CHAPTER 7
CORRELATION OF THE MUTATIONAL PROFILE OF THE GENOME WITH THE VIRAL LOAD, DISEASE SEVERITY AND FINAL OUTCOME OF THE DISEASE
CHAPTER 7
CORRELATION OF THE MUTATIONAL PROFILE OF THE GENOME
WITH THE VIRAL LOAD, DISEASE SEVERITY AND FINAL
OUTCOME OF THE DISEASE

The proportion of cases with and without substitution were compared between different groups formed on the basis of viral load, prothrombin time, bilirubin, and final outcome of the disease i.e survival or expiry status of the patient using Fisher's Exact /Chi-square test.

Table 5.R12: Hepatic Encephalopathy Grade in ALF survived and expired patients

<table>
<thead>
<tr>
<th>Encephalopathy Grade</th>
<th>ALF (N = 52)</th>
<th>ALF-Expired (N = 29)</th>
<th>ALF-Survived (N=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4 (7.69%)</td>
<td>0</td>
<td>4(17.39 %)</td>
</tr>
<tr>
<td>3</td>
<td>6 (11.53%)</td>
<td>0</td>
<td>6 (26.08 %)</td>
</tr>
<tr>
<td>4</td>
<td>42 (80.76%)</td>
<td>29 (100 %)</td>
<td>13 (56.52 %)</td>
</tr>
</tbody>
</table>

Table 4.R13: Table showing survival of the patients

<table>
<thead>
<tr>
<th>Outcome</th>
<th>AVH(n=60)</th>
<th>ALF(n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Survived</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Expired</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

7.1 Comparison of the disease severity according to the nucleotide substitutions in the Methyltransferase region in the ORF 1 region of the HEV genome:

Histidine 105Arginine (H105 R), Aspartate29Asparagine (D29N), Valine27Alanine (V27A) were the mutations detected in this region.
Correlation between the H105 R mutation and viral load:

For the 32 sequences tested, 16 had a mutation at the 105 position and the viral load corresponding to these 16 samples were significantly lower compared to the remaining 16 where the mutation was absent (3166 ± 3756.47 copies/ml vs. 9278.063 ±11068.31 copies/ml, (p<0.05).

![H105R vs Viral Load](image)

**Figure 11.R13** Correlation between the H105 R mutation and viral load

In the pregnant acute liver failure category there were only 2 patients carrying this mutation in which the viral load was 9546 ± 9110 copies/ml compared to the other 5 pregnant patients with a viral load of 17641 ± 17285.5 copies/ml who lacked this mutation. However there was no significant difference between this two categories (p=0.57) of patients.

**H105 R mutation in relation to total bilirubin:**

The mean bilirubin in the patients carrying the mutation was 17.19±5.94 mg/dl compared to 10.86± 7.2 in whom this mutation was absent and was found to be statistically significant (p<0.01) between the two.
H105R vs Total bilirubin

<table>
<thead>
<tr>
<th></th>
<th>With Mutation</th>
<th>Without Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin</td>
<td>17.19</td>
<td>10.86</td>
</tr>
</tbody>
</table>

**Figure 11.14:** Correlation between the H105R mutation and bilirubin

**H105R mutation in relation to patient discharge status (dead/alive):**

The proportion of patients carrying this mutation had a significantly lower survival rate (p<0.01) of 10 patients compared to the other 16 patients who lacked this mutation and survived. Though it was a significant mutation it was observed that a higher number of mutations survived carrying this mutation.

**H105R mutation in relation to prothrombin time:**

The proportion of the cases with the mutation with a normal prothrombin time (<60 seconds) was significantly higher compared to the cases with abnormal prothrombin time who had the mutation.

**H105R mutation in relation to age:**

The patients carrying this mutation had a similar median age of 25.56 ±12.49 years corresponding to the 25.25 ± 10.26 years in 16 patients.

