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RESEARCH ARTICLE
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STUDY OF CHANGES IN BIOLOGICAL AND HEAMOTOLOGICAL MARKERS IN PAKISTANI PATIENTS OF AGES ABOVE 50 YEARS WITH CHRONIC HEPATITIS C GENOTYPES 1-5 IN THE ERA OF DIRECT ACTING ANTIVIRAL DRUGS

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Abstract
The Hepatitis C virus is RNA containing virus which damages liver at molecular level and causes inflammatory conditions such as fibrosis and cirrhosis and can also lead to cancer. Various direct-acting anti-viral drugs available in local area were administered to patients. 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 using relevant biological parameters. 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 as evidenced by biological and haemotological markers.

Keywords: Hepatitis C virus; genotype 3; direct-acting antiviral drugs; liver function tests; renal function tests.

Introduction

Hepatitis C virus is RNA containing virus which can spread via blood transfusion and blood contact (1, 2). Flaviviridae is the family to which this virus belongs and genus is *Hepacivirus* (3). This virus spreads in its host which are humans and liver affects in this disease (4). When infection spreads, it

also disturbs immune system of host (2). Inspite of blood contact, other ways can also spread this disease such as use of unsafe drugs (5) and via sexual intercourse (rarely) (3). Measurement of HCV RNA via serum is regarded as diagnostic procedure for this disease, additionally genotypes can also be measured (6). Before discovery of vaccine, this disease was incureable in past (5). After onset of viral disease, it was very difficult to manage the case and mostly patients died of disease, especially at advanced stages (7). Even complications still resided after treatment of disease and there are equal chances of reoccurrence in patients in future (8). Patients having this disease for 30 or more years, they develop liver complications (9). These complications make treatment difficult and unable to manage, resulting in death of patients (10). Due to chronic nature of disease, this inflammation is more lethal in old age and hence, many old aged people died on annual basis as compared to young people (8). Many patients are suffered from this disease with annual increase in their percentages in different regions of world (9). Cancer can also occur at later stages of disease and this risk is increased in developing countries for many years as compared to more developed countries due to poverty and lack of awareness and medical facilities (1).

There are nearly 1000 nucleotides in RNA of virus which can make proteins of 10 types and elongate about 3000 amino acids (9). Eight genotypes have been found in different patients of hepatitis C in different regions of world (1-3). These genotypes have differences on base of number of nucleotides in them (11). Some genotypes have also sub-catagories or as their subtypes exist in different patients, based on their genotype and ethnicity (1), especially genotypes 1, 2, 3, 4 and 6 have many sub-types with minor differences at genetic level, whereas genotype 5 has only 1 sub-type. Genotype 1 is more common i.e., 46% in developed countries such as Australia, North America and Europe (12). Genotype 3 is more common in South Asia about nearly 50-80% (13). Genotype 7 has two sub types named as 7a and 7b and they were discovered in Congo in 2006 (14). Most common genotype is genotype 3, followed by genotypes 2, 4 and 6, followed by genotypes 5 and 7 (3).

Many factors contribute to pathogenesis of disease such as environmental factors, host-virus relationship, immunity of patients, life style, gender based, age differences and stage of disease (15). Development of infection is somewhat related to age of patients (16). Young people have less chances of suffering from this infection as compared to old aged people (1, 17).

Currently, there are many direct acting antiviral drugs available in market to combat disease (8). In past, interferons were used to cure disease but now currently available direct acting antiviral durgs are free from these interferons which were regarded as antiviral proteins (18). Those regions of world which have availability of these drugs are managing this disease well (9) and in some cases these drugs are administered in combination (19). When mechanism is observed, it is found that these drugs actually damage proteins which are necessary for life cycle of hepatitis C virus and ultimately killed viruses and decreased viral load in blood of affected patients with passage of time (20). Some proteins are named as NS3/4A proteases, NS5A proteins and RNA dependent polymerase NS5B proteins (5). NS3/4A protease is very necessary for processing of viral polyprotein (2, 21). NS5A phosphoprotein is much necessary for regulation of replication of viral assembly (2, 22). Viral RNA-dependent polymerase is necessary for genome replication catalysis (2). For development of these antiviral drugs, identification of some non-structural proteins is important to act as inhibitors for proteins of viruses (23). Some inhibitors of NS5B nucleotide polymerase are named as sofosbuvir and MIV-802. Some inhibitors of NS5B non-nucleotide polymerase are named as dasabuvir. Some NS3/4A protease inhibitors are named as imeprevir, paritaprevir, grazoprevir, glecaprevir and voxilaprevir. Some inhibitors of NS5A are named as daclatasvir, ledipasvir, ombitasvir, velpatasvir, elbasvir and pibrentasvir (9). Direct acting antiviral drugs are very successful in controlling this disease so far in different regions fo world (2). This study is aimed to evaluate the outcome of direct-acting antiviral drugs after 12 week of treatment in hepatitis C patients of different genotypes on base of evaluation of differences in levels of different biological and haemotological markers, especially in genotype 3 patients.

