Lamivudine resistance in children with chronic hepatitis B

Erhun Kasırga

Erhun Kasırga, Department of Pediatric Gastroenterology, Celal Bayar University, 45030 Manisa, Turkey

Author contributions: Kasırga E designed research, performed research, contributed new reagents or analytic tools, analyzed data and wrote the paper.

Conflict-of-interest: None.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Correspondence to: Erhun Kasırga, Professor, Department of Pediatric Gastroenterology, Celal Bayar University, Uncubozköy Mh., Mimarsinan Bulv., No:173, 45030 Manisa, Turkey. hekasirga@hotmail.com

Telephone: +90-236-4444228
Fax: +90-236-2338040
Received: August 26, 2014
Peer-review started: August 27, 2014
First decision: September 16, 2014
Revised: October 31, 2014
Accepted: January 18, 2015
Article in press: January 20, 2015
Published online: April 28, 2015

Abstract

Currently, although lamivudine (LAM) has a low genetic barrier, only interferon-alpha and LAM are available as a first-line treatment in children with chronic hepatitis B (CHB). LAM is a potent inhibitor of hepatitis B virus-deoxyribonucleic acid (HBV-DNA) polymerase replication by termination of the proviral HBV-DNA chain. LAM has a good safety and tolerability profile in CHB patients with hepatic decompensation. However, the main disadvantages of this HBV reverse transcriptase inhibitor are: (1) pre-existing covalently closed circular DNA cannot be eradicated by LAM, thus relapse after therapy withdrawal is frequent; and (2) although the longer LAM treatment induced the higher seroconversion rate, the risk of viral resistance increased through the selection of YMDD (tyrosine, methionine, aspartate, aspartate) motif. Insufficient suppression of viral replication leads to the emergence of resistant strains that could result in virological breakthrough which is usually followed by biochemical breakthrough. Mutant strains affects additional resistance and cross resistance, leading to drug resistance in a significant number of CHB patients. In this case, efficacy of more powerful anti-viral agents with higher genetic barrier against development of resistance is diminished. Furthermore, strains that are resistant to LAM could bring about vaccine escape mutants, decreasing the efficacy of HBV vaccine. A more potent drug with a high genetic barrier to resistance needs to be approved as the first-line treatment option for CHB in children.

Key words: Children; Chronic hepatitis B; Lamivudine; Lamivudine-resistant mutants; YMDD mutation

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In present day, antiviral drugs with higher genotypic barrier to resistance cannot be used for children with chronic hepatitis B since these drugs are not covered by the general health insurance in many countries. Therefore, lamivudine (LAM) which is not used for adults due to its many drawbacks has been used as a first-line of treatment for children out of necessity. Even though long term treatment results with LAM appear to be good, long term treatment increases the possibility of occurrence of resistant strains. These strains which are resistant to LAM could develop cross resistance to other anti-viral agents.
INTRODUCTION

Approximately 400 million human are globally affected by chronic hepatitis B virus (HBV) infection. There is a high risk of developing serious complications such as cirrhosis and liver cancer in these people. Despite the development of new therapies using antiviral agents fighting chronic hepatitis B (CHB) remains to be a major clinical challenge. Interferon-alpha (IFN-α), lamivudine (LAM), adefovir, entecavir and lately tenofovir are all amongst the approved drugs for medical care of children affected by CHB. IFN-α for 12 mo and older children; LAM initiating at 3 years of age; adefovir and tenofovir in children 12 years and older; and entecavir initiating from 16 years of age are used. Even though LAM is the primary antiviral drug officially accepted in present day for children with CHB less than 12 years old, use of antiviral drugs with a high genetic barrier against the emergence of resistance (such as entecavir and tenofovir) are not practiced for children with HBV because these drugs are not covered by the general health insurance in many countries.

