



A Study on the Distribution of HCV Genotypes Across the North Indian Population

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ABSTRACT

The Hepatitis C virus has globally affected the population through cases of liver cancer and cirrhosis. It has been seen that the genotype that is witnessed most commonly are the genotype 1 and genotype 3. In order for the patient to go for successful antiviral therapy and ensure suitable and adequate recovery from the disease, the study of the genotype is extremely crucial. This genotype study determines what the treatment of the disease. It can also be used as a reference to future diagnostic measures to not just detect the disease but also enable suitable measure to study the prognosis of the disease.

The Hepatitis C viral infection can be detected through both serological and with molecular methods. However, serological methods of testing can also be employed, that is not as sensitive and accurate as the ones done on a molecular level with reference to whether or not the immune antibody response is adequate. Molecular methods of diagnosis detect the specific gene of the pathogen which produces significantly reliable results. Studying the distribution of the genotype of the virus especially over the North Indian subcontinent can enable one to identify and determine suitable therapeutic methods that can be availed to prevent the spread of the disease. This creates reference data for approaches such as area specific immunization along with a study of a suitable environment for the pathogen to thrive in. The data obtained through this study can be used along with already present data to generate an epidemiological analysis with respect to diagnostic implications and prevention strategies.

Keywords: Genotype, Immunization, Therapy, Molecular, Serological, Diagnosis, Distribution.

Introduction

In a number of cases of liver cancer and other diseases of similar nature it has been found that the root cause of infection is the Hepatitis C virus. This means that for the successful detection of cirrhosis causing diseases especially those of the hepatic origin, it is important to check for the presence of the Hepatitis C virus. This makes it easier to correlate and diagnose factors such as viral load and the presence of viral infection. The genotype study and identification of the virus causing the disease is crucial in this case as it enables for the usage of appropriate antiviral therapies that can help to eradicate the disease from the system of the host effectively.

Hepatitis C disease

Hepatitis is primarily an infection that is caused by the presence of the Hepatitis C virus. This disease usually affects the liver and results in major health issues such as the cancer of the liver, liver failure, major liver diseases or in some cases, even death. The infections of the virus can range from both acute to chronic. To break it down, the individuals that have an acute Hepatitis Virus infection are usually able to get clear of it within the first six months of the infection without any significant treatment. The average percentage of individuals at that are able to do this is thirty percent; with the overall ranging being from fifteen percent to thirty- five percent [2]. The individuals that develop the chronic HCV virus infection are within the percentage ranges of fifty- five percent to eight- five percent. Within the individuals that do develop chronic liver infection due to the hepatitis C virus, around thirty percent to fifteen percent of individuals are likely to develop cirrhosis within the liver. This usually develops within the first twenty years of acquiring the infection.

Global Epidemiology of the HCV virus

Out of the overall population of the world, roughly about 71.1 million individuals have been affected across the world chronically [6]. This could be estimated to be at an incidence of 23.7 individuals per 1,000,000 individuals. This is an ongoing increase from the prior incidence rate and may carry the chances of HCV becoming a global epidemic. However, the incidence varies strongly across regions and this primarily is dependent upon the healthcare practices that are employed in regions. For example, in the south-East Asia region, the incidence rate for the viral infection of HCV is 14.8 individuals per 100,000 individuals. The spread of the virus also depends on the level of development of the country. For example, in developing countries and countries undergoing transition, the spread of the Hepatitis C viral infection is resultant of the use of unsterile medical equipment. Unsafe medical injections are most commonly responsible for the transmission of this infection. According to the data based on the geographical incidence of the Hepatitis C virus, higher incidence rates have been reported from individuals in areas of the WHO European region as well as the Eastern-Mediterranean region. In cases of co-infection with HIV and HBV; HIV and HCV co-infection is seen to be more prevalent. The presence of the HBV virus is seen to inhibit the progression of the HCV infection. However, HBV and HCV co-infection is seen in cases where there is more severe liver disease.

