Japanese Journal of Gastroenterology and Hepatology

Research Article

# Epidemiological Characteristics of De Novo Hepatitis B Infection in Liver Transplant Recipients - An Experience from a Tertiary Care Centre in Qatar

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Received: 09 June 2020

Accepted: 20 June 2020

Published: 22 June 2020

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## 1. Abstract

**1.1. Background:** Emergence of Hepatitis B Surface Antigen (HBsAg) in a patient with previously negative Hepatitis B Virus (HBV) serology post Orthotropic Liver Transplant (OTLX) is known as De-novo Hepatitis B (DNHB).

**1.2. Aim:** To study the clinical profile and epidemiology of patients with DNHB in Qatar.

**1.3. Patients and Methods:** This descriptive epidemiological study was done by retrospectively reviewing records of 159 post-OTLX patients. Baseline epidemiological characteristics of DNHB cases were analysed statistically using the chi-square test and Kaplan-Meier Curve.

**1.4. Results:** The overall incidence of DNHB was 10.7%, with transplants in China having significantly higher incidence compared to transplants from all other countries. The mortality rate was 23.5% in DNHB cases compared to 2.8% in non-DNHB. 67% of patients survived at least 64 months after diagnosis of DNHB. 5-year survival did not vary significantly between those with DNHB and those without.

**1.5. Discussion and Conclusion:** OTLX in centers selecting donors liberally without screening for HBV poses a risk of DNHB. We recommend having protective levels of Hepatitis B Surface Antibodies (HBsAb) before OTLX. Prophylactic antiviral treatment should be considered until peri-operative HBV transmission has been excluded by screening hepatic tissue for HBV DNA.

**2. Keywords:** Hepatitis B; Liver Transplantation; Antiviral agents; De novo B; Immunosuppression

## 3. Introduction

The appearance of Hepatitis B Surface Antigen (HBsAg) in a patient with previously negative hepatitis B serology post orthotropic liver transplantation (OTLX) is known as De novo Hepatitis B (DNHB). The aetiology of DNHB may be a transfusion of blood product, infection in donor liver or occult pre-transplant infection in the recipient [1]. Internationally, there is an increasing demand and burden of shortage for donor livers, which is more pronounced in the Middle Eastern countries due to the high prevalence of hepatitis B and C. Hence, liver from hepatitis B core antibody (HBcAb) positive donors is being increasingly used, which however has the risk of higher hepatitis B virus (HBV) reactivation post-OTLX due to immunosuppressive therapy [2]. Many international studies have shown that HBcAb positive grafts can be donated safely, and adequate antiviral prophylaxis decreases post-OTLX reactivation of HBV significantly [3-5]. Risk of DNHB post OTLX have been reported as highly variable, ranging from 16- 88% in international studies [6, 7] and 5-7% in studies from the middle east region [8]. However, there are no studies available indexing the clinical profile and epidemiology of patients with DNHB post-OTLX from the state of Qatar.

## 4. Patients and Methods

A retrospective analysis of a prospectively maintained medical record database was conducted in a cohort of 159 patients who were citizens or residents in Qatar, underwent transplantation in various parts of the world, and were followed up in Liver Transplantation Clinic at Hamad Medical Corporation, Doha, Qatar. The period of transplantation was from 1986 to 2018, and the period of follow-up evaluated was from 2011 May to 2018 May. Liver transplantation program in Qatar was officially started in 2011 and the first transplantation was performed in December 2011. Patients from Qatar who needed liver transplantation before 2011 had to approach centers in various parts of the world. Even after 2011, many patients had to travel abroad for transplantation because of the limited capacity of local transplant facilities, lack of compatible donors, and long waiting lists. However, most of these patients had post-operative follow up in Liver Transplantation Clinic at Hamad Medical Corporation, which was started in 2008. Patients fit for liver transplantation, were evaluated by a multi-disciplinary team involving experts from hepatobiliary surgery, gastroenterology, infectious diseases and radiology. A complete set of investigations including blood type, antibody screen, viral hepatitis profile, serum hepatitis C virus (HCV)- ribonucleic acid (RNA) titres, HCV genotype, hepatitis B virus (HBV) - deoxy-ribonucleic acid (DNA), hepatitis B envelope antigen (HBeAg) and antibody, autoimmune markers, iron and copper studies, immune protein electrophoresis, tumour markers, complete blood count, complete metabolic panel, coagulation studies, fibrinogen levels, cytomegalovirus status, quantiferon testing, varicella titters, and cryptococcal antibodies were performed pre-operatively. They were vaccinated against hepatitis A and B, if there was no evidence of prior immunity. Our program recommended pre-transplant vaccination, however for those who underwent transplantation without vaccination or those who failed to achieve protective antibody level, post-transplant vaccination was administered. A protective level of hepatitis B antibody titre was defined as ≥ 10 IU/L. Annual influenza and pneumococcal vaccines were administered to all patients. The availability of donor information was limited as most patients (138 out of 159) underwent transplantation in centres abroad (70 in China, 20 in USA, 16 in Egypt, 10 in India, 9 in UK, 2 each in Austria, Iran and 1 each in Turkey, Belgium, Canada, Philippines, France, Jordan and Saudi. Hence, the donor profile was excluded from the scope of this study. The most common indication for liver transplantation was hepatitis C virus-related cirrhosis in 95 patients, followed by HBV liver disease in 13, alcoholic hepatitis in 12, cryptogenic liver cirrhosis in 15, autoimmune hepatitis in 5, Wilson's disease in 4, non-alcoholic steatohepatitis in 3, primary biliary cirrhosis and hepatocellular carcinoma in 2 patients and Budd-Chiari syndrome, Carolis disease, cholangiocarcinoma, cholestatic hepatitis, chronic graft dysfunction, congenital hepatic fibrosis, biliary atresia and primary hyper-oxaluria in 1 patient each.

