Hepatology Research 2007; **37**: 498–502 doi: 10.1111/j.1872-034X.2007.00079.x

Original Article

The rate of hepatitis B and C virus infections and the importance of HBV vaccination in children with acute lymphoblastic leukemia

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Aim: The aim of the study was to evaluate the rate of hepatitis B and C virus infection and emphasize the importance of hepatitis B virus (HBV) vaccination in leukemic children.

Methods: One hundred and sixty children who were treated for acute lymphoblastic leukemia (ALL) at Hacettepe University Faculty of Medicine, Pediatric Hematology Unit were included in the study. They were 71 (44.4%) girls and 89 (55.6%) boys with a mean age of 6.45 ± 3.87 years.

Results: Of these 160 children, 22 (13.8%) were anti-HBs-positive and 138 (86.2%) were anti-HBs-negative at the diagnosis of ALL. Among the 138 anti-HBs-negative children, 67 (41.9%) were vaccinated for HBV during maintenance chemotherapy, and 71 (44.3%) could not be vaccinated. Two (2.9%) vaccinated and 22 (30.9%) unvaccinated children developed HBV infection during the follow-up period ($P < 0.001$). Among 160 children treated for ALL, 24 (15.0%) had HBV, three (1.9%) had hepatitis C virus (HCV) infections, and 29 (18.1%) had toxic hepatitis. The majority of patients with HBV or HCV infections had high risk (HR) protocol, whereas most of the patients with toxic hepatitis had low risk (LR) protocol, especially St Jude Total XIII LR protocol.

Conclusion: Viral hepatitis and toxic hepatitis were observed more commonly in the HR and LR group, respectively, of ALL patients. This could be explained by intensive chemotherapy and more heavy blood product administration in the HR group and the chemotherapeutic agents of methotrexate and 6-mercaptopurine, basic drugs used in the LR group. In respect to protection from these complications, periodical liver function tests, serological tests for HBV and HCV, and vaccination for HBV should be performed for all children with ALL.

Key words: hepatitis B virus infection, hepatitis C virus infection, HBV vaccination, acute lymphoblasic leukemia, children

INTRODUCTION

HEPATITIS WITH ELEVATION of aminotransferase levels is common during the treatment of children with acute lymphoblastic leukemia (ALL). Toxic hepatitis may result from chemotherapeutic agents, especially methotrexate (Mtx) and 6-Merkaptopurine (6-MP). In addition to chemotherapy, hepatitis B and C virus infections, occasionally leukemic infiltration, bacterial infections and antibiotics used during febril neutropenia periods, are also responsible for the elevation of hepatic transaminases. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are more common in children with ALL than in the general population because of transfusions given during the treatment and the immunosuppressive effect of the chemotherapy.[1–3] The aim of this study was to evaluate the hepatitis B or C virus infections of children treated for ALL in our center and to emphasize the importance of HBV vaccination in leukemic children.

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Received 1 October 2006; revision 8 February 2006; accepted 15 February 2007.

METHODS

BETWEEN MARCH 1991 and January 2001, 204 children were treated for ALL at Hacettepe University Faculty of Medicine, Pediatric Hematology Unit. One hundred and sixty children who were still in remission status comprised the study group. Of these 160 children with ALL, 71 (44.4%) were girls and 89 (55.6%) were boys. The mean age of the children was 6.45 ± 3.87 years (range: 0.5–15 years). Eighty-seven patients (54.4%) received modified St Jude Total XI protocol,[4,5] whereas 73 (45.6%) received modified St Jude Total XIII protocol (Table 1).[6] In March 1997, St Jude

Table 1 Children with toxic hepatitis, HBV and HCV infections

	St Jude Total XI		St Jude Total XIII		Total *n*
	LR *n* = 30 (%)	HR *n* = 57 (%)	LR *n* = 40 (%)	HR *n* = 33 (%)	
Toxic hepatitis	6/30 (20.0%)	8/57 (14.0%)	14/40 (35.0%)	1/33 (3.0%)	29
HBV infection	4/30 (13.3%)	15/57 (26.3%)	–	5/33 (15.2%)	24
HCV infection	–	1/57 (1.8%)	–	2/33 (6.1%)	3

HBV, hepatitis B virus; HCV, hepatitis C virus; HR, high risk; LR, low risk.

