12. Helicobacters & hepatobiliary diseases

12.01 Association between Helicobacter bilis in bile and biliary tract malignancies: H.bilis in bile from Japanese and Thai patients with benign and malignant diseases in the biliary tract

N. Matsukura1, T. Tajiri1, S. Yokomuro1, S. Yamada2, K. Morino1, J. Yamasato1, A. Togashi1, S. Kamiya3, J.G. Fox4, 5, Nippon Medical School, Tokyo, Japan, 2Chiang Mai University, Chiang Mai, Thailand, 3Kyorin University, Tokyo, Japan, 4Massachusetts Institute of Technology, Cambridge, MA

Background: Japan and Thailand have high incidences of bile duct carcinoma and gallstones. The presence of Helicobacter bilis detected by PCR and 16S rRNA analysis in bile samples from Chileans with chronic cholecystitis has been reported. An association between H.bilis in bile and biliary tract malignancies has not been investigated. The aim of this study was to determine whether malignant diseases of the biliary tract was associated with the presence of H.bilis in bile samples obtained from two high-risk populations.

Methods: Bile samples from 45 Japanese and 40 Thai patients were subjected to PCR analysis using H.bilis specific primers, and six of the H.bilis amplicons were sequenced.

Results: Thirty out of 15 (87%) Japanese and 11 out of 14 (79%) Thai patients with bile duct or gallbladder cancer tested positive for the presence of H.bilis in their bile. Eight out of 16 (50%) Japanese and 10 out of 26 (38%) Thai patients with gallstone and/or cholecystitis tested positive for H.bilis. Only 4 out of 14 (29%) subjects without biliary disease tested positive for H.bilis in Japanese. Six of the H.bilis amplicons sequenced had a high homology with H.bilis sequences in the database. Bile duct and gallbladder cancer showed significantly higher positive rates for H.bilis than did the non-biliary diseases in Japan (p<0.01) and the odds ratios for bile duct or gallbladder cancer with H.bilis in comparison with gallstone and/or cholecystitis were 6.50 (95% CI 1.09-38.63) in the Japanese and 5.86 (1.31-26.33) in the Thai patients.

Conclusion: H.bilis infection in bile was associated with biliary tract and gallbladder cancers in two high risk populations, Japanese and Thai.

12.02 Expression and activity of the cytolethal distending toxin of Helicobacter hepaticus

P. Avenaud, N. Bouges, F. Mégraud, A. Ménard, Laboratoire de Bactériologie, Université Victor Segalen Bordeaux 2, Bordeaux, France

Helicobacter hepaticus has been recognized as the causal agent of hepatocellular carcinoma in mice. A toxin, namely cytolethal distending toxin (cdt), may lead to cell cycle arrests and apoptosis. The low quantity of toxin produced by H. hepaticus and the difficulty in obtaining purification from bacterial lysate did not allow a comprehensive study of this putative pathogenic factor.

Our aim was to express the recombinant protein in a sufficient amount to allow pathogenic studies. The rapid translation system (RTS) (Roche) which allows protein expression in vitro was used. ORFs of the 3 Cdt were cloned in the plasmid pVE2.4a and expressed. Briefly, CdtA, CdtB and CdtC were independently amplified from strain CCUG33637 using primers which also included SacII and SacI restriction sites. After successive digestions with these restriction enzymes, the 3 amplicons were ligated to plasmid pVE2.4a and put in phase with the plasmid sequence between the T7 RNA polymerase promoter and terminator. The product expressed is a fusion protein including a linker and a histidine tag, the cleavage site of factor Xa, and H. hepaticus CdtA, CdtB or CdtC with the same sequence as the original protein but with an additional glycine residue at the N terminal end.

Each Cdt was found independently to have a low toxic activity for CCL9.1, a murine hepatic cell line, whereas the reconstructed complex (with histidine tag) induced cell death. In conclusion, a recombinant active H. hepaticus Cdt complex can be obtained in sufficient quantities using the RTS. A two-step purification of the protein is now being conducted, employing a nickel affinity column and hydrolysis by Factor Xa, in order to carry out pathogenicity studies.