CLINICAL ASPECTS OF LAM RESISTANCE IN CHILDREN

LAM, a nucleoside analogue has been officially accepted for treatment of CHB infection by United States Food and Drug Administration in 1998. LAM is found to be effective in suppression of HBV-DNA, normalizing aminotransferase values and improving histologic activity index. However, hepatitis B e antigen (HBeAg) seroconversion is not always resulted from LAM treatment. The probability of response to LAM treatment increases with high aminotransferase levels and high histologic activity index at baseline. Hom et al found that there is no significance of age, gender, previous IFN therapy, baseline weight, HBV-DNA, and body mass index in prediction of response to LAM treatment in children with CHB. However, Hong et al showed that high aminotransferase levels affect the HBeAg seroconversion as well as younger age in children with long-term LAM treatment. Figlerowicz et al reported that pretreatment serum HBV-DNA level is related to seroconversion of HBeAg and sustained viral response rate. Although LAM is a potent antiviral drug in the treatment of HBV, it does not help to purify liver from covalently closed circular DNA integrated into the cell nuclei. Covalently closed circular DNA brings about continued presence of HBV in liver cells. Therefore, after stopping LAM treatment HBV replication may return to pretreatment levels. In fact, it has been reported that relapse rates varied from 19% to 62% after cessation of treatment with LAM. Kansu et al reported that relapse rates of 6.8% in children treated with combined IFN-α2a and LAM. Jonas et al determined a relapse rate of 17.5% in a placebo controlled LAM trial in children. Hagmann et al found relapse rate of 25% after cessation of LAM treatment. It is likely that duration of LAM treatment would be a culprit for the variations in relapse rates. Choe et al reported long term LAM treatment increased HBeAg seroconversion rates more than IFN treatment. This is especially seen in pre-school children. High relapse rates have been observed when LAM treatment is discontinued before and right after HBeAg seroconversion. Because of this, treatment should continue possibly 12 mo after HBeAg seroconversion is observed. Nevertheless, major limitation of prolonged LAM therapy is formation of resistant mutants. It is recognized that resistance to LAM develops as a result of emerging mutations which are formed in catalytic part of the reverse transcriptase YMDD [(Y) tyrosine, (M) methionine, (D) aspartate, (D) aspartate]. In YMDD mutation formations, methionine is replaced with valine (rtM204V), isoleucine (rtM204I) or rarely serine (rtM204S). In these mutations, rtM204V is always together with rtL180M which is a compensating mutation. This mutation partially restores replication fitness of HBV. However, it has been shown that rtM204I differentiation is independent from rtL180M. In addition, rtV173L differentiation which is found in some samples resistant to LAM, increased replication capacity of HBV. Resistance to LAM causes absence of HBV-DNA suppression and eventually advancement of liver disease. However, replication capacity of YMDD mutants is less than the wild virus. Because of this, lower aminotransferase and HBV-DNA levels can be found in YMDD mutant virus infections. After development of LAM resistance, usual serum HBV-DNA becomes positive (virologic breakthrough) and then serum alanine aminotransferase level increases (biochemical breakthrough). Mutant strains generally emerge after 6 mo of therapy with LAM. Resistance rates of 38%, 49% and 65% have been reported at 2, 3 and 5 years of therapy with LAM. In a multicenter trial carried out by Jonas et al, the YMDD mutation was detected in 19% of children who had undergone LAM therapy for 52 wk. No LAM resistance mutations were identified in the placebo group during the first year of this study. Sokal et al found YMDD mutation rates of 49% and 64% in second and third year of treatment, respectively. Hartman et al found YMDD resistant mutants in 11 of 17 (65%) children at the end of the first year of LAM treatment. Interestingly, YMDD mutation rate of this study was extremely higher than other studies. Hagmann et al reported development of clinical resistance to LAM in 3 children (19%) in the first year of therapy. Furthermore, in this study, frequency of drug resistance is found to be low in children with high HBV-DNA suppression level. Hong et al reported breakthrough in 25.9% (21 out of 81) of patients treated with LAM. These patients were followed up for more than 1 year. Lee et al reported viral breakthrough in 12 children (27%) during the therapy and documented.
YMDD mutation in 11 children (25%). In this study, average time for development of mutation was 22.7 mo. Ni et al[19] found mutant strains in 34% of the children after 12 mo of therapy with LAM. In this study, higher resistance rates were found compared to other studies. Akman et al[20] reported YMDD mutants in 58.4% of the total 24 children treated with LAM for 30 ± 10 mo. Choe et al[21] found viral breakthrough developed 10% in the first year and 23% in the second year of LAM treatment. In this study, YMDD mutation was found in 9 of 11 patients who have developed breakthrough. Liberek et al[22] determined mild and temporary aminotransferase increase in 4 out of 59 children with CHB and 2 children with YMDD mutation between third and twelfth months of LAM treatment. Koh et al[23] reported breakthrough and relapse rates in 10% and 3.3% of children with CHB after 52 mo with LAM therapy. In this study, although the exact reason of lower breakthrough and relapse rates are not known, clinical characteristics of patients and differences in treatment schedule could be reasons for this phenomenon.