MATERIALS AND METHODS

Materials

Specimen (patient's plasma), Gloves (latex or nitrile), blood collection tubes, pipette, centrifuge, micropipettes to dispense volumes 1-1000µl, with compatible sterile filtered tips, Roche® COBOS e411 auto analyzer for ELISA, Sysmex® KX-21 / Mindray® BC5000 automated hematology analyzers, Roche® COBAS c311 auto analyzer for routine chemistry, Roche® COBOS e411 auto analyzer for ECLIA special/hormonal assays, Roche® AMPLIPREP for automated Nucleic acids extraction), in association with COBAS TaqMan® and the CEPHID Smart Cycler by Thermofisher®.

Sampling

These include patient's serum, plasma, EDTA and citrated whole blood. All the samples were subjected to the relevant diagnostic work-up. Peripheral blood was collected from each participant, and serum/plasma was stored at -80^0C for molecular assays.

Follow-up

Patients started on treatment with direct-acting antiviral drugs were reviewed after an evaluation based on the clinical, hematological, biochemical and molecular assays depending on upindividual criteria. They were followed up after 3 months of treatment.

Laboratory Methods

Hematology

CBC on Sysmex® KX-21 or Mindray® BC5000 automated analyzers.

Clinical Chemistry

Routine chemistry tests like LFTs, RFTs etc. were performed with Roche® COBAS c311 auto analyzer for routine chemistry.

Direct Acting Antiviral Drugs

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

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 (genotypes mentioned).

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

Genotypes	Duration of Treatment	Medicine Names
1, 3, 5	12 weeks	Sofomac 400mg + Maclinza
3, 5	12 weeks	Vierof 400mg + Ecavir
3, 4, 5	12 weeks	Zoval 400mg + Dakvir
3, 4, 5	12 weeks	Maclusa 400mg +1000mg
3, 5	12 weeks	Tefod Tablet
3	12 weeks	Sofosbuvir 400mg
3	12 weeks	Zoval 400mg + Daklana
2, 3	12 weeks	Vierof 400mg + 100mg

Complete Blood Count

High prevalence of different tests of complete blood count was found in post-treatment positive group compared to post-treatment negative group, having data of patients with age above 50 years, observed after 3 months of study (figure 1).