**7.1.2 Correlation between the D29N mutation and disease severity parameters:**

**Correlation between the D29N mutation and viral load**

For the 32 sequences tested, 16 had a mutation at the 29 position and the viral loads corresponding to this 16 samples were significantly higher than those of the other 16 (median viral load of the patients with this mutation was
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

80062.44 ± 48420.24 copies/ml which was significantly higher (p<0.0001) compared to 4384.73±5469.532 copies in the patients who lacked this mutation (Figure 11R15). Interestingly all the patients with acute liver failure 100%(16/16) of the patients harboured the mutation compared to all the patients with AVH who lacked the mutation.

![Figure 11.R15 Correlation between the D29N mutation and viral load](image)

In the pregnant acute liver failure category 11 patients carried this mutation in whom the viral load was 94532.64 ± 32209.07 copies/ml compared to the other 6 pregnant patients with a median viral load of 8010.5 ± 6775.59 copies/ml who lacked this mutation and was found to be significantly higher (p<0.0001). However there was no significant difference (p=0.484) between the pregnant patients with acute liver failure and the 2 non pregnant patients with acute liver failure who carried a HEV viral load of 111816 ±15013.29 copies/ml.

**D29N mutation in relation to total bilirubin:**

The mean bilirubin in the patients carrying the mutation was 17.19 ± 5.94 mg/dl compared to 10.86 ± 7.2 mg/dl in whom this mutation was absent and was found to be statistically significant (p<0.01) between the two.
D29N mutation in relation to patient discharge status (dead/alive):

The proportion of patients carrying this mutation had a significantly lower survival rate (p<0.01) of 10 patients compared to the other 16 patients who lacked this mutation and survived. Though it was a significant mutation, it was observed that a higher number of mutations survived carrying this mutation.

D29N mutation in relation to prothrombin time:

The proportion of the cases with the mutation with a normal prothrombin time (<60 seconds) was significantly higher compared to the cases with abnormal prothrombin time who had the mutation.

D29N mutation in relation to age:

The patients carrying this mutation had a similar median age of 27.35±4.93 years compared to the 25.25 ± 5.65 years in 16 patients in whom the mutation was absent. But it was not a significant difference (p=0.26) between the categories of patients.

7.1.3 Correlation between the V27A mutation and disease severity:

Correlation between the V27A mutation and viral load

For the 32 sequences tested, 16 had a mutation at the 27 amino acid position and the viral loads corresponding to this 16 samples was 72989.25 ± 43099.2 copies/ml which was significantly higher (p<0.0001) than those of the
other 16 patients who lacked this mutation in whom it was 9134.05 ± 11018.3 copies/ml. Interestingly all the patients with acute liver failure 100% (16/16) of the patients harboured the mutation compared to all the patients with AVH who lacked the mutation.

![V27A mutation vs viral load](image)

**Figure 11.R17** Correlation between the V27A mutation and viral load

In the pregnant acute liver failure category 11 patients carried this mutation in whom the viral load was 85894.2 ± 31504.69 copies/ml compared to the other 8 pregnant patients with a median viral load of 13104.5 ± 14489.77 copies/ml who lacked this mutation and was found to be significantly higher (p<0.0001). However there was no significant difference (p=0.484) between the pregnant patients with acute liver failure and the 2 non pregnant patients with acute liver failure who carried a HEV viral load of 102740.5 ± 27847.91 copies/ml.

**V27A mutation in relation to total bilirubin:**

The mean bilirubin in the patients carrying the mutation was 19.38 ± 6.42 mg/dl compared to 10.86 ±7.2 in whom this mutation was absent and was found to be statistically significant (p<0.01) between the two.
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

Figure 11.R18 Correlation between the V27A mutation and bilirubin

V27A mutation in relation to patient discharge status (dead / alive):

The proportion of patients who expired having this mutation was 87.5% (14/16) compared to none (0/16) of the patients in whom the mutation was lacking. Interestingly, 2 of the patients survived with this mutation but it was insignificant since it appeared to be a lethal mutation.

The proportion of patients carrying this mutation had a significantly lower survival rate (p<0.01) of 10 patients compared to the other 16 patients who lacked this mutation and survived. Though it was a significant mutation it was observed that a higher number of patients accumulating this mutation survived carrying this mutation.

