Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.4194

CORRELATION OF GENOTYPES, ANTIVIRAL DRUGS, GENDER AND AGE DIFFERENCES IN OLD AGED HCV PATIENTS OF SIALKOT, PAKISTAN

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Abstract
The Hepatitis C virus contains RNA as its genome that can cause liver injury at the cellular level. Inflammation caused by viruses can progress to lethal conditions such as fibrosis, cirrhosis and cancer. Various direct-acting anti-viral drugs are available in the market in many regions of the world. These drugs work on principle by targeting specific proteins which damage the virus. Many drugs are administered in combination and produce a sustained virological response in 12 weeks. A follow-up research was performed to evaluate 3 months of treatment outcome of some direct-acting antiviral drugs in 131 patients (110 patients of HCV genotype 3) above 50 years and they were compared related to type of genotypes, administration of antiviral drugs, gender and age differences. 115 patients yielded a pooled SVR of more than 85% after twelve weeks of commonly used antiviral drug evaluation. Commonly used antiviral drugs in Sialkot, Pakistan, showed effectiveness in HCV patients having genotypes 1-5.

Keywords: Hepatitis C virus; genotype 3; direct-acting antiviral drugs; age; gender

Introduction

Hepatitis C virus is a blood-borne infection (1) caused by the hepatotropic RNA virus (2). It is a major worldwide pathogen belonging to the Flaviviridae family*ae* in the genus *Hepacivirus* (3). Infection can lead to fatal conditions. Humans are the only hosts of the hepatitis C virus, and the liver is their primary target (4). Under infection, adaptive and innate immunity are both affected (2). Unsterile medical procedures, unsafe drug use (5) and sexual intercourse (rarely) (3) can lead to a higher

incidence of infection. For diagnostic purposes, serum HCV antibody testing, HCV RNA measurement, genotyping of viral population and assessment of resistance-associated substitutions are used (6). In the past, the majority of HCV cases were difficult to treat until the availability of the prophylactic vaccine discovery (5). A person containing hepatitis C virus is a major challenge in treatment because it remains undiagnosed for a long time and reaches an advanced stage, which is difficult to manage (7). Even treated cases of hepatitis C can also suffer from fibrosis, cirrhosis and cancer in future (8). Patients with a history of 30 years of infection are more at risk of developing hepatic fibrosis and cirrhosis (9). Annually, many deaths are reported globally due to hepatitis viral infection, especially at chronic stages (10). These deaths are more common in old age as compared to young age (8). There is an annual increase of 3-5% risk of liver cancer in those patients who are diagnosed with liver cirrhosis (9). Hepatocellular carcinoma development can occur in the advanced stage of hepatitis C viral infection, with an incidence rate of 20% in developing countries and less than 20% in developed countries (1).

The genome of hepatitis C virus contains nearly 1000 nucleotides, which encode 3000 amino acids to make 10 types of proteins by cleavage action of both host and viral proteases (9). Seven genotypes (1-7) have been discovered so far related to hepatitis C (1-3), although another was also discovered in India (eighth genotype) (3), with many differences among each other. These genotypes are different based on the difference of one-third of nucleotides among them (11). Even the main genotypes have sub-types such as 1a, 1b, 2a, 3a, etc., especially in developed countries[1]. Genotypes 1-4 and 6 contain multiple sub-types with 15-25% differences at the genomic level, whereas genotype 5 has only 1 sub-type identified. Genotype 1 is more prevalent in Australia, North America and Europe, with a percentage of 46% (12). Genotype 3 is more prevalent in South Asia, with a percentage of 45-79% (13). Genotypes 7a and 7b were discovered in 2006 in Congo (14). 23% of cases revealed genotypes 2, 4 and 6, followed by less than 1% in genotypes 5 and 7 (3).

