Prevalence of hepatitis B virus genotype in Saudi Arabia: a preliminary report

The partially double-stranded circular genome of the hepatitis B virus (HBV) consists of four overlapping genes encoding the viral envelope (pre-S and S), nucleocapsid (pre-core and core), polymerase with an error-prone reverse transcriptase activity, and X protein. Because of the spontaneous error rate of viral reverse transcriptase, the HBV genome evolves with an estimated rate of nucleotide substitution at 1.4–3.2 x 10^{-5}/site/year. Based on an inter-group divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into eight genotypes A–H. Different HBV genotypes are dominant in various parts of the world.1

Saudi Arabia has been considered an endemic area for HBV infection. A population-based survey of HBV markers among Saudi children showed an overall prevalence of HBsAg in 6.7% in 1992.2 By 1997, the prevalence of HBV infection in children had declined to 0.3%.3 The objective of this study was to determine HBV genotypes among Saudi Arabian nationals.

The study was conducted in 2006 at a tertiary referral center, which serves population groups resident in different regions of Saudi Arabia. Serum samples of 54 chronic hepatitis B patients, who were native Saudi Arabsians, were randomly selected from the serum sample pool in our Molecular diagnostic laboratory. The following parameters were determined for each patient: gender, age, alanine amino-transferase (ALT) level, hepatitis B e antigen (HBeAg) and HBV viral load. HBV viral load was measured using the Bayer Quantiplex bDNA system (Bayer Diagnostics, Tarrytown, NY) and quantification detection of this system ranged between 2000 copies/mL to 100 million copies/mL. HBsAg, HBeAg and ALT were measured using standard commercial assays.

HBV genotypes were determined using the INNO-LiPA methodology (LiPA, INNO-LiPA HBV genotyping assay, Innogenetics NV, Ghent, Belgium). This genotyping assay is a line probe assay designated to identify hepatitis B virus genotypes A–G by detection of type-specific sequences in the HBV-pol gene domain B to C. This method is based on the reverse hybridization principle. DNA was amplified by nested polymerase chain reaction (PCR) according to the instructions of the manufacturer to provide a biotinylated product. PCR products were visualized on a 1.5% agarose gel. The developed strips were read manually against a chart provided with the kit and the genotype was determined.

Fifty-four patients with mean age 39.6 (SD 16.2) years (40 men) were included; 34 (63%) of them were HBeAg antigen negative. Mean ALT was 144.2 IU/L (range 13–1135); forty-four patients had an ALT level >40 IU/L.

Forty-six patients (85.2%) had genotype D, 3 (5.6%) had genotype A, 3 (5.6%) had genotype E, and 2 patients (3.7%) had mixed genotype A/E. Univariate analysis showed no difference in genotypes between HBeAg-positive and -negative patients, or ALT level in relation with the HBV genotype (Table).

<table>
<thead>
<tr>
<th>Variable</th>
<th>D</th>
<th>A</th>
<th>E</th>
<th>A/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>46</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Gender Male (n)</td>
<td>34</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>39.5</td>
<td>30.3</td>
<td>42.7</td>
<td>50</td>
</tr>
<tr>
<td>ALT (mean) U/L</td>
<td>156</td>
<td>95</td>
<td>59</td>
<td>72</td>
</tr>
<tr>
<td>ALT (median) U/L</td>
<td>60.50</td>
<td>129</td>
<td>57</td>
<td>71.5</td>
</tr>
<tr>
<td>ALT &gt;40 U/L (n)</td>
<td>38</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HBV viral load*</td>
<td>1.7 X 10^6</td>
<td>1.6 X 10^4</td>
<td>2.4 X 10^3</td>
<td>1.0 X 10^a</td>
</tr>
<tr>
<td>HBeAg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive (n)</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-reactive (n)</td>
<td>28</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* number of HBV copies/mL

References

Table: Details of HBV genotype distribution with relation to gender, age, HBV viral load, ALT values and HBeAg status
Relationship between ABO blood groups and seroprevalence of Helicobacter pylori

Helicobacter pylori is the most common chronic bacterial infection in the world.1 We investigated the prevalence of H. pylori and its association with ABO/Rh blood groups in non-symptomatic young adults in Tehran, Iran.