Post-operatively, they were seen every month during the first 6 months and then every 2 months. During each visit, serum transaminase levels, HBsAb titre, and liver imaging were done. Biopsy of the transplanted liver was done annually or if any changes in serum transaminase levels were detected. The DNHB patients in this study were defined as those who were tested negative to HBsAg before transplantation but positive to the same at any time after the procedure, provided other possible modes of transmission like blood transfusion, contact with hepatitis B positive individuals, dental procedures, tattooing, and hijjama were ruled out. Patients who were DNHB positive were evaluated further with HBV DNA titre, elastography, and liver biopsy to assess the grade of hepatitis and stage of fibrosis. They were offered multiple anti-viral agents, including lamivudine, adefovir, entecavir, and tenofovir. Hepatitis B immunoglobulin was not used in any of the cases. We identified the patients from the prospectively maintained records in the Liver Transplant Clinic. Each patient was given a Unique Identification Number (UIN) after concealing their personal information. The clinical records of these patients were thoroughly reviewed in the electronic medical record system, and the following information was collected: age, sex, nationality, blood group, date and indication of transplant, the country in which transplant was done, immunosuppression received and hepatitis serological status of the patient before and after transplant. Based on the serological status, DNHB positive cases were identified. The patients were divided into infected and non-infected groups. Subjects in the infected group were further evaluated to find out the date of infection, HBV-DNA level at the time of infection, transaminase and bilirubin levels, the grade of hepatitis, and stage of fibrosis. From this data, time taken for HBsAg, HBeAg, and HBV DNA to become undetectable was calculated.

## 5. Statistical Analysis

Descriptive statistics were used to summarize demographic, clinical, infection biomarkers, and other related parameters and characteristics of the participants. The normally distributed data and results were reported with mean and Standard Deviation (SD); the remaining results were reported with median and interquartile range (IQR). Categorical data were summarized using frequencies and percentages. Preliminary analyses were conducted to examine the distribution of the data variables using the Kolmogorov-Smirnov test. Associations between two or more qualitative variables were assessed using the Chi-square ($\chi^2$) test, Fisher Exact, or Yates corrected Chi-square tests as appropriate. Quantitative data and outcome measures between the two independent groups were analyzed using unpaired't' test (or Mann Whitney U test for skewed data). Survival functions were estimated with the Kaplan–Meier survival curve method. Additionally, the mean duration of follow-up with 1 and 5-year survival proportions was also calculated, and for those who did not survive, the cause of mortality was recorded. This was further classified into liver-related and unrelated causes of mortality and included in sta-

tistical analysis. Pictorial presentations of the key results were made using appropriate statistical graphs. All P values presented were two-tailed, and P values <0.05 were considered as statistically significant. All Statistical analyses were done using statistical packages SPSS 22.0 (SPSS Inc. Chicago, IL) and Epi-info (Centers for Disease Control and Prevention, Atlanta, GA) software.

## 6. Results

One hundred and fifty-nine patients from Qatar underwent liver transplantation during the period from 1986 to 2019. Among them, 65% were males, and 35% were females. The mean age of the patients was 57.4 ± 12.5 years. These patients were of various nationalities: Bahrain (1), Bangladesh (2), Britain (2), Canada (1), Egypt (57), Philippines (1), India (5), Iran (1), Iraq (1/), Jordan (3), Lebanon (1), Oman (1), Pakistan (7), Portugal (1), Qatar (64), Saudi Arabia (3), Somalia (2), Sudan (2), Syria (1) and Yemen (3). Most of them underwent OTLX in China. A flowchart describing the outcome in these patients is shown in (Figure 1).