Due to complex interactions among environmental factors, virus and host, the rate of liver cancer varies in chronic hepatitis C patients, depending upon multiple factors such as lifestyle, chronic stage, gender and age factors (15). Similarly, the progression of infection towards cirrhotic conditions also depends upon the age of patients (16). Below 40 years, patients have less chance (5%) of developing cancer as compared to those above 40 years (20%) (1). Another study revealed the prevalence of HCV antibodies as 10% in patients aged 15-59 years compared to 0.4% in patients aged 1-14 (17).

Administration of direct-acting anti-viral drugs regimen revolutionized the treatment of hepatitis C in the current era (8). Interferon-free direct-acting antiviral drugs increase the chances of HCV elimination from the body and can prevent infection progression (18). Countries having the availability of direct-acting antiviral drugs ceased the use of interferons as a treatment for hepatitis C (9). Various direct-acting antiviral drugs are effective in combination for a complete cure (19). They target three proteins that are necessary for the life cycle of the hepatitis C virus and inhibit their life cycle, leading to decreased viral load in the blood of patients (20). These proteins include NS3/4A proteases, NS5A proteins and RNA dependent polymerase NS5B proteins (5). NS3/4A protease is necessary for viral polyprotein processing (2, 21). NS5A phosphoprotein is necessary for regulation of replication of virus assembly (2, 22). Viral RNA-dependent polymerase is necessary for catalysis of genome replication (2). Identifying some non-structural proteins played a key role in developing direct acting antiviral drugs which act as inhibitors to proteins of hepatitis C virus (23). Some inhibitors of NS5B nucleotide polymerase include sofosbuvir and MIV-802. Some inhibitors of NS5B non-nucleotide polymerase include dasabuvir. Some NS3/4A protease inhibitors include simeprevir, paritaprevir, grazoprevir, glecaprevir and voxilaprevir. Some inhibitors of NS5A include daclatasvir, ledipasvir, ombitasvir, velpatasvir, elbasvir and pibrentasvir (9). Direct acting antiviral drugs displayed more 95% cure rate, although infection can also reoccur in later conditions (2). This study is aimed to evaluate the outcome of direct-acting antiviral drugs after 12 week of treatment in hepatitis C patients, especially in genotype 3 patients.

MATERIALS AND METHODS

Direct Acting Antiviral Drugs

Current direct acting antiviral drugs available in the local area are mentioned in table 1.

Follow-up

Patients started on treatment with direct-acting antiviral drugs were reviewed after an evaluation based on the clinical data. They were followed up after 3 months of treatment.

Inclusion criteria

Patients reporting to the outpatient department of Pak Medical Centre, Sialkot were interviewed and examined by the medical officers offering registration to the research enrolment. Presumptive Hepatitis C positive cases of ages above 50 years, identified by using the standardized WHO/Hepatitis Control Program (HCP) clinical diagnostic algorithms were enrolled. Consent in writing was obtained from all the participants. Patients with reactive HCV on ELISA and ages above 50 were enrolled in this study. Participants with high ALT levels (1.5 times more than the normal range) with a difference of 6 months and patients with co-morbidities like well-controlled diabetes and hypertension were included in this study.

Exclusion criteria

Not agree to participate in research work at any stage of treatment. Patients having platelets count less than 50,000/cubic mm. Patients with moderate to severe hepatic or renal insufficiency. Patients co-infected with HBV. Pregnant females were not enrolled in this study. Patients having either extrahepatic malignancy or hepatocellular carcinoma.

Statistical analysis

Statistical analysis was performed with Graph Pad software, and all data of groups were expressed as mean ± SEM. For statistical analysis, groups were compared by unpaired t- test (two-tailed) with 95% confidence interval. $P \leq 0.05$ was the threshold for statistical significance.

RESULTS

Available Direct Anti-Viral Drugs in Local Area

Locally available direct acting anti-viral drugs have been mentioned in table 1 with their administration on patients (ages and genotypes mentioned).