V27A mutation in relation to prothrombin time:

The proportion of the cases with the mutation 85% (17/20) with a normal prothrombin time (<60 seconds) was significantly higher compared to the cases with abnormal prothrombin time who had the mutation.

V27A mutation in relation to age:

The patients carrying this mutation had a similar median age of 27.06± 4.85 years compared to the 25.25 ± 10.26 years in 16 patients in whom the
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

mutation was absent. But it was not a significant difference (p=0.26) between the categories of patients.

7.2 Comparison of the disease severity according to the nucleotide substitutions in the RdRp region in the ORF 1 region of the HEV genome:

7.2.1 Correlation between the C1483W mutation and disease severity

Correlation between the C1483W mutation and viral load:

For the 55 sequences tested, 45.45% (25/55) had a mutation at the amino acid position 1483 and the viral loads corresponding to this 25 samples were significantly higher than those of the other 30 (median viral load of the patients with this mutation was 58627.56 ± 47029.83 copies/ml which was significantly higher (p<0.0001) compared to 5682.16 ± 4982.74 copies/ml.

![C1483W vs Viral Load](image)

**Figure 11.R19** Correlation between the C1483W mutation and viral load in the patients who lacked this mutation. Interestingly all the patients with acute liver failure 100% (25/25) of the patients harboured the mutation compared to all the patients with AVH who lacked the mutation. Besides, the 30 patients who lacked these mutations belong to the group with self limiting disease.

In the pregnant acute liver failure category, 15 patients harbored this mutation in whom the viral load was 82515.53 ± 35647.466 copies/ml compared to the other 9 pregnant patients with a median viral load of 9027.5 ± 5790.52 copies/ml who lacked this mutation and was found to be significantly higher.
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease (p<0.0001). However there was not a single non pregnant patient with acute liver failure harbouring this mutation.

**Cysteine1483Tryptophan (C1483W) mutation in relation to total bilirubin:**

The mean bilirubin level of the patients 25/55(45.5%) carrying this mutation of 18.50 ± 5.88 mg/dl was significantly higher (p<0.0001) compared to 30 patients without this mutation in whom it was 10.30 ± 7.41 mg/dl.

![Figure11.R20 Correlation between the C1483W mutation and bilirubin](image)

**C1483W mutation in relation to patient discharge status (dead/alive):**

The proportion of patients who expired having this mutation was 34.54 % (19/55) compared to none (0/55) of the patients in whom the mutation was lacking. Interestingly, 6 of the patients 10.9 % (6/55) survived with this mutation but it was insignificant. 54.54% (30/55) of the patients lacking this mutation survived and all these patients belonged to AVH category. It was observed that the mutation was found to be significant associated with the final outcome of the disease.

**C1483W mutation in relation to prothrombin time**

The proportion of the cases with the mutation 80% (20/25) with an abnormal prothrombin time (>60 seconds) was significant higher compared to the cases 20% (5/25) with a normal prothrombin time who had the mutation. Further
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

100% (30/30) of the patients who had normal prothrombin time lacked the mutation which was extremely significant.

**Cysteine1483Tryptophan (C1483W) mutation in relation to age:**

The 25 patients carrying this mutation had a similar median age of 28.28 ± 10.06 years compared to the 29.4 ± 9.015 years in 30 patients in whom the mutation was absent. The median age in the patients harbouring this particular mutation had no significant difference compared to the age of the patients in whom the mutation was absent.

**7.2.2 Correlation between the N1530T mutation and disease severity**

**Correlation between the N1530T mutation and viral load**

For the 55 sequences tested, 45.45% (25/55) had a mutation at the amino acid position 1530 and the viral loads corresponding to this 25 samples was 42619.4 ± 48237.78 copies/ml which was higher compared to 4739.8 ± 5218.687 copies/ml in the 30 patients who lacked this mutation. But there was no significant difference (p=0.591) between the two categories of patients. Hence this mutation had similar viral loads when compared.