According to the definition, 17 patients were deemed to be DNHB positive. The overall incidence of DNHB was 10.7%. 16 out of these 17 patients underwent OTLX in China, and one in Qatar. OTLX in China had a higher incidence of DNHB compared to all other countries [22.6% v/s 1.12%, Relative Risk (RR) = 20.34; CI 2.7, 149.7]. The mean age of DNHB cases was 56, and male: female ratio was 2:3. Most of them were Qataris (10/17), followed by Egyptians (6/17) and Yemeni (1/17). The most common indication for liver transplantation in DNHB cases was hepatitis C in 10 patients, followed by autoimmune hepatitis in 2, cholestatic hepatitis in 1, and cryptogenic liver cirrhosis in 3. One patient had both HCV and alcoholic liver disease. Six of them had A (+) ve blood group, six O (+) ve, one B (+)ve, and one B (–)ve. The mean duration of follow-up was 3041 days, and the mean onset of infection was 1395 days after OTLX. Average HBsAb before the transplant was 24, with five of them having HBsAb >10. HBsAb titre > 10 IU/L and > 100 IU/L were present in 29.4% and 11.7% of DNHB cases respectively. None of them had HBsAb titer>1000 IU/L before transplantation. Only one patient had HBsAb levels of more than 1000 IU/L, whereas all other patients had levels < 10 IU/L post-transplantation. Two of them had HBcAb positive before transplant. Four out of nine patients with liver biopsy had hepatitis grade II and fibrosis stage II according to the Scheuer score, with a mean elastography score of 6.7 ± 1.9. The complete epidemiological profile of infected cases is given in (Table 1), and a complete serological profile before and after transplant is given in (Table 2).

Most of the patients were treated with Entecavir or Tenofovir. DNHB cases had a mortality rate of 23.5% compared to 2.8% for non-DNHB cases (RR 8.4, 95% CI 2.3 to 30.4, P = 0.0002). However, 67% of patients survived at least 64 months after the diagnosis of DNHB (Figure 2). 93.8% of those with DNHB survived five years after OTLX compared to 96.4% of those without (P = 0.605).

Eight out of the total of 159 patients passed away during the period of follow-up, and six were due to liver-related causes. There were three liver-related deaths in both infected and non-infected groups. Various factors that might affect the mortality rate among DNHB cases were statistically analyzed and presented in (Table 3).

Comparative statistical findings indicate that both the mean/median AST and ALT were significantly higher in patients who died compared to those who survived (P < 0.05) (Figure 3). Similarly, mortality was found to be considerably higher in patients with a severe degree of fibrosis compared to mild to moderate degrees of fibrosis (P < 0.05). Other characteristics such as age, gender, nationality, blood group, time from OTLX to the detection of DNHB, and antiviral treatment did not show any significant association with mortality. Moreover, younger age and female gender were positively associated with higher proportions of survival; however, these differences were not statistically significant (P > 0.05), as shown in (Table 3). A scatter diagram describing the linear relationship of total bilirubin, AST, and ALT with the time of onset of infection is shown in figure 4 and a ROC curve to determine optimal cut-off values for bilirubin, AST and ALT in predicting mortality is shown in (Figure 5).

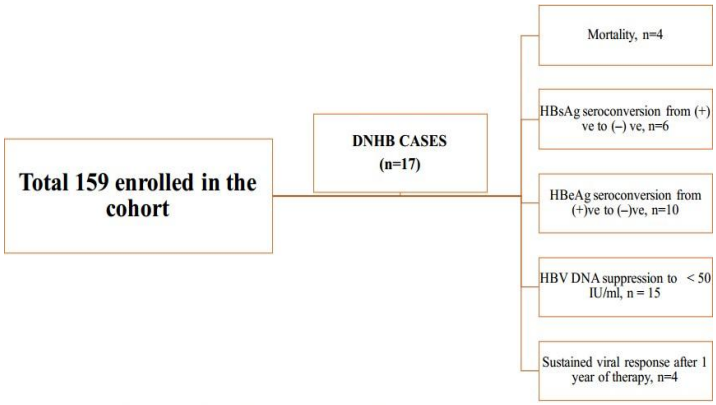
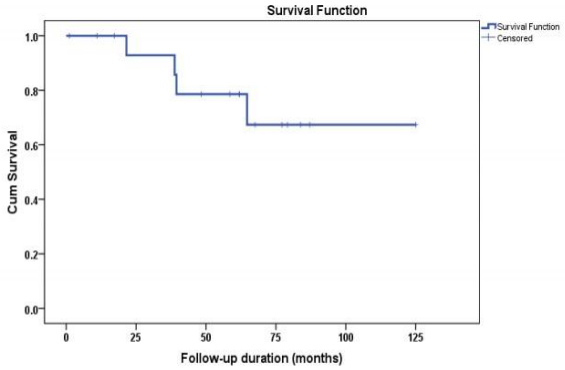



**Figure 1:** Flow chart describing the final out-comes of DNHB cases.




Kaplan Meier survival curve presented in Figure 3 shows that 67.3% of patients survived at least 64.7 months after diagnosis of DNHB. Longer follow-up is needed to estimate exact median survival time on such cases. Furthermore, DNHB cases had a significantly higher mortality rate of 23.5% compared to 2.8% in non-DNHB cases (RR 8.4, 95%CI 2.3 to 30.4, P = 0.0002).