Resistance to LAM increases with longer treatment periods. Therefore, LAM therapy should be discontinued 6 mo after HBeAg seroconversion or appearance of YMDD mutations[1]. On the other hand, higher proportion of LAM resistance is associated with higher viral load after first 6 mo of therapy[22]. It has been shown that complete virologic response reduces the risk of resistance to LAM. Yuen et al[24] established a relationship between high HBV-DNA level and alanine aminotransferase level at beginning with the emergence of YMDD mutations. Paik et al[25] determined a significant relationship between YMDD mutations emerging at three months with viral breakthrough. In another study, after 12 mo of LAM treatment, Yuen et al[26] showed no significant differences exist in virologic response and YMDD mutant rates between patients with genotypes B and C. Contrary to this study, Kobayashi et al[26] showed development of YMDD mutants was influenced by HBV genotypes in patients with CHB. Numerous studies have been performed to determine whether a combination regimen with LAM and IFN-α prevents or delays the emergence of YMDD mutants. There are conflicting results in literature regarding this matter. In accordance with a study conducted by Chan et al[27], a lower LAM resistance was found in combination treatment with pegylated-IFN and LAM (21%) compared with LAM monotherapy (40%). However, Marrone et al[28] showed risk for emergence of LAM resistance was not reduced with IFN and LAM combination treatment. It is possible that older patients and moderately high aminotransferase levels prior to treatment in the study of Chan et al[27] could have caused the differences between these two studies. Furthermore, results may have been affected in favor of the combination therapy since Chan et al[27] conducted combination of LAM with IFN eight weeks longer than monotherapy with LAM. Ozgenc et al[29] determined high breakthrough incidence in children with partial response to long-term LAM therapy. In this study, reported breakthrough rates of LAM were 13.3%, 69.4%, and 82.4% in 1, 2, and 3 years, respectively. Kansu et al[30] reported breakthrough rates of 17.9% in simultaneous therapy group and 24.6% in sequential therapy group. Yilmaz et al[31] did not find breakthrough in any patient that could suggest YMDD mutation. Selimoglu et al[32] reported breakthrough in 11 (23.4%) children treated with IFN-α and LAM combination therapy. In another combination treatment of IFN-α and LAM, Dikici et al[32] demonstrated no viral breakthrough with the exception of one patient during the follow-up period after the treatment. The viral breakthrough for this child was accepted as an YMDD mutation. Kuloğlu et al[33] reported breakthrough and YMDD mutant rates of 65.8% and 55.2% respectively with combined IFN-α and long term LAM therapy. Saltik-Temizel et al[34] provided no information about viral breakthrough rates in their article on combination therapy with LAM and high-dose IFN-α.

Results from different treatment regimens are presented in Table 1.