![N1530T vs Viral Load](image)

Figure 11.R21 Correlation between the N1530T mutation and viral load

In the pregnant acute liver failure category, 11 patients harbored this mutation in whom the viral load was 69931.55 ± 46261.17 copies/ml compared to the other 11 pregnant patients with a median viral load of 7313.45 ± 5826.53
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

copies/ml who lacked this mutation and was found to be significantly higher (p<0.0002). Further, the 14 non pregnant patients with acute liver failure without mutation with a mean viral load of 21119.86±39018.34 copies/ml was significantly lower (p<0.008) compared to their pregnant counterparts who possessed the mutation.

**N1530T mutation in relation to total bilirubin**

The mean bilirubin level of the patients 25/55 (45.5%) carrying this mutation of 16.54 ± 5.86 mg/dl was significantly higher (p<0.0001) compared to 30 patients without this mutation in whom it was 9.08 ± 5.71 mg/dl.

![N1530T vs Total bilirubin](image)

**Figure 11. R22** Correlation between the N1530T mutation and bilirubin

**N1530T mutation in relation to prothrombin time**

The proportion of the cases with the mutation 68% (17/25) with an abnormal prothrombin time (>60 seconds) was significantly higher compared to the cases 32% (8/25) with a normal prothrombin time who had the mutation. Further 100% (30/30) of the patients who had normal prothrombin time lacked the mutation which depicted that a significant association between prothrombin time and mutation.
N1530T mutation in relation to final outcome of the patients

The proportion of patients who expired having this mutation was 27.27% (15/55) compared to none (0/55) of the patients in whom the mutation was lacking and which was extremely significant (p<0.0001). Interestingly, 10 of the patients (6/55) survived with this mutation but it was insignificant. 54.54% (30/55) of the patients lacking this mutation survived and all these patients belonged to AVH category. It was observed that the mutation was found to be significant associated with the final outcome of the disease.

N1530T mutation in relation to age:

The 25 patients carrying this mutation had a similar median age of 26.37 ± 9.73 years compared to the 24.13 ± 6.11 years in 30 patients in whom the mutation was absent. The median age in the patients harbouring this particular mutation had no significant difference (p=0.3) compared to the age of the patients in whom the mutation was absent.

7.3 Comparison of the disease severity according to the nucleotide substitutions in the capsid gene of the ORF 2 region of the HEV genome

7.3.1 Correlation between the P259S mutation and disease severity

Correlation between the P259S mutation and viral load:

For the 55 sequences tested, 51.02% (25/49) had a mutation at the amino acid position 259 and the viral loads corresponding to this 25 samples was 74018.84 ± 43952.21 copies/ml which was higher compared to 7206.915 ± 9801.183 copies/ml in the 24 patients who lacked this mutation. The viral load in these patients carrying this mutation was significantly higher compared to the patients who lacked this mutation.
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

**Figure 11.R23** Correlation between the P259S mutation and viral load

In the pregnant acute liver failure category, 18 patients harbored this mutation in whom the viral load was $89136.44 \pm 29566$ copies/ml compared to the other 12 pregnant patients with a median viral load of $9994.67 \pm 12939.18$ copies/ml who lacked this mutation and was found to be significantly higher ($p<0.0001$). Further, the 3 non pregnant patients with acute liver failure without mutation with a mean viral load of $78877.33 \pm 58030.74$ copies/ml was significantly lower ($p<0.008$) compared to their pregnant counterparts who possessed the mutation.

**Correlation between the P259S mutation and prothrombin time:**

The proportion of the cases with the mutation with a abnormal prothrombin time (>60 seconds) with 88% (22/25) was significantly higher ($p<0.0001$) compared to none of the cases 0/30 who lacked the mutation with abnormal prothrombin time. 12%(3/25) with a normal prothrombin time who had the mutation. Further 100%(30/30) of the patients who had normal prothrombin time lacked the mutation which depicted that a significant association between prothrombin time and mutation.