**Figure 2:** Kaplan Meier Survival curve showing estimation of overall survival time.

**Table 1:** Epidemiological profile of patients diagnosed with de novo hepatitis B

| No. | Sex/ Age | Indication For OTLX* | Type of LT | Immuno-suppression | Onset of DNHB (Mo.) | Rx | F/U (Mo.) | TB | AST | ALT | Liver Biopsy** | | Elastography# | Survival$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Hepatitis | Fibrosis | | |
| 1 | 48/M | HCV | DDLT | Tac+MMF | 9 | TDF | 10.9 | 10 | 18 | 74 | III | IV | 4.51 | + |
| 2 | 38/F | CLD | DDLT | Tac+MMF | 13.7 | ETV | 16.9 | 16 | 57 | 53 | I | I | 3.6 | + |
| 3 | 50/M | HCV | DDLT | Tac+MMF | 15.9 | 3TC | 61.1 | 14 | 22 | 24 | NA | NA | NA | + |
| 4 | 56/F | HCV | DDLT | Tac+MMF | 21.9 | ETV | 78 | 16 | 36 | 35 | NA | NA | 7.78 | + |
| 5 | 54/M | HCV | DDLT | Tac+MMF | 23.4 | ETV | 82.8 | 8 | 10 | 20 | II | II | 6.44 | + |
| 6 | 57/M | ALD+ HCV | DDLT | Tac+MMF | 25.9 | ETV | 63.9 | 8 | 154 | 169 | III | III | NA | - |
| 7 | 57/M | CC | DDLT | Tac+MMF | 26.8 | ETV + TDF | 21.2 | 114 | 483 | 222 | NA | NA | NA | - |
| 8 | 60/F | HCV | DDLT | Tac+MMF | 26.8 | ETV | 61.2 | 9 | 115 | 106 | I | I | 5.61 | + |
| 9 | 76/M | HCV | DDLT | Tac+MMF | 38.1 | ETV | 38.9 | 31 | 42 | 62 | NA | NA | NA | - |
| 10 | 54/F | HCV | DDLT | Tac+MMF | 49.9 | ETV + 3TC | 76.1 | 6 | 20 | 17 | II | II | 6.91 | + |
| 11 | 53/M | HCV | DDLT | Tac+MMF | 50.1 | TDF | 85.9 | 33 | 39 | 60 | II | II | 8.18 | + |
| 12 | 61/M | HCV | DDLT | Tac+MMF | 51.1 | ETV | 38 | 13 | 77 | 89 | II | II | NA | - |
| 13 | 74/F | CC | DDLT | Tac+MMF | 54.5 | TDF | 66.6 | 21 | 29 | 24 | NA | NA | 7.42 | + |
| 14 | 38/M | CC | DDLT | Tac | 60.1 | ETV | 123.3 | 11 | 94 | 140 | I | I | NA | + |
| 15 | 67/F | HCV | DDLT | Tac+MMF | 79.9 | Nil | 1.1 | 21 | 98 | 92 | NA | NA | 10.14 | + |
| 16 | 44/M | AI | DDLT | Tac | 83.4 | TDF | 57.7 | 10 | 27 | 41 | NA | NA | NA | + |
| 17 | 65/M | AI | DDLT | Tac+MMF | 92.4 | ETV+ TDF | 47.7 | 11 | 46 | 78 | NA | NA | 6.28 | + |

Table 1 describes the complete epidemiological profile of all patients diagnosed with de novo hepatitis B post liver transplant.
*Reason for undergoing liver transplantation: HCV – Hepatitis C Virus related cirrhosis, CLD – Cholestatic liver disease, ALD – Alcoholic liver disease, AI – Auto-Immune liver cirrhosis, CC – Cryptogenic liver cirrhosis.
DDLT – Dead Donor Liver Transplant, Tac- Tacrolimus, MMF- Mycophenolate Mofetil, Mo-Months, TDF- Tenofovir Disoproxil Fumarate, ETV- Entecavir, 3TC- Lamivudine, TB – Total Bilirubin in µmol/L, AST- Aspartate Amino Transferase in U/L, ALT – Alanine Amino Transferase in U/L
**Grade of hepatitis and stage of fibrosis is determined based on the Scheuer scoring system
# Mean elastography score of liver determined by ultrasound examination
$Patients who survived till end of the study are represented by '+' and those who died during the period of follow up are represented by '-'