Three hundred healthy young adults (96 men), who were referred to our clinic for a routine check-up, underwent testing for H. pylori antibody detection and ABO/Rh blood group typing. Subjects complaining of dyspepsia or any other gastrointestinal problems were excluded. Serum IgG against H. pylori infection was tested by a commercial ELISA kit (Trinity Biotech, USA), which had sensitivity of 96.4% and specificity of 96.1%. Patients were categorized into three groups: seronegative (IgG <0.9), equivocal (0.9< IgG<1.1) and seropositive (IgG ≥1.1). ABO/Rh blood group status was determined by monoclonal antibody testing (Blood Fractionation and Research Co, Iran).

The mean age of 300 subjects was 23.9 (3.8) years. The overall distribution of blood groups was: O (123; 41.0%), A (93; 31.0%), B (59; 19.7%) and AB (25; 8.3%). Two hundred sixty-eight individuals (89.3%) were Rh-negative. Four subjects with blood group O, 3 with blood group A and 4 with blood group B had equivocal IgG titers and were excluded from further analysis. One hundred and fifty-five individuals (53.6%) were H. pylori seropositive. The positivity rate was similar among men (n=93 [37.6%]) and women (n=102, [50%]; p=0.38). Seropositive individuals were older than seronegative ones (24.5 [3.7] years vs 23.3 [3.9] years; p=0.006). The frequency of H. pylori infection was similar among various blood groups: 51.2%, 49.5%, 52.5%, and 60.0% in blood groups O, A, B and AB (p=0.84), respectively. It was 53.9% (n=139) in the Rh (+) subgroup and 51.6% (n=16) in the Rh (−) subgroup (p=0.85).

Few studies have evaluated the epidemiology of H. pylori infection in Iran. A previous study in an Iranian province with high incidence of gastric carcinoma reported a prevalence of 89.2% in adults as assessed by the rapid urease test and/or histology.2 Another study demonstrated a 30.6%–47.5% seroprevalence in an Iranian population aged 6–20 years.3 We found a seropositivity of 51.7% among those 18–30 years of age.

Most studies have revealed no association between the ABO blood groups and H. pylori serological status either in healthy4–5 or in symptomatic subjects.6–9 In contrast, Kanbay et al observed that individuals with blood groups A and O were more prone to H. pylori infection, and those with AB blood group were less prone.10 We did not find any association between seropositivity and ABO/Rh antigens. Considering the known association between blood group O and peptic ulcer disease, it may be concluded that H. pylori and blood group antigens are independently linked to peptic ulcer disease.

Iman Khodarahmi, Armin Rashidi, Parvin Khodarahmi Tehran University of Medical Sciences; and Department of Physiology, Islamic Azad University, Parand Branch, Tehran, Iran

References

7. Sharara AI, Abdul-Baki H, ElHajj I, Kreidieh N, Kfoury Baz EM. Association of gastroduodenal disease phenotype with ABO blood group and *Helicobacter pylori* virulence-specific sero-
Diabetes mellitus after liver transplantation in Iranian patients

Diabetes mellitus (DM) is one of the complications occurring after liver transplantation (LT). Early studies had demonstrated decreased survival if transplant recipients developed diabetes.1,2 However, the impact of post-transplant DM on patients’ survival has remained controversial.1,3 The prevalence of and risk factors for DM after LT are not well understood.4 In one study, approximately 20% of LT recipients developed DM.5

The World Health Organization (WHO) and American Diabetes Association (ADA) have established criteria for the diagnosis of diabetes in the general population.6 Similar criteria are used for transplant recipients.7 We studied the prevalence of post-transplant DM and risk factors associated with this condition among Iranian liver transplant recipients.