12.03 Optimising a PCR-DGGE assay for Helicobacter species in paraffin embedded pancreatic tissue

H. Nilsson1, *M. Vicencio1, *J. Ihse2, U. Steenram1, T. Wadström1, Lund University, Dept of MMDI, Lund, Sweden, 2Lund University, Dept of Surgery, Lund, Sweden, 3Lund University, Dept of Pathology, Lund, Sweden

Intestinal- and H. pylori-like Helicobacter species are increasingly being detected by PCR and DNA sequencing in liver tissue- and gallbladder specimens in patients with chronic liver diseases, hepatocellular carcinoma, and cholecystitis. Since Helicobacters are fastidious, non-culture based assays are necessary for detection of these and other microbes difficult to identify by culture. Paraffin embedded pancreatic tissue samples (n=6) with normal histology, were de-embedded and each tissue sample (5 mg) was extracted using Quagen Tissue DNA protocol and tested by PCR for the genus Helicobacter. Negative samples were homogenised in PBS and pooled at 10% (w/v). Subsequent to experimental inoculation, using 5%, 1% and 0.2% of pancreas tissue homogenates, by 10-fold dilutions of H. pylori, the efficiency of different primers and DNA-polymerases were evaluated. The sensitivity of PCR was compared with nested PCR. PCR-inhibition was tested by adding a pancreatic tissue homogenate directly to PCR reaction mixtures. 39 embedded samples of pancreatic carcinoma were analysed using the optimised assay. Eight positive samples were further analysed by DGGE.

The H. pylori-inoculated 1% homogenate showed the highest signal intensity with Helicobacter genus-specific primers. Nested PCR was 10 times as sensitive as one-step PCR. Different DNA-polymerases showed large variation regarding ability to amplify H. pylori DNA in pancreatic tissue. Of the polymerases tested, rTth was the most efficient, detecting 50 CFU H. pylorinl tissue homogenate. Applying the assay on patients with pancreas carcinoma, 29 of 39 (74%) samples were Helicobacter positive. By DGGE, 8 of 8 positive samples contained H. rappini. 5 samples contained more than one Helicobacter species. H. pullorum, H. pylori and H. bizzozeroni was also detected. A PCR-protocol for detecting Helicobacter in paraffin embedded pancreatic tissue was optimised. The method showed an equal sensitivity to a method previously used on fresh pancreas samples (H-O Nilsson et al. J Nat. Cancer. Inst. 2002;94:632-633).

12.04 Helicobacter ganmani 16S rDNA detected in pediatric hepatic diseases

H. Nilsson1, V. Tolia2, A. Wuerth3, R. Rabah4, T. Wadstrom1, Lund University, Dept of MMDI, Lund, Sweden, 2Childrens Hospital of Michigan, Dept of Gastroenterol, Detroit, MI, 3Childrens Hospital of Michigan, Dept of Pathol, Detroit, MI, United States

Intestinal Helicobacter species, such as H. hepaticus, can infect the murine liver and cause chronic hepatitis and hepatocellular carcinoma in susceptible strains of mice. By PCR and DNA sequence analysis, H. pylori-like 16S rDNA was detected in patients with chronic liver diseases and primary liver cancer. Studies to examine the prevalence of Helicobacter species in the liver of children with hepatic diseases have not yet been performed.

Paraffin embedded sections of 42 liver biopsies of pediatric patients with miscellaneous diseases such as autoimmune chronic active hepatitis (AHI, n=15), biliary atresia (n=6), steatohepatitis (n=3), chronic hepatitis C (HePC, n=5), sclerosing cholangitis (SC, n=4), chronic hepatitis B (HePB, n=3), neonatal giant cell hepatitis (n=3), and biliary hypoplasia (n=1), were de-embedded, extracted by the Quagen Tissue Protocol (Qiagen, Hilden, Germany), and examined for the presence of Helicobacter species by a genus-specific semi-nested 16S rDNA PCR assay. DNA sequencing was subsequently performed on positive samples using ABI 310 and the BigDye Kit (Applied Biosystems). Sequences were aligned in BioEdit and compared with sequences in public databases by BLAST searches at NCBI. Of the 42 liver tissue samples, 22 (52.4%) were positive for the genus Helicobacter. The positive specimens were found at approximately the same rate (around 50%) in the different patient groups. The nucleotide sequences of partially sequenced 16S rDNA fragments of 8 patients were 98-100% similar to H. ganmani, 16S rDNA similar to H. canis was found in one patient.