Affect on the Infected Individual

The damage caused by the HCV virus is mostly evident through the liver of the infected individual. The exact mechanism of the infection is not properly known yet. However, it is safe to believe that the immune affect that is caused on the system is due to the immune response of the individual itself on coming into interaction with the Hepatitis C virus [23]. A majority of individuals that showed a high level of Hepatitis C virus replication, a very insignificant amount of liver damage was detected. However, those that did show signs of liver damage also, showed a greater susceptibility to being re-infected by the Hepatitis C virus.

Cirrhosis of the liver is resultant of inflammatory cells that activate hepatic stellate cells in order to release collagen through the use of chemokines. The stellate cells that are quiescent cells of the liver that are crucial for all the normal functions of the liver undergo injury. This injury makes them differentiate to a state in which their phenotype becomes activated. This transition leads to reprogramming of the metabolic function of the liver. This reprogramming is resultant to autophagy. Additionally, it has also been seen that this reprogramming results in the increase of parenchymal injury. Myofibroblasts or hepatic stellate cells that are activated result in the depiction of the altered phenotypic features through cirrhosis of the liver. This leads to the development of cirrhosis. It has been seen that as the number of activated hepatic stellate cells is reduced, it can lead to a state in which the fibrosis that is taking place in the liver undergoes regression. The activated stellate cells can be reduced or eliminated through senescence, undergoing apoptosis or they can be reversed into an inactivated phenotype. Of these, the cells that have reached a senescent state are cleared away. This is usually carried out by a natural killer cell. The inactivated stellate cells reach a state where they become primed to respond in an appropriate manner to additional injury that may be taking place in the liver [22].

Evidence has been found to show that once treatment begins against the Hepatitis C virus; it is found that they are found in large amounts (almost in a reservoir like fashion) in leukocytes. This viral strain has undergone transformation by the intake of treatment. This suggests that though the compartments of the leukocyte, there may be an additional route that enables the spread of the Hepatitis C virus which puts it in a position where it is able to directly affect the immune system of the host. This route is not fully understood. However, it could also explain the manner through which this Hepatitis C virus is able to avoid eradication.

Significance of HCV Genotyping

For the usage of therapies that are antiviral in nature it is crucial to go for the determination of the genotype of the strain of virus that is responsible for the cause of infection in an affected individual. This is all the more significant because the different subtypes of the Hepatitis C virus show different levels of resistance to different antiviral drugs. The difference in the subtypes of the virus is caused by the regions that encode the core protein of the virus as well as the NS5B region. Both of these regions provide differences that are accounted for by the variances in the subtypes and genotypes. These are non-overlapping regions of the gene in the different strains [25].

Methods for HCV Genotyping

Genotyping is a way of establishing the genotype or the sequence of the nucleic acids of the viral pathogen in question. Knowing the sequence of genes can be crucial in identifying and determining the type of therapeutic system that can be employed in order to counteract the spread of the infection. It is also key in being able to come up with a suitable immunization to curtail the spread of this viral infection on the whole. Broadly speaking there are two methods of Genotyping [26]:

Molecular methods-based genotyping: This is based on the principle of the Polymerase Chain Reaction. Upon the detection of these certain segment and whether or not they are established to be of a certain concentration, the genotype can be detected. The genotype that shows the greater concentration in the bloodstream will be determined as the viral strain the is responsible for the infection on the individual. It is also for this strain that the individual will receive treatment for. The quantification is usually done through the usage of specific primer probes that enable in the detection of the signal that comes from the amplification that is taking place [26].

Serological methods of genotyping: The serological method is based primarily on the usage of antibodies for the detection of the presence of the Hepatitis C virus in the bloodstream. These antibodies that are utilized are usually specific to the strain on the basis of the altered genotype of the Hepatitis C viruses. This method has a number of factors that make it particularly of an advantage to large scale studies that are used to monitor epidemics. In addition to this it is quite simple to perform the test which makes it a much more preferable option while carrying out the testing of the genotype [26]. However, the drawback remains that it is not as sensitive and specific as a PCR test would be. This test is given a greater preference when a case of chronic Hepatitis C viral infection is under question.

This study that has been carried out has focused on the methods of detection of Hepatitis C Viral infection based on the Molecular Method of Diagnostics.