**Table 2:** Timeline of hepatitis B viral seroconversion in 17 DNHB cases

| PT. NO | SEROLOGICAL MARKERS | BEFORE OTLX | AT SEROCONVERSION | | MOST RECENT | MORTALITY$ |
|---|---|---|---|---|---|---|
| | | | TIME(Months)* | Markers | | |
| 1 | HbsAg/Ab | -/- | 9 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | +/- | |
| | HBV DNA | Undetectable | | 170000000 | 42 | |
| 2 | HbsAg/Ab | -/- | 13.7 | +/- | +/- | - |
| | HBcAb | - | | - | - | |
| | HBeAg/Ab | -/- | | +/- | +/- | |
| | HBV DNA | | | >110000000 | <20 | |
| 3 | HbsAg/Ab | -/- | 19.9 | +/- | -/- | - |
| | HBcAb | + | | + | + | |
| | HBeAg/Ab | -/- | | -/- | -/- | |
| | HBV DNA | Undetectable | | Undetectable | Undetectable | |
| 4 | HbsAg/Ab | -/+ | 21.9 | +/- | -/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 110000000 | Undetectable | |
| 5 | HbsAg/Ab | -/- | 23.4 | +/- | -/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 87451944 | Undetectable | |
| 6 | HbsAg/Ab | -/- | 25.9 | +/- | -/- | + |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 49658203 | Undetectable | |
| 7 | HbsAg/Ab | -/- | 26.8 | +/- | +/- | + |
| | HBcAb | - | | - | - | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | >110000000 | 21 | |
| 8 | HbsAg/Ab | -/+ | 26.8 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | Undetectable | Undetectable | |
| 9 | HbsAg/Ab | -/- | 38.1 | +/- | +/- | + |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | -/- | -/- | |
| | HBV DNA | Undetectable | | <60 | 412 | |

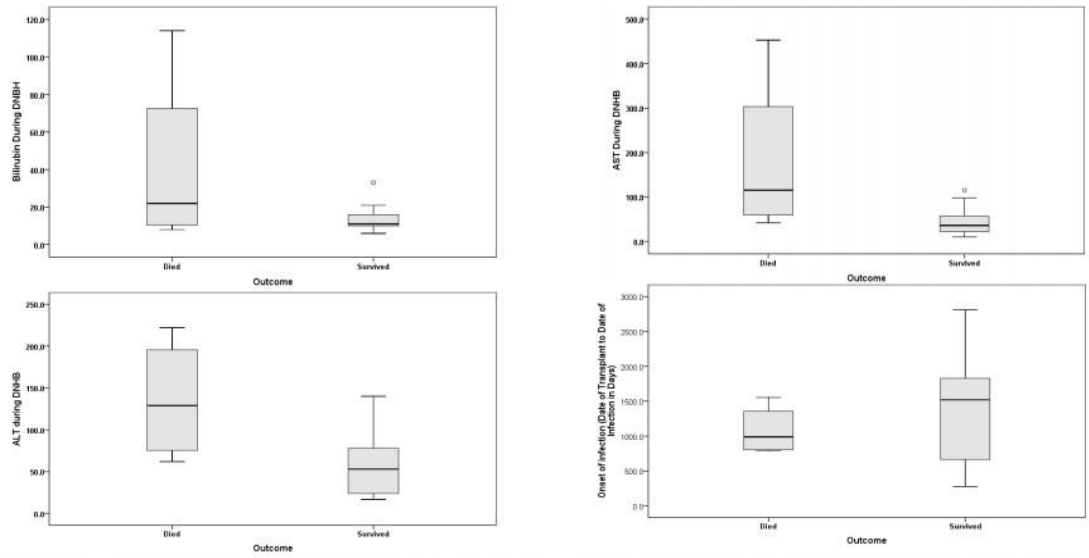
| | | | | | | |
|---|---|---|---|---|---|---|
| 10 | HbsAg/Ab | -/- | 49.9 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 57818 | <20 | |
| 11 | HbsAg/Ab | -/- | 50.1 | +/- | -/- | - |
| | HBcAb | + | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 25031310 | Undetectable | |
| 12 | HbsAg/Ab | -/- | 51.1 | +/- | +/- | + |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | >110000000 | Undetectable | |
| 13 | HbsAg/Ab | -/+ | 54.5 | +/+ | -/+ | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 1204431 | Undetectable | |
| 14 | HbsAg/Ab | -/+ | 60.1 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | +/- | |
| | HBV DNA | Undetectable | | >110000000 | 58 | |
| 15 | HbsAg/Ab | -/- | 79.9 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | +/- | |
| | HBV DNA | Undetectable | | Undetectable | Undetectable | |
| 16 | HbsAg/Ab | -/- | 83.4 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | +/- | |
| | HBV DNA | Undetectable | | 1310 | Undetectable | |
| 17 | HbsAg/Ab | -/+ | 92.4 | +/- | +/- | - |
| | HBcAb | - | | + | + | |
| | HBeAg/Ab | -/- | | +/- | -/+ | |
| | HBV DNA | Undetectable | | 156910859 | Undetectable | |

