Mutant problems in Turkey

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HBV and HCV have a very high rate of mutation.

- HBV reverse transcriptase and HCV RNA polymerase have no proof-reading capacity to correct the misincorporated nucleotides.
- $10^{10}$ and $10^{11}$ mutations may enter the virus pool in HBV and HCV infection, respectively.
Mutations may accumulate through the evolution and may diversify the subtypes and genotypes. New species may evolve by the accumulation of mutations.

HBV genotypes have 8%, HCV genotypes have 30% dissimilarities of nucleotide sequence.
Genotypes have different patterns of clinical progression and treatment response.

However, we will focus on the importance and consequences of mutations.
HBV mutations are clinically more relevant comparing to HCV mutations.
- Vaccination → Vaccine escape mutations
- Resistance-prone drugs in the treatment
- Compact genomic structure

HCV-ISDR (interferon sensitivity determining region) mutations were subjects of several studies, but there has been no consistent results.
Clinically relevant HBV mutations

- Precore, core promoter mutations which prevent HBeAg secretion.
- Immune-, vaccine-escape mutations of HBsAg,
- Mutations leading nucleos(t)ide resistance.
- Mutations associated with HCC.
Precore and core promoter mutations

- They should be considered as variations, instead of mutation.
- These mutants are more common than the wild type.
- Because of the widespread genotype D infection, precore stop codon mutation is highly prevalent in Turkey.
Genotype A

ATGCAACTTTTTCACCTCTGCCATACTCAT

HBeAg(−)

1858 → 1896

Tryptophan

HBeAg(+) Stop

Genotype A Genotype D
Result of precore stop codon mutation

ATG…. TGG…ATG…

ATG…. TAG…ATG…

HBcAg

HBeAg

HBcAg
What are the advantages of HBeAg negative mutants for the virus?

- Escape from immune recognition.
- More effective replication and viral mechanisms
  - Enhance the strength of $\varepsilon$ encapsidation signal sequence
  - Restore the replication competence of nucleoside resistant mutants
  - HBeAg precursor may diminish the encapsidation by competition with HBcAg (dominant negative effect). Absence of HBeAg may increase the replication capacity.
  - Core promoter mutants may increase the expression of core antigen, while reduce the synthesis of HBeAg mRNA.
Characteristics of HBeAg negative infection

- **HBeAg negative infection:**
  - Represents the advanced stage of the disease.
  - An immune tolerogen (HBeAg) does not exist.
  - Other mutations in HBV genome are more likely to be present.

- For these reasons prognosis and response to treatments of HBeAg negative patients is poor.
  - At the time of diagnosis 30% have cirrhosis.
  - Mortality for 4 years is 20%, Risk of HCC is found to be 14%.
HBeAg negativity in Turkey

- 65-80% of the patients with CHB are HBeAg negative.
- These figures are lower comparing to other Mediterranean country.
- Higher rate of vertical transmission and young age of CHB may be the cause.
  - Median age of treated patients was 37, more than 10 years ago
  - Now it is 43
Nucleotide divergences in the core promoter and precore region of genotype D hepatitis B virus in patients with persistently elevated or normal ALT levels

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Table 2
Patient characteristics and core promoter and precore mutations (N:Normal; H: High)

<table>
<thead>
<tr>
<th></th>
<th>A1 (HBeAg positive)</th>
<th>A2 (HBeAg positive)</th>
<th>A1+A2 (HBeAg positive)</th>
<th>B1 (HBeAg negative)</th>
<th>B2 (HBeAg negative)</th>
<th>B1+B2 (HBeAg negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT: N</td>
<td>ALT: H</td>
<td></td>
<td>ALT: N</td>
<td>ALT: H</td>
<td></td>
</tr>
<tr>
<td>No of Patients</td>
<td>13</td>
<td>16</td>
<td>29</td>
<td>20</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>Male/Female</td>
<td>8F/5M</td>
<td>2F/14M</td>
<td>10F/19M</td>
<td>10F/10M</td>
<td>4F/14M</td>
<td>14F/24</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>30 ± 10</td>
<td>34 ± 12</td>
<td>32 ± 11</td>
<td>37 ± 11</td>
<td>45 ± 11</td>
<td>41 ± 11</td>
</tr>
<tr>
<td>ALT (mean ± SD)</td>
<td>20 ± 10</td>
<td>149 ± 220</td>
<td>49 ± 8</td>
<td>104 ± 66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion 1763–1770</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1762 A→T</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1764 G→A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1762/1764 (T/A)</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1896 stop codon</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>1762/1764 (T/A) + 1896 (G→A) stop</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>12</td>
<td>17</td>
<td>29</td>
</tr>
</tbody>
</table>
Vaccine escape mutations in Turkey
Loop 1 of ‘a’ determinant

Loop 2 of ‘a’ determinant

(Caman, W. J Viral Hepatitis 1997 4: 11-20)
Clinical consequences of S-gene mutations

- HBV infection in postexposure prophylaxis
- HBV recurrence under HBIG prophylaxis in postransplant patients
- Diagnostic inaccuracy in ELISA tests
Vaccine coverage of mutants is assumed zero.
Mutants are assumed to infect vaccinees.

