

Mutations in Hepatitis Virus Subgenotypes Structure in Patients with Chronic Hepatitis CGhulam Mustafa¹, Mohammad Zafar Majeed², Mohammad Bilal Ghafoor³, Abida Parveen⁴**ABSTRACT**

Background: It has been studied that the most common cause of chronic hepatitis in Pakistan is hepatitis C infection. Similarly studies have shown that the commonest etiology of cirrhosis liver in our country is hepatitis C. Usually virus's genotype remains the same. Mutations in the sub genotypes can occur at random. Mutations can also occur in response to circumstances. These mutations are responsible for the response towards treatment. The aim of this study was to find out any mutations in sub genotypes in patients of chronic hepatitis during the course of their treatment.

MATERIALS AND METHODS: The study was conducted in the Department of Medicine, Sheikh Zayed Medical College, RYK in collaboration with community medicine dept. Eight hundred patients who were suffering from chronic hepatitis C were included in the study. There were 365 males and 435 females. Their ages ranged from 26 to 64 (mean 38)

RESULTS: Most of the non-compliant patients were women and the reasons they give for omitting the doses were forgetfulness, illiteracy, lack of money and poverty, side effects of the drugs. This resulted into failure to attain the cure rate and could not attain the desired decrease in viral load. This also increased the no. of undetected sub genotypes. In some patients only the undetected sub genotype was found while in others there were undetected sub genotypes in addition to the previously detected variety. This lead to either resistance to the medication or prolonged time taken to achieve the desired result.

CONCLUSION: Genotype 3a had the highest rate among the study subjects followed by 1b. Non compliant patients showed mutations and resistance to the treatment

Keywords: Chronic hepatitis, hepatitis C, genotype

INTRODUCTION:

The frequency of hepatitis C viral infection is increasing day by day globally (1). Hepatitis C

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virus belongs to family Flavivirida and genus Hepacivirus (1). It is single-stranded RNA virus and is enveloped. There are six genotypes (1,2,3,4,5,6) known which have further been divided into various sub genotypes (2). About hundred subtypes of hepatitis C virus have been identified. Some researchers claim that there are 11 genotypes while others prove that they are actually the subtypes of genotype 6. Many studies are being done on sub genotypes which reveal that there is different geographical distribution and prevalence of genotypes and their subtypes in various parts of the world (3). Due to its diversity in structure and continuous mutations, no vaccine has been developed against hepatitis C virus to date (3).

Manifestations of hepatitis C virus vary. Most of the patients are asymptomatic. Others present with acute, acute on chronic or chronic hepatitis. Chronic hepatitis may progress to cirrhosis liver or fulminant hepatitis (4). During course of the disease almost 80-90% develop chronic hepatitis. Out of these about 15-20% develop cirrhosis and ultimately the devastating sequel.

In patients with chronic hepatitis C 1-4 % develop approximately 3% people throughout the world are suffering from hepatitis C infection (6). Usually virus's genotype remains the same. Mutations in the sub genotypes can occur at random. Mutations can also occur in response to circumstances. These mutations are responsible for the response towards treatment. New HCV treatments are considering all these problems and include more than one drug to be safe from drug resistance (7). Missing the doses during therapy can cause genetic mutations, which cause resistance to medicine. Genotype 3 shows more rapid progression to cirrhosis liver and hematoma . It is more common in Europe and South Asia. People carrying genotype 3 are the most difficult patients to treat if they have been previously been treated, have cirrhosis or have decompensated liver disease (8). Genotype 3 requires longer duration of treatment and rarely gets complete cure especially patients with cirrhosis (9).

It has been studied that the most common cause of chronic hepatitis in Pakistan is hepatitis C infection. Similarly studies have shown that the commonest etiology of cirrhosis liver in our country is hepatitis C (9, 10).

Real – time PCR is the gold standard method in identifying hepatitis C virus and its genotypes. It is cost effective and technically easy to perform. It is important to know the genotype before starting antiviral therapy (11) . The diagnosis of certain genotype and its specific subtype is necessary to assess the type of treatment, the patient needs . It also helps to chose the duration of treatment . Some studies have proved that if the physician knows the exact subgenotype the patient benefits a lot (12). But studies have shown that during the course of treatment some patients may have mutations of hepatitis C subgenotype which may effect the treatment strategy and so it is important to get the genotyping done after 2-3 months of the start of the treatment (13). The aim of this study was to find out any mutations in subgenotypes in patients of chronic hepatitis during the course of their treatment .