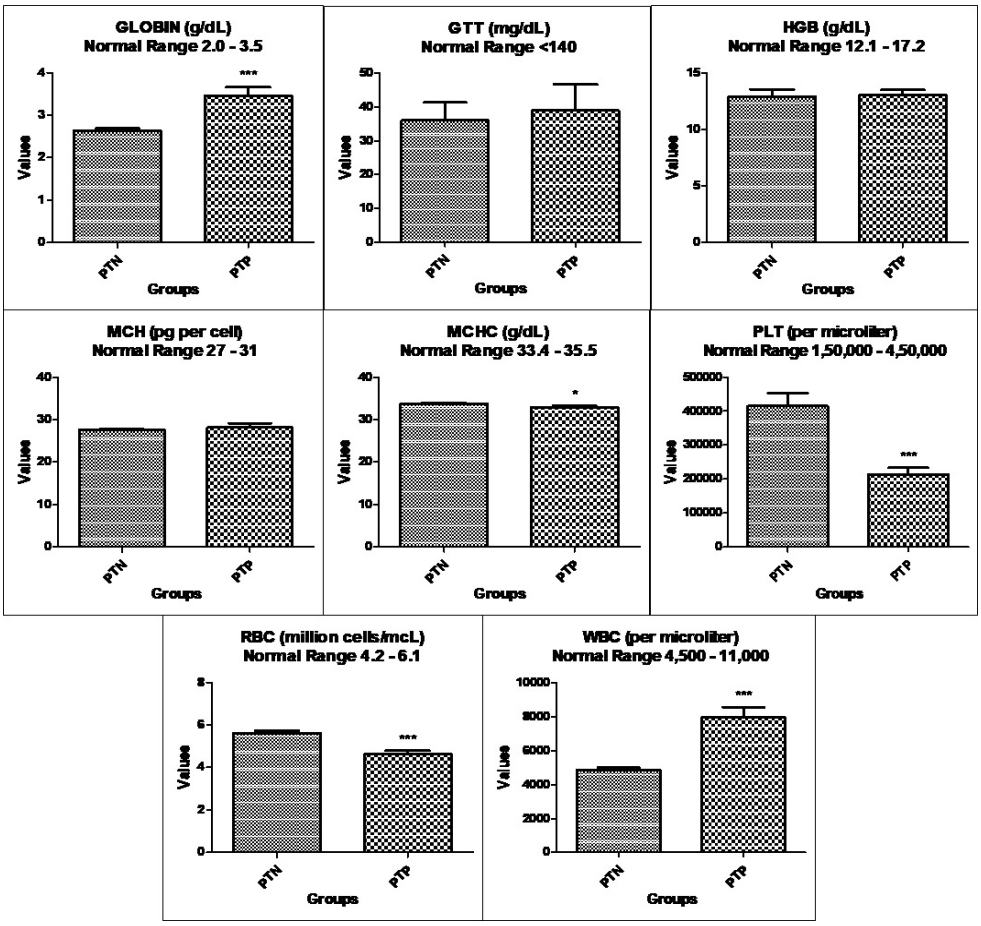

Figure 1: Complete blood count including different blood tests comparison between post-treatment positive and post-treatment negative groups. Data was calculated as mean ± SEM where $p \leq 0.05$ and * shows significance levels between two groups.

Blood Chemistry Panels

A high prevalence of different tests of blood chemistry panels (including renal function tests) was found in the post-treatment positive group compared to the post-treatment negative group, with data of patients above 50 years of age, observed after 3 months of study (figure 2).

