammatory cells, ductular reactions, fatty deposit in part, and sinusoidal dilation. Immunohistochemistry revealed geographic positive for serum amyloid A (SAA), focal positive for glutamine

synthetase, diffuse and strong positive for C-reactive protein, and positive for liver-type fatty acid binding protein. These pathological features corresponded to that of an inflammatory HCA. However, we could not make a clear diagnosis, because HCAs were defined as not to arise in cirrhotic liver. Finally, this tumor was diagnosed as a SAA positive hepatocellular neoplasm.

Key words: Hepatocellular adenoma; Contrast-enhanced ultrasonography; Serum amyloid A; Serum amyloid A-positive hepatocellular neoplasms; Alcoholic cirrhosis

Core tip: Hepatocellular adenoma (HCA) was classified into four pathological subtypes. And HCA usually arises in the absence of significant fibrosis. Recently, some reports about serum amyloid A (SAA) positive hepatocellular neoplasm were published. All tumors shared features with inflammatory HCA arising in alcoholic cirrhosis. We describe the contrast-enhanced ultrasonographic findings of SAA positive HCA.

Kumagawa M, Matsumoto N, Watanabe Y, Hirayama M, Miura T, Nakagawara H, Ogawa M, Matsuoka S, Moriyama M, Takayama T, Sugitani M. Contrast-enhanced ultrasonographic findings of serum amyloid A-positive hepatocellular neoplasm: Does hepatocellular adenoma arise in cirrhotic liver? *World J Hepatol* 2016; 8(26): 1110-1115 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i26/1110.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i26.1110

INTRODUCTION

Hepatocellular adenoma (HCA) was recently classified into four pathological subtypes; hepatocyte nuclear factor 1 alpha inactivated HCA, beta catenin activated HCA, inflammatory HCA, and unclassified HCA[1-4]. Although contrast-enhanced ultrasonographic features of HCA have been reported in several literatures till now[5-7], there have been little studies that described those of each type of HCA[8]. HCAs usually arise in the liver without steatosis, because a nodule arising in fibrotic/cirrhotic liver was not to be a HCA according to World Health Organization classification 2010[1]. Recently, some reports about serum amyloid A (SAA) positive hepatocellular neoplasm were published. All nodules shared features with inflammatory HCA arising in alcoholic cirrhosis[9-11]. In this report, we describe contrast-enhanced ultrasonographic findings of SAA positive hepatocellular neoplasm which had features similar to inflammatory HCAs.

CASE REPORT

A 78-year-old man had undergone routine medical check-ups for hepatitis C over 21 years. He received interferon therapy 21 years ago, but could not achieve complete remission. In these years, he had compensatory hepatic cirrhosis and was gave medication of glycyrrhizin formulation. Abdominal ultrasonography showed 20 mm, hypo-echoic in the segment 4 of the liver 3 mo ago. Because the tumor increased in diameter to 28 mm, he was admitted to our hospital for further examinations. He had no history of other disease. He drank two glasses of whisky and one glass of beer from 20 till 66-year-old. His integrating amount of drinking was 670 kg convert into ethanol. He had no symptoms. Physical examination showed untoward features. Blood examination demonstrated thrombocytopenia, mild hyper-bilirubinemia, elevated liver enzymes (aspartate aminotransferase, 36 IU/L; alanine aminotransferase, 35 IU/L), positive for HCV-antibody, and 6.4 log IU/mL for HCV-RNA (Table 1).