Total XI protocol was changed to St Jude Total XIII protocol in our center. Therefore, before March 1997 the patients received St Jude Total XI protocol and after March 1997 the patients received St Jude Total XIII protocol. St Jude Total XIII protocol is still used in our center. Among the 87 patients who received St Jude Total XI protocol, 30 (34.5%) had low risk (LR) and 57 (65.5%) had high risk (HR) protocols. Among the 73 patients who received St Jude Total XIII protocol, 40 (54.8%) had LR and 33 (45.2%) had HR protocols. The mean follow-up period, which extended from the diagnosis of ALL to the last follow-up examination, was 51.22 ± 23.50 months (range: 30–145 months) for all of the patients. Hepatitis B surface antigen (HBsAg), anti-hepatitis B surface antibody (anti-HBs), anti-hepatitis B core antibodies (anti-HBc), and anti-hepatitis C antibody (anti-HCV) were studied by the ELISA method (Axsym Diagnostics, Abbott Park, IL). Hepatitis C virus RNA in serum and plasma was detected by PCR assay (TaqMan; Perkin Elmer/Applied Biosystems, Foster City, CA). Serological markers for HBV and HCV and liver function tests were performed at diagnosis and at 3-month intervals during the follow-up period. Children seronegative for HBV were vaccinated according to our institutional vaccination protocol.[7] Serological markers for HBV were repeated six weeks after the vaccination. Anti-HBs >10 IU/L was defined as a protective antibody level.

Serum transaminase elevation >300 IU/L was accepted as severe toxic hepatitis,[8] and serum transaminase elevation was also detected in viral hepatitis. We performed the differential diagnosis of toxic and viral hepatitis as follows: negative serological markers for HBV and HCV associated with elevated transaminases during chemotherapy suggested a diagnosis of toxic hepatitis. If cessation of chemotherapy resulted in a gradual drop in the elevated transaminases, this was diagnostic for toxic hepatitis. However, in the cases of viral hepatitis, positive serological markers for HBV or HCV and elevated transaminases, especially alanine aminotransferase (ALT) elevation, were both necessary for the diagnosis. ALT is highly liver-specific, whereas aspartate aminotransferase (AST) can also be elevated after injury to other organs. Marked increases in aminotransferase activities, especially ALT elevation, best reflect the degree of liver cell injury. After the diagnosis of viral hepatitis had been established, cessation of chemotherapy was not enough for transaminases to return to normal.

The diagnosis of acute hepatitis B is based on the detection of HBsAg and AntiHBc-IgM. During the initial phase of infection, the markers of HBV replication (HBe Ag and HBV–DNA) were also present. Past HBV infection was diagnosed by the detection of anti-HBs and AntiHBc-IgG. Immunity to HBV infection after vaccination was determined by the presence of anti-HBs only. The diagnosis of chronic HBV infection was based on the detection of HBsAg. Additional tests for HBV replication (HBe Ag and HBV–DNA) were also present to determine if the patient should be considered for antiviral therapy. HbsAg-positive, but HBV replication markers (HBe Ag and HBV–DNA) negative individuals were described as inactive HbsAg carriers. If positive serological markers for HBV and HCV had persisted for more than six months, the diagnoses of chronic hepatitis B and C infections were established.

Additionally, liver biopsy was performed to confirm the diagnosis of chronic hepatitis B and C infections. Histopathological confirmation of the diagnosis was established, especially before the beginning of antiviral treatment including lamivudine and interferon therapies. Since 10 of the 24 patients with HBV infection and all three with HCV infection developed chronic hepatitis B and C infections, they underwent liver biopsy and received antiviral treatment.