DNA-sequencing of 16S rDNA in patients positive for the genus Helicobacter was similar to H. ganmani in a majority of the analysed samples.
The role of Helicobacter species, and other microaerophilic organisms, in pediatric liver disease should be further studied.

12.05 Helicobacter pylori survives in native human bile but not in growth medium supplemented with physiological concentrations of deoxycholic acid


Objective: Recently, several authors reported that Helicobacter pylori (HP) has been detected in human bile. This observation seems to be contradictory to the finding that bile acids possess antibacterial activity against HP. Here, we present data which are compatible with both findings.

Methods: The HP type strain ATCC 43504 and three HP isolates from clinical samples were incubated either with samples of native human bile or with growth medium supplemented with ascending concentrations of deoxycholic acid (2.5 mM, 5 mM and 10 mM). After 4 hours, aliquots were plated on blood agar plates, and the viability of the bacteria was determined by counting the number of colonies after 3 to 5 days of incubation.

Results: All four HP strains survived a 4 hours incubation in native human bile from three individual patients. In contrast, the same incubation period in growth medium supplemented with 2.5 to 10 mM deoxycholic acid resulted in a complete loss of growth.

Discussion: These data indicate that HP is able to survive in native human bile, however, physiological concentrations of deoxycholic acid in vitro possess strong antibacterial activity against HP. Further studies are needed to explain this phenomenon. It will be important to know if HP is moreover capable to grow in bile, and therefore is able to colonize the biliary system.

12.06 Possible role of Helicobacter sp as a risk factor for the evolution of HCV infection toward cirrhosis and hepatocellular carcinoma

M. Rocha1, A. Méndez1, P. Avenaud1, B. LeBall1, C. Balabaud1, P. Boulic-Sage2, D.M.M. Queiroz2, F. Mégraud1, Laboratoire de Bactériologie, Université Victor Segalen Bordeaux 2, Bordeaux, France.

A limited number of patients infected with HCV develop a cirrhosis and finally a hepatocellular carcinoma (HCC). The risk factors currently recognized cannot fully explain this evolution and a bacterial coinfection could be the cause. Our goal was to study the prevalence of Helicobacter sp infection in the liver of HCV+ patients with cirrhosis and without HCC as well as in patients with HCV hepatitis alone, in comparison to controls.

The presence of Helicobacter sp in the liver of the patients was detected by PCR, after surgical resection or liver biopsy. The tissue fragments were maintained at -70°C. After DNA extraction (Qiagen), HS1 and HS2 primers amplifying DNA of all Helicobacter species (399bp) were used as well as primers specifically amplifying H. pylori DNA. Controls were comprised of patients with hepatic resection for benign tumors or metastasis.

Helicobacter DNA was found in 21/23 HCC tumor samples from HCV positive patients (91%); in samples from the cirrhotic liver of 13/23 (56%) of the same patients, as well as in samples from the cirrhotic liver of 20/29 patients (69%) without HCC. Helicobacter DNA was also found in 1/13 samples with HCV hepatitis alone (3.2%) and 2 of 27 controls (7.4%).

An H. pylori specific PCR was performed in all positive cases and turned out to be positive in 41 out of 44 cases. In 2 negative cases, sequencing indicated the presence of H. pullorum.

This study shows that Helicobacter sp DNA, essentially from H. pylori, can be present in the liver of HCV+ patients with cirrhosis and HCC. It does not allow a conclusion to be drawn on causality. Indeed, the presence of these bacteria could be the result of morphological changes of the liver. However, Helicobacter sp could also be a cofactor in HCV infection and warrant prospective studies.

12.07 Helicobacter pylori infection in patients with liver cirrhosis

S. Naumovski-Milajic, M. Katicic, V. Colic-Cvrlje, B. Srbesc, B. Papa, B. Sabaric. Clinical Hospital Merkur, Zagreb, Croatia

Background: HP infection currently is the most important etiologic agent in the development of chronic active gastritis, gastric and duodenal ulcers,carcinoma and Malt-lymphoma of the stomach.Moreover HP infection has also been associated with various extradigestive diseases.