Sonographic examination showed a homogenous, hypo-echoic, round mass in the segment 4b of the liver (Figure 1A). Color Doppler sonography revealed no signals in the lesion (Figure 1B). Contrast-enhanced ultrasonography (CEUS) with 0.5 mL of Sonazoid (Daiichi Sankyo, Tokyo, Japan) demonstrated mild global hyper-enhancement with inflow of microbubbles from the periphery of the tumor in the arterial phase (Figure 1C and D), persist enhancement in the portal venous phase (Figure 1E), and heterogeneous hypo-enhancement in the post vascular phase (Figure 1F). Plain computed tomography (CT) showed a hypodense tumor (Figure 2A). Contrast-enhanced CT showed iso-enhancement in the arterial phase (Figure 2B) and slight hypo-enhancement in the portal phase (Figure 2C). Magnetic resonance imaging (MRI) demonstrated slightly high intensity in the T1 weighted image (Figure 3A), and slightly low intensity in the T2 weighted image (Figure 3B). Contrast-enhanced MRI using Gadolinium ethoxybenzyl diethylene triamine pentaacetic acid revealed slightly high intensity in the hepatobiliary phase (Figure 3C).

Because the mass increased in size, it was suspected as being a well differentiated hepatocellular carcinoma. Considering the risk of hemorrhage and dissemination, partial segment 4 resection was performed without biopsy. Because the mass was adjacent to horizontal portion of the left portal vein and pathological diagnosis was needed, percutaneous ablation was not chosen. Microscopic findings showed homogeneous cell proliferation with low grade atypia, infiltration of inflammatory cells, ductular reactions, fatty deposit in part, and sinusoidal dilation (Figure 4). Immunohistochemistry revealed geographic positive for SAA, focal positive for glutamine synthetase, diffuse and strong positive for C-reactive protein, and positive for liver-type fatty acid binding protein (Figure 5). These pathological features corresponded to that of an inflammatory HCA.

DISCUSSION

Classification of HCA is based on molecular, pathologic, and immunohistochemical features[1]. Inflammatory HCA accounts for 45%-60% of all HCAs[1-4], and has

Table 1 Patient's laboratory results (the normal ranges)

WBC (3.3-8.6)	6200/μL	Total protein (6.6-8.1)	8.0 g/dL
Hgb (13.7-16.8)	15.0 g/dL	Albumin (4.1-5.1)	4.7 g/dL
Platelet (148-348)	108×10^3 /μL	HBs antibody	(-)
PT% (> 80)	99%	HBc antigen	(-)
INR	0.92	HCV antibody	(+)
Total bilirubin (0.4-1.5)	1.41 mg/dL	HCV-RNA	6.4 logIU/mL
Direct bilirubin (0.05-0.4)	0.37 mg/dL	Alpha-fetoprotein (< 20)	6.4 ng/mL
Aspartate transaminase (13-30)	36 IU/L	PIVKA-Ⅱ (< 40)	91 mAU/mL
Alanine transaminase (10-42)	35 IU/L	ICG (15 s) (< 15.0)	14.0%

WBC: White blood cell; INR: International normalized ratio; HCV: Hepatitis C virus; PIVKA-Ⅱ: Prothrombin induced by vitamin K absence-Ⅱ; ICG: Indocyanine green.