In accordance with the results of these studies, avoiding unnecessary use of antiviral drugs can help to reduce resistance. Therefore, LAM should be prescribed only for patients with good predictors of response. If there is no finding for resistance to LAM, children should be treated for one year. However, there may be a need for longer treatment[35]. Although the optimal duration of therapy is not well-established, patients should be treated for at least six more months after HBeAg seroconversion[36]. Treatment may be discontinued in those who have HBV-DNA replication or mutant strains[37]. High HBV-DNA load before treatment was shown to be an important factor causing virologic breakthrough. Early suppression of viral replication plays a key role for prevention of LAM resistance. Insufficient response to LAM therapy with persistence of viremia can increase the resistance[38]. On the other hand, an elevated pretreatment alanine aminotransferase level (more than twofold the upper normal limit) is a key factor reducing the LAM resistance[39]. Patients who have not achieved a complete virologic response (partial response) to LAM at week 24, switching to a more potent antiviral agent or add-on another antiviral agent without cross-resistance profile is the only useful treatment approach[40]. Treatment guidelines for children have not been established yet. However, in case of failure with LAM therapy, addition of adefovir or switching to either adefovir or entecavir therapies should be considered in older children.

**LAM RESISTANCE IN PREVIOUSLY UNTREATED PATIENTS WITH CHB**

Because HBV polymerase lacks of proofreading mechanism, spontaneous polymerase mutations occur naturally[1,2]. Therefore, YMDD motif variants can develop not only as secondary to LAM usage, but also...
it can naturally occur with a relatively high incidence in previously untreated patients with CHB\textsuperscript{[43]}. Recently, the incidence of YMDD mutants in previously untreated patients from eight countries was found to be 12.2\%\textsuperscript{[42]}. It is important to investigate these mutations in primary LAM-nonresponsive patients. Although some correlation between virologic breakthrough during LAM therapy and previously presence of LAM-resistant mutants in untreated patients has been found, its clinical significance during LAM therapy is still unknown. However, there is a small possibility for these mutants to be dominant during HBV infection and CHB can effectively be treated with LAM. Lee et al\textsuperscript{[43]} indicated that previously presence of LAM resistant mutants was rapidly cleared with LAM therapy in untreated CHB patients. Further researches are necessary to evaluate the influence of LAM-resistant mutants in previously untreated patients with CHB.

\section*{CROSS-RESISTANCE}

Presently, medications such as adefovir, entecavir and, recently, tenofovir have been used for the treatment of adolescents with CHB\textsuperscript{[1]}. However, only IFN-\alpha and LAM are still available as a first-line treatment especially in young children at this time. In patients with LAM-resistance, sufficient suppression of HBV-DNA is not obtained and the incidence of resistance to adefovir is increased. It has been observed that adding adefovir to continued LAM therapy is found to be linked with lower adefovir resistance rates. Because only one additional substitution at T184, S202, and/or M250 is enough to emergence of entecavir resistance, the development of entecavir resistance occurs more easily in LAM-resistant patients than treatment-naïve patients\textsuperscript{[31]}. After two years therapy, the resistance rates of entecavir have been increased (8\%) in LAM-resistant patients\textsuperscript{[1]}. Tenney et al\textsuperscript{[44]} reported a low rate of entecavir resistance (0.8\%) and a high rate of entecavir resistance (43\%) in LAM-resistant patients after five years of therapy.