Aim: The aim of our study was to investigate the possible pathogenetic role of Helicobacter pylori (HP) infection in patients with cirrhosis of the liver.

Patients and Methods: 235 patients (M/F 155/80, aged 24-86 yo) suffered from liver cirrhosis, were hospitalised during five years in ICU, University Hospital Merkur Zagreb, and were included in the study. They were divided into three groups according to the Child’s classification for cirrhotic severity (A - good, B - fair and C poor). In addition, the patients were divided according to the presence or absence of each of the following:ascites, splenomegaly, oesophageal varices, bilirubin level and known risk factor for hepatic encephalopathy (gastrointestinal bleeding, azothemia, hepatorrenal syndrome, infection and severity of disease). All patients had upper gastrointestinal endoscopy and the gastroduodenal pathology was identified. Helicobacter pylori (HP) infection was confirmed by gastric histology.

Results: 143 (60,85%) patients were HP positive. 79 (33,61%) patients were admitted because of upper GI bleeding. In this group, 61 (77,21%) were HP positive (χ2 test 13.38, p = 0.003, CI = 0.32-0.42). The highest rates of HP infection were found among patients in Child’s class C. We found significant difference in HP positive rate between the patients with and without oesophageal varices too. 113 (69,36%) patients had encephalopathy, and 88 (77,87%) of those were infected with HP, compared with only 48,3% patients without encephalopathy (χ2 test 17.58, p = 0.0001, CI = 0.54-0.62).

Conclusion: According to our results we found that HP infection was higher among patients with cirrhosis and acute GI bleeding, as well as with Child’s class C group. HP positive patients have been in higher risk for hepatic encephalopathy too.

12.08 Comparison of immune response to H. hepatitis, H. bilis, H. pullorum and H. pylori in children and adults

T. Vorobjova1, M. Granholm1, M. Lyrra1, T. Porkka1, S. Terjärv1, O. Ananieva1, I. Nilsso2, T. Wadström2, R. Uibo1, 1Dept. of Immunology, Univ. of Tartu, Tartu, Estonia, 2Dept. of Medical Microbiology, Dermatology and Infection, Univ. of Lund, Lund, Sweden

Background: Bile-tolerant Helicobacter sp may be a risk factor for chronic liver diseases. Although data about the seroprevalence of antibodies to these Helicobacter spp in adults are available, data about their presence in children are limited.

Aim: To compare immune response to bile-tolerant Helicobacter spp. and H. pylori in children and in adult population and in blood donors (Ananieva et al. IJMM 291; Suppl.31, G-06, 58, 2001). We found significant difference in HP positive rate between the patients with and without oesophageal varices too. 113 (69,36%) patients had encephalopathy, and 88 (77,87%) of those were infected with HP, compared with only 48,3% patients without encephalopathy (χ2 test 17.58, p = 0.0001, CI = 0.54-0.62).

Methods: Sera from 103 consecutive paediatric patients (age range 1-14 y., 46 m, 57 f) were tested for IgG to the antigens of H. hepatitis (strain CCUG 33637), H. bilis (strain CCUG 38995), H. pullorum (strain CCUG 33388) and H. pylori (stain CCUG 17874) by ELISA.

Results: The results are presented as the mean values of relative antibody activity (RAA).

<table>
<thead>
<tr>
<th>Study populations</th>
<th>n</th>
<th>Mean age</th>
<th>H. hepatitis RAA</th>
<th>H. bilis RAA</th>
<th>H. pullorum RAA</th>
<th>H. pylori RAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paediatric patients</td>
<td>103</td>
<td>8.0±0.4</td>
<td>27.2±10.5*</td>
<td>32.6±10.5*</td>
<td>20.8±3.3*</td>
<td>23.0±28.0*</td>
</tr>
<tr>
<td>Adult population</td>
<td>189</td>
<td>41.9±16.2</td>
<td>17.5±16.4*</td>
<td>29.8±17.3*</td>
<td>37.1±15.0*</td>
<td>60.8±31.0*</td>
</tr>
<tr>
<td>Blood donors</td>
<td>100</td>
<td>37.7±11.4</td>
<td>36.9±11.4*</td>
<td>26.9±12.2*</td>
<td>37.3±29.6*</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant difference: *p < 0.001; cc-c; p < 0.001; cc-c-c; p = 0.02; ds-d; p < 0.001; ddd-d; p = 0.04.