Table 2 shows the complete serological profile of patients diagnosed with DNHB, before and after transplant.
HBV DNA is expressed as international units per millilitre
*Number of months after liver transplantation, for HBsAg to seroconvert from -ve to +ve
$Patients who survived till end of the study are represented by '+' and those who died during the period of follow up are represented by '-'

**Table 3:** Association of demographic and various other parameters with mortality

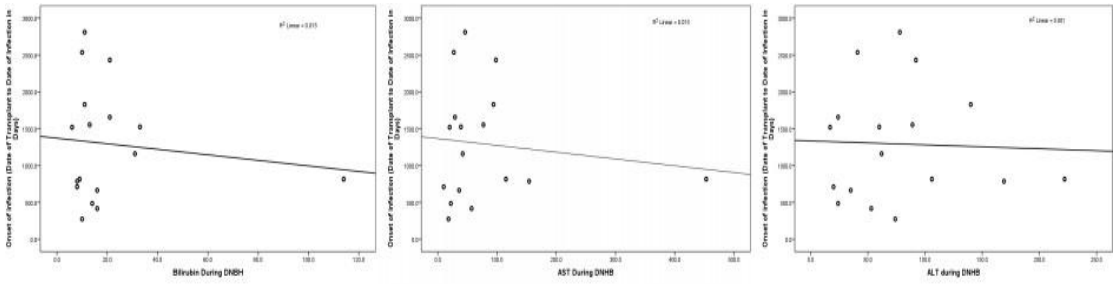
| | | | | | |
|---|---|---|---|---|---|
| No. of Patients | | 13 | 4 | | |
| Age (in years) | | 53.92±10.78 | 62.75±9.03 | 1.47 | 0.16 |
| Gender | Male | 7 (63.6%) | 4 (36.4%) | 2.85 | 0.091 |
| | Female | 6 (100%) | 0 (0%) | | |
| Nationality | Qatari | 7 (70%) | 3 (30%) | 0.7 | 0.706 |
| | Egyptian | 5 (83.3%) | 1 (16.7%) | | |
| | Yemeni | 1 (100%) | 0 (0%) | | |
| Blood Group | A (+)ve | 4 (66.7%) | 2 (33.3%) | | |
| | B (+)ve | 4 (100%) | 0 (0%) | 2.18 | 0.536 |
| | O (+)ve | 4 (66.7%) | 2 (33.3%) | | |
| | B (-)ve | 1 (100%) | 0 (0%) | | |
| Country of Transplant | China | 12 (75%) | 4 (25%) | 0.33 | 0.998 |
| | Qatar | 1 (100%) | 0 (0%) | | |
| Indication for Transplant | HCV Cirrhosis | 8 (61.5%) | 3 (75%) | 1.17 | 0.76 |
| | Cryptogenic Liver Cirrhosis | 2 (66.7%) | 1 (33.3) | | |
| | Autoimmune Hepatitis | 2 (100%) | 0 (0%) | | |
| | Cholestatic Liver Disease | 1 (100%) | 0 (0%) | | |
| Number of Patients with HbsAb titre >10 IU/L before OTLX | | 5 (38.4) | 0 (0%) | 2.18 | 0.416 |
| Elapse time from OTLX to DNHB | | 44.7±28.5 | 35.5±11.8 | 0.62 | 0.546 |
| LFT at detection of DNHB | Total bilirubin | 14.3±7.3 (median 11, range 6 to 33) | 41.5±49.3 (median 22, range 8 to 114) | 18.5 | 0.394 |
| | AST | 47.0±34.2(median 36, range 10 to 115) | 181.5±186.9 (median 115, range 42 to 453) | 8 | 0.042 |
| | ALT | 58.8±37.8 (median 53, range 17 to 140) | 135.5±73.4 (median 129, range 62 to 222) | 80 | 0.041 |
| Degree of Fibrosis | Normal- Mild | 4 (100%) | 0 (0%) | 6.63 | 0.036 |
| | Mild-Moderate | 9 (90%) | 1 (10%) | | |
| | Severe | 0 (0%) | 3 (100%) | | |
| Antiviral Treatment | Lamivudine | 1 (100%) | 0 (0%) | 1.32 | 0.517 |
| | Entecavir | 7 (71%) | 4 (29%) | | |
| | Tenofovir | 5 (100%) | 0 (0%) | | |

Table 3 compares various demographic, clinical, serological and radiological features between patients with DNHB who survived and who died.
*This group represents the patients with DNHB who survived till the end of follow-up
**This group represents the patient with DNHB who died during the period of follow-up
***Chi-square Fisher Exact test was used for 2x2 tables and for tables more than 2x2, Yates corrected Chi-square test was applied in case of small cell frequencies(50% or more cells have expected frequencies <5), whereas quantitative outcome measures were compared by using t test or Mann Whitney U test (for skewed data) as appropriate to compute respective statistical P-value.