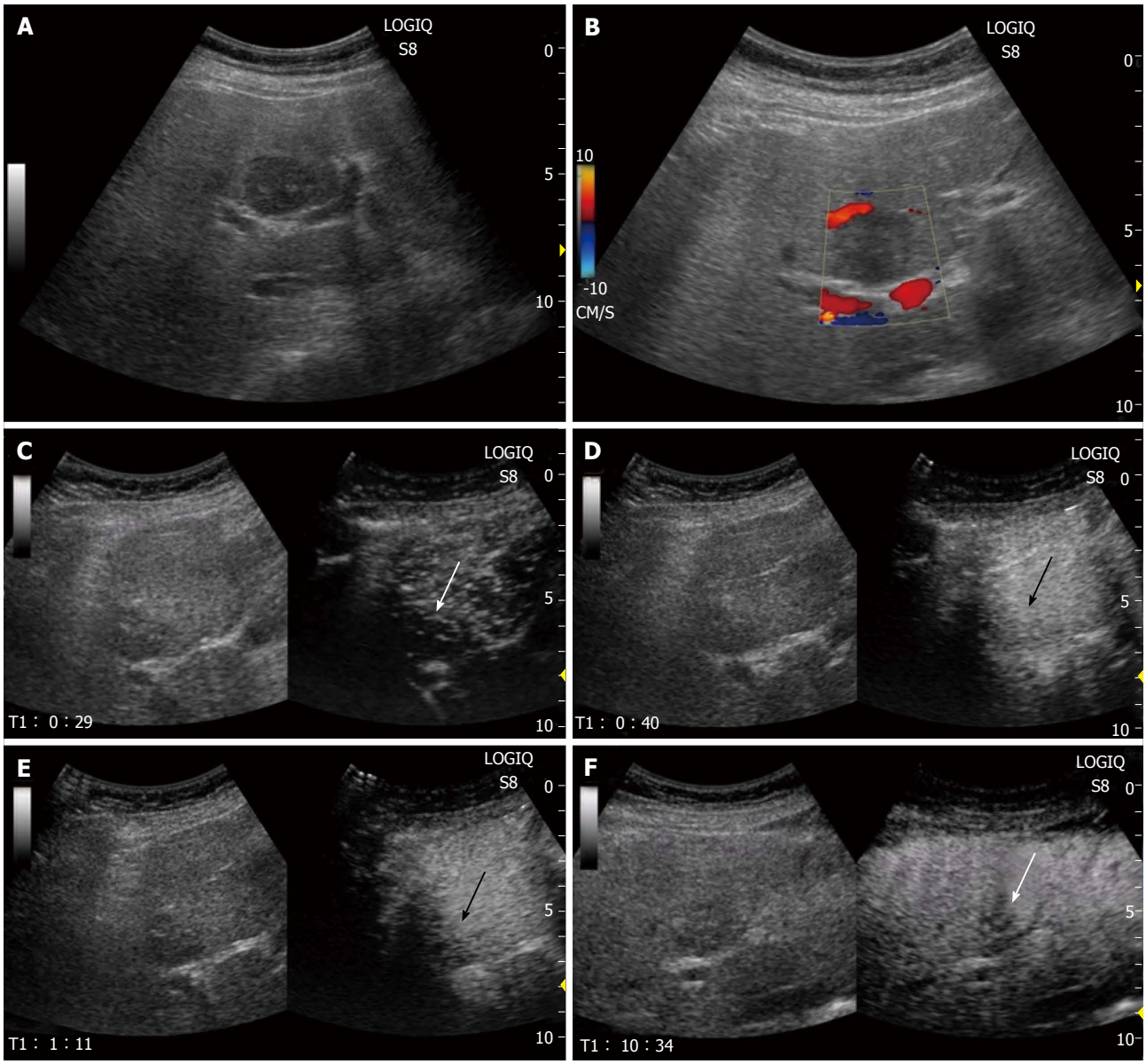

Figure 1 Sonography. Sonographic examination showed a homogenous, hypo-echoic, round mass in segment 4 of the liver (A). Color Doppler sonography revealed no signals in the lesion (B). CEUS demonstrated mild global hypo-enhancement (D, arrow) with inflow of microbubbles from peripheral of the tumor (C, arrow) in the arterial phase, persist enhancement in the portal venous phase (E, arrow), and heterogeneous hypo-enhancement in the post vascular phase (F, arrow). CEUS: Contrast-enhanced ultrasonography.

mutations of the *IL6ST* gene[12-14]. Alcohol intake and obesity are association with inflammatory HCA[9,15-17]. The rate of malignant transformation is unknown.