Immune response to H. pullorum and to H. bilis was significantly lower in children, whereas to H. hepatitis it was significantly higher in children, compared with the adults. A significantly higher mean value of RAA for H. hepatitis (29.7±26.9) and H. bilis (29.6±30.5) was in 1-5 y. children compared with those aged 6-10 y. (18.3±14.9 and 15.9±17.1; p=0.03; p=0.02). The mean RAA to H. pylori was significantly lower in children and in the youngest age group of 1-5 y. (16.5±2.17) compared with 11-14 y. (30.1±32.6; p=0.04).

Conclusion: Immune response to all Helicobacter spp. studied occurred in paediatric patients, while a significantly lower response to H. pullorum,
Association between the presence of Helicobacter in gallbladder tissue and cholelithiasis

C.P. Silva, A.G. Oliveira, J.B. Guerra, J.C. Pereira-Lima, D.L. Marques, L. Santos, D.M.M. Queiroz, Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Porto Alegre, Brazil, 2Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 3Faculdade de Medicina do Triângulo Mineiro, Uberaba, Brazil

Helicobacter species, including H. pylori, have been detected by PCR in the human bile and gallbladder tissue. These bacteria have been associated with some hepatobiliary diseases. In this study we have investigated the presence of Helicobacter species in patients with cholelithiasis and controls by culture and PCR. Bile and gallbladder tissue was obtained from 45 patients with (mean age 51.5 yr., SD 16.4, 31 female) and 18 patients without (mean age 39.5 yr., SD 17.1, 6 female) cholelithiasis at surgery. All samples were stored at -20°C before processing. The samples were cultured onto BHI in a microaerophilic atmosphere at 37°C. Genomic DNA was extracted using the QIAamp Tissue kit (Qiagen), and the 16S rRNA gene was amplified by PCR. The amplicons obtained of 1200 or 400 bp were purified and directly sequenced with an Applied Biosystems DNA automated sequencer using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and sequencing primers. No Helicobacter species were cultured from the analyzed specimens. Helicobacter DNA was detected by PCR in the bile of 24 patients (16 with cholelithiasis) and in the gallbladder of 20 patients (18 with cholelithiasis). In a logistic regression model, cholelithiasis was independently associated with the gender (p=0.04) and the presence of Helicobacter DNA in the gallbladder tissue (p=0.03, OR=11.1), but not in the bile (p=0.8). The sequences of 16S rRNA gene were >99% similar to that of H. pylori. In conclusion, our results suggest that Helicobacter may be associated with pathogenesis of cholelithiasis. Grants: FAPESP/MIG, FINEP, CNPq/Brasil.

Helicobacter pylori infection in patients with chronic hepatitis B: Preliminary report

R. Vilaiachine, V. Mahachau, O. Hanwiwatwong, D. Thong-Gyam, C. Prempatches, P. Kullanivajaya, 1Helicobacter Study Group, Chulalongkorn University Hospital, Bangkok, Thailand, 2Helicobacter Study Group, Chulalongkorn University Hospital, Bangkok, Thailand

Purpose: The aim of this case-control study was to demonstrate that Helicobacter pylori infection is correlated with the degree of hepatic inflammation in patients with chronic hepatitis B.

Method: Clinical information, liver function test, pathological report of liver biopsy and H. pylori infection were collected from chronic hepatitis B patients between June 2001 and April 2002. Chronic hepatitis B was defined as HbsAg positive with persistent transaminases for more than six months. The blood for liver function test was collected from each patient within one week before doing liver biopsy. The hepatic inflammation was defined as HbsAg positive with persistent transaminitis for more than six months. The blood for liver function test was collected from each patient status was Child Class A, 23 (37,7%) as Child class B and 8 (13,1%) as Child Class C. There was no statistical difference in H. pylori prevalence among the three groups (54,1% (40/74) and the control group (60%) 39/65 (P=0,4801). Only one patient in the control group was resistant (A-G at position 2143) (0,67%). There was no macrolide-resistance in cirrhotic group.