MATERIALS AND METHODS:

The cross sectional study was conducted in the Department of Medics, College RYK in

hematoma (5). WHO has estimated that College in collaboration with medical ward. Ethical committee of the college approved the study. Verbal consent was taken from all patients . Study was explained to them. Researchers bore the expenses of the study.

Eight hundred patients who were suffering from chronic hepatitis C were included in the study. There were 365 males and 435 females. Their ages ranged from 26 to 64 (mean 38).

Inclusion Criteria : Patients who were detected to be suffering from hepatitis C for more than 6 months and were being prescribed medication for chronic hepatitis C.

Exclusion Criteria : Patients who were already on treatment were not included in the study. Similarly patients who were detected to have hepatitis B in addition to hepatitis were also excluded from the study.

The patients were examined in the medical OPD and were diagnosed to have chronic hepatitis were sent to pathology department for the diagnosis of viral load of hepatitis C virus, genotyping and sub genotyping. Treatment was prescribed and patient was asked to visit OPD every month. The patient was asked whether they took the medicine regularly or skipped any dose. Genotyping and viral load was advised every month and record of follow up was kept. PCR was done by conventional method so as to finf the sub genotyping by gel electrophoresis.

RESULTS:

The distribution of sub genotype of hepatitis C virus in patients having chronic hepatitis is shown in Table 1. The most common type of sub genotype is 3a followed by genotype1b in our study subjects. However 30 males were found to have undetected genotype.

Table 1: Distribution of sub genotypes in study subjects.

Gender		Sub genotype (n)				
		1a	1b	2		3a
Male	20	85	15	200	15	30
Female	30	65	20	300	20	
Total	50	150	35	500	35	

All patients were asked to get their genotyping and viral load done after every 4 weeks. They were advised not to miss their doses. But unfortunately 75% of our study subjects were found to have undetected novel genotypes after 16 weeks of the treatment (Table 2) . Most of the non-compliant patients were women and the reasons they give for omitting the the doses were forgetfulness, illiteracy, lack of money and poverty , side effects of the drugs .This resulted into failure to attain the cure rate and could not attain the desired decrease in viral load. This also increased the no. of undetected sub genotypes. In some patients only the undetected sub genotype was found while in others there were undetected sub genotypes in addition to the previously detected variety. This lead to either resistance to the medication or prolonged time taken to achieve the desired result.

Table 2: Mutations found in genotypes of study subjects

Genotype repeated tests (weeks)	Mutations in sub Genotypes in compliant patients	Mutations in sub genotypes in noncompliant patients (type 1)	Mutations in sub genotypes in noncompliant patients (type 2)	Mutations in sub genotypes in noncompliant patients (type 3)
04 weeks	None	None	None	0.1 %
08 weeks	None	0.25%	None	0.5 %
12 weeks	None	0.25%	0.1%	1.0 %
16 weeks	None	0.4%	0.2%	1.0%

DISCUSSION:

It has long been known that what is the importance of identifying the exact genotype and subtype of hepatitis C virus . If the doctor knows the genotype , it gives important information and this helps patients find the most effective treatment (14). All six types of genotypes can cause chronic hepatitis . However, cirrhosis liver is commonly seen in people suffering from genotype 1 (especially subtype 1b) and 3. Studies have proved that genotypes 1b and 3 may also cause hepatoma (15). Hepatitis C Virus the has capability of replicating within the body and makes its own copies. Direct-acting antivirals stops the hepatitis C virus to make its copies within the human body . The medicine binds to proteins in the virus and blocks the viral replication (16). Then immune system of the body removes the virus

out of the patients body. However, pangenotypic direct acting antiviral have lately been introduced which are almost equally effective in all genotypes . But the problem in the treatment of chronic hepatitis due to HCV infection is not simple. Many patients do not take the drugs regularly this leads to the occurrence of mutations while replication of the HCV in the body (17) . This may lead to the formation of novel sub genotypes . These novel sub genotypes are either resistant to the treatment being prescribed or show delayed cure rates. This change in the firstly diagnosed genotype has hazardous effects on the patient treatment regimen (18) . So, it is necessary that the patients should be monitored properly and should be ensured that they are taking medicine according to the prescribed regimen. Non-compliant patients result in the development of mutated subgenotypes which may hinder the mechanism of action of the antiviral drug. Thus, it is necessary to get the viral load and genotyping done regularly during the course of treatment (19). People of different races and ethnic groups may carry any genotype or subtype (20) . However, some genotypes may be more prevalent in some races e.g; approx 90% of Africans have genotype 1 and about 60% of Caucasians carry genotype 1. People can be exposed to varying subtypes . Those who frequently travel between regions can carry different subtypes or may carry mixed infections i.e; more than one genotype may be present in them . Another reason for having been contracted with varying strains is through contaminated blood products transfusions, renal dialysis, or the sharing of needles by drug abusers (21). Usually virus's genotype remains the same. Mutations in the sub genotypes can occur at random. Mutations can also occur in response to circumstances (22). These mutations are responsible for the response towards treatment. New HCV treatments are considering all these problems and include more than one drug to be safe from drug resistance. Missing the doses during therapy can cause genetic mutations, which cause