CEUS findings of HCA were described in previous

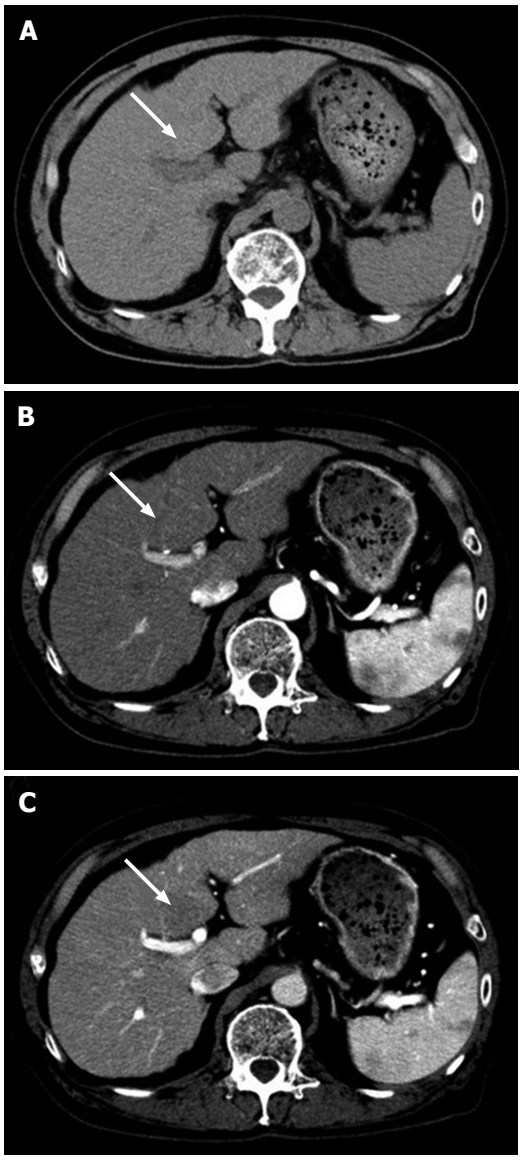

Figure 2 Computed tomography. Plain computed tomography (CT) showed a hypodense tumor (A, arrow); Contrast-enhanced CT showed iso-enhancement in the arterial phase (B, arrow); and slightly hypo-enhancement in the portal phase (C, arrow).