Conclusion: A very low macrolide-resistance rate was found in our study. (0,67%). The high cost of antimicrobial agents in Brazil is likely to constrain their indiscriminate use.

Lack of Helicobacter species in biliary atresia, an inflammatory cholestatic liver disease in infancy

P. Vincent, K. Mention, E. Leteurtre, S. Armand, F. Gottrand, Bacteriologie, Centre Hospitalier Régional Universitaire de Lille, France, Pédieatrie, Centre Hospitalier Régional Universitaire de Lille, France, Histopathologie, Centre Hospitalier Régional Universitaire de Lille, France

Bile-tolerant Helicobacter species have been reported in animal models, and the possible role of Helicobacter species in human liver pathology is a raising question.

Aim: to look for an association between Helicobacter species and biliary atresia, that is the most frequent cause of cholestasis in infancy, resulting from an unexplained sclerosing inflammatory lesion initiated in the first days of life.

Patients and Method: Liver biopsies were studied in ten sequential cases of biliary atresia (5 males, 5 females, aged 19 to 92 days; median 50 days). In order to control for a possible natural colonisation of biliary tract in infant by Helicobacter species, biopsies of 10 other infants presenting with non inflammatory intrahepatic cholestasis were included as controls (7 males, 3 females, aged 43 to 514 days; median 80 days). Presence of Helicobacter species was tested by 16S rRNA PCR, using genus specific primers 5’ AAG CAT GAT GAA GCT GCT AGC TTG CTA G 3’ and 5’ GTG CTT ATT CST NAS AIA CGG TCA T 3’. Potential presence of polymerase inhibitors in hepatic tissue was tested with an H. pylori suspension control. When they were found, inhibitors were removed using low melting point agarose blocks.

Results: In spite of good amplification of H. pylori controls, no Helicobacter species could be found, neither in biliary atresia nor in other cholestasis syndrome of infancy.

Conclusion: none of Helicobacter species seem implied in the inflammation of biliary atresia. These species cannot be retained as aetiological agents of this affection. Moreover, it appears that none of the presently known Helicobacter species (bile-tolerant or not) can be considered as coloniser in infants with cholestasis. This suggest that entero-hepatic Helicobacter species are not common natural coloniser of normal gut flora during the first months of life.
12.13 Helicobacter spp. found in gallbladder stones

V.M. Govorun 1, K.T. Monynialiev 1, V.V. Cheleishva 1, V.A. Isakov 2
1 Research Institute Physics Chemistry Medicine, Moscow, Russian Federation, 2MONIKI, Moscow, Russian Federation

Bacteria can possibly take part in bile stones formation and its DNA is well preserved in crystal structure of bile stones.

Aim: to identify fragments of bacterial DNA from bile stones.

Methods: Bile stones were obtained from 7 patients during cholecystectomy, then washed in normal saline and dissolved in 1 ml of guanidine thiocyanate during 2 h at 55°C. Then DNA was extracted and amplification of fragment of 16S rRNA gene was performed with primers HPL 288 (5'-ACACGGTCCAGACTCCTACG-3') and HPL 636 (5'-CAGGATTGCTCTACACCA-3'). All 7 amplicons were cloned in plasmid vectors of several clones that was produced by cloning each initial amplicon: 231 (5'-GTITTTCCGATCGACGTCG-3') and 232 (5'-CAGGAGAAACAGCTATGAC-3').

Results: In all 7 stones bacterial DNA was identified. Sequence analysis has shown that in all cases at least DNA fragments of 3 different bacterial genera were found. They were presented by anaerobes (Bacteroides, Eubacterium, Porphyromonas, Prevotella spp.), by microaerophiles (Helicobacter, Campylobacter spp.) and facultative anaerobes (Enterobacter, Klebsiella, Corynebacterium spp.) (table).