resistance to medicine. Genotype 3 shows more rapid progression to cirrhosis liver and hematoma . It is more common in Europe and South Asia. People carrying genotype 3 are the most difficult patients to treat if they have been previously been treated, have cirrhosis or have decompensated liver disease. Genotype 3 requires longer duration of treatment and rarely gets complete cure especially patients with cirrhosis(23).

A physician should confirm whether the HCV infection is chronic and whether they have been cured of the virus. Genotypes 1(subtypes 1a and 1b) and 3 requires additional blood tests to diagnose whether the viral genotype has any resistance or not (24). Hepatitis C Viral infection treatment and laboratory diagnosis has become cost effective and safer . The medicines are freely available throughout the world . Various studies have proved the following effective medicines for different genotypes:

- Genotypes 1 through 4: Sovaldi, Viekira XR and Technivie , Harvoni , Olysio
- Genotypes 1 or 4: Zepatier
- Genotypes 2 or 3: Sovaldi , Daklinza
- Genotype 6: Harvoni

Birth defects and abortions are caused by Ribavirin. So pregnant ladies cannot use it. Ribavirin stays in the blood for many months (25). Scientists have researched that” With hepatitis C treatment, the most important thing a person can do to be cured is not to miss taking any of their medication—and to finish all of it” (26).The other studies have proved that,”Just like people, viruses do not live forever; they are constantly reproducing. Hepatitis C drugs work by blocking different steps in the virus life cycle; this prevents HCV from making more of itself. Once the virus stops reproducing, it dies off. After both of these things happen, a person is cured. People need to stay on HCV treatment for a certain amount of time to make sure that drugs can get the job done. Hepatitis C treatment lasts from

eight to 24 weeks. (Researchers are looking at even shorter treatment.)”(27).Research work has proved that “Each day, HCV makes billions of copies of itself. Some of these copies are not the same as the original virus. They have changes, called mutations. Some mutations are harmless, but others can stop hepatitis C drugs from working (called drug resistance).When people miss doses of their hepatitis C treatment, the virus gets a chance to reproduce. Some of the copies it makes might have mutations that cause drug resistance. Drugs can stop working if changes in the hepatitis C virus make it resistant to the drugs.Some people have drug resistance even though they have never been on hepatitis C treatment. Many of them have been cured anyway. But most people who are not cured will have resistance to one or more of the hepatitis C drugs they took. Resistance to certain hepatitis C drugs can disappear within months. But resistance to other drugs can last for years and might prevent re-treatment from working.”(28). Non – compliance is the major cause mutations during the treatment of chronic hepatitis. One of the major problem has been found out in various studies is to simply forget to take medicine. Studies have also revealed other reasons of lack of maintaining the optimal levels of medicine in the body, including: being sick of side effects from medication; feeling better before the completion of the course of treatment; illiteracy and irresponsible behavior; to hide from other people that one is taking medication; not wanting to take several different drugs at various times, with or without food; poverty or loss of employment; frequent traveling to different regions for employment and undiagnosed mental problems (29). Same problems were found in our study.In our study, the conventional method was used to identify genotype and its subtypes. While in most of the studies automated RT-PCR method was used (13, 17, 23) . Both methods give results in a short time and a user independent and standardized system. Both of the methods give precise and accurate results. In a study, it was determined that the commonest genotype in the CHC patients was genotype 1b and

that genotype 3 followed it (14) . This was contrary to our study in which commonest variety was genotype 3a followed by genotype 3b. When our genotype 3 detection frequency (Table 2)was compared with other studies (14, 18,22), it was most similar to the rate reported in our study. In Northern areas of Pakistan, Karachi and Lahore being the leading areas that attract tourists from all over the world , where genotype 1b is dominant in recent years may explain the high detection rate of this genotype in our study. In our study, genotype 3 was found in 63.39%, genotype 1 in 30.98% and almost same are our results in chronic hepatitis C patients undergoing treatment.

Conclusion:

Genotype 3a had the highest rate among the study subjects followed by 1b . Non compliant patients showed mutations and resistance to the treatment. This important finding indicates that this study may be effective in the migration and tourism genotype change and needs repeat testing in HCV-infected patients at regular intervals in our country.

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