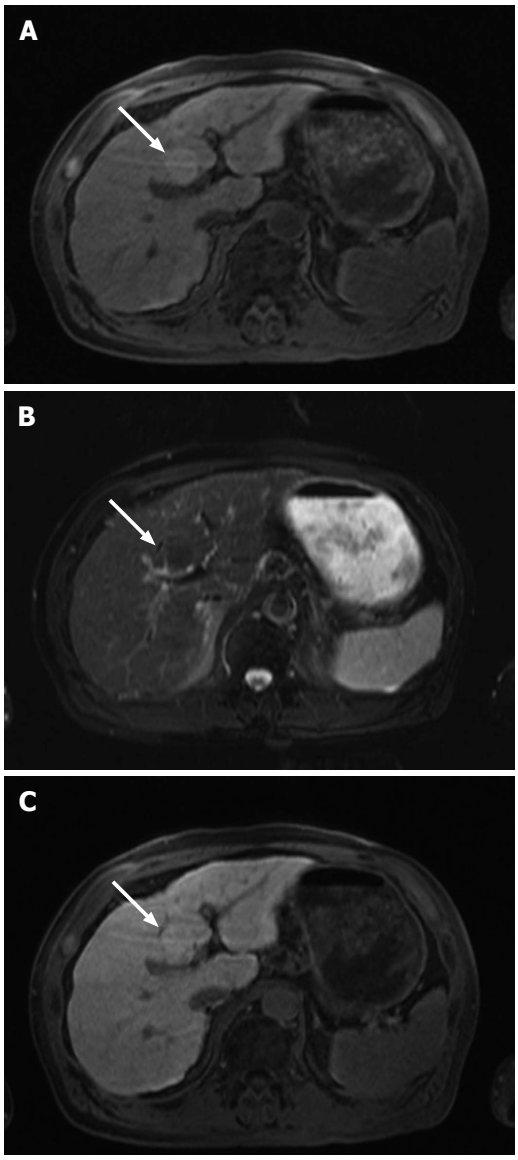

Figure 3 Magnetic resonance imaging. Magnetic resonance imaging (MRI) demonstrated slightly high intensity in T1 weighted image (A, arrow), and slightly low intensity in T2 weighted image (B, arrow). Contrast-enhanced MRI using Gd-EOB-DTPA revealed slightly high intensity in the hepatobiliary phase (C, arrow). Gd-EOB-DTPA: Gadolinium ethoxybenzyl diethylene triamine pentaacetic acid.

studies[5-8,18]. In one study which investigated 18 lesions, which were iso-hypoechoic in 9, hyper-enhancing in all in the arterial phase, and iso or hypo-enhancement in all in the late phase[18]. Ricci *et al*[19] emphasized homogeneous and centripetal enhancement during artery phase was showed in almost all. In accordance with previous studies[19-21], HCA lesions had some typical features, including early, homogeneous, centripetal, and strong enhancement in the arterial phase and the lack of a portal vein supply. According to a study[22], a number of HCAs demonstrated persistent enhancement. Dong *et al*[22] said "slow wash-out" (persistent enhancement during portal venous and late phase) may be a discriminant sign for HCAs in CEUS. In our case, the arterial phase findings were not so strong but homogenous and centripetal hyper-enhancement and some peripheral vessels were showed. The contrast medium that used in that study

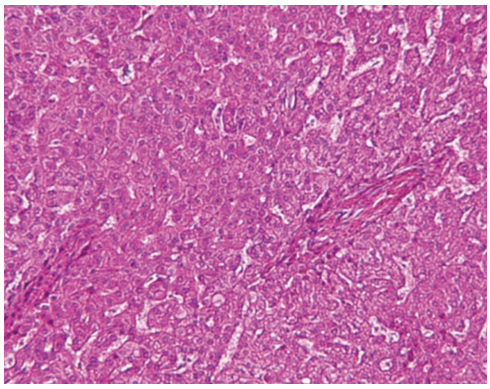

Figure 4 Microscopy showed homogeneous cell proliferation with low grade atypia, infiltration of inflammatory cells, ductular reactions, fatty deposit in part, and sinusoidal dilation.