\section*{TREATMENT OF LAM-RESISTANT CHB IN CHILDREN}

In case of virologic breakthrough, to avoid the emergence of cross resistance, a second antiviral agent without cross-resistance is added to LAM\textsuperscript{[1]}. There have been no beneficial effects of using adefovir in children between 2 and 12 years of age. Therefore, adefovir was licensed for use in adolescents. Jonas et al\textsuperscript{[45]} reported that early virologic response was a good predictor for emergence of resistance against adefovir. Both the combination of LAM with adefovir and entecavir monotherapy were found to be more effective by Chu et al\textsuperscript{[46]} in suppressing HBV replication compared to adefovir monotherapy in LAM-resistant children. Ryu et al\textsuperscript{[47]} reported that high baseline viral load was rapidly declined with entecavir monotherapy in LAM refractory children. However combination of LAM with adefovir was more effective in suppressing the viral load than entecavir.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Ref. & Therapeutic regimen & Duration of treatment & HBeAg seroconversion rate (%) & Relapse rate (%) & Breakthrough rate (%) \\
\hline
Jonas et al\textsuperscript{[9]} & LAM & 12 mo & 26 & 18 & 19 \\
Hagmann et al\textsuperscript{[32]} & LAM & 24 mo & 25 & 0 & 64 \\
Sokal et al\textsuperscript{[33]} & LAM & 12 mo & 6 & 0 & 65 \\
Koh et al\textsuperscript{[8]} & LAM & 12 mo & 60 & 0 & 27 \\
Lee et al\textsuperscript{[29]} & LAM & 24 mo & 38 & 0 & 34 \\
Ni et al\textsuperscript{[4]} & LAM & 12 mo & 20.8 & 0 & 58.4 \\
Liberek et al\textsuperscript{[3]1} & LAM & 12 mo & 65 & 0 & 10\% \textsuperscript{2} \\
Kansu et al\textsuperscript{[5]} & LAM & 12 mo & 60 & 0 & 23\% \textsuperscript{3} \\
Akman et al\textsuperscript{[17]} & LAM & 12 mo & 27 & 0 & 3.38 \\
Choe et al\textsuperscript{[18]} & LAM & 12 mo & 42 & 3.3 & 10 \\
Kuloğlu et al\textsuperscript{[19]} & LAM & 6 mo IFN & 60 & 39.4 & 17.9 \\
Ozgenç et al\textsuperscript{[3]} & LAM & 6 mo IFN & 0 & 6.8 & 24.6 \\
Dikici et al\textsuperscript{[34]} & LAM & 12 mo IFN & 15.6 & 5.6 & 69.4 \\
Kuloğlu et al\textsuperscript{[31]} & LAM & 6 mo IFN & 0 & 6.8 & 82.4 \\
Sulik-Temizel et al\textsuperscript{[33]} & LAM & 6 mo IFN & 37 & 3.3 & 65.8 \\
Liberek et al\textsuperscript{[31]} & LAM & 6 mo IFN & 34.2 & 3.3 & 65.8 \\
Liberek et al\textsuperscript{[32]} & LAM & 12 mo IFN & 60 & 0 & 3.3 \\
\hline
\end{tabular}
\caption{Outcomes of different therapeutic regimens in children with chronic hepatitis B}
\end{table}
LAM-ASSOCIATED VACCINE-ESCAPE MUTATIONS

Currently, there are two types of LAM-associated HBV mutants with antigenically modified HBsAg. In the genome organization of HBV, surface and polymerase genes overlap; and changes in the polymerase reverse transcriptase which involve LAM resistance substitutions may cause mutations [first type hepatitis B surface antigen (HBsAg) mutant] in the surface gene of HBV. A triple substitution pattern (V173L + L180M + M204V) of LAM resistance is associated with the changes (sE164D + sI195M) in the overlapping surface gene. These mutants may act as a vaccine escape mutants (sG145R). As a result, those viruses which have mutated cannot be recognized and eliminated by existing monoclonal antibodies (anti-HBs). Because of the prolonged viral suppression with LAM treatment, the second type HBsAg mutants are emerged from the selection of surface antigen escape mutants. The development of LAM resistant and HBsAg escape mutants is associated with decreased attachment of anti-HBs antibodies to HBsAg. LAM-resistant HBV mutants with the capability to escape from anti-hepatitis B surface antibodies have the ability to infect individuals both vaccinated and unvaccinated for HBV. Therefore, it is imperative that physician weigh up the possible benefits and harms of treatment with LAM carefully.

CONCLUSION

Currently, LAM monotherapy is not used in adults because of very high recalcitrance rates. Similarly, most potent antiviral agents with optimal resistance profile should be used as first-line therapy in children. It is important to monitor early detection of virologic breakthrough and determine genotypic resistance to decide the optimal intervention. Monitoring the levels of HBV-DNA and determination of types of resistant strains would be necessary to establish therapeutic strategies. Because the LAM resistant viruses appear to be more prevalent in population, these mutants may become a potential serious public health problem.

In conclusion, there is a need to conduct further studies and new arrangements in general health insurance policies for use of the antiviral drugs which have strong antiviral effects and low resistance rates as first-line treatment in children with CHB.