One hundred and seventy patients (mean age at LT 31.4 [13.3] years; 108 men) who underwent LT from 1994 to 2006 in the Organ Transplantation Center of the Shiraz University of Medical Sciences, and survived at least 6 months after LT, were studied. Information about age, sex, body mass index (BMI), underlying liver disease, graft type, and immunosuppressive medications was recorded, and blood tests for diabetes were performed monthly. The current standard definition of diabetes as proposed by the international expert panel on new-onset diabetes after transplantation was applied. DM was defined by the reference value as two consecutive fasting blood sugar (FBS) values >126 mg/dL and/or the use of medications for DM.7

The data were analyzed by the Pearson and chi square tests using the SPSS software. A p value <0.05 was considered significant.

The mean duration of follow up was 25.9 (23.5) months (range 6–156). The main indications for LT were cryptogenic cirrhosis (n=42; 24.8%), hepatitis B infection (n=34; 20%), cirrhosis due to autoimmune hepatitis (n=30; 17.6%), Wilson’s disease (n=21; 12.4%), primary sclerosing cholangitis (n=18; 10.6%), hepatitis C infection (n=7; 4.1%), and primary biliary cirrhosis (n=5; 2.9%). All patients received immunosuppression with prednisolone 1 mg/kg/day, mycophenolate mofetil 10–40 mg/kg/day, and cyclosporin 2–5 mg/kg/day (n=132; 77.6%) or tacrolimus 0.05–0.15 mg/kg/day (n=38; 22.4%); 20 patients (11.8%) receiving cyclosporin were switched to tacrolimus.

Out of 170 patients, 44 patients (25.8%; 33 men, p<0.05) developed DM. Hepatitis B infection and older age were significantly associated with the development of diabetes after LT (p<0.05), while hepatitis C infection and type of immunosuppressive medications were not predictors for post-LT DM (Table).

Further, post-transplant DM was significantly correlated with obesity (p=0.048). The mean BMI in patients with DM was 22.9 (4.5) and in non-DM patients it was 21.3 (4.9). Also, family history of DM was significantly correlated with the development of DM after LT (p=0.002).

Post-transplant DM occurred in 43 recipients of whole grafts from deceased donors, and 1 case occurred in a recipient of a live-related partial LT.

We treated all post-transplant diabetic patients with insulin and all of them are under control.

Identification of risk factors for development of DM after LT is very important for better surveillance of patients. These risk factors may vary in different populations.

The prevalence of DM after LT was 25.9% in our study. Male gender was identified as a risk factor for the development of post-transplant DM. This finding is consistent with those from a number of other studies, suggesting that gender is an independent risk factor for post-transplant DM.2,4

Several studies have demonstrated that HCV infection may be associated with the development of post-transplant DM.1,3,8 In contrast, in our study, HBV, but not HCV

<table>
<thead>
<tr>
<th>Gender M:F</th>
<th>Developed DM</th>
<th>Did not develop DM</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>33:11</td>
<td>75:51</td>
<td></td>
<td>0.047</td>
</tr>
<tr>
<td>Mean age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.7 (12.5)</td>
<td>28.8 (12.7)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>HBV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/44</td>
<td>19/126</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>HCV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/44</td>
<td>5/126</td>
<td></td>
<td>0.425</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34/44</td>
<td>98/126</td>
<td></td>
<td>0.549</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/44</td>
<td>30/126</td>
<td></td>
<td>0.575</td>
</tr>
</tbody>
</table>
infection, was a risk factor for post-transplant DM. This difference may be due to a larger number of cases who underwent transplantation for HBV cirrhosis than HCV cirrhosis in our center, but this needs further investigation in larger centers.

The data on the effect of calcineurin inhibitors (cyclosporin or tacrolimus) on the development of DM in liver transplant recipients have been equally inconsistent, and most studies have not found either calcineurin inhibitor more diabetogenic.1,2,3,8,9

This may be due to different definitions of DM, different population characteristics (such as BMI), and variable doses and combinations of immunosuppressive medications. Some studies reported that tacrolimus is more diabetogenic than cyclosporin,7,10 but we did not reach the same conclusion. The reason for this variability may include the use of lower doses of tacrolimus in our center as we do not regularly monitor serum levels of tacrolimus in our center.

We conclude that DM is prevalent after LT, and all transplant recipients should be screened for DM, particularly men, those with HBV infection, obesity, and a family history of DM.