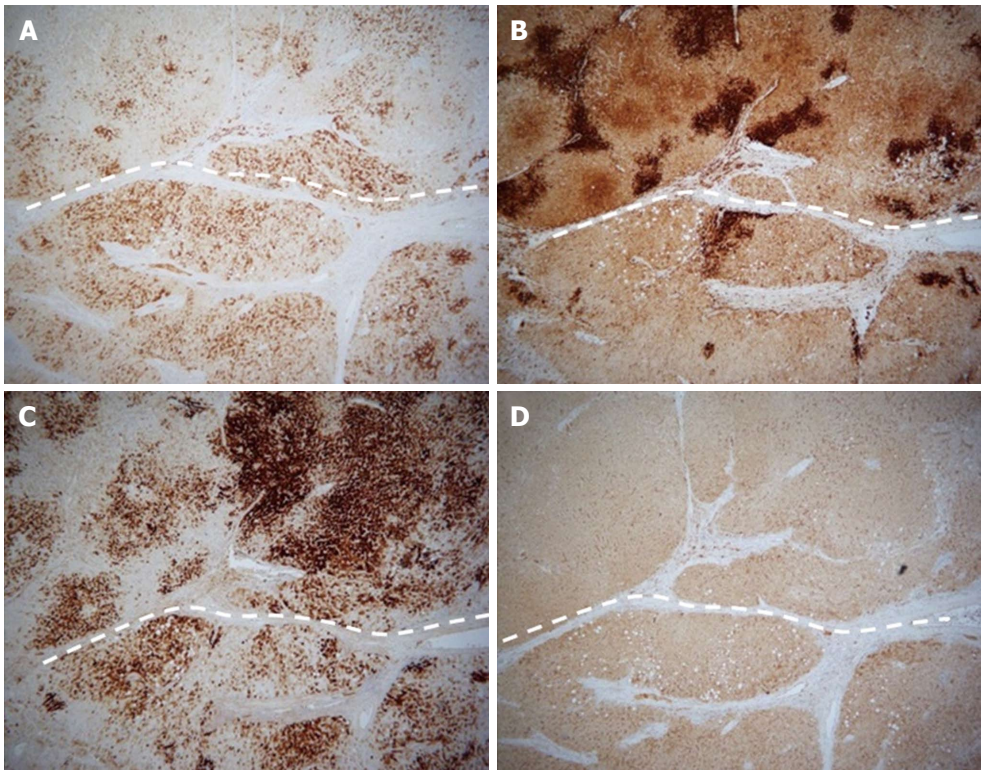

Figure 5 Immunohistochemistry revealed geometric positive staining for serum amyloid A (A), focal positive for glutamine synthetase (B), diffuse and strong positive for C-reactive protein (C), positive for liver-type fatty acid binding protein (D). Upper side is tumor area.

was Sonovue (Bracco, Milan, Italy) whereas Sonazoid was administered to our patient. Sonovue and Sonazoid are phagocytosed by Kupffer cells, and visualize clearly malignant tumor as defect. CEUS using Sonazoid revealed hypo-enhancement in the post vascular phase in our patient which was interpreted as lack of Kupffer cells in the tumor.

Recently SAA-positive hepatocellular neoplasms were proposed[10,11]. Generally, HCA arises from normal liver[1,23]. Although SAA-positive hepatocellular neoplasms have similar features to inflammatory HCA, they arise from alcoholic cirrhosis. Sasaki *et al*[10,11] suggested, considering that the patient exposed to alcohol in inflammatory HCA, it may be not be surprising that inflammatory HCA arise in alcoholic hepatic disease or cirrhosis. Our case had liver cirrhosis with HCV infection, moreover had a history of excessive amounts-alcohol consumption. We considered that our case is SAA-positive hepatocellular neoplasm.

In conclusion, CEUS revealed homogeneous mild hyper-enhancement in the arterial phase and heterogeneous hypo-enhancement in the post vascular phase in our case. Some of CEUS findings corresponded to features of HCA. Our patient had both HCV infection and alcohol abuse, and it was not typical for inflammatory HCA. It may be a case of so-called SAA-positive hepatocellular neoplasm.

COMMENTS

Case characteristics

A 78-year-old man with hepatitis C and hepatic cirrhosis received abdominal ultrasonography, in which 20 mm, homogenous, hypo-echoic, round mass was shown in the segment 4b of the liver.

Clinical diagnosis

Because the mass increased in size and he had hepatic cirrhosis, a well differentiated hepatocellular carcinoma was suspected.

Differential diagnosis

Dysplastic nodule, large regenerative nodule, hepatocellular adenoma (HCA), and focal nodular hyperplasia.

Laboratory diagnosis

Hepatic pre-cirrhosis with early hepatocellular carcinoma.

Imaging diagnosis

A well differentiated hepatocellular carcinoma was suspected.

Pathological diagnosis

Inflammatory HCA.

Treatment

Segment 4 partial resection.

Related reports

HCA was classified into four pathological subtypes and usually arises in the absence of significant fibrosis. Serum amyloid A (SAA)-positive hepatocellular neoplasm shares features with inflammatory HCA arising in alcoholic cirrhosis.

Term explanation

SAA-positive hepatocellular neoplasms have similar features to inflammatory HCA, they arise from alcoholic cirrhosis.

Experience and lessons

Considering that the patient exposed to alcohol in inflammatory HCA, it may

be not be surprising that it arise in alcoholic hepatic disease or cirrhosis. Recognizing SAA-positive hepatocellular neoplasm as differential diagnosis is important in the case that had both HCV infection and alcohol abuse.