Methodology

The methodology involved for the diagnosis of the viral strain within the infected individual is can be summarized as the selection of the samples, the determination of the assay kit and further the performance of the Polymerase Chain Reaction of the product. The procedures for testing are further discussed below:

Selection of samples: Plasma samples are usually preferred for the testing of the samples. This is because; serum or plasma samples have the ability to solubilize certain components that need to undergo analysis in liquid form. Quantities such as hormones and proteins are a lot easier to work with when they are in the form of serum. Extraction of nucleic acids can happen from the nucleus of leukocytes which are present in serum therefore making serum a preferable source.

Extraction of the DNA: The MagMax extraction system is employed for the extraction of the nucleic acids from the affected serum samples. This method is done using the extraction kit (Kingfisher) of the ThermoFisher brand. The principle of extraction is based on the usage of magnetic beads to extract the electronegative nucleic acids that bind to the positively charged ferric cation that is present in the magnetic beads.

Genotype Based PCR of the extraction elute: The kit that has been used for the genotype study of the PCR amplification system is the kit from MyLabs. This contains a Master Mix solution, multiple Primer Probe solutions for each of the various genotypes, positive and Negative controls and the sample under question. For the kit that is used, four mixtures for each sample are taken. This is because each well contains a mixture of a separate primer probes. The primer-probe mixtures contain two or more genotypes.

The results of the amplification are shown in the form of a sigmoidal curve based on the Cycle threshold value. The curve must be sigmoidal in shape to ensure that the run has taken place perfectly and there is minimal to no presence of any contamination or noise. The following is an image of how the results look like. Each genotype has a different reporter dye; it makes it easier to understand the amount of amplification that has taken place and for what genotype.

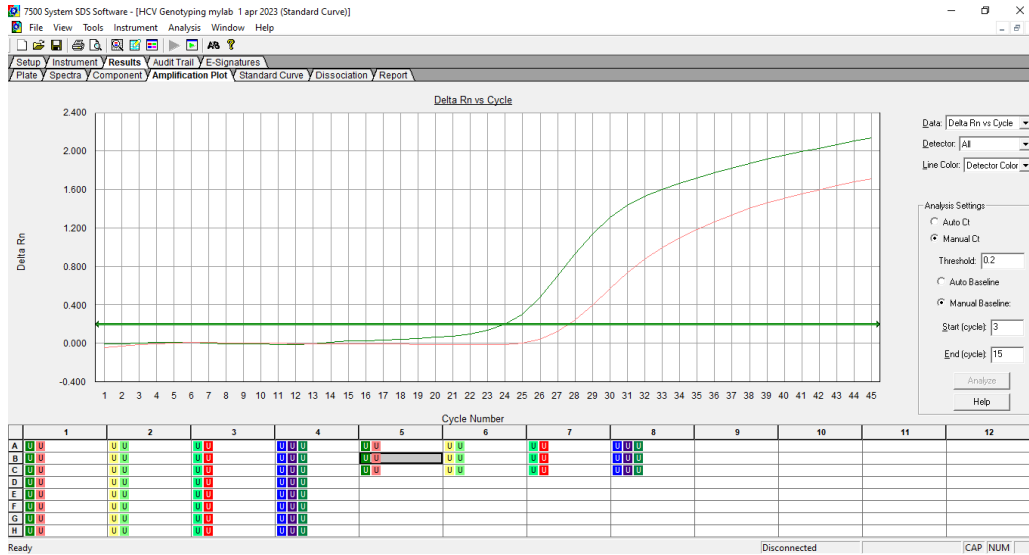


Image 1: Sigmoidal curve showing the HCV genotype results

Observations

The genotype assay was conducted for over 4 months for a total of about 309 samples in total. This sample quantity is usually quite limited in number. This is because some cases include the genotype assay as a reflex to the general molecular diagnostic tests for the HCV infection.