**Correlation between the P259S mutation and final outcome**

The expired cases with this mutation 88%(22/25) was found to be significantly higher ($p<0.0001$) compared to none of the cases 0/30 who lacked the
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

Mutation. Besides the survival rate with this mutation was only 12% (3/25) compared to 100% (30/30) who lacked this mutation.

**Correlation between the P259S mutation and bilirubin**

The mean bilirubin level of the patients 25/49 (45.5%) carrying this mutation of 17.67 ± 5.85 mg/dl was significantly higher (p<0.0004) compared to 24 patients without this mutation in whom it was 10.72 ± 6.77 mg/dl.

![Figure 11.R24](image)

Correlation between the P259S mutation and bilirubin

**Correlation between the P259S mutation and age**

The 25 patients carrying this mutation had a similar median age of 26.52 ± 6.35 years compared to the 26 ± 9.26 years in 24 patients in whom the mutation was absent. The median age in the patients harbouring this particular mutation had no significant difference (p=0.81) compared to the age of the patients in whom the mutation was absent.

7.3.2 Correlation between the Phenylalanine 356 Leucine (F356L) mutation and Lysine 350 Glutamate (K350 E) mutation and disease severity:

**Correlation between the F356L mutation and K350 E mutation and viral load:**

For the 32 sequences tested, 5 patients had a mutation at the 356 and 350 amino acid position and the viral loads corresponding to this 5 samples was 74054.8 ± 61465.87 copies/ml which was significantly higher (p<0.0001) than those of the other 25 patients who lacked this mutation in whom it was
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

7206.91±9801.18 copies/ml. Interestingly, only 20% (5/25) of the patients with acute liver failure harboured the mutation compared to all the patients with AVH who lacked the mutation. The same mutations were also present in 5 of those samples who also possessed the P259S mutation.

![F356L vs Viral Load](image)

**Figure 11.R25** Correlation between the F356L mutation and K350 E mutation and viral load

In the pregnant acute liver failure category 2 patients carried this mutation in whom the viral load was 115671.5 ± 24538.5 copies/ml compared to the other 12 pregnant patients with a median viral load of 9994.67 ± 12939.18 copies/ml who lacked this mutation and was found to be significantly higher (p<0.0001). However there was no significant difference (p=0.484) between the pregnant patients with acute liver failure and the 3 non pregnant patients with acute liver failure who carried a HEV viral load of 46310.33 ± 66094.24 copies/ml.

**F356L mutation and K350 E mutation in relation to total bilirubin:**

The mean bilirubin in the patients carrying the mutation was 19.5 ± 5.35 mg/dl compared to 10.72 ± 6.77 in 24 patients in whom these mutation was absent and was found to be statistically significant (p<0.05).
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

Figure 11.R26 Correlation between the F356L mutation and K350E mutation and bilirubin

F356L mutation and K350E mutation in relation to patient discharge status (dead/alive):

The proportion of patients who expired having this mutation was 80% (4/5) compared to none (0/16) of the patients in whom the mutation was lacking. Interestingly, 1 of the patient survived with this mutation but it was insignificant since it appeared to be a lethal mutation.

The proportion of patients carrying this mutation had a significantly lower survival rate (p<0.01) of 1 patient compared to the other 24 patients who lacked this mutation and survived. Though it was a significant mutation it was observed that a higher number of patients expired carrying this mutation.

F356L mutation and K350E mutation in relation to prothrombin time:

The proportion of the cases with the mutation 60% (3/5) with a abnormal prothrombin time (<60 seconds) was significant higher (p<0.0001) compared to none of the case (0/24) with abnormal prothrombin time who lacked the mutation.
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

F356L mutation and K350 E mutation in relation to age

The patients carrying this mutation had a similar median age of \(25 \pm 2.91\) years compared to the \(26 \pm 9.26\) years in 24 patients in whom the mutation was absent. But it was not a significant difference \((p=0.26)\).

