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Original Research Article

Molecular differentiation of dengue virus serotypes using RT-PCR and estimation of their effect on liver function

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ABSTRACT

Dengue is one of the common and leading cause of mortality and morbidity in tropical countries specially in India. Its hepatotoxic effects have been demonstrated by the researchers over a long time. Dengue virus is a RNA virus, and known to have four serotypes and recently a fifth variant is also demonstrated in India. The effect of the different serotypes is seldomely investigated by the researchers. So we try to estimate the effect on Liver due to infection via different serotypes of Dengue virus. After taking the ethical clearance, from the ethical committee, A hospital based prospective study was conducted from August 2017 to November 2018, on patients attending OPD and IPD, of Medicine Department at Shri Mahant Indiresh Hospital Dehradun. 60 Dengue Positive cases were selected. RNA gets extracted using Reverse Transcriptase PCR, Conventional PCR and Real Time PCR techniques and further visualized using Agarose gel electrophoresis techniques at the CMRL (Central Molecular Research Laboratory), and liver Enzymes SGPT, SGOT, ALP, GGT were analyzed at Central Clinical Laboratory of SMI Hospital using the vitros 5600 fully autoanlyzer of the Orthoclinical diagnostics. Results were analyzed using suitable statistical tools.

1. Introduction

Dengue is a common febrile illness, and also one of the leading cause of morbidity and mortality particularly in developing countries. Dengue virus, an arthropod-borne, RNA virus that infects humans by Aedes mosquitoes and cause of major disease burden in tropical and subtropical countries worldwide.[1] Dengue Virus seems to have hepatotoxic effects.[2] DENV, is a major human pathogen, and are of four antigenically different serotypes.(although there is a report of 2013 that claims a fifth serotype has been found). These four subtypes are different strains of dengue virus that have 60-80% homology but which is most hepatotoxic is not focus of the researchers. In India, dengue is an endemic, and begins after 2 to 14 days incubation period from the bite of the mosquito, with high fever, headache, retroocular pain, lumbosacral pain, conjunctival congestion, and/or facial flushing with biphasic fever etc. The major difference is in the surface proteins of the different dengue subtypes. Infection induces immunity against the infecting serotype, but it gives only a short time cross protective immunity against the other types, which explains the phenomenon of Antibody-Dependent Enhancement.[3] So we conduct this study to estimate the hepatotoxic effects of different dengue serotypes on the basis of liver transaminases, ALP and GGT.

2. Aims and Objective

1. To evaluate the Liver enzymes of the patients suffering from dengue fever.
2. To find the most hepatotoxic serotype of dengue virus.

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3. Materials and Methods

The study design does not include the direct intervention to the patients as we collected the ELISA confirmed dengue patients sample for the study, we get the ethical clearance for the same. After taking the ethical clearance from the ethical committee, A hospital based prospective study was conducted from August 2017 to November 2018, on patients attending OPD and IPD, of Medicine Department at Shri Mahant Indiresh Hospital Dehradun. Informed consent was taken by the patients for the same. 60 ELISA confirmed Dengue Positive patients samples were selected.

3.1. Method for RNA isolation for serotyping

In 140μl of serum sample, 560μl of Lysis buffer and 5.4μl carrier RNA added, and incubated with intermittent vortex for 10 minutes. Then 560μl of absolute ethanol was added and vortexed. Out of this MCT (micro centrifugation tube) 700 μl of sample was transferred to the silica column and centrifuged at 10000 rpm for 1 minute in cooled centrifuge and decant the supernatant. Column dry washed and transferred to the new freshly labeled MCT. 60μl of elusion buffer was added and incubated at room temperature for 4 minutes. MCT with the column was centrifuged at 10000 rpm for 1 minute in cooling centrifuge. The silica column was discarded, and the extracted nucleic acid was used as a template for detection. The extracted template was utilized using Reverse Transcriptase PCR, Conventional PCR and Real Time PCR techniques and further visualized using Agarose gel electrophoresis techniques at the CMRL and liver Enzymes SGPT,[4] SGOT.[5] ALP,[6] GGT[7] were analyzed at Central Lab of SMI Hospital using the vitros 5600 fully autoanlyzer of the Orthoclinical-diagnostics.

4. Results

Out of 60 samples DENV-1 was 15, DENV-2 was 29 and DENV-3 was 16.

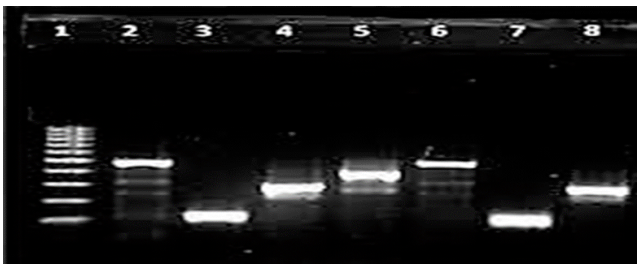


Fig. 1: Dengue virus aAmplicons in agarose gel

Well 1: 100 bp DNA difference ladder
Well 2: PCR amplicon of 482 for DENV-1 serotype
Well 3: PCR amplicon of 119 for DENV-2 serotype
Well 4 and 5: PCR amplicon of 482 for DENV-1 serotype
Well 6: PCR amplicon of 482 for DENV-1 serotype
Well 7: PCR amplicon of 119 for DENV-2 serotype
Well 8: PCR amplicon of 290 for DENV-3 serotype