Peer-review

This is a very interesting case, well investigated and presented with also pathological comparison images.

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LETTERS TO THE EDITOR

Predictive potential of *IL-28B* genetic testing for interferon based hepatitis C virus therapy in Pakistan: Current scenario and future perspective

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Abstract

In Pakistan which ranked second in terms of hepatitis C virus (HCV) infection, it is highly needed to have an established diagnostic test for antiviral therapy response prediction. Interleukin 28B (*IL-28B*) genetic testing is widely used throughout the world for interferon based therapy prediction for HCV patients and is quite helpful not only for health care workers but also for the patients. There is a strong relationship between single nucleotide polymorphisms at or near the *IL-28B* gene and the sustained virological response with pegylated interferon plus ribavirin treatment for chronic hepatitis C. Pakistan is a resource limited country, with very low per capita income and there is no proper social security (health insurance) system. The allocated health budget by the government is very low and is used on other health emergencies like polio virus and dengue virus infection. Therefore it is proposed that there should be a well established diagnostic test on the basis of *IL-28B* which can predict the antiviral therapy response to strengthen health care set-up of Pakistan. This test once established will help in better management of HCV infected patients.

Key words: Diagnostics; Hepatitis C virus; Interferon therapy; Polymorphisms; *IL-28B*; Genetic testing; Pakistan

Core tip: Pakistan has a very heavy burden of hepatitis C virus (HCV) infection with around 11 million positive cases; however, in spite of well established prognostic value, the data regarding the role of interleukin 28B (*IL-28B*) single nucleotide polymorphisms (SNPs) in HCV antiviral therapy response are very limited. There are only six reports on the topic and it can be concluded from this limited information that *IL-28B* could be a good prognosis marker for HCV patient management in Pakistan. The major prevalent HCV genotype in Pakistan is 3a and *IL-28B* SNP rs12979860 showed a good prediction for interferon based antiviral therapy response against this viral genotype. It can be predicted that inclusion of *IL-28B* genetic testing in

routine diagnostic set-up of Pakistan will help in better management of the disease. A well directed antiviral therapy based on personalized *IL-28B* genotyping along with virus genotyping will help in lessening of therapy cost and better management of the disease.

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TO THE EDITOR

Recent advancements in molecular biology techniques help in identification of various host and pathogenic factors influencing the disease prognosis and therapeutic outcomes. One example is identification of various genetic factors through genome wide analysis studies (GWAS). In the field of gastroenterology and hepatology, an example is the discovery of an association between single nucleotide polymorphisms (SNPs) at or near the interleukin 28B (*IL-28B*) gene and the sustained virological response (SVR) rate with pegylated interferon (IFN) plus ribavirin treatment for chronic hepatitis C (CH-C)[1-3]. IL-28B (IFN-λ3) is produced by many immune cells like neuronal cells, alveolar epithelial cells, and hepatocytes in response to viral infection. IFN-λ showed antiviral activity against many viruses. It not only inhibits viral replication but also has immune-modulatory functions[4]. It has been shown by four autonomous GWAS that SNPs of the *IL-28B* gene, which is located on chromosome 19q13, are strongly associated with treatment response to interferon based therapy and spontaneous viral clearance in chronic hepatitis C virus (HCV)-infected patients[4]. After these studies, the predictive potential of *IL-28B* genetic variations has been investigated and verified throughout the world in patients infected with HCV of all viral genotypes and currently IL-28B SNPs are in commercial use for antiviral therapy response prediction around the world.