REFERENCES

1. Williams JN. A window on the future: can computerization help your practice? Ky Dent J 1990; 42: 8-9 [PMID: 2370736 DOI: 10.1016/j.jhep.2013.05.016.7]
2. Palumbo E. Lamivudine for chronic hepatitis B in children. Infect Dis Clin Prac 2008; 16: 13-15 [DOI: 10.1097/ ipc.0b013e31815aa2dd]
3. Choe BH, Lee JH, Jang YC, Jang CH, Oh KW, Kwon S, Hyun MC, Ko CW, Lee KS, Lee WK. Long-term therapeutic efficacy of lamivudine compared with interferon-alpha in children with chronic hepatitis B: the younger the better. J Pediatr Gastroenterol Nutr 2007; 44: 92-98 [PMID: 17204960 DOI: 10.1097/01. mgn.0000243439.47334.4e]
4. Hom X, Little NR, Gardner SD, Jonas MM. Predictors of virologic response to Lamivudine treatment in children with chronic hepatitis B infection. Pediatr Infect Dis J 2004; 23: 441-445 [PMID: 15131468 DOI: 10.1097/01.inf.0000126412.93562.65]
5. Hong SJ, Kim YH, Choe BH, Park HJ, Tak WY, Kweon YO. Current role of Lamivudine regarding therapeutic response and resistance in children with chronic hepatitis B. Pediatr Gastroenterol Hepatol 2013; 16: 80-88 [PMID: 24010111 DOI: 10.5223/pgih.2013.16.2.80]
6. Figlerowicz M, Kowala-Piaskowska A, Filipowicz M, Bujnowska A, Mozer-Lisewska I, Sluzewski W. Efficacy of lamivudine in the treatment of children with chronic hepatitis B. Hepatol Res 2005; 31: 217-222 [PMID: 15799860 DOI: 10.1016/j.hepres.2005.02.003]
7. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001; 34: 1225-1241 [PMID: 11732013 DOI: 10.1053/jhep.2001.29401]
8. Kansu A, Doğancı T, Akman SA, Artan R, Kuyucu N, Kalaycı AG, Dikici B, Dalığiz B, Selimoglu A, Kasırge E, Ozkan TB, Kologlu Z, Aydoğdu S, Boşnak M, Ertekian V, Tanir G, Haspolat K, Girgin N, Yağcı RV. Comparison of two different regimens of combined interferon-alpha2a and lamivudine therapy in children with chronic hepatitis B infection. Antivir Ther 2006; 11: 255-261 [PMID: 16640166]
9. Jonas MM, Mizerzki J, Badia IB, Areias JA, Schwarz KB, Little NR, Greensmith MJ, Gardner SD, Bell MS, Sokal EM. Clinical trial of lamivudine in children with chronic hepatitis B. N Engl J Med 2002; 346: 1706-1713 [PMID: 12037150 DOI: 10.1056/NEJMa021452]
10. Haggman S, Chung M, Rochford G, Jani M, Triash-Shevrin C, Sitińska-Takahashi Y, Neumann AU, Pollack H. Response to lamivudine treatment in children with chronic hepatitis B virus infection. Clin Infect Dis 2003; 37: 1434-1440 [PMID: 14614646 DOI: 10.1086/378739]
11. Zoulif M, Locarnini S. Management of treatment failure in chronic hepatitis B. J Hepatol 2012; 56: Suppl 1: S112-S122 [PMID: 22300461 DOI: 10.1016/S0168-8278(12)60012-9]
12. Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, Brown N, Woesnner M, Boehme R, Condraey L. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003; 36: 687-696 [PMID: 12627352 DOI: 10.1086/368083]
13. Hartman C, Berkowitz D, Shouval D, Eshach-Adov O, Hino B, Rimon N, Satinger I, Kra-Oz T, Daudi N, Shamir R. Lamivudine treatment for chronic hepatitis B infection in children unresponsive to interferon. Pediatr Infect Dis J 2003; 22: 224-229 [PMID: 12634582 DOI: 10.1097/01.inf.0000055062.64695.2e]
14. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology 2003; 125: 1714-1722 [PMID: 14724824 DOI: 10.1053/j.gastro.2003.09.033]
15. Sokal EM, Kelly DA, Mizerzki J, Badia IB, Areias JA, Schwarz KB, Végnente A, Little NR, Gardner SD, Jonas MM. Long-term lamivudine therapy for children with HBeAg-positive chronic hepatitis B. Hepatology 2006; 43: 225-232 [PMID: 16440364 DOI: 10.1002/hep.21020]
16. Hartman C, Berkowitz D, Eshach-Adov O, Hino B, Rimon N, Satering I, Kra-Oz T, Shamir R. Long-term lamivudine therapy for chronic hepatitis B infection in children unresponsive to interferon. J Pediatr Gastroenterol Nutr 2006; 43: 494-498 [PMID: 17033525 DOI: 10.1097/01.mgn.0000225982.34323.67]
17. Lee EH, Jang YJ, Kim KM. Efficacy of lamivudine therapy for chronic hepatitis B in children. Korean J Pediatr Gastroenterol Nutr 2008; 11: 130-136
18. Ni YH, Huang FC, Wu TC, Kong MS, Jeng YM, Chen PJ, Tsuei DJ, Chen HL, Hsu HY, Chang MH. Lamivudine treatment in maternally transmitted chronic hepatitis B virus infection patients. Pediatr Int 2005; 47: 372-377 [PMID: 16091071 DOI: 10.1111/
A systematic review and meta-analysis. PLoS One 2012; 7: e32789 [PMID: 22479339 DOI: 10.1371/journal.pone.0032789]