The following describes the distribution of the Hepatitis C virus with regards to the states that are part of the North Indian region. In total the number of samples received for the detection of the genotype was 309. Out of these samples, the number of samples that belonged to the Southern Indian states and the Central Indian states was accounted to be 80 in total. Therefore, only 228 cases belonged to the North Indian population and fulfilled the criteria of the study. Out of these 228 cases, only 151 cases showed a positive result for the detection of the Hepatitis C virus. The distribution according to the North Indian States is described in the table below.

State	Genotype detected	Number of Samples
Assam	3	2
Assam	4	1
Assam	5	1
Bihar	4	1
Chattisgrah	3	3
Gujarat	1 (a)	1
Haryana	3	4
Haryana	4	1
Haryana	1 (a)	1
Jammu and Kashmir	3	20
Jammu and Kashmir	4	6
Jammu and Kashmir	5	2
Jammu and Kashmir	1 (a)	5
Jammu and Kashmir	1 (b)	4
Madhya Pradesh	3	1

Madhya Pradesh	1 (a)	1
Manipur	3	21
Manipur	4	5
Manipur	5	15
Manipur	6	5
Manipur	1 (a)	2
Manipur	1 (b)	3
Uttar Pradesh	3	11
Uttar Pradesh	1 (a)	3
Uttar Pradesh	5	1
Punjab	3	2
Tripura	3	5
Tripura	4	1
Tripura	6	1
Uttrakhand	3	1
West Bengal	3	3
West Bengal	4	3
West Bengal	1 (a)	3
West Bengal	1 (b)	3

Table 1: Distribution and frequency of the genotype based on various North Indian states.

- The presence of genotypes 3,4 and 5 are seen in Assam; with the presence of the genotype 3 viral strain being the most common.
- The lack of cases shows that the virus is not severely widespread in the state of Bihar. It shows only the prevalence of genotype 4.
- The prevalence of Hepatitis C virus strain 3 is shown to be the only strain prevailing in the region of Chattisgarh.
- In the North Indian state of Gujarat. It is seen that the only prevalent genotype in this region is the subtype of the genotype 1 which is 1 (a).
- The only prevalent genotype in the state of Haryana is the genotype 3 followed by equal prevalence of genotype 4 and the subtype of the genotype 1 which is 1 (a).
- In the North Indian state of Jammu and Kashmir. It is seen that the only prevalent genotype in this region is the genotype 3 followed by equal prevalence of genotype 4, the 1 (b) and 1(a) followed by the genotype 5.
- In the distribution in the North Indian state of Madhya Pradesh. It is seen that the prevalent genotype in this region is the genotype 3 and the subtype of the genotype 1 which is 1 (a).
- In the distribution in the North Indian state of Manipur. It is seen that the major prevalent genotype in this region is the genotype 3 followed by equal prevalence of genotype 4,5 and the subtype of the genotype 1 which is 1 (a) and 1 (b). Even the presence of genotype 6 is witnessed here. This shows that all strains of the Hepatitis C virus are quite evenly distributed in this region.
- In the North Indian union territory of New Delhi. It is seen that the major prevalent genotype in this region is the genotype 3 followed by prevalence of the subtype of the genotype 1 which is 1 (a).
- The only genotype present in the state of Punjab is the genotype of the HCV viral strain containing the genotype 3.
- The data shows the lack of prevalence of the Hepatitis C virus in the region of Rajasthan. Thus there is only one case present.

- In most of the infected cases, the genotype that has shown great prevalence is from the genotype 3 in the state of Tripura. However, the lack of positive cases shows that the region is not severely affected by the Hepatitis C virus.
- In Uttar Pradesh, the genotype 3 is the most prevalent with little to no prevalence of alternative genotypes.
- Distribution of Viral Genotype in Uttarakhand with singular case shows a spread of the genotype 3
- In West Bengal, the genotype detected shows an equal spread of all types of data spread across the region. However, the rarest type of genotype 6 is not present here.

General distribution of genotype	
Genotype	Total number of samples
3	73
4	18
5	19
6	6
1 (a)	16
1 (b)	10

Table 2: General distribution of the genotype data.