7.3.3 Correlation between the Serine 209Proline (S209P) mutation and disease severity

Another significant mutation Serine 209Proline (S209P) was detected in 6 patients with AVH and absent in patients with acute liver failure. This mutation had a significantly lower viral load \((p<0.001)\) compared to the patients who lacked this mutation. The mean age of the patients with this mutation was \(23 \pm 5.2\) years compared to \(27 \pm 6.2\) years and was insignificant compared to the patients who lacked this mutation. Comparatively the bilirubin levels were significantly lower compared to the patients who lacked the mutations. In regard to the prothrombin time the patients had no association with abnormal prothrombin time. With regard to the final outcome of the patients there was not a single mortality in the patients accumulating this mutation.

7.4 Comparison of the disease severity according to the nucleotide substitutions in the ORF 3 region of the HEV genome:

7.4.1 Correlation between the Serine108 Alanine(S108A) mutation and disease severity

Serine108 Alanine(S108A) was the most significant mutation in this region whereas the other 6 mutations Threonine 105 Glutamine(T105Q),Proline 110 Alanine(P110 A),Glycine 100 Alanine (G100A) and insertion of Arginine at position 101,Aspartate at 102,Glutamine at 103 were also present in this region. The G100A was a synonymous mutation and hence was not a significant one in this region.
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

Correlation between the S108A mutation and viral load

From the 40 sequences tested, 20 had a mutation at the 108 amino acid position and the viral loads corresponding to this 20 samples was 76466.25 ± 45415.7 copies/ml which was significantly higher (p<0.0001) than those of the other 20 patients who lacked this mutation in whom it was 8091.762 ± 10123.91 copies/ml. Interestingly all the patients with acute liver failure 100%(20/20) of the patients harboured the mutation compared to all the other patients with AVH who lacked the mutation.

In the pregnant acute liver failure category 14 patients carried this mutation in whom the viral load was 91616.21 ± 31796.34 copies/ml compared to the other 10 pregnant patients with a median viral load of 11069.4 ± 13864.34 copies/ml who lacked this mutation and was found to be significantly higher (p<0.0001). However there was not a single patient without pregnancy who carried the mutation.

S108A mutation in relation to patient discharge status (dead / alive):

The expired cases with this mutation 95%(19/20) was found to be significantly higher (p<0.0001) compared to none of the cases 0/20 who lacked the mutation. Besides the survival rate with this mutation was only 5%(1/20) compared to 100%(20/20) who lacked this mutation.

S108A mutation in relation to prothrombin time:

The proportion of the cases with the mutation with a abnormal prothrombin time (>60 seconds) with 85% (17/20) was significantly higher (p<0.0001) compared to none of the cases 0/20 who lacked the mutation with abnormal prothrombin time.

S108A mutation in relation to total bilirubin

The mean bilirubin level of the patients 20/40(50%) carrying this mutation of 17.67 ± 5.85 mg/dl was significantly higher (p<0.0004) compared to 24 patients without this mutation in whom it was 10.72 ± 6.77 mg/dl.
Correlation of the mutational profile of the genome with the viral load, disease severity and final outcome of the disease

S108A mutation in relation to age:

The patients carrying this mutation had a similar median age of 25.85±5.63 years compared to the 24.66 ± 3.66 years in 20 patients in whom the mutation was absent. But the age did not differ significantly between the two groups (p=0.43).

7.4.2 Correlation of insertion of Arginine at position 101, Aspartate at 102, Glutamine at 103 and disease severity

Correlation of insertion of Arginine at position 101, Aspartate at 102, Glutamine at 103 and the viral load:

From the 40 sequences tested, 20 patients had a insertion at the 101,102 and 103 amino acid position and the viral loads corresponding to this 20 samples was 73767.55±45870 copies/ml which was significantly higher (p<0.0001) than those of the other 20 patients who lacked this mutation in whom it was 8091.76 ± 10128.91 copies/ml. Interestingly all the patients with acute liver failure 100% (20/20) of the patients accumulated the mutation compared to all the other patients with AVH who lacked the mutation.