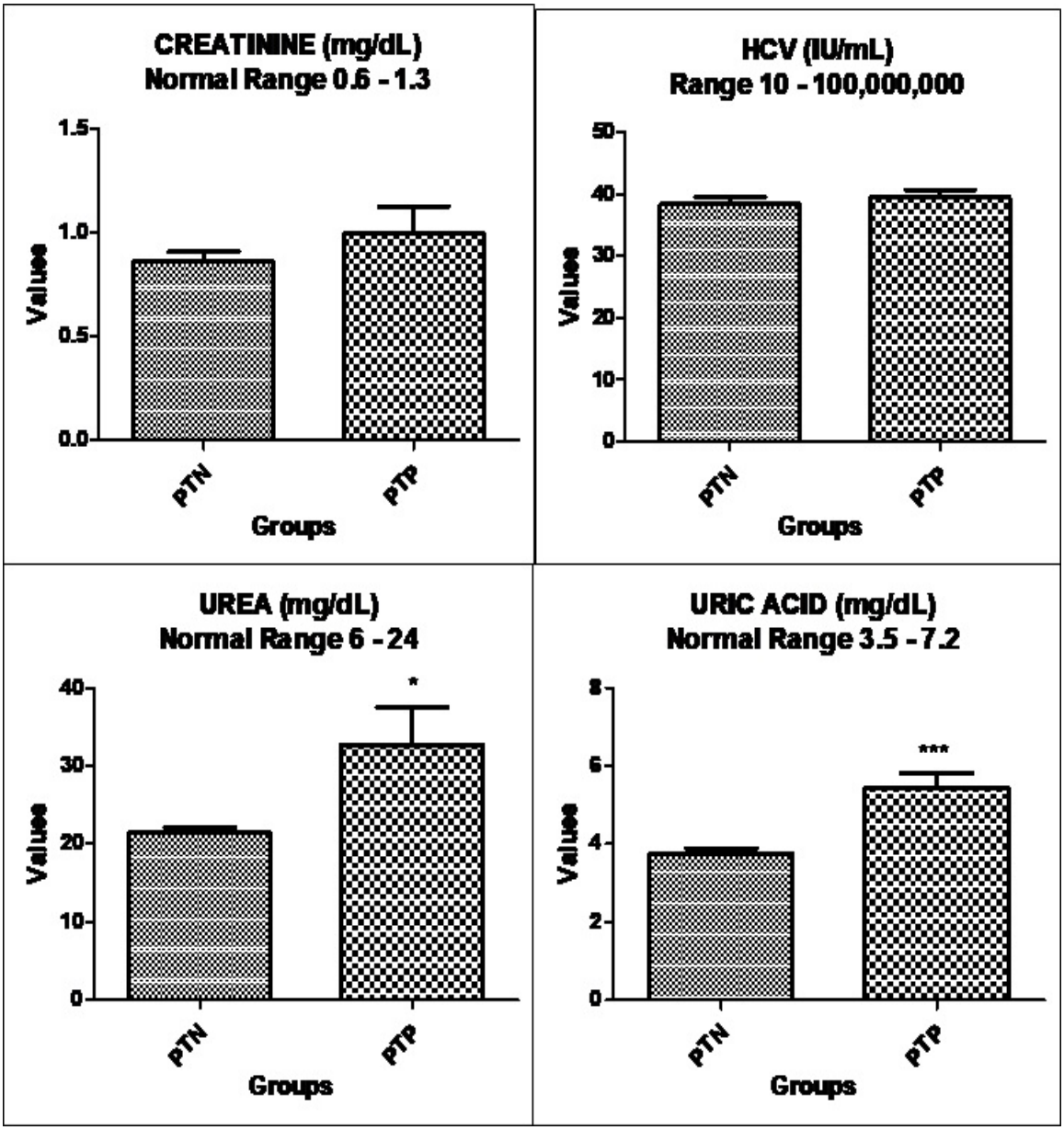

Figure 2: Blood Chemistry Panels including renal function tests comparison between post-treatment positive and post-treatment negative groups. Data was calculated as mean ± SEM where $p \leq 0.05$ and * shows significance levels between two groups.

Liver Function Tests

High prevalence of liver function tests was found in the post-treatment positive group compared to post-treatment negative group, having data of patients above 50 years of age, observed after 3 months of study (figure 3).