Wilson, JVH 1998
Publications related to HBsAg mutants from Turkey

A new hepatitis B virus vaccine escape mutation in a renal transplant recipient

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A new mutation at a determinant, sS143L, was described. It can not be detected by commercial assay of HBsAg.
41 inactive HBV infection

40 patients with CHB were studied.

22 patients have aa substitutions.

- 42.5% in CHB
- 12.2% in inactive infection

None of the mutations result in drug resistant changes in rt gene.
<table>
<thead>
<tr>
<th>Sample code</th>
<th>HBsAg subtype</th>
<th>Antigenic regions of MHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HBs1 101</td>
</tr>
<tr>
<td>Reference seq. AY796032</td>
<td>ayw2</td>
<td>Gln</td>
</tr>
<tr>
<td>A11</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>A16</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>A35</td>
<td>ayw3</td>
<td></td>
</tr>
<tr>
<td>A38</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>A40</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>ayw3</td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>ayw3</td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B9</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B13</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B14</td>
<td>ayw3</td>
<td></td>
</tr>
<tr>
<td>B17</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B18</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B20</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B22</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B25</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B31</td>
<td>ayw3</td>
<td></td>
</tr>
<tr>
<td>B32</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B35</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B38</td>
<td>ayw2</td>
<td></td>
</tr>
<tr>
<td>B40</td>
<td>ayw3</td>
<td></td>
</tr>
<tr>
<td>Number of substitutions at the position (total: 26)</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>
Mutations in the S gene region of hepatitis B virus genotype D in Turkish patients

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2Department of Gastroenterology, Ankara Numune Education and Training Hospital, 06100 / Ankara, Turkey
3Department of Gastroenterology, University of Gaziantep, 27310/ Gaziantep, Turkey

- Isolates from 40 patients were sequenced (nt250-715)
- Mutations were detected at 10 different points.
- C498A, A531G and T536C were observed in all of the isolates.
  - Met125Thr, Thr127Pro aa changes
- C501A point mutation was described in 82.5% of the isolates.
- T496C, C517T, G523A, C479T, T320C (Ser55Phe), C296G (Val47Ala) were found in some patients.
- Most of the family members have the same sequence
Drug resistant mutations in Turkey
498 lamivudine-treated patients’ data were retrospectively analyzed.

Patients who had two consecutive normal ALT values at least 1 month apart were defined as responsive to treatment.

Biochemical breakthrough was defined as the presence of two consecutive ALT values more than the upper limit of normal, at least 1 month apart, after ALT normalization.

Primary resistance was defined as the persistent ALT elevation during lamivudine treatment.

To investigate the parameters which were related to breakthrough, Kaplan-Meier analysis and log-rank test were performed after categorization of the above parameters. Variables reaching a statistical significance were introduced into Cox’s multiple regression analysis.
## Characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>498</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>344 (69.1%)</td>
</tr>
<tr>
<td>Age</td>
<td>40±13</td>
</tr>
<tr>
<td>Follow-up (weeks)</td>
<td>105 (0-377)</td>
</tr>
<tr>
<td>Response</td>
<td>153 (30.7%)</td>
</tr>
<tr>
<td>Basal ALT (IU/l)</td>
<td></td>
</tr>
<tr>
<td>median (range)</td>
<td>113 (34-480)</td>
</tr>
<tr>
<td>&lt;2 x ULN</td>
<td>31%</td>
</tr>
<tr>
<td>2-5 x ULN</td>
<td>45%</td>
</tr>
<tr>
<td>&gt;5 x ULN</td>
<td>24%</td>
</tr>
<tr>
<td>Basal HBV DNA (pg/ml)</td>
<td>312 (5-8935)</td>
</tr>
</tbody>
</table>
HBeAg and biochemical breakthrough

- HBeAg(-)
- HBeAg(+)

$p < 0.0001$
HBV DNA and biochemical breakthrough

- HBV DNA < 2000 pg/ml
- HBV DNA ≥ 2000 pg/ml

p < 0.0001
Age and biochemical breakthrough

P=0.0019
ALT and biochemical breakthrough

![Graph showing breakthrough rate (%)]

- **ALT<2xULN**
- **ALT 2-5xULN**
- **ALT>5xULN**

p<0.0036
Multivariate analysis indicated that HBV DNA \((p<0.0001)\) and categorized ALT \((<2\times\text{ULN}, 2-5\times\text{ULN}, >5\times\text{ULN})(p=0.006)\) were independently related to cumulative biochemical breakthrough rate. Decreased rate of lamivudine resistance in HBeAg negative patients did not reach a statistically significant difference \((p=0.158)\).
Conclusions