In the pregnant acute liver failure category 15 patients carried this mutation in whom the viral load was 93304.33 ± 32393.92 copies/ml compared to the other 10 pregnant patients with a median viral load of 11069.4 ±13864.34 copies/ml who lacked this mutation and was found to be significantly higher (p<0.0001). However there was not a single patient without pregnancy who carried the mutation.

Insertion of Arginine at position 101,Aspartate at 102,Glutamine at 103 in relation to patient discharge status(dead /alive):

The expired cases with this mutation 95%(19/20) was found to be significantly higher(p<0.0001) compared to none of the cases 0/20 who lacked the mutation .Besides the survival rate with this mutation was only 5%(1/20) compared to 100%(20/20) who lacked this mutation.
Insertion of Arginine at position 101, Aspartate at 102, Glutamine at 103 in relation to prothrombin time:

The proportion of the cases with the mutation with a abnormal prothrombin time (>60 seconds) with 85% (17/20) was significantly higher (p<0.0001) compared to none of the cases 0/20 who lacked the mutation with abnormal prothrombin time.

Insertion of Arginine at position 101, Aspartate at 102, Glutamine at 103 in relation to total bilirubin

The mean bilirubin level of the patients 20/40 (50%) carrying this mutation of 17.405 ± 5.7 mg/dl was significantly higher (p<0.05) compared to 24 patients without this mutation in whom it was 11.33 ± 7.31 mg/dl.

Insertion of Arginine at position 101, Aspartate at 102, Glutamine at 103 mutation in relation to age

The patients carrying this mutation had a similar median age of 27.6 ± 4.2 years compared to the 24.66 ± 3.66 years in 20 patients in whom the mutation was absent. But the age did not differ significantly between the two groups (p=0.43).

7.4.3 Correlation of Threonine 105 Glutamine (T105Q), Proline 110 Alanine (P110 A) and disease severity

Correlation of T105Q, P110 A and viral load:

From the 40 sequences tested, 20 patients had mutations of Threonine 105 Glutamine (T105Q), Proline 110 Alanine (P110 A) at the positions 105 and 110 amino acid position and the viral loads corresponding to this 20 samples was 64811.1 ± 46584.25 copies/ml which was significantly higher (p<0.0001) than those of the other 20 patients who lacked this mutation in whom it was 8091.76 ± 10123.91 copies/ml. Interestingly, all the patients with acute liver failure 100% (20/20) of the patients harboured the mutation compared to all the other patients with AVH who lacked the mutation.
In the pregnant acute liver failure category 14 patients carried this mutation in whom the viral load was $91513.79 \pm 24645.41$ copies/ml compared to the other 10 pregnant patients with a median viral load of $11069.4 \pm 13864.34$ copies/ml who lacked this mutation and was found to be significantly higher ($p<0.0001$). However there was not a single patient without pregnancy who carried the mutation.

**T105Q, P110 A in relation to total bilirubin**

The mean bilirubin level of the patients 20/40 (50%) carrying this mutation of $16.75 \pm 5.48$ mg/dl was significantly higher ($p<0.05$) compared to 24 patients without this mutation in whom it was $11.33 \pm 7.31$ mg/dl.

**T105Q, P110 A in relation to prothrombin time**

The proportion of the cases with the mutation with an abnormal prothrombin time (>60 seconds) with 75% (15/20) was significantly higher ($p<0.0001$) compared to none of the cases 0/20 who lacked the mutation with abnormal prothrombin time.

**T105Q, P110 A in relation to age**

The patients carrying this mutation had a similar median age of $24.15 \pm 4.86$ years compared to the $24.66 \pm 3.66$ years in 20 patients in whom the mutation was absent. But the age did not differ significantly between the two groups ($p=0.43$)

**T105Q, P110 A in relation to final outcome**

The expired cases with this mutation 95% (19/20) was found to be significantly higher ($p<0.0001$) compared to none of the cases 0/20 who lacked the mutation. Besides the survival rate with this mutation was only 5% (1/20) compared to 100% (20/20) who lacked this mutation.