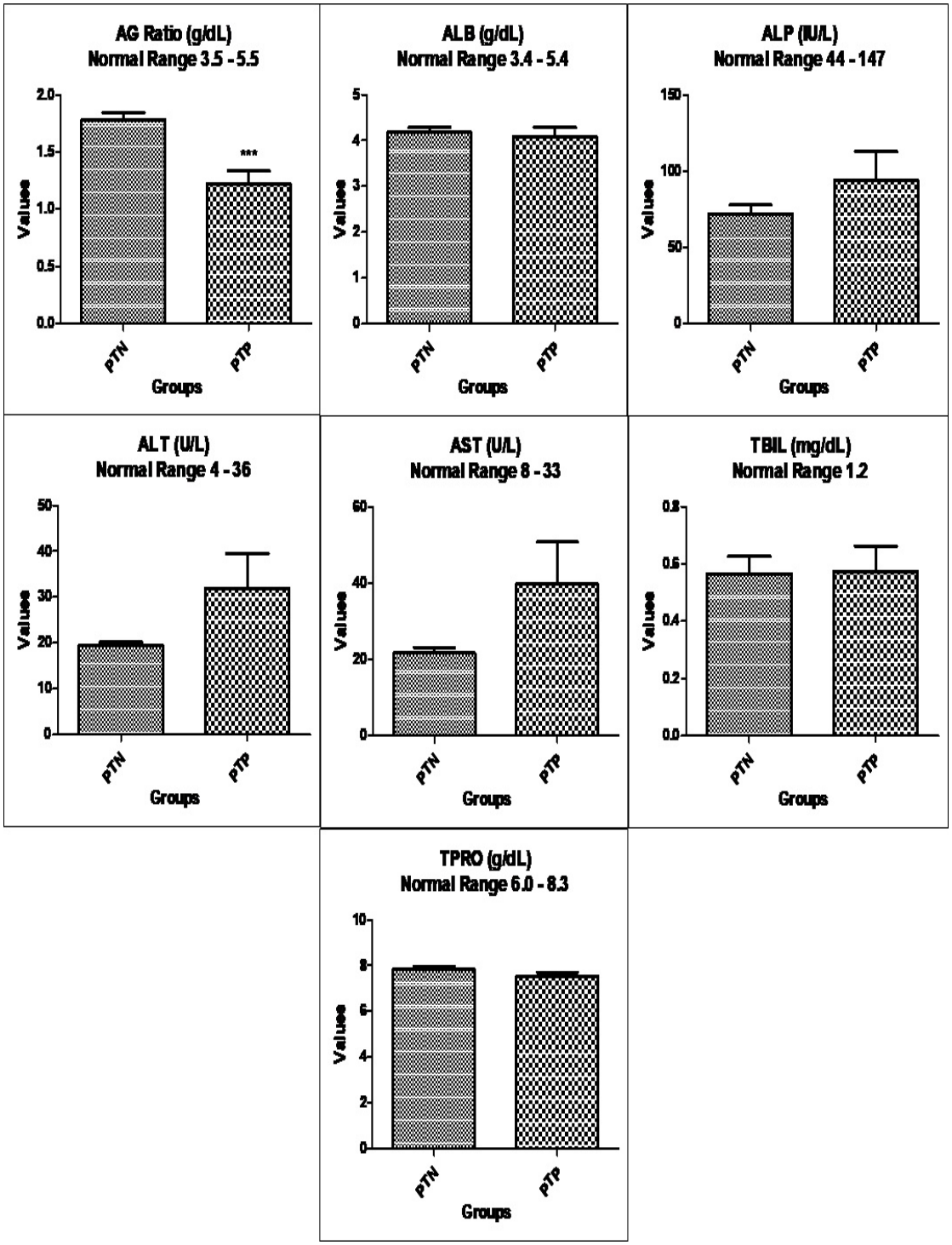

Figure 3: Liver function tests comparison between post-treatment positive and post-treatment negative groups. Data was calculated as mean ± SEM where $p \leq 0.05$ and * shows significance levels between two groups.

Prevalence of Genotypes, Medicines and Post-Treatment Evaluation

Compared with genotypes 1, 2, 4 and 5, genotype 3 patients were more found. Total six kinds of groups of direct acting antiviral drugs were used in the study. More than 85% of patients were post-treatment negative after 3 months of treatment of direct acting antiviral drugs, having data of patients over 50 years, observed in study in 3 months (figure 4).