Statistical analysis was performed with a statistical package program, SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL). The χ^2-test was used to compare the patients with different risk and chemotherapy groups. The rate of the patients who received St Jude Total XI and St Jude Total XIII protocols were compared in respect to the development of toxic hepatitis, HBV and

HCV infections. Statistical significance was established at a *P*-value <0.05.

RESULTS

AMONG THE 160 children treated for ALL, 24 children (15.0%) had HBV infection, three (1.9%) had HCV infection, and 29 (18.1%) had toxic hepatitis during the follow-up period.

Of these 160 children with ALL, 22 (13.8%) were vaccinated for HBV (anti-HBs positive) and 138 (86.2%) were not vaccinated for HBV (anti-HBs negative) at the diagnosis of ALL. For the 22 children who were anti-HBs positive at the diagnosis of ALL, only two became seronegative, with the others remaining seropositive at the end of the ALL treatment. Anti-HBs antibody levels of the seropositive patients at the diagnosis were 440 ± 377 U/L (42–1000), but decreased to 156 ± 251 (18–898) at the end of the treatment ($P < 0.05$). All 22 anti-HBs-positive children at the diagnosis of ALL did not have HBV infection during the follow-up period.

Among the 138 seronegative children, 67 (41.9%) were vaccinated for HBV during maintenance chemotherapy and 71 (44.3%) could not be vaccinated. Some of the seronegative patients (n = 71) could not be vaccinated because HBV vaccination was not free of charge at that time in Turkey and their parents could not afford the vaccination and/or did not comply with the recommended vaccination programme. Among the vaccinated patients, 35.8% (24/67) gained the protected antibody level. However, two children (2/67, 2.9%) who were vaccinated during maintenance chemotherapy and 22 (22/71, 30.9%) unvaccinated children developed HBV infection ($P < 0.001$). The mean follow-up durations of vaccinated (n = 67) and unvaccinated groups (n = 71) were 53.20 ± 22.40 months (range: 36–158 months) and 52.60 ± 20.80 months (range: 38–146 months), respectively. The difference between the mean follow-up durations of vaccinated and unvaccinated groups was not statistically significant ($P > 0.05$).

Among 24 children with HBV infection, 10/24 children developed chronic HBV infection and 14/24 were inactive HBsAg carriers during the follow-up period. All three children with HCV infection also developed chronic HCV infection. Only four of 24 children with HBV infection developed clinical manifestations of acute hepatitis. After the acute phase of the disease, one developed chronic HBV infection and the other three were inactive HBsAg carriers. Nineteen of 24 patients with HBV infection had received St Jude Total XI protocol (4 LR and 15 HR protocol) and the remaining five patients received St Jude Total XIII-HR protocol. Of three patients with HCV infection, one had St Jude Total XI-HR and two had St Jude Total XIII-HR protocols. Thus the majority of patients with HBV and HCV infections (23/27, 85.2%) had HR protocols ($P < 0.001$) (Table 1).

All of the donors were negative for the serological markers of HBV, HCV and HIV and all of the patients were transfused from the same blood bank in Hacettepe University Faculty of Medicine. Retrospective chart review of the patients revealed that the HR patients received 8.5 ± 2.3 times eritrocyte and 6.5 ± 2.2 times platelet transfusions, whereas LR patients received 4.5 ± 2.2 times eritrocyte and 3.4 ± 2.0 times platelet transfusions during the treatment. The differences between eritrocyte and platelet requirements of the HR and LR groups were statistically significant ($P < 0.001$).