Seyed Mohsen Dehghani, a,b,d Seyed Ali Reza Taghavi, a,c,d Mohammad Hadi Imanieh, b,h,d Siavash Gholami, a Ahad Eshraghian, d Hamed Jalaean, a

a Shiraz Organ Transplantation Center, Departments of
bPediatric Gastroenterology, cInternal Medicine, and
dGastroenterohepatology Research Center, Nemaze Hospital, Shiraz University of Medical Sciences
Shiraz, Iran

References


Correspondence to: Dr Dehghani, Assistant Professor of Pediatric Gastroenterology, Gastroenterohepatology Research Center, Nemaze Hospital, Shiraz University of Medical Sciences, Shiraz, 71937-11351, Iran. Fax: 98 (711) 6265024
E-mail: dehghanism@sums.ac.ir

Acknowledgments: We thank Professor GH Amirhakimi for editorial assistance and Dr. Shayan at Center for Development of Clinical Research of Nemaze Hospital for statistical assistance.

Rectal perforation after injection sclerotherapy for hemorrhoids: Case report

Injection sclerotherapy is a widely practised, effective, and generally safe method of treating first- and second-degree hemorrhoids. We describe possibly the first reported case of rectal perforation after injection sclerotherapy.

A 21-year-old man had symptoms of bleeding from hemorrhoids for 7 months for which he received injection sclerotherapy, using 5% phenol in almond oil from a private practitioner. Within the next 48 hours he developed pain in the perianal region, which increased on defecation, along with high-grade fever and vomiting. The perianal pain became progressively more severe over the next 5–6 days and from the 7th day onwards the patient also developed bleeding per rectum and foul-smelling discharge from the perianal area.

On general physical examination he was febrile, dehydrated, and had a pulse rate of 100/min. On local examination, there was blood at the anal opening and multiple pilonidal sinuses along the natal cleft which emitted a foul-smelling discharge. Digital rectal examination (DRE) revealed two irregular mucosal defects, one 3 cm × 3 cm at 9 o’clock position around 3 cm from the anal verge, and another 7 cm × 5 cm at 4 o’clock position, leading into the pararectal space around 3.5 cm from anal verge. On proctoscopy, the findings on DRE were confirmed.

Laboratory investigations showed hemoglobin 11.5 g/dL and WBC count 12 × 10³/mL, with neutrophilia. Blood urea and blood sugar were within normal limits. X-ray of the soft tissue did not reveal any gas in the subcutaneous plane. An MRI of the pelvis (with gadolinium) revealed anorectal wall perforation at 5 o’clock position and a collection in the left ishiorectal fossa with an air-fluid level (Figure).
The patient was given intravenous antibiotics (ceftriaxone 1 g q 12 hourly and metronidazole 500 mg q 8 hourly). At surgery, about 25–30 ml of purulent fluid was drained from the pararectal space. Intraoperative examination revealed rectal ulceration (3 cm × 3 cm) at 9 o’clock and perforation of the left lateral rectal wall (7 cm × 5 cm) at 3 o’clock position, which extended into the left pararectal space. The sigmoid colon and rectum were copiously lavaged with antibiotic solution and a proximal defunctioning sigmoid colostomy was done. The associated pilonidal sinuses were laid open.

The postoperative course was uneventful. Clinical examination and distal cologram revealed that at 16 weeks post surgery, rectal injuries had completely healed. The colostomy was closed 5 months after the initial surgery.

Many different substances have been used for injection sclerotherapy but the most popular sclerosant is 5% phenol in almond oil. Retroperitoneal and subcutaneous abscesses, injection ulcers, necrotizing fasciitis, anal stricture, prostatic abscess and fistula, and superficial necrosis following sclerotherapy have been described previously. In the present case, the patient developed severe reaction to the sclerosant in the form of fever and local sepsis, which caused necrosis of the wall possibly due to impairment of vascularity. To the best of our knowledge, no similar complication has been described before.5

References

Correspondence to: Professor Lal, B-90, Swasthya Vihar, Delhi 110 092, India. Fax: 91 (11) 2323 5574
E-mail: drplal@vsnl.net, drplal@bol.net.in

Erratum

In the above article, the figure is incorrect. The correct Figure is published here.