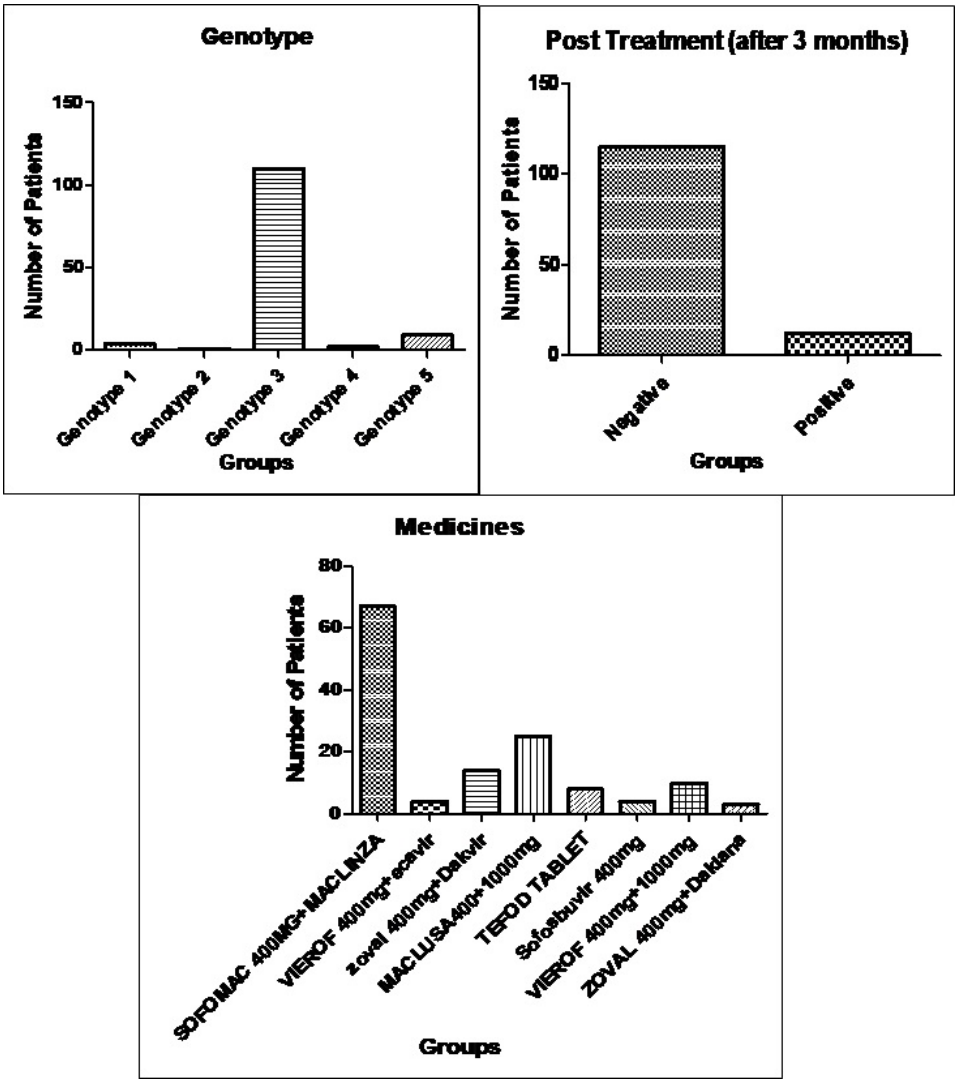

Figure 4: Comparison between post-treatment positive and post-treatment negative groups regarding prevalence of HCV genotypes, kinds direct acting antiviral drugs and number of patients in both groups taking direct acting antiviral drugs in duration of 3 months.

Discussion

Hepatitis C is fatal infection of liver in humans which become worse in advanced stages (24). Patients suffered from this disease are at risk of death, especially in old age (25). Entry and spread of virus in human body determines severity of symptoms and presence of viral load in patient's blood (24). Viruses have different types of genotypes due to different genetic makeup and different penetration ability into humans and genotype 1 was more studied as compared to other genotypes in past studies and genotype 3 was least studied (26) and least work was done on follow up results of genotype 3 patients. Genotype 3 patients are more affected from cirrhosis as compared to other genotypes, especially in Asian regions in which 50% patients of hepatitis C exist in this region. Similarly chances of formation of hepatocellular carcinoma is more in genotype 3 patients in comparison with genotype 1 and 2 patients. Genotype 3 mechanism is correlated with mechanism of lipids and insulin but their pathogenesis is not fully understood yet. These mechanisms may have connection between genotypes and onset of liver complications (27) and due to these factors this disease is regarded as difficult to cure and still this disease is challenge to medical science despite much advancements in drug development.

Old age people are more prone to this disease (27). As serological and biological markers revealed increased levels of parameters taken samples from blood of hepatitis C patients, results displayed

increased rate of SVR and patients found much recovery after administration of direct acting antiviral drugs. 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.

This study was conducted to screen potential outcomes of available direct-acting antiviral drug administration in hepatitis C patients using several biological tests and analysis approaches for 12 weeks with genotypes ranging 1-5. However the main purpose of the study was to evaluate the outcome of direct-acting antiviral drugs genotype 3 hepatitis C patients. Some biochemical and serological markers have been discussed in this study to estimate HCV prevalence among patients above 50 years of age in Pakistan and to analyze high-risk populations for disease.

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 as evidenced by different serological and biological markers. 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 and 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.

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Competing interests

Authors have declared that no competing interests exist among them.

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