Table 1: Treatment administered to hepatitis patients of different genotypes with ages in local area

Genotypes	Ages of patients (Years)	Duration of Treatment	Medicine Names
1, 3, 5	51, 52, 53, 54, 55, 56, 57, 60, 61, 62, 63, 65, 67, 68, 70, 71, 72, 75, 79, 88	12 weeks	Sofomac 400mg + Maclinza
3, 5	51, 54, 55, 58	12 weeks	Vierof 400mg + Ecavir
3, 4, 5	52, 55, 56, 57, 58, 60, 65, 67, 70, 72, 80	12 weeks	Zoval 400mg + Dakvir
3, 4, 5	51, 52, 53, 55, 56, 58, 60, 62, 63, 65, 70, 75	12 weeks	Maclusa 400mg + 1000mg
3, 5	52, 55, 60, 63, 65, 70, 75	12 weeks	Tefod Tablet
3	55, 64, 66, 70	12 weeks	Sofosbuvir 400mg
3	52, 60, 62	12 weeks	Zoval 400mg + Daklana
2, 3	52, 53, 54, 55, 56, 57, 60, 72, 75	12 weeks	Vierof 400mg + 100mg

Efficiency Rate of administration of Drugs in Patients

Table 2 shows 3 months of treatment outcomes of 131 patients with numbers and percentages related to both groups, one which had developed SVR (115 patients) and the other had not (12 patients).

Table 2: Available direct acting anti-viral drugs in local area with their numbers and percentages of administration of drugs to Hepatitis C patients

DAA Regimen	All Patients (n = 131)	SVR (n = 115)	No SVR (n = 12)
Sofomac 400mg + Maclinza	65 (49.61%)	60 (52.17%)	5 (41.66%)
Vierof 400mg + Ecavir	4 (3.05%)	2 (1.73%)	2 (16.66%)
Zoval 400mg + Dakvir	14 (10.68%)	12 (10.43%)	2 (16.66%)
Maclusa 400mg + 1000mg	25 (19.08%)	23 (20.00%)	2 (16.66%)
Tefod Tablet	8 (6.10%)	8 (6.95%)	0 (0%)
Sofosbuvir 400mg	4 (3.05%)	4 (3.47%)	0 (0%)
Zoval 400mg + Daklana	3 (2.29%)	3 (2.60%)	0 (0%)
Vierof 400mg + 100mg	10 (7.63%)	8 (6.95%)	2 (16.66%)
Follow-up lost		4 (3.05%)	

Age Wise Distribution of Genotypes in Hepatitis C Patients

Table 3 shows age wise distribution of genotypes in hepatitis C patients. Genotype 1 patients range from 60 to 61 years. Age of genotype 2 patient is 75 years. Ages of genotype 3 patients range from 51 to 80 years. Ages of genotype 4 patients range from 56 to 58 years. Ages of genotype 5 patients range from 52 to 88 years.

Table 3: Age wise distribution of genotypes

Ages of patients (Years)	Genotypes
60, 61	1
75	2
51, 52, 53, 54, 55, 56, 57, 58, 60, 62, 63, 65, 66, 67, 68, 70, 72, 75, 79, 80	3
56, 58	4
52, 58, 60, 63, 67, 75, 88	5

Gender Wise Distribution of Genotypes in Hepatitis C Patients

Table 4 is showing gender gender-wise distribution of genotypes in hepatitis C patients. There were 4 patients (50% males and 50% females) of genotype 1 with a mean age of 60.05 years. There was 1 patient of genotype 2 with mean age of 75 years. There were 110 patients (49.09% males and 50.90% females) of genotype 3 with mean age of 63.15 years. There were 2 patients (50% males and 50% females) of genotype 4 with mean age of 57 years. There were 10 patients (70% males and 30% females) of genotype 5 with mean age of 66.14 years.