Analysis of the Observations

With reference to the data, it is evident that the genotype 3 is most commonly widespread across the North Indian region of the Indian subcontinent. This is followed by the spread of the genotype 4 and 5. This figure is followed by the distribution by the subtype of the genotype 1 which is 1 (a). Genotype 1 is known to be very common globally. However, this figure undergoes variance based on the location and the mutation rate of the virus. Then it is seen that the genotype 1(b) is quite prevalent. The rarest and least commonly occurring genotype is genotype 6, as is evident by the table above. Thus, Most of the North Indian population can be said to have a viral spread of the genotype 3 and is affected greatly by the same.

Significance of the Study

Future Prospects of the Genotype Study: Immunization of a community is one of the first and most crucial steps that must be taken into account if the spread of the virus is to be stopped on the whole. In order to ensure successful immunization, it is crucial to have a complete in-depth knowledge of the virus and the manner in which it infects the individuals the come into contact with it. Having suitable knowledge about the receptors that the virus targets, in addition to the combination of the proteins of the viral coat can help come up with a suitable immune response that helps to eradicate the virus. The genotype information of the HCV virus in an area specific manner can greatly help through the usage of the making area specific immunization strategies [23]. The genotype data can also assist in determining how therapy has worked for each of these individuals based on the disease prognosis. This can help in the assessment of the area specific risk factors pertaining to the Hepatitis C virus that the individuals may face. This may give rise to mass therapeutic schemes that help to curtail the entire spread of the virus in the region itself eventually curtailing epidemics. Also, similar modes of infection as well as similar pathways can be identified and targeted in a number of cases in order to come up with broadly specific therapeutic approaches in order to tackle the infection of the Hepatitis C virus on the whole. Therefore, the study of the distribution of the genotype of the hepatitis C virus can have a number of positive implications for the current and future incoming therapeutic areas in the zone of anti viral therapeutic procedures.

Limitations of the study: It must be taken into account that in a period of four months a very minimal sample size can undergo assessment. There is no specific control over the data that may come into the system of diagnostics. This means that the data that can be accounted for may not always have the result that the analyst is looking for. For example, in this case it was difficult to make sure that all the patients that were opting of the genotype detection method of Hepatitis C virus diagnosis were actually positive for the infection in the first place. A lot of patients with negative analysis would only increase the sample size but contribute minimal to no significant input in the study of the genotype information. Another issue that could raise concern is that fact that there is limitation to the data which can be obtained about the entire health history of the patient. This makes it difficult to understand whether the infection was the result of a viral co-infection or whether it was individually acquired. This could pose a barrier in coming up with significant conclusions about the data provided.

A way in which this can be improved can be to take into consideration all the factors including patients' medical history as well as the genetic history of the family of the patient. This can also give information such as susceptibility to infection based on the history that the patient and their family. Furthermore, another point of improvement could be using a Polymerase Chain Reaction kit that actually maps all 8 genotypes of the data that have been standardized. This can give further insights into mutation rate and can help predict the subunits of the population that are exposed to certain types of viral infections.

Regardless of all of the above, the genotype data would still be valuable information when designing area specific remediation strategies to make sure therapeutic measures are able to reach all individuals of a specific location.

Conclusion

Therefore, in conclusion it is important to account for the significance of the genotype data of the Hepatitis C virus as it gives a valuable understanding not just about the spread of the epidemic but also the mutation rate of the virus. Having a good understanding of the mutation rate of the virus can also easily help individuals to study the genetic makeup of the other virus strains that may be prevalent in the areas. In addition to these suitable environmental conditions for the spread of the viral infection can be determined. Even if a significant study of the genetic build-up of other viral strains belonging to the same region may not be completed; at least suitable predictions can be made about the nature of the viruses of the region. The goal of therapeutics is always to eradicate or at least minimize the spread of an infection and it can be effectively done through the collection of data that can be analyzed. This can be of great significance to the therapeutic and antiviral industry on the entirety [26]. The distribution of the genotypes of Hepatitis C virus therefore can help to assess the data that can be used for a number of therapeutic interventions in the future. Thus, the genotype distribution of the virus can have a significant impact on the current and future implications in the areas of therapeutic approaches.

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