Lee SH, Kim HS, Byun IS, Jeong SW, Kim SG, Jang JY, Kim YS, Kim BS. Pre-existing YMDD mutants in treatment-naive patients with chronic hepatitis B are not selected during lamivudine therapy. J Med Virol 2012; 84: 217-222 [DOI: 10.1002/jmv.23191]

Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichtroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. Hepatology 2009; 49: 1503-1514 [PMID: 19280622 DOI: 10.1002/hep.22841]

Jonas MM, Kelly D, Pollack L, Mizerski J, Sorbel J, Frederick D, Mondou E, Rousseau F, Sokal E. Safety, efficacy, and pharmacokinetics of adefovir dipivoxil in children and adolescents (age 2 to & lt; 18 years) with chronic hepatitis B. Hepatology 2008; 47: 1863-1871 [PMID: 18433023 DOI: 10.1002/hep.22250]

Chu M, Cho SM, Cho BH, Cho MH, Kwon S, Lee WK. Virologic responses to add-on adefovir dipivoxil treatment versus entecavir monotherapy in children with lamivudine-resistant chronic hepatitis B. J Pediatr Gastroenterol Nutr 2012; 55: 648-652 [PMID: 22688509 DOI: 10.1097/MPG.0b013e318262a737]

Ryu JJ, Lee JM, Ahn SH, Kim Do Y, Lee MH, Han KH, Cho Y, Park JY. Efficacy of adefovir add-on lamivudine rescue therapy compared with switching to entecavir monotherapy in patients with lamivudine-resistant chronic hepatitis B. J Med Virol 2010; 82: 1835-1842 [PMID: 20872709 DOI: 10.1002/jmv.21898]

Sheldon J, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. J Antimicrob Chemother 2008; 61: 766-768 [PMID: 18218641 DOI: 10.1093/jac/dkn14]

Yeh CT. Development of HBV S gene mutants in chronic hepatitis B patients receiving nucleotide/nucleoside analogue therapy.

Clinical importance of lamivudine resistance
Sayan M, Akhan SC. Antiviral drug-associated potential vaccine-escape hepatitis B virus mutants in Turkish patients with chronic hepatitis B. *Int J Infect Dis* 2011; 15: e722-e726 [PMID: 21784687 DOI: 10.1016/j.ijid.2011.05.019]

**Kasırga E. Clinical importance of lamivudine resistance**