In Pakistan, data regarding the role of IL-28B SNPs in HCV antiviral therapy response are very limited. To our knowledge, there are only six studies that investigated the role of IL-28B in HCV patients regarding interferon therapy response and disease prognosis (Table 1)[5-10]. These studies investigated the predictive potential of either IL-28B protein level or IL-28B SNPs (rs12979860, rs8099917, rs12980275). It can be concluded from existing limited data that IL-28B could be a good prognosis marker for HCV patient management. Recent studies by Shaikh *et al*[9] (2014) and Imran *et al*[8] (2015) reported significant existence (47.5% and 6.4%, respectively) of circulation of diagnostically untypable HCV variants in local populations of Sindh Province of Pakistan. We have lately highlighted the issue of diagnostically untypable HCV circulation in Pakistan and recommended immediate need to resolve this problem for the better management of HCV patients as course and fate of antiviral therapy are viral genotype dependent[11]. The resolution of this problem will also help in understanding the potential role of IL-28B SNPs in antiviral therapy response prediction against each viral genotype.

HCV is highly endemic in Pakistan with around 11 million infections[12-14]. The major prevalent viral genotype is 3a along with 2a, 3b, 1b, 2b, 2a and a large number of untypable ones[11,15,16]. It is observed that irrespective of the HCV genotype, SVR rate of interferon plus ribavirin is quite good (80%-97%) in Pakistan[7,8,17,18]. Pakistan is a resource limited country with much low per capita income in the general population. According to the World Health Organization, the total expenditure on health is only 2.8% of GDP, which means total expenditure on health per capita is only 126 $[19]. Other medical emergencies like polio virus and dengue virus endemics shift the government priorities and funds are becoming less available for HCV management. There is no health insurance for the general population in Pakistan, which also affect the patient's ability to bear therapy cost. *IL-28B* genetic test is an established diagnostic test for interferon based antiviral therapy response prediction across the world. In the current scenario of Pakistan, it is highly needed to have an established diagnostic test on the basis of IL-28B which can predict the antiviral therapy response.

The currently available literature on the role of IL-28B in HCV interferon therapy response in Pakistan shows that rs12979860 is a good predictor of therapy response against HCV 3a genotype. In the era of direct acting antivirals (DAAs), interferon based therapy against HCV will remain the major choice in Pakistan due to higher SVR and low cost compared with DAAs[20]. On the basis of the above discussion, we propose future studies across the country on different ethnic groups infected with all viral genotypes so that the results could be generalized for diagnostic purpose. It is also suggested that the forthcoming studies should include a comparatively larger number of patients so that the results could be applicable for commercial purpose. It is highly anticipated that inclusion of *IL-28B* genetic testing in routine diagnostic tests will help health care professionals in better management of the patients. Well directed antiviral therapy on the basis of personalized *IL-28B* genotyping along with viral genotyping will help in reduction of therapy cost and better management of the disease.

Table 1 Summary of interleukin 28B and interferon based therapy response in hepatitis C virus patients in Pakistan

Year	Viral genotype	Patients (*n*)	Objective/SNP investigated	Findings/conclusion	Ref.
2015	3a	66	IL-28B protein levels	IL-28B protein levels were significantly associated with therapy response	[5]
2015	3	105	rs8099917	TT genotype favors RVR	[6]
			rs12979860	CC genotype favors SVR	
2015	1a,1b, 3a	111	rs12979860	CC genotype favors SVR in HCV 3a genotype	[7]
2015	1a, 1b, 3a, 3b, 4, UT	140	rs8099917	No association was observed with therapy response	[8]
			rs12979860	CC genotype favors SVR	
2014	(2a, 3a, UT)	220	rs8099917	No association was observed with therapy response	[9]
			rs12979860	No association was observed with therapy response	
			rs12980275	AA genotype favors SVR	
2014	3a	200	rs12979860	TT genotype favors SVR	[10]

Genotyping performed only for non-responders patients. SVR: Sustained virological response; RVR: Rapid virological response; UT: Untypable; IL-28B: Interleukin 28B; HCV: Hepatitis C virus; SNP: Single nucleotide polymorphism.

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