Fourteen of 29 patients with toxic hepatitis had St Jude Total XI protocol (six LR and eight HR) and 15 patients had St Jude Total XIII protocol (14 LR and one HR). Most of the patients with toxic hepatitis (20/29, 68.9%) had LR protocol, especially St Jude Total XIII LR protocol ($P < 0.05$) (Table 1). The mean AST level was 388 ± 284 U/L (range: 332–1012) and ALT level was 424 ± 316 U/L (range: 310–900) in patients with toxic hepatitis. After cessation of chemotherapy, the high AST and ALT levels were decreased to normal levels in all of the patients with toxic hepatitis.

DISCUSSION

HEPATITIS REVEALED BY a marked transaminase elevation may be seen in patients with leukemia.[8–11] Especially, HBV and HCV infections may cause viral hepatitis in leukemic children who are heavily transfused after the onset of their hematological disease. In developing countries where HBV and HCV infections are common in the general population of the country, such as Turkey, children with ALL will be at a greater risk for HBV and HCV infections. In Turkey the prevalence of HBV infection is put at 5.4–8.2% in different nationwide surveys.[12,13] The prevalence of HBV infection in our study (15.0%) is higher than that of the general population in the country. However, it has been reported in different studies from Turkey that the prevalence of HCV infection is 0.5–2.4% and in our study the prevalence of HCV infection is 1.5%, which is almost equal to that of the general population in our country.[14,15] Kocabas *et al.*[16] reported that HBV and HCV

infections are also common among Turkish children with cancer. In that study, 47.4% of the patients with cancer were positive for HbsAg and anti-HCV was detected in 5.8% of the cancer patients.

Toxic hepatitis is also common during combined chemotherapy, including methotrexate (Mtx) and 6-mercaptopurine (6-MP). Pharmacodynamic and pharmacogenetic properties of the patients may be responsible for the development of toxic hepatitis. Several studies have been performed to understand the mechanism of toxic hepatitis induced by these drugs. Methotrexate and its polyglutamates, 6-thioguanine nucleotides (the major cytotoxic metabolites of 6-MP) and methylated metabolites of 6-MP generated by thiopurine methyltransferase in competition with the formation of 6-thioguanine nucleotides were postulated to be responsible for toxic hepatitis in children with ALL.[1–3,8–11] Berkovitch *et al*.[2] conducted a study related to the toxic hepatitis of 6-MP in childhood ALL. In that study, they found that the time to achieve peak 6-MP levels was significantly longer in the symptomatic patients with toxic hepatitis compared to the asymptomatic patients. These results suggest the accumulation of 6-MP and its metabolites in the liver of the patients with gastrointestinal symptoms, leading to toxic hepatitis.[2]

In our study, the majority of patients with HBV or HCV infections had HR protocols, whereas most of the patients with toxic hepatitis had LR protocol, especially St Jude Total XIII LR protocol.

Hepatotoxic agents were equal for St Jude Total XI-LR and HR protocols except cranial radiotherapy in St Jude Total XI-HR protocol.[4,5] However, St Jude Total XIII-LR protocol was based on consecutive administration of Mtx and 6-MP. St Jude Total XIII-HR protocol includes these drugs at 4-week intervals. Thus, chemotherapy-related hepatotoxicity was much more common in children who received St Jude Total XIII-LR protocol.[6] However, HBV or HCV infections were more common in children who received HR protocols. The children on HR protocols were more heavily transfused and immunosuppressed than the others.

CONCLUSION

IN CONCLUSION, CHEMOTHERAPY-RELATED hepatotoxicity might vary principally with the intensity of Mtx and 6-MP treatment. The effect of chemotherapy-related hepatotoxicity on the liver seems to be reversible and does not result in chronic liver disease. However, HBV and HCV infections occur on the basis of the severity of immunosuppression and the amount of administered blood products, which were much more common in HR ALL patients. Additionally, HBV or HCV infections may cause chronic hepatitis and the patients should be followed up for this respect. Periodical liver function tests and serological tests for HBV and HCV should be performed for leukemic children to monitor for these complications.

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