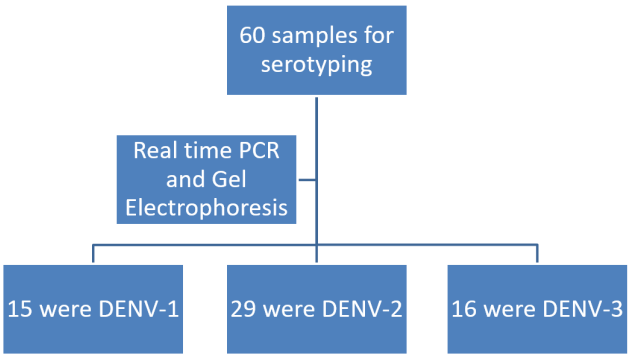
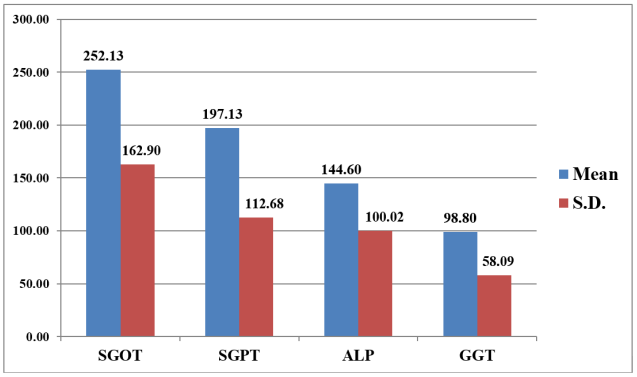


Chart 1:

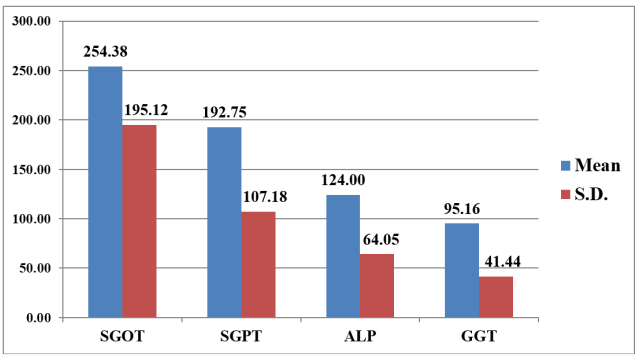
The results of serotyping are described above in Chart 1 and Figure 1.

The results of the involvement of the Liver enzymes are tabulated below in Table 1 and represented graphically below in Graphs 1, 2 and 3.

In our results the DENV-3 was found to be most hepatotoxic among the different dengue viruses.



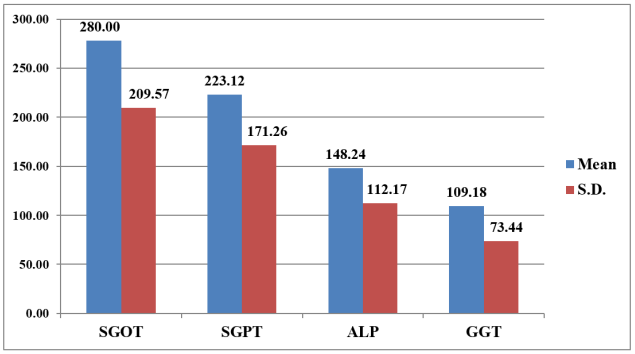
Graph 1: (a) Effect of DENV-1Serotype



Graph 2: Effect of DENV-2 serotype

Table 1: Effect of the dengue viruses on the liver enzymes

Dengue Virus	SGOT (Mean±SD)	SGPT (Mean±SD)	ALP (Mean±SD)	GGT (Mean±SD)
DENV-1	252±162	197±112	144±100	98±58
DENV-2	254±195	192±107	124±64	95±41
DENV-3	280±209	223±171	148±112	109±73



Graph 3: Effect of DENV-3 serotype

5. Discussion

Till date there are two hypothesis that explains the damage of liver in dengue patients. The first is immune enhancement hypothesis. In 2004, a strong correlation was found between T-cell activation and hepatic cellular infiltration in immune competent mice infected with dengue virus. It was noted that kinetics of liver enzymes elevation was also correlated with that of T-cell activation, which suggest a relationship between T cell activation and elevations of liver enzymes.[8] One of the study detected the appearance of different helper cells and cytokines in human WBC's cultures, infected invitro with dengue virus. They reported that during infection WBC's (monocytes, B cells, T cells and mast cells) produced a large amount of cytokines.[9] The second hypothesis relates the damage in the liver to direct virulence of the virus.[10] According to these studies, we hypothesized the same mechanism responsible for the liver damage which occurred in our study. The aspartate aminotransferase (AST) levels in dengue infection tend to be greater than alanine aminotransferase (ALT) levels.[11,12]

6. Conclusion

We concluded that dengue virus serotype 3 affects liver transaminases more than other serotypes. So it clears that the dengue virus serotype 3 is more hepatotoxic as compared to other serotypes.

7. Source of Funding

None.

8. Conflicts of Interest

There are no financial or non-financial conflict of interests that are directly or indirectly related to the work.

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