DNA fragments of non-cultured proteobacteria were found in all stones, Campylobacter in 6/7, and Helicobacter in 5/7 stones. In 3/5 stones 16S rRNA gene fragments were verified as: in one stone as H. pylori, in another one as H. mustelae and H. musesae with equal homology of 89% and in the rest one as H. felis and H. salmonis with equal homology of 95%.

In conclusion, DNA fragments of Helicobacter spp. are the third most common among other bacterial genera that could be found in bile stones. Further studies are needed to confirm their role in bile stones formation.

12.14 Prevalence of Helicobacter pylori infection in chronic liver disease: systematic review and meta-analysis

J.P. Gibert, R. Moreno-Otero, J.M. Pajares. La Princesa University Hospital, Madrid, Spain

Aim: To systematically review and perform a meta-analysis of the prevalence of H. pylori (Hp) infection in chronic liver disease (CLD).

Methods: Bibliographical searches were performed in Medline until January 2002. Key words: pylori and [“chronic liver disease”] (CLD) or cirrhosis or hepatitis. In epidemiological studies, individual and mean Hp prevalence was calculated. In case-control studies, meta-analysis was performed combining the Odds Ratios (OR) of the individual studies in a global OR (Random-Effects-Model).

Results and Discussion: Mean Hp prevalence in 29 studies including 3,036 patients with CLD was 61%. From fourteen comparative studies, including 1,437 patients with CLD and 2,821 controls, mean Hp prevalence was 65% (95%CI=63-68%) and 53% (51-55%), respectively [(the OR for the effect of CLD on Hp infection was 1.21 (0.68-2.17)]. Results were heterogeneous and subanalyses were consequently performed. When only 6 studies including dyspeptic patients as controls were considered, Hp prevalence in CLD was lower than in controls [51% (47-55%) vs. 67% (63-71%), OR=0.14 (0.24-0.72)]. In contrast, when 7 studies including healthy asymptomatic controls (true controls) were evaluated, Hp prevalence was higher in CLD [68% (64-71%) vs. 48% (46-50%), OR=2.92 (1.47-4.33)]. When studies determining Hp infection by serology (in contrast with biopsy-based methods) were separately analyzed, a higher infection rate was demonstrated in CLD: 78% (75-81%) vs. 51% (49-53%) in controls (OR=3.06; 1.93-4.85). However, serology to Hp has been reported to have frequent false positive results in CLD. As almost all comparative studies including healthy controls used serological methods to detect Hp, and almost all case-control studies including dyspeptic controls performed biopsy-based methods, both subanalyses (depending on the type of control and the type of diagnostic method) are almost equivalent. Therefore, it cannot be concluded for sure whether the higher prevalence reported in CLD is real or consequence of the type of diagnostic method used.

12.15 Helicobacter pullorum and Mycobacterium paratuberculosis in inflammatory bowel disease

R. Andersson 1, I. Kornilovska 2, T. Melin 1, Å.H. Ljungb 1, 2Dept Med Microbiology, Dermatal, Infection, Lund University, Sweden, 3Dept Med Microbiology, Dermatology, Infection, Lund University, Sweden, 4Dept Medicine, Lund University, Sweden, 5Med Microbiol, Dermatal Infection, Lund, Sweden.

The aetiology of Morbus Crohn (CD) and Ucerative Colitis (UC) are still unknown. Attempts have been made to link a microbial agent to either UC or CD. Mycobacterium paratuberculosis and Listeria monocytogenes have been proposed to cause CD, and specific Escherichia coli strains UC but conclusive evidence are lacking. This may partly be due to their multifactorial aetiologies, as shown e.g. by the establishment of UC in mice which lack the interleukin-12 (IL-12) gene. Some of the more recently identified Helicobacter species, like H. pullorum, are classified as enterohelial with a possible relation to enteric and hepatobiliary diseases.

In the present study, sera from 24 patients with UC and 24 patients with CD since several years were analysed by immuno blot with antigens from H. pylori and H. pullorum (crossadsorbed with H. pylori) and by EIA with specific M. paratuberculosis antigen (Svanova AB, Uppsala, Sweden). Ileostomy fluid from some of the patients were analysed.