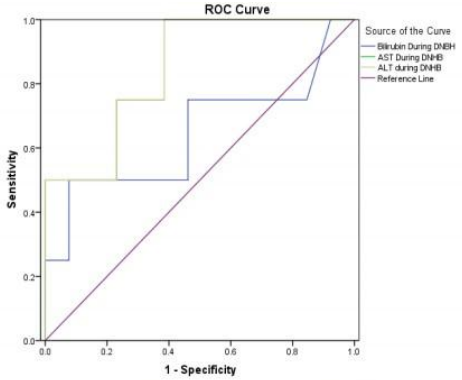

Box plot depicts the distribution of total bilirubin, AST*, ALT** and onset of infection in both groups (mortality and survival) and it clearly shows that the median and inter quartile range (IQR) values of these parameters are significantly higher in patients who died compared to those who survived.
*AST – Aspartate Amino Transferase, **ALT – Alanine Amino Transferase

**Figure 3:** Box plot depicting distribution of Bilirubin, AST*, ALT** and Onset of infection in demised and survived groups.




Scatter diagram depicting the linear relationship of Total Bilirubin, AST* and ALT** with onset of infection and it is evident from figure 4 that all three LFT parameters were inversely but weakly correlated with onset of infection (Pearson (r) range: -0.124 to -0.039; P>0.05).
*AST – Aspartate Amino Transferase, **ALT – Alanine Amino Transferase

**Figure4:**Scatterdiagram showinglinearrelationshipbetween A)TotalBilirubinandonsetofinfection,B)AST*andonsetofinfectionandC) ALT**andonsetofinfection.




**Statistical Method-ROC:** The receiver operating characteristic (ROC) curve was calculated using significant predictors AST, ALT and total bilirubin to derive best suitable cut-off values and to assess model discrimination and predictive accuracy. The ROC curves provide a comprehensive and visually attractive way to summarize the accuracy of predictions. The ROC curve shows the trade-off between sensitivity and specificity and is a better method to detect the performance of a developed test, which classifies subjects into two categories such as survival and mortality.
**Result Interpretation:** The discriminative ability of the model including predictors AST, ALT and total bilirubin was found to be good with an area under the ROC curve value of 0.846 (AST), 0.846 (ALT) and 0.644 (total bilirubin). The cutoff score point of ≥67 had sensitivity 75% and specificity 76.9% (for AST), cutoff score point of ≥83 had sensitivity 75% and specificity 76.9% (for ALT), and at a cutoff score point of ≥12 (total bilirubin). had sensitivity 75% and 53.8%, respectively as shown in Figure 5.

**Figure 5:** ROC curve to determine an optimum cut-off value for Bilirubin, AST and ALT in predictive mortality.

## 7. Discussion

Chronic hepatitis B caused by the HBV affects 350 million people worldwide, causing mortality in 20% of them every year due to complications such as cirrhosis and liver cancer [8]. In patients with end-stage liver disease, whether acute or chronic, liver transplantation is the treatment of choice. With the introduction of liver-transplantation, the survival of patients with fulminant hepatic failure has improved from 50% to 85% at 1 year. The most frequent source of liver is donation after brain death (DBD), followed by living donor liver transplantation (LDLT) [9]. However, there is a growing shortage of donor liver worldwide, leading to a prolonged waiting time. 15% of these patients on the waiting list die every year [10]. Efforts to overcome the shortage has resulted in expanding the donor pool by using grafts from elderly donors, steatotic donors, donors with malignancies, donors with viral hepatitis, donation after circulatory death (DCD), use of split liver grafts and donors with infections or metabolic derangements [11]. The long waiting list forced 86.8% of the patients in our study to choose transplantation centers outside Qatar. 44% of the patients went to china for OTLX. It is estimated that 20-30% of the population of the People's Republic of China is infected with the hepatitis B virus (HBV). There is limited regulation or oversight of organ donation and there was no national organ registry or network till 2005[10]. The incidence of DNHB post liver transplantation in our study (10.7%) is much higher compared to international data (1.7%- 3.5%) [1, 12]. This can be attributed to the fact that many patients chose to undergo OTLX in centers outside Qatar with liberal donor selection criteria such as China.