Table 4: Gender wise distribution of genotypes

Gender of Patients	Genotypes	Average Age (Years)
4 (2 Males + 2 Females)	1	60.05
1 (1 Male)	2	75
110 (54 Males + 56 Females)	3	63.15
2 (1 Male + 1 Female)	4	57
10 (7 Males + 3 Females)	5	66.14
4	Missed data	

Discussion

Hepatitis C is one of the fatal diseases of the liver in which the organ suffers from infection, which leads to inflammation, fibrosis, cirrhosis and cancer in later stages (24). During infection of hepatitis virus, the risk of morbidity and mortality is determined by the stage of fibrosis of the liver (25). The severity of the disease depends upon the entry and spread of the virus inside liver tissues (24). Different viruses have different genotypes, so their penetration capability in the liver varies. Only genotype 1 was studied via successful development of in-vitro and in-vivo models compared to other

genotypes, especially genotype 3 (26). In addition, genotype 3 was the least studied regarding follow-up outcomes in patients. Cirrhosis occurs in one-third of hepatitis patients of genotype 3, especially in Asian countries, and more than half of hepatitis patients with a diagnosis of liver cancer were genotype 3 as compared to genotypes 1 and 2. The mechanism of insulin resistance and lipids are interlinked with genotype 3; hence, much work is required to determine the complete pathogenesis of the genotype 3 virus in the body. For further studies, genetic mechanisms may reveal a link between risks of liver cirrhosis/cancer and HCV genotypes, as evidenced by a study (27). Due to this link, hepatitis C is difficult to cure. Though new treatments have evolved but still hepatitis C is still a major challenge to medical science.

Insulin resistance and lipid metabolism modifications are distinct characteristics of genotype 3, which may lead to a lower rate of cure in hepatitis C patients. Multiple potential aspects of genotype 3 of HCV are still unknown.

Age has a close connection with the progression of liver problems, especially in viral hepatitis (27). Higher age patients are difficult to cure compared to lower or young-age patients. This study included patients above 50 years of age and was divided into two groups based on treatment outcome after 12 weeks. In elder age, genotype 3 is more common than other genotypes (27). Similarly, in the current study, genotype 3 patients were more common in old age than other genotypes. In different studies, genotype 3 was also found to be more common in the same region in lower age groups (28, 29). For the treatment of viral hepatitis, the development of direct-acting antiviral agents is of much interest and improved the prognosis of HCV patients, especially chronic cases (26, 30). Virulence and disease persistence depend upon the virus penetration ability into host liver cells via blood (26). Much work has been done on genotype 1, 2 (28) and 4 (30) patients, but little work was done in genotype 3 cases. Main purpose of the study was to evaluate the outcome of direct-acting antiviral drugs genotype 3 hepatitis C patients. The study resulted in the efficient formation of sustained virological response in most patients after 12 weeks of treatment. Treatment was successful in more than 85% of patients compared to the old study (31). Most patients in the current study were given either SOFOMAC 400mg + MACLINZA (n=65) or MACLUSA 400mg + 1000mg (n=25) for 3 months. Direct-acting antiviral drug administration, especially in genotype 3 patients, as most of the patients in the study were genotype 3 (n=110), showed SVR compared to clinical trials conducted earlier (32-35).

Conclusion

In conclusion, direct-acting antiviral drugs were very effective in treating patients of Sialkot region of Pakistan with HCV genotypes 1, 2, 3, 4 and 5, and an overall more than 85% post-treatment negative result was found after 3 months of treatment duration in patients age above 50 years. Based on the current study, long-term follow-up studies can be planned with a large perspective. Our analysis had several limitations, such as the small number of HCV-positive patients after 12 weeks of treatment is small. Genetical studies can also help to link the association between HCV genotypes and disease. It is concluded that currently available therapies for the treatment of hepatitis C are highly and productive, and it means that the progression of the disease in Pakistan is not due to weak drugs but due to limited access of people to treatment services.

Funding

Self-funding.

Competing interests

Authors have declared that no competing interests exist among them.

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