Conclusions: Patients with UC, and a subset of patients with CD had antibodies against H. pylori, in serum as well as ileostomy fluid, and 10/24 against H. pullorum, 5 of which had no significant antibody levels against H. pylori. Six of the 24 HD patients had antibodies against H. pylori, and 7/24 against H. pullorum, 4 of which were negative for H. pylori. With M. paratuberculosis EIA, antibodies were detected in 11/23 of CD patients and 4/23 of UC patients.

This is the first report of specific antibodies to M. paratuberculosis in patients from Nordic countries. The source of infection is unknown since domestic and wild animals do not harbour M. paratuberculosis in Sweden.

12.16 Is it really low the prevalence of Helicobacter pylori in patients with chronic liver disease?

A. Rodrigues Jr 1, M. Reber 2, 3, A.P. Ferrari 1, M.S. Cury 1, R. Artigiani Neto 1, M.M.M.B. Leite-Mor 1, E.R. Parise 1, 1Gastroenterology Section and Pathology Department - Federal University of São Paulo, São Paulo, Brazil, 2Gastroenterology Section and Pathology Department - Federal University of São Paulo, São Paulo, Brazil, 3Brasistol, São Paulo, Brazil

Patients with chronic liver disease (CLD) are believed to have a low prevalence of Helicobacter pylori (Hp) detected by rapid urease test, only performed in macosal fragments from antrum. In order to access the real prevalence of Hp in CLD, 53 patients with hepatic cirrhosis of different etiologies and portal hypertension were included in this study where Hp was detected through Urea Breath Test (UBT) and urease rapid test (URT) and histology (Giemsa-stained slides) in biopsy specimens from gastric antrum, body and fundus, collected during esophageal gastroduodenoscopy (EGD). UBT test was performed in a period of time no longer than four weeks after EGD. Patients receiving antibiotic therapy or taking proton pump inhibitors (PPI) or H2-receptor antagonists in the last eight weeks and patients with actual digestive bleeding were excluded.

The overall prevalence of Hp was 52.8% (28/53) according to URT, while this prevalence with UBT was 64% (32/53). In 4 patients with negative antrum UBT (16% of the total of such cases), Hp was found in the gastric antrum and fundus by UBT and/or histology. In two others Hp was the only positive test found.

In conclusion at least part of the believed low prevalence of Hp in CLD can be associated to the false positive antrum urease test used to identify the presence of the microorganism. Part of these patients present migration of Helicobacter pylori to other areas of gastric mucosa, that could be associated with the gastric hypochloridria frequently found in CLD or with the usual intake of gastric acid inhibitors by these patients.
Antibodies to Helicobacter pylori (Hp) have been found in 89% of 254 Italian hepatitis C (HCV) patients, all cirrhotic (1), however, only in 29 of 45 HCV Veteran population (64.4%) (2). It has also been suggested that differences in progression of chronic HCV may be due to a cofactor stemming from coinfection by Hp bacteria (3).

Object: To determine: 1) the prevalence of Hp in Israeli HCV patients; 2) are there similar factors; 3) if natural history of HCV differs in Hp-infected patients.

Methods: 72 serologic positive HCV patients were tested for HCV-RNA (by nucleic acid amplification), and genotype (by sequencing), Hp IgG (ELISA) and 2 of the following: rapid urease test, Giemsa staining, $^{13}$C-UBT. Liver disease was assessed as usual while sociodemographic survey was conducted.

Results: Cohort included 35 males, 37 females (mean age: 52.9 years; range 18-80). Six patients born in Israel: 66 immigrated from Eastern Europe (52), North Africa (6), and Asia (8). HCV-RNA was positive in 58/72 (80.5%) and Hp in 62/72 (86.2%) as overall and respectively in 11/16 (68.7%) and 51/56 (91.1%) in those aged less or more than 35 years. Eight, all Hp infected, had cirrhosis (11.1%), of which 3 decompensated.

Conclusions: In these 72 HCV patients, prevalence of Hp which rises with increasing age, is very high (86.2%); low socioeconomic level is a common factor; the 2 diseases do not interact, however, eradication of Hp is advised.

References