It is crucial to expand the donor pool. DNHB infections usually develop after LT using HBcAb positive grafts, especially in patients with no prior exposure to HBV. The risk of transmission of hepatitis B to a liver transplant recipient is between 60% and 80% when the donor is HBcAb positive. Hence HBcAb positive donors haven't been preferred [5]. However, recent reports suggest that the use of HBcAb positive grafts may not be independently associated with poor outcomes [4]. In our study, Seventeen patients with new-onset hepatitis B were identified among the 159 who received liver allografts. There was no sex or age related predispositions. 94% of those with DNHB underwent OTLX in China. The majority of these patients are expected to have received HBcAb positive donor livers, but donor information from these centers was not available. The outcome of patients with DNHB was not inferior compared to those without DNHB. 67% of patients survived at least 64 months after the diagnosis of DNHB. The 5-year survival of those with DNHB was 93.8%, and those without was 96.4 % (p=0.605). International data recommend HBsAb titer more than 1000 IU/L before transplantation [13]. In our cohort, none of the patients had this level before OTLX. It is recommended to maintain HBsAb titer more than 100 IU/L post-transplantation [14]. 94% of the patients in our study had loss of HBsAb post-transplantation, and their titer was less than 10 IU/L. None of our patients received Hepatitis B Immunoglobulin (HBIG) or Nucleoside Analogues (NA) as prophylaxis post-transplantation since donor data was not available. Liver Transplantation (LT) from HBcAb positive donors is being increasingly used due to the shortage of organs. In these patients, the risk of HBV reactivation is high after LT due to immunosuppressive therapy. In a study by Cholongitas et al., HBV recurrence was found to be 11% in HBsAg positive LT patients who received HBcAb positive grafts compared to HBcAb negative grafts, but overall survival was the same in both groups. They also noted that without prophylaxis, HBV reactivation was 48% in naïve patients [15]. In the early days, prophylaxis for recurrent HBV infection was given to HBsAg positive patients using monotherapy with HBIG or lamivudine (LAM). This caused significant improvement of graft survival after LT, but the re-infection rates continued to be 30%-40% [16]. Also, LAM monotherapy resulted in the development of HBV reverse transcriptase mutations that lead to antiviral drug resistance [17]. Combination therapies of HBIG with NA were successful in controlling HBV infection by reducing the HBV recurrence rate to less than 5%. DNHB infection rates in HBsAg negative patients were reduced to 19%, 2.6% and 2.8% using HBIG, LAM and combination, respectively [18]. HBsAg sero-clearance was observed in 35.2% (6/17) of DNHB patients in our cohort. Two each received treatment with entecavir and tenofovir monotherapy, one with their combination and one with lamivudine monotherapy. A meta-analysis by Zheng et al. showed that entecavir was the best prophylactic option for reducing the risk of HBV recurrence when compared against 5 other regimens (entecavir, tenofovir, adefovir, lamivudine, lamivudine plus tenofovir, and lamivudine plus adefovir) [19] concurred that long-term entecavir monotherapy resulted in a durable HBsAg sero-clearance rate of 92%, undetectable HBV DNA rate of 100% at 8 years, and excellent long-term survival of 85% at 9 years [20].

## 8. Limitations

The determination of potential sources of HBV infection is of the utmost importance. Three possible routes (blood transfusions, recipient sources, and environmental factors) have been analyzed in this study. However, the non-availability of donor data made the evaluation of potential sources incomplete. This was not a controlled prospective work. Being retrospective in nature, follow-up and treatment strategies varied among patients. Our small number of patients limits the statistical power afforded by our data. Large, prospective, multicentre studies with long-term follow-up are required to provide statistically significant conclusions.

## 9. Conclusion

OTLX in centers selecting donors liberally without screening for HBV poses risk of DNHB. However, the 5-year survival of those with DNHB is comparable to those without DNHB. As there is a

considerable demand for donor livers worldwide, patients can still be referred to such centers. However, it is prudent that accurate donor information with a clinical and serological profile should be made available by these centers. Recipients should be vaccinated and have protective levels of HBsAb more than 1000 IU/L before OTLX, and more than 100 IU/L after OTLX. It is safe for patients with positive HBcAb and HBsAb to receive HBcAb positive liver. If either of them is negative antiviral prophylaxis with NA is recommended post LT. HBV naïve patients (Negative for both HBcAb and HBsAb) ideally should not receive a liver from HBcAb positive donors, but in-case they do, prophylaxis with HBIG and life-long NA is recommended post LT.

## 10. Acknowledgment

The authors would like to acknowledge Department of Gastroenterology, Department of Infectious Diseases and Internal Medicine Residency Program of Hamad Medical Corporation for scientific support.

This study was funded by Medical Research Center of Hamad Medical Corporation.

## 11. Conflicts of Interest

The authors declare no conflicts of interest. Abstract of this study was presented as poster at 29th Annual Conference of Asian Pacific Association for the Study of Liver (APASL 2020) at Bali, Indonesia on 5th March 2020.

## 12. Ethical Approval

Ethical approval was obtained from Medical Research Center at Hamad Medical Corporation (Approval Number: MRC-01-18-333).

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