- HBeAg negative patients have a lower rate of biochemical breakthrough during lamivudine therapy comparing to HBeAg positive patients. However, this may be related to lower HBV DNA levels comparing to HBeAg positives.
Long-term results of lamivudine treatment for chronic hepatitis B virus infection: 10 year data in HBeAg-positive and -negative patients.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>HBe Ag (+)</th>
<th>HBe Ag (-)</th>
<th>p value HBe Ag (+) versus HBe Ag (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>407</td>
<td>131</td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Age, mean (years ± SD, range)</td>
<td>41.1 ± 12.8  (14–76)</td>
<td>31.9 ± 13.2 (14–76)</td>
<td>43.8 ± 9.3  (18–70)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Male sex (n)</td>
<td>281 (68 %)</td>
<td>87 (65 %)</td>
<td>194 (69 %)</td>
<td></td>
</tr>
<tr>
<td>Number of patients treated with prior interferon therapy</td>
<td>194 (47 %)</td>
<td>71 (53 %)</td>
<td>123 (44 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mean lamivudine therapy duration (months)</td>
<td>41.5 ± 26.1</td>
<td>33.8 ± 22.4</td>
<td>45 ± 27</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Number of patients with cirrhosis</td>
<td>57 (14 %)</td>
<td>11 (8 %)</td>
<td>46 (16 %)</td>
<td>= 0.02</td>
</tr>
<tr>
<td>Mean baseline HBV DNA (copy /ml)</td>
<td>1.7 x 10^8 ± 2.4 x 10^8</td>
<td>3.4 x 10^8 ± 2.9 x 10^8</td>
<td>7.8 x 10^7 ± 1.7 x 10^8</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mean baseline fibrosis score (knodell)</td>
<td>2 ± 1.2</td>
<td>1.6 ± 1.1</td>
<td>2.2 ± 1.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mean baseline ALT levels (IU/L) (range)</td>
<td>87.7 ± 53.3 (10-425)</td>
<td>88.6 ± 57.5</td>
<td>87.4 ± 51.5</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
Cumulative proportion of virologic resistances in HBeAg positive and HBeAg negative patients
Cumulative proportion of virologic resistances in cirrhotics and non-cirrhotic patients
Types of lamivudine-resistant mutations

<table>
<thead>
<tr>
<th>Polymerase mutation</th>
<th>rt204V (n)</th>
<th>rt204I (n)</th>
<th>rt204I+V (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rt180L (wild)</td>
<td>6</td>
<td>37</td>
<td>6</td>
</tr>
<tr>
<td>rt180M</td>
<td>27</td>
<td>34</td>
<td>8</td>
</tr>
</tbody>
</table>

Patterns of lamivudine resistant mutations in lamivudine-treated patients (Bozdayı et al, TASL Meeting 2005)

<table>
<thead>
<tr>
<th>Mutation patterns</th>
<th>N (315)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L180M + M204V</td>
<td>82</td>
<td>26</td>
</tr>
<tr>
<td>L180M + M204V/I</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>L180M/L + M204M/V/I</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>L180M + M204I/V</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>M204I</td>
<td>79</td>
<td>25</td>
</tr>
<tr>
<td>L180M + M204I</td>
<td>45</td>
<td>14</td>
</tr>
<tr>
<td>L180M/L + M204I</td>
<td>37</td>
<td>12</td>
</tr>
<tr>
<td>M204M/I</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>L180M</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>L180M/L + M204M/I</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>L180M + M204S</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>L180C + M204I</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>L180M + Y203C + M204I</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Adefovir resistance

- 201 lamivudine-resistant patients have been treated with adefovir for more than 1 year.
  - 109 received adefovir monotherapy
  - 92 adefovir add-on to lamivudine
- Median duration of follow-up was 140 weeks
  - 10 pts followed up >240 weeks
Adefovir resistance rate

One Minus Survival Functions

p = 0.07
Adefovir resistance

- 17 patients had adefovir resistant mutations
  - rtN236T 8
  - rtA181V + rtN236T 3
  - rtA181V 3
  - Wt + rtA181T + rtN236T 2
  - Wt + rtA181T/V + rt236T 1
Drug-resistant mutations in treatment-naive patients

YMDD motif variants in inactive hepatitis B carriers detected by Inno-Lipa HBV DR assay
Mesut Akarsu, * Aylin Sengonul, † Ethem Tankurt, * Ayca Arzu Sayiner, † Omer Topalak, * Hale Akpinar* and Yusuf Hakan Abacioglu†

Departments of *Gastroenterology and †Microbiology and Clinical Microbiology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

- YMDD variants were detected in 13 (18.3%) of the 71 anti-HBe positive inactive HBV carriers. Of the 13 patients, 10 (76.9%) also had accompanying L180M mutation.
- This study used INNO-LIPA. Entecavir or adefovir mutations were not investigated.
Summary

- HBV mutation patterns and problems are not different in Turkey from the rest of the world.
- Dominance of genotype D determines the higher prevalence of HBeAg negative infection.
- Precore stop codon mutation is mostly responsible for HBeAg negativity.
- Vaccine escape mutations of surface gene seem not to be an important public health problem.
- Drug resistant mutations have the same